MRI-BASED BRAIN MORPHOMETRY CORRELATES OF CHRONIC PAIN IN KNEE OSTEOARTHRITIS

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The aim of the wise is not to secure pleasure, but to avoid pain

Aristotle

Dedicated to my family…

ABSTRACT

Chronic pain is a complex experience that involves sensory, emotional, and cognitive aspects. The neurobiological mechanisms are therefore expected to be complex, widespread and largely maladaptive. Recent research of neuroimaging in chronic pain suggests cerebral re-organization on a structural level as a consequence of chronic pain. However, a combined and large-scale brain morphological profile in chronic pain to investigate its neural substrates has not been elucidated. The research presented aims to investigate morphological brain correlates and putatively related behavioural and cognitive aspects of chronic pain due to primary nociceptive knee osteoarthritic disorder using advanced imaging techniques for manual, voxel-based, and surface-based analysis, and questionnaire-based participants' characterization.

31 participants with chronic painful knee osteoarthritis (age= 64.6± 8.4 years, 15 females, mean duration of pain=9.6 years) and 22 healthy controls (age= 61.3± 7.5, 13 females) underwent high-resolution anatomical MRI at 3 Tesla, and detailed pain characterization and psychometric assessment.

Findings from this thesis challenge the common belief that chronic pain leads to hippocampal volume reduction and allegedly cognitive dysfunction. Indeed, general cognitive function and delayed recall memory were normally preserved in the studied cohort, and

moreover the hippocampal volume was significantly enlarged. The volume of the rostral part (emotional) of anterior cingulate showed significant positive correlation with pain catastrophizing behaviour suggesting that it may underlie the pain catastrophizing tendency in patients with chronic knee pain. Higher scores of mechanical pain sensitivity correlated with reduced cortical thickness in the anterior cingulate indicating its potential key role in the process of central pain sensitization.

Sufferers of chronic knee OA pain exhibited less grey matter volume in the left dorsolateral prefrontal cortex, which has a modulatory role in nociceptive transmission namely, pain perception inhibitory effect. Although the mechanism of this reduction is unknown, such a change may suggest functional disturbance with subsequent aberrant contribution to pain sustainability and chronification.

Whole brain cortical thickness was investigated in patients and results revealed wide spread cortical thinning progresses with pain duration, preferentially in females, and in areas largely outside the known pain matrix, but including the posterior default mode network.

Finally, preliminary results from investigating the potential mechanism of chronic pain related neocortical plasticity will be presented that may provide framework for future studies.

List of accepted conference abstracts, and prizes:

1. **Alshuft H**, Auer D, Dineen R. Hippocampal volume and asymmetry: An analysis of 3 Tesla data from healthy adults. European Society of Neuroradiologists ESNR, Scotland (September 2012)
2. **Alshuft H**, Dixon J, Condon L, Dineen R, Auer D. Brain cortical thickness correlates of chronic pain. 1st Osteoarthritis research day, Loughborough UK (October 2012)
3. **Alshuft H**, Dixon J, Condon L, Dineen R, Auer D. Brain cortical thickness correlates of chronic pain duration in knee OA. The British Pain Society annual scientific meeting, Bournemouth UK (April 2013)
4. **Alshuft H**, Dixon J, Condon L, Dineen R, Auer D. Morphometric cortical correlates of pain catastrophizing behaviour in patients with chronic painful knee OA. The International Society for Magnetic Resonance in Medicine (ISMRM) 21st annual meeting, Salt Lake city-UTAH USA (April 2013)
5. **Alshuft H**, Auer D, Dineen R. Hippocampal volume asymmetry: Is it explained by inter-hemispheric volume variation? The British Society of Neuroradiologists, Oxford UK (September 2013)
6. **Alshuft H**, Wheeler M, Kurien T, Scammell B, Dineen R, Auer D. Pain progression in knee OA: associations with morphological brain changes. 8th Congress of the European Federation of IASP Chapters, Florence ITALY (October 2013)
7. **Alshuft H**, Auer D, Dineen R. Hippocampal volume is enlarged in chronic pain due to knee OA. 15th World Congress on Pain, Buenos Aires ARGENTINA (2014)- awaiting decision

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LIST OF ABBREVIATIONS

ACC	Anterior cingulate cortex
AI	Asymmetry index
BDI	Beck's depression inventory
CT	Cortical thickness
DMN	Default mode network
DTI	Diffusion tensor imaging
DLPFC	Dorsolateral prefrontal cortex
FDR	False-discovery-rate
FOV	Field of view
fMRI	Functional magnetic resonance imaging
GM	Grey matter
HC	Healthy control
HV	Hippocampal volume
ICC	Intraclass correlation coefficient
KOA	knee osteoarthritis
LGI	Local Gyrification Index
LPD	Long pain duration
MRI	Magnetic resonance imaging
MD	Mean diffusivity
Ms	Millisecond
MoCA	Montreal cognitive assessment
MSK	musculoskeletal
OA	Osteoarthritis
QST	Quantitative sensory testing
PIF	posterior inferior frontal
PPT	Pressure pain threshold
ROI	Region of interest
SPD	Short pain duration
SE	Spin echo
SPGR	Spoiled gradient recalled echo
SPSS	Statistical package for social sciences
T	Tesla
TE	Echo time
TI	Inversion recovery
UK	United Kingdom
USA	United States of America
VBM	Voxel based Morphometry

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1 PART ONE: INTRODUCTION

1.1 Overview of the concept of pain

1.1.1 Background

Pain is essential for life. Under normal physiological conditions and in its acute form pain warns us against danger to tissue damage or life-threatening events. It also teaches us to avoid such damage. However, in contrast to acute pain, chronic pain is a maladaptive syndrome that does not serve any protective function. It is the most common reason people seek medical care. Chronic pain is a leading cause for long-term disability with direct impacts on individual's health and overall quality of life. Epidemiological risk factors include age, sex, socioeconomic factors, psychological stress (McBeth and Jones, 2007), and there is an association with increased cardiovascular risks (Cimmino et al., 2011).

It was revealed in a large European survey (Breivik et al., 2006a), that about 19% of adult Europeans had chronic pain of moderate to severe intensity affecting the quality of their social and working lives. In Europe alone it is estimated that chronic pain costs over €200 billion per year, and in the United States the cost is estimated around $ 150 billion (Tracey and Bushnell, 2009b). According to the British Pain Society, 7.8 million people in the UK live with chronic pain. Prescriptions for pain are estimated to cost around £584 million annually. Back pain alone costs the British economy around £12.3 billion per year. Additionally 49% of patients with

chronic pain experience depression, 25% lose their jobs and 16% of sufferers feel their chronic pain is unbearably bad that they sometimes want to die (BPS, 2009).

It is not understood why only some people develop chronic pain despite the fact that pain is a universal experience (Rodriguez-Raecke et al., 2009). Exhibiting discrepancy with the degree of tissue damage further complicates our understanding of chronic pain. The pain intensity in knee osteoarthritis for instance is quite variable ranging from hardly perceptible to immobilizing, and the severity of pain in knee osteoarthritis cannot often be predicted from the degree of structural damage at the joint. Several studies (Kornaat et al., 2006, Link, 2009, Phan et al., 2006) have reported no significant correlation between the severity of pain and structural knee osteoarthritic lesions.

Chronic pain is increasingly recognized as a disease in its own right (Tracey and Bushnell, 2009b, Niv and Devor, 2004), with subsequent strong research interest to characterize its neurobiological substrates. Neuroimaging has played a major role in investigating chronic pain mechanisms at supraspinal levels. Continuous advances in technology and availability of magnetic resonance imaging and dedicated image analysis software have enabled an unprecedented insight into the structural, functional and neurochemical central correlates of chronic pain.

1.1.2 Pain and chronicity definitions

The current definition endorsed by the International Association for the Study of Pain (IASP) states that pain is *"an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage"* (Merskey and Bogduk, 1994). The definition addressed the shortcomings of previous attempts that were confined to sensory aspects or limited to linguistic expressions. For example, Mountcastle (Mountcastle, 1974) defined pain as "*that sensory experience evoked by stimuli that injure or threaten to destroy tissues, defined introspectively by every individual as that which hurts*". Another more of nursing or clinical use definition was offered by McCaffrey and Beebe (McCaffery M and Beebe A, 1989) who described pain as "*whatever the experiencing person says it is, existing whenever the experiencing person say it does*".

Pain is a universal experience that is very subjective, and multidimensional in nature that involves sensory, emotional and cognitive aspects. It is important that a proper definition of pain is agreed in order to enable a meaningful scientific research and to provide adequate medical care (Price and Barrell, 2012). The IASP definition has been arguably recognized as the current international and comprehensive definition of pain.

The standard definition of pain chronicity also comes from the International Association for the Study of Pain, which describes it as pain without apparent biological value that persists beyond the normal time for healing (Bonica, 1953, cited in Merskey and Bogduk 1994). However, the healing time can vary between different types of tissue. Under optimum conditions, skin wound, for instance heals in 3-7 days, bone fracture takes about 6 weeks, and tendons and ligaments can take up to 3 months to achieve normal healing (Marcus. et al., 2009). A fixed time of persistent pain has also been proposed. For example 3 and 6 months of persistent pain in post herpetic neuralgia and back pain respectively would entitle the condition to be characterized under chronic pain state (Apkarian et al., 2009). Loeser and Melzack (Loeser and Melzack, 1999) emphasise that it is not the duration that distinguishes acute from chronic pain but rather the inability of the body to restore its normal physiological functions. In the case of painful osteoarthritis a definition of pain for most days of the prior month was used (Altman et al., 1991). However and from a neurobiological correlations perspective, it is conceivable that structural adaptive changes may take several years to develop. Moreover, duration alone regardless the persistency status may indicate episodicity rather than chronicity. Therefore the definition of chronic pain used in the experimental work presented in this thesis is: ‘primarily knee pain that lasted more than a year and

experienced most of the day on most days of the week for at least the last month' (Adapted from (Peat et al., 2006a).

1.1.3 Pain characteristics and dimensions

Chronic pain, tends to be less localized than acute pain and it often fluctuates in severity (Marcus. et al., 2009). With no clear biological function, chronic pain can persist long after the normal healing time.

As previously stated pain is a complex and multidimensional in nature that involves sensory, affective and cognitive dimensions. This model was first conceptualized by Melzack and Casey (Melzack R. and Casey K., 1968), with subsequent descriptions of 1) sensory-discriminative; concerned with the intensity, location, quality and duration of pain, 2) affective-motivational; refers to the emotional and motivational changes experienced as suffering and unpleasantness and the urge to avoid and escape it, and 3) cognitive-evaluative; relates to the judgemental aspects. Socio-cultural dimension has been considered by some as a fourth dimension, which suggests that the responses to pain is in part due to the person's social identity and expectations others may have (Lynch and Vasudevan, 1988).

1.1.4 Ascending pathways and central pain mechanisms

Noxious signals are transmitted from nociceptors mainly via afferent A-δ and C fibres to the dorsal root of spinal cord (or medulla for cranial regions) and subsequently to the dorsal horn from thence via the ascending tracts to supraspinal centres involved in the conscious appreciations of pain. Psychogenic pain, pain arising from damage to the nervous system tissue (Boivie et al., 1989), as well as vicarious pain (Danziger et al., 2009) do not however require stimulation to the peripheral nociceptors. Of note that some nociceptors called '*silent*' will become activated only if sensitized by, for example inflammation. These kinds of nociceptors are abundant in the joints and they were first recorded, in preclinical studies, from afferent nerves in the knee joint (Schaible and Schmidt, 1988). In very rare cases nociceptors can be congenitally defected (Cox et al., 2006) leading to inability of the affected person to experience pain with subsequent dramatic threatening effects.

The main ascending spinal pathways (Fig. 1), through which nociceptive information is transmitted from the body to the brain include the spinothalamic tract (STT) that is further subdivided into lateral and medial parts, spinoreticular (SRT), and spinomesencephalic (SMT) tracts. The lateral STT is considered

phylogenetically recent, and hence called neospinothalamic tract (nSTT) whereas, the medial STT, SRT, and SMT, are collectively called paleospinothalamic tract (pSTT) (Raj, 1996). Axons in the nSTT terminate in ventro-postero-lateral nucleus of the thalamus (VPL), from which, third order neurons take over and project to the somatosensory cortex. Axons in the pSTT project to the reticular formation, periaqueductal grey, hypothalamus, and medial and intralaminar thalamic nuclei, which then interconnect with neurons that are interlinked with limbic forebrain structures and further widespread projections to other areas in the brain (Raj, 1996). The precise role of each pathway remains largely obscure, and indeed a putative multiplicity of ascending pain pathways is proposed (Villanueva and Bernard, 1999). In addition to the main ascending pathways other tracts are involved, which collectively are considered to subserve two major pain systems namely the lateral and medial systems (Albe-Fessard et al., 1985).

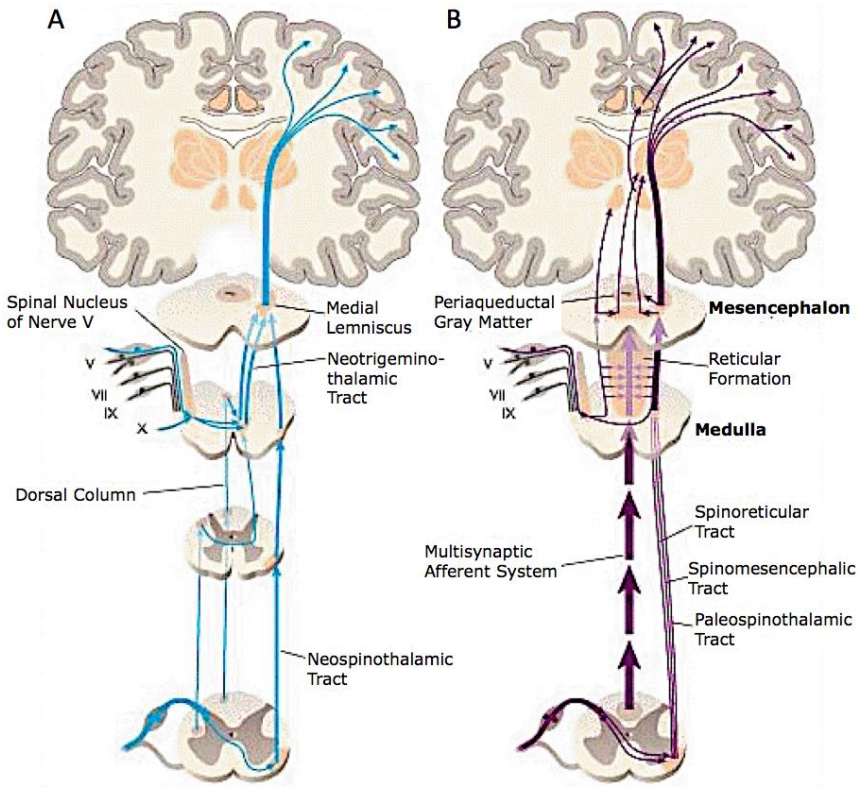


Figure 1: Lateral (A) and medial (B) pain transmission systems [Image modified from http://www.pharmacology2000.com/]

The lateral pain system comprises long tracts that project to the ventral posterior lateral nucleus of the thalamus (VPL), thence to the primary and secondary sensory cortices. These fibres are relatively thick allowing for fast conduction, and hence the lateral system is thought to be involved in processing acute pain where rapid transmission of information regarding the sensory-discriminative aspects (onset, location, intensity, duration) of pain is required (Jones and Derbyshire, 1996). On the other hand the medial pain system is composed mainly of relatively thin fibres with slower conduction rate projecting to the medial nuclei of the thalamus with subsequent important connections with the anterior

cingulate cortex (ACC) (Vogt et al., 1993), prefrontal cortex (Tsubokawa et al., 1981), insula, periaqueductal grey (PAG), and brain stem (Jones and Derbyshire, 1996). The medial pain system is concerned with the affective-motivational and cognitive-evaluative components of pain response and it is thought to be largely involved in processing chronic pain (Jones and Derbyshire, 1996). Cortical centres, which receive projections from thalamic nuclei where pain fibres end, have been recently reinforced with findings from a plethora of functional brain imaging techniques.

Neuroimaging studies of brain areas that are involved in pain processing have produced varying results. However a meta-analysis of pain imaging studies revealed that by large convergence the most commonly activated areas include primary somatosensory (S1), secondary somatosensory (S2), ACC, insula, prefrontal cortex, thalamus, basal ganglia, and cerebellum (Apkarian et al., 2005). It is not unexpected for pain processing with its complex multidimensionality to recruit extensive network in the brain which is arguably referred to as the “pain matrix”.

1.1.5 Descending pain modulatory systems: inhibition and facilitation

Several factors are thought to affect the pain experience either by inhibition 'antinociception', or further enhancement 'pronociception'. Emotional state, anxiety, attention and distraction, expectations, past memories and experiences, and other factors can modulate the pain experience and may explain the variability between subjects in levels of pain perception to same noxious stimuli (Ossipov et al., 2010). One of the earliest reported observations that pain can be modulated (endogenously inhibited), was by Henry Beecher (Beecher, 1946), who noted that, strikingly reduced rates of pain perception were reported by conscious soldiers in the battlefield with complicated fresh wounds. Similar observations were seen in other stressful conditions, and further investigated in, for example, athletes in competitions (Geva and Defrin, 2013). The suppression of pain during stressful or fearful circumstances is considered as an in-built system in mammals leading to stress-induced analgesia (Butler and Finn, 2009).

The neurobiological mechanisms of the descending pain modulatory circuits (Fig. 2) have been widely explored. According to preclinical and clinical studies, the pain modulatory system involves inputs from higher centres including the hypothalamus, amygdala, rostral

ACC, and prefrontal cortex, interconnecting with the periaqueductal grey, with outputs to the medulla (Ossipov et al., 2010, Bingel and Tracey, 2008). In addition to the PAG, other structures including the locus ceruleus, the nucleus raphe magnus (NRM), and several nuclei of the bulbar reticular formation are involved in the descending pathways down to the neurons in the spinal cord. Several different neurotransmitters such as opioids, serotonin, and catecholamines are utilized (Willis and Westlund, 1997).

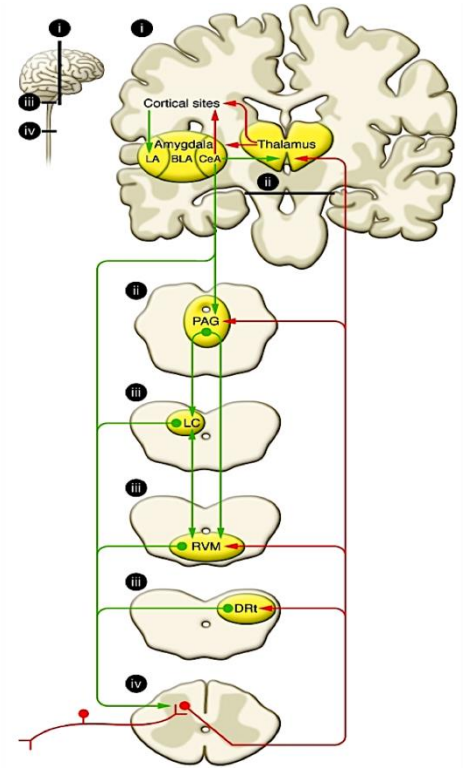


Figure 2: Schematic representation of pain modulatory circuits [Image from (Ossipov et al., 2010, Bingel and Tracey, 2008)]

PAG has been shown to play a key role in the descending pain inhibition system. Microinjection of opiates or focal electric stimulation of PAG can lead to remarkable pain inhibition. Morphine

microinjection into PAG showed a profound analgesic effect in rabbits (Tsou and Jang, 1964). David Reynolds in 1969 was able to strikingly induce antinociceptive effect in a number of rats by electrically stimulating the PAG enabling him to perform laparoscopic procedure apparently painlessly without the use of chemical anaesthetics. In humans, focal simulation of PAG also exerted some analgesic effects, but accompanied by side effects such as anxiety and distress and therefore it has been discontinued (Ossipov et al., 2010). Similarly, placebo analgesia is considered as an example of cognitive modulation of pain that has shown to recruit the rACC, PAG, and amygdala to induce endogenous pain control (Bingel et al., 2006b).

Conversely, activation of the descending facilitation system can lead to enhanced nociception. Indeed, sustained activity of the descending modulatory system with subsequent pain transmission facilitation has been suggested to contribute to pain chronicity (Fields, 1992). Early in the twentieth century, Sherrington noticed an enhancement in the nociceptive reflexes following spinal cord transection (Sherrington, 1906). Over the following years a large body of evidence has emerged revealing that several brain regions including the frontal lobe, ACC, insula, thalamus, amygdala, as well as PAG and nucleus cuneiforms (NCF) and rostroventromedial medulla (RVM) are involved in this descending modulation (Tracey

and Mantyh, 2007). The proposed key relay structure in descending pain facilitation has been the rostroventromedial medulla (RVM), though its role is controversial with some lines of evidence suggesting that under different circumstances it can accordingly have a bidirectional contribution i.e. inhibition and/or facilitation (Porreca et al., 2002).

Functional MRI studies in humans had a crucial role in elucidating the normal and aberrant functional connectivity of pain inhibitory network in health (Bingel et al., 2006a, Scheef et al., 2012) and disease (Jensen et al., 2012, Song et al., 2006) respectively. The descending pain modulatory system, in both its inhibitory and facilitatory aspects, enables the subject to regulate nociceptive processing according to different circumstances and demands. However dysfunction of this powerful and dedicated endogenous system may underlie some states of chronic pain (Tracey and Bushnell, 2009a).

1.1.6 Central sensitization

Central sensitization as defined by the International Association for the Study of Pain is "*an increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input*" (Merskey and Bogduk, 1994). In contrast to peripheral sensitization, which involves local pain hypersensitivity restricted to the damaged area, central sensitization can further produce pain hypersensitivity in remote non-injured areas and may persist without clear peripheral nociceptive conduction. The abnormal state of exaggerated responsiveness is reflected by a triad of pain sensitivity, which includes 1) hyperalgesia- amplified nociception response, 2) allodynia- perception of pain to innocuous stimulus, and 3) pain expansion- spread of pain beyond local area of pain (Marcus. et al., 2009). The change in the properties of nociceptive neurons in CNS is modulated by certain neurotransmitters with subsequent enhancement of postsynaptic transmission to higher centres include thalamus, ACC, insula, and somatosensory cortex (Meeus and Nijs, 2007).

Central sensitization, indexed by reduced pain thresholds (as a possible biomarker) at remote sites, has been widely reported in different chronic pain conditions (Banic et al., 2004, Staud et al., 2001, Bendtsen et al., 1996), particularly osteoarthritic chronic

pain (Suokas et al., 2012). Central sensitization can also be introduced experimentally e.g. following induced peripheral injury or inflammation (LaMotte et al., 1991).

The mechanistic investigation of central sensitization has been widely studied in animal models and early evidence of central component was presented over thirty years ago (Woolf, 1983). It was found in Woolf's experiment that under normal conditions in rats 1) there was no spontaneous activity from motor neurons as recorded from flexor reflex withdrawal response, 2) activation of neurons required high threshold nociceptive stimulus and 3) the receptive field was spatially limited. However after a repeated noxious stimulation-induced peripheral inflammation, long lasting increased excitability of motor neurons was noted as well as a reduction in pain threshold (e.g. a non-noxious stimulus like touch could induce pain), and expansion of cutaneous receptive field. These findings were further reproduced preclinically, and some mechanistic explanation later emerged in humans (LaMotte et al., 1991). LaMotte and colleagues showed that hyperalgesia to punctate stimuli following intradermal injection of capsaicin in the volar forearm can be maintained long after anesthetizing the injection site. The cutaneous hyperalgesia could be prevented by locally anaesthetising the site before capsaicin injection or by anaesthetically blocking the peripheral nerve proximal to the

injection site. These findings suggest that secondary hyperalgesia develops after noxious tissue damage though the latter is not needed for its maintenance.

The persistence of pain hypersensitivity long after the healing time cannot be explained simply by peripheral sensitization. Nonetheless, peripheral neural mechanisms are likely to contribute during early stages of tissue damage and inflammation. It is now known that different kinds of CNS plasticity (molecular, structural, functional) are involved in the process of central sensitization. Following noxious stimulation, neurons are sensitized in the dorsal horn of spinal cord as well as in other areas along the somatosensory pathways (Coderre et al., 1993). Direct measurements of CNS neuronal activity in humans cannot be made and therefore direct evidence of neuronal hyper-excitability cannot be provided (Curatolo et al., 2006). However, with the expanding use of advanced functional neuroimaging techniques such as functional MRI and magnetoencephalography (MEG), the contribution of supra-spinal structures to central sensitization can be elucidated. Studies have reported increased activities in structures including the brain stem, thalamus, cingulate, insula, middle frontal, posterior parietal, and primary and secondary somatosensory cortices, in association with experimentally induced

mechanical hyperalgesia (Lee et al., 2008, Maihofner et al., 2010, Mainero et al., 2007, Zambreanu et al., 2005).

Central sensitization may provide explanation for many intractable chronic diseases including musculoskeletal and as such offer therapeutic approaches such as pain physiology education, which has reportedly proven effective for changing pain cognitions and health status in patients and showing better outcomes prior to active treatment (Nijs et al., 2011b). Pharmacological options aiming at desensitizing the CNS in chronic unexplained pain conditions is getting a great deal of awareness and research interest (Nijs et al., 2011a).

1.2 Chronic Pain in knee OA

1.2.1 Overview

Osteoarthritis has been known as the most common form of arthritis with substantial impact on individual's quality of life and health care economics (Arden and Leyland, 2013). A report by the world health organization states that globally the knee OA is likely to become the fourth most important cause of disability in women and the eighth in men (Murray and D., 1997). Moreover, it was found to be one of the most disabling disorder in the elderly, along with stroke, depression, hip fracture, and heart disease (Guccione et al., 1994). Risk factors include age, sex, obesity, joint injury or instability as well as genetic and occupational factors (Felson et al., 2000). The prevalence of OA is higher in women and increases with age in both sexes, although OA is uncommon before the age of 40 years (Office for National Statistics, 2006). The peripheral pathophysiological mechanism of OA arises mainly from a disruption of the dynamic equilibrium, which is maintained by articular chondrocytes, between synthesis and degeneration of extracellular matrix components, resulting in progressive loss of cartilage and apoptosis of cells (Lee et al., 2013).

Despite other debilitating symptoms, pain remains as the major presenting problem of OA that is activity-related and induced mainly by specific weight bearing activities such as walking up and down stairs, as in case of knee OA (Felson, 2009). However, the experience of pain in OA is far more complicated as revealed by a large qualitative study (Hawker et al., 2008) showing that two distinct types of pain were described by hip and knee OA patients: a chronic dull aching pain with less changeability over time, which did not affect patients' activities but increasingly intercalated by a flickering unpredictable pain that is intense, stressful and emotionally draining. Furthermore, the non-linear relationship of pain severity with joint pathology makes it difficult to fully explain the degree of perceived pain (Kornaat et al., 2006, Link, 2009, Phan et al., 2006). Among other knee joint problems osteoarthritis is considered as the most common cause of knee pain.

1.2.2 Definitions

1.2.2.1 Knee OA

The American College of Rheumatology (ACR) (Altman et al., 1986), defined OA as "a heterogeneous group of conditions that lead to joint symptoms and signs which are associated with defective integrity of articular cartilage, in addition to related changes in the underlying bone and at the joint margins". ACR also developed clinical criteria of classifying primary knee OA based on clinical and radiological findings. The classification algorithm involved the presence of pain experience irrespective of quality, duration, or periodicity, and a selective combinations of findings from other co-existing variables such as radiographic, laboratory and medical history or physical examination (Altman et al., 1986). However these criteria have been criticized for being insensitive to early and mild OA in primary care (Peat et al., 2006b), and therefore no standard definition of OA is agreed to be used in research especially in epidemiological studies (Lawrence et al., 1998).

1.2.2.2 Chronic painful knee OA

In the Literature of chronic pain imaging, the definition of pain chronicity in OA and eligibility criteria has varied widely between studies. Parks and colleagues (Parks et al., 2011a) used a duration

of ≥3 months and pain severity of ≥ 30/100, whereas Rodriguez-Raecke et al (Rodriguez-Raecke et al., 2009) used a duration of longer than 12 months, and Gwilym and co-workers (Gwilym et al., 2010b) adopted a definition of pain that is of sufficient magnitude to warrant joint replacement surgery. However and from a neurobiological correlations perspective, it is conceivable that neurobiological adaptive changes may take several years to develop. Moreover, duration alone regardless the persistency status may indicate episodicity rather than chronicity. Therefore the definition of chronic pain that I will use in the experimental work presented in this thesis is: 'primarily knee pain that lasted more than a year and experienced most of the day on most days of the week for at least the last month' (Adapted from (Peat et al., 2006a).

1.2.3 Epidemiology

It was estimated in a large study 'the Framingham osteoarthritis study' that involved 1805 subjects, with knee radiographs available in 1424 (Felson et al., 1987), that the prevalence of radiographic-evidenced knee OA in elderly ranged between 27% in subjects under 70 years, to 40% in those over 80. The rate was however higher in females, and in particular and significantly the symptomatic OA. The incidence rate was also recorded as higher

in females (1.7 times) as well as the frequency of disease progression rate (Felson et al., 1995).

As the perceived pain and degree of knee joint pathology are often discordant, radiographic changes thus constitute only a modest risk factor for the pain reported in knee OA (Felson and Schaible, 2009). Indeed other factors such as psychosocial and demographic variables play important roles (Davis et al., 1992). Creamer and other researchers (Creamer et al., 1999) investigated the association between anxiety and depression and self-reported knee pain in 374 volunteers aging ≥ 40 years. Anxiety levels correlated significantly with the reported pain in women but not in men participants (adjusted for age), whereas depression had no predictive relation in either sex. Other factors, which have been found to predict pain and/ or OA changes, include hypochondriasis, health phobia or health anxiety (Lichtenberg et al., 1986), aging (Blagojevic et al., 2010), sex (Jones et al., 2000, Srikanth et al., 2005), obesity (Hart and Spector, 1993), race (Valdes et al., 2007), educational level (Hannan et al., 1992), genetics (Spector and MacGregor, 2004), occupational activity (Cooper et al., 1994), and lower extremity muscular strength (Slemenda et al., 1998).

1.2.4 Individual and socioeconomic impact

Knee osteoarthritis is a major cause of pain and disability in elderly with profound impact on quality as well as quantity of life, and there is an association with cardiovascular diseases (Philbin et al., 1996). The immobility associated with knee OA may impose significant life-shortening effects as shown in a study by Landi and colleagues (Landi et al., 2010). The disability in elderly patients exerted significant influence on mortality independent of age and other clinical and functional variables. In another recent large population study (Nüesch et al., 2011) authors investigated cause and disease specific mortality in 1163 patients ≥ 35 years, with knee or hip OA and found excess mortality in OA patients compared with the general population irrespective of the cause of death, and furthermore the risk of mortality from cardiovascular causes was higher in those with walking disability.

The pain in knee OA is known for its typical transition from an intermittent weight-bearing to a more persistent chronic pain (Neogi, 2013). In OA and similar conditions, pain probably is the most concerning issue from a patient's perspective as revealed in a large survey conducted in a Norwegian cohort (Heiberg and Kvien, 2002). Around 70% of patients preferentially wanted to see improvement in pain as a top priority, followed by function (45%)

walking and bending (33%), household tasks (25%), social activity (13%), self care (11%), and work (9%). Health care practitioners may not always appreciate the patients' prioritization of improvements in areas of health, which importantly calls for awareness that the patient's and physician's general views of the disease are drawn from different perspectives (Studenic et al., 2012).

In an eight-year prospective follow-up study (Dieppe et al., 2000) of 500 patients with peripheral OA including knee OA (111 knee OA alone, and 87 with hand and knee OA at baseline), knee OA patients showed deterioration in physical activity, with higher levels of anxiety and depression and an increase in the utilization of health care resources such as joint replacement, medications, and walking aids was noted.

The impact of chronic pain in OA can extend to sufferer's spouse and negatively affect their emotional and physical health and marital relationship (Flor et al., 1987) as well as sleep quality putting the spouse's health at risk too (Martire et al., 2013).

OA results in major economic costs with estimations that the musculoskeletal disorders, of which OA is the most prevalent, costs between 1 and 2.5% of the gross national product (GNP) of some

developed countries including USA, Canada, UK, France, and Australia (March and Bachmeier, 1997). The costs of OA can be direct as a result of medical care and management expenses or indirect from e.g. loss of productivity or expenditures on disease-related dependency consequences (Bitton, 2009). In a Canadian study (Maetzel et al., 2004) the indirect costs were estimated at $1760 per person per year as opposed to the direct costs of $3952. In the UK the economic burden of OA has a major negative impact and is estimated as equivalent of 1% of GNP per year. In year 1999/2000 the British economy lost £3.2 billion, as 36 million working days were recorded as lost days due to OA alone, and moreover £43 million and £215 million were spent on community and social services respectively, for OA (National Collaborating Centre for Chronic Conditions, 2008).

1.2.5 Peripheral mechanisms and correlates of pain in knee OA

The osteoarthritic disease involves a local tissue damage and inflammation including a cartilage loss, but the cartilage itself is a non-innervated tissue. Thus the peripheral mechanism and correlates of pain can be quite complex. The intriguing question therefore is; what structures in the knee that have nociceptors and

able to conduct pain and what triggers nociception in these structures?

A useful review on the sources of pain in knee OA was presented by David Felson (Felson, 2005). Evidence from the reviewed studies suggests that type 3 (myelinated) and type 4 (C-unmyelinated) fibres innervate most joint structures, though with inconsistent findings on the existence of nociceptive fibres in the synovium or in the inner avascular portion of the meniscus, and no evidence of their presence in hyaline articular cartilage. These pain fibres are high threshold fibres and can be activated by noxious mechanical stimulation of the joint that is mediated by a range of pain molecular substances. The pain-innervated structures include the periostium, the subchondral bone and the marrow underneath, the fat pad, the capsule, and at the junction between the bone and cartilage. There is also some evidence that the internal retinaculum of the patella and ligamentous insertions are also pain-innervated (Felson, 2005).

Research studies suggest that the presence of synovitis and bone marrow lesions are likely to mediate pain in knee OA (Sofat et al., 2011). Felson et al (Felson et al., 2001) examined 401 patents with radiographic knee OA, 351 had knee pain and 50 had no knee pain. Of those with pain 78% presented with bone marrow lesions as

opposed to only 30% of non-painful knee OA group ($p<0.001$). Furthermore larger sizes of bone marrow lesions were associated with the knee pain.

In a recent systematic review by Yusuf and co-workers (Yusuf et al., 2011), authors evaluated the association between pain and a wide range of knee OA MRI-based findings: (cartilage defects, bone marrow lesions, osteophytes, meniscal lesion, effusion or synovitis, ligamentous abnormalities, subchondral cysts and bone attrition). Researchers found that the pain in knee OA is associated with bone marrow lesions and effusion or synovitis.

1.2.6 Central mechanism of knee OA pain

As mentioned above, from reviewing the peripheral correlates of pain in knee OA, the exact pathological elements in knee OA pain remain largely unclear. The disparity between peripheral changes and pain as well as other observations such as the placebo effect- where placebo surgery resulted in a significant long-term pain relief (Moseley et al., 2002), and the unresponsiveness to surgical treatment (Wylde et al., 2011), taken together all suggest additional causative factors in chronic OA pain beyond the direct nociceptive tissue damage, such as neural plasticity or predisposing factors.

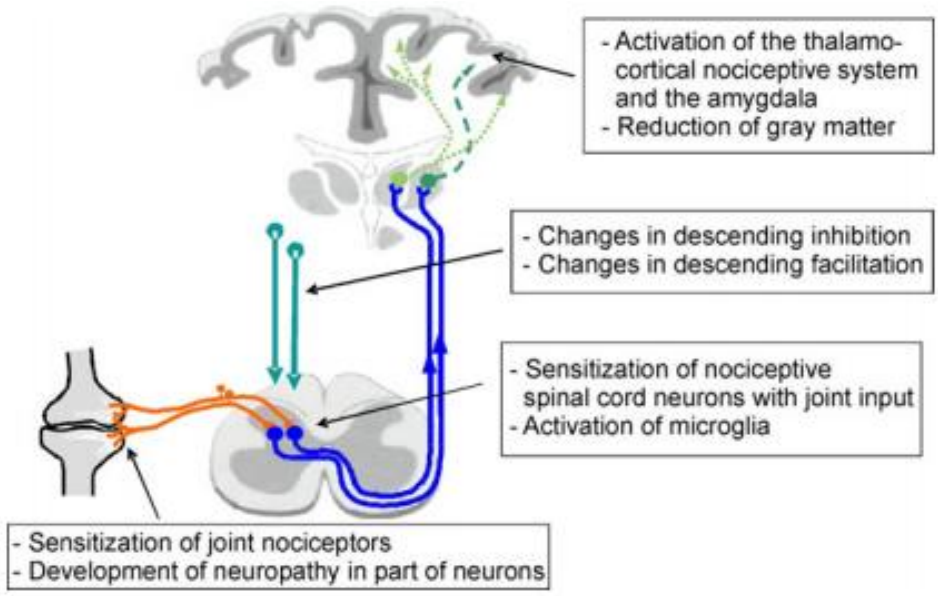


Figure 1: The major nociceptive system in knee OA: from periphery to higher centres. (Image from Hans-Georg Schaible, 2012)

Pain that is generated from nociceptive activation in the knee joint is transmitted to central areas via two types of neurons that are functionally different (Felson, 2005). The first is responsible for producing nociception and has relatively small receptive field in the periphery but bridges both the knee joint and deep soft tissue e.g. muscle. The second type of nociceptive neuron with a receptive field in the periphery, which covers the joint and overlying skin and a wide territory of skin and joint, is a wide-dynamic-range neuron that produces pain in response to both noxious and innocuous mechanical stimuli. However the transmitted signal is interpreted as one or the other based on the frequency of firing (Felson, 2005).

The ascending pain pathways have been previously described (Chapter 1), and further illustrated as a simple schematic

representation in Fig. 1. The inflammatory processes at the joint, mediated by inflammatory compounds (Schaible et al., 2011), cause complex changes in the CNS with subsequent enhancement of postsynaptic transmission to higher centres include thalamus, ACC, insula, and somatosensory cortex (Meeus and Nijs, 2007). This amplification of nociceptive processing has both spinal and supraspinal components, yet the mechanism is not fully understood in humans but has been extensively investigated in animal models (Schaible, 2012). The abnormal state of exaggerated responsiveness at the affected joint can be reflected by a triad of pain sensitivity, which includes hyperalgesia, allodynia, and pain expansion- spreading beyond local area of pain (Marcus. et al., 2009).

Functional MRI has been widely implemented in neuroscience and brain imaging to measuring specific task- (or in some occasions task-free) related activities in the brain. Neuroimaging studies of pain in knee OA to investigate the cortical and subcortical areas involved in pain processing have been scarce in the literature. The difficulty in conducting such studies in knee OA arises partially from the fact that pain is not always feasibly possible to induce while in the scanner. Kulkarni and colleagues (Kulkarni et al., 2007) used positron emission tomography of the brain in knee OA patients and

recorded an increased brain activity as a result of both spontaneous arthritic and experimentally induced pain, in the cingulate cortex, the thalamus, and the amygdala suggesting that arthritic pain is processed in centres concerned with emotions and fear. Baliki et al (Baliki et al., 2008b) were able to induce pain (via MRI compatible machine that delivered controlled pressure) in a preliminary study involved 5 knee OA patients. Pain perception was associated with higher brain activity in bilateral thalami, S2, insula, and cingulate cortices, and unilaterally in the putamen, and amygdala. Of note that these areas are part of what is know as the pain matrix (Derbyshire et al., 1997), and overlaps largely with areas involved in acute pain processing (Apkarian et al., 2005).

As pain is a multi-dimensional experience involving sensory, emotional, and cognitive aspects it is expected that it would recruit multiple areas in the brain with different functional domains. Recently, a study was conducted to dissociate the brain activity induced by evoked pain from that induced by spontaneous pain, in chronic knee OA patients (Parks et al., 2011b). It was found that the pain in both cases recruited different networks. Evoked pain activated areas similar to those observed for acute pain in healthy subjects, whereas spontaneous pain was particularly engaged in

activating prefrontal-limbic areas, which indicates involvement of emotional components in chronic pain.

Both fMRI techniques and pain neuroimaging are relatively recently developed fields but expanding rapidly with promising findings that will help, from a neurobiological perspective, better understanding how the brain process arthritic pain as well as other intractable chronic pain conditions.

1.2.7 Treatment options and outcome

There seem to be no effective preventive strategies for OA, and therefore treatment options are directed towards relieving symptoms, maintaining function, and preventing further damage (Kidd, 2006). The European League Against Rheumatism (EULAR) (Jordan et al., 2003) identified three lines of management of knee OA: non-pharmacological (e.g. education, exercise, life style adjustments), pharmacological (medications such as non-steroidal anti-inflammatory, tropical treatment), and invasive interventional (e.g. intra-articular injections, lavage, joint replacement). EULAR concluded that there is a wide range of treatment to knee OA however there is no single right or wrong approach and the evidence-based recommendation was a combination of pharmacological and non-pharmacological interventions in order to achieve better results of knee OA treatment. The evidence for the

efficacy of physical interventions (e.g. electro-acupuncture EA, low level laser therapy LLLT, pulsed electromagnetic field, transcutaneous electrical nerve stimulation TENS, ultrasound) in knee OA pain was evaluated and presented in a systematic review and meta-analysis (Bjordal et al., 2007). Researchers concluded that TENS, EA, and LLLT under optimum administrational conditions could offer short-term pain relief.

The efficiency of pharmacological treatment of knee OA including the use of topical non-steroidal, capsaicin, and intra-articular steroid injections, is controversial (Kidd, 2006), however some studies have shown significant benefit of Paracetamol and systemic non-steroidal anti-inflammatory drugs over placebo (Felson, 2006). Moore and co-workers (Moore et al., 2013a) reviewed relevant studies and examined rates of success and failure (defined as 50% pain relief or its equivalent) of commonly used pain relief medications. In OA it was found that the failure rate of non-steroidal anti-inflammatory drugs is up to 70%, and in a combined analysis for OA and chronic back pain, the failure rates for opioids (tapentadol and oxycodone), were 90% and 100% respectively. On top of the list of medications that show promise for pain relief in OA comes Tanezumab (10mg) with a success rate of 29% followed by Etoricoxib 60mg and Naproxen 1000mg (27% each), Celecoxib

200 (22%), Topical Diclofenac 1.5% (20%), Ibuprofen 2400mg (16%), and Duloxetine 60/100mg (14%) (Moore et al., 2013a).

Other non-pharmacological options, though with smaller effect sizes of pain relief are available such as complementary and alternative medicine reviewed by Lapane et al (Lapane et al., 2012), and Tai Chi (a series of dance-like movements) and medical leech therapy reviewed by Lauche et al (Lauche et al., 2013); (Lauche et al., 2014).

Joint replacement surgery is the only known effective treatment with large effect size, but is only appropriate for a small percentage of patients who have advanced disease and severe symptoms (Juni et al., 2006). The outcome of total knee arthroplasty, indicated by primary knee OA, has been studied in 1244 procedures (with loss of follow up of 1.4% one year post-operatively) by Hamilton and colleagues (Hamilton et al., 2012). The satisfaction rate reached 81% with significant improvements in pain and function. Beswick and co-workers systematically reviewed published studies of knee replacement for the treatment of OA focusing mainly on reporting proportions of people by pain intensity (Beswick et al., 2012). Researcher found that the percentage of patients with adverse long-standing pain outcome ranged between 10-34% (as compared to 7-23% in hip arthroplasty). Authors concluded that these

proportions of unfavourable pain outcomes were significant, which requires further awareness.

1.2.8 The problem and knowledge gap

Patients with chronic pain are in desperate need of pain relief (Heiberg and Kvien, 2002) that tends to be under-estimated by care providers (Moore et al., 2013b). The available analgesic drugs may work well, but only in a small percentage of patients (Moore et al., 2013a), as do surgical interventions however with larger effect size. Moore et al argue that focusing on average pain relief response in research studies is not as useful as seeking out what works for each patient (Moore et al., 2013a).

Although some intractability of chronic pain in knee OA after arthroplasty can be ascribed to surgical techniques and implant factors (Mont et al., 1996), much of persistent pain and disability seen post-operatively remains largely unexplained. Several risk factors may contribute to developing the un-explained persistency of chronic pain, which include socio-demographic (e.g. low social support, low social class, female sex and older age), psychological (depression, pain catastrophizing), and biological factors (e.g. central sensitization) (Wylde et al., 2007). The bio-psycho-social model is now increasingly recognized as an important perspective that will help better understanding chronic pain aetiology and

management (Roth et al., 2012). The *bio* part of the model from an imaging-based neurobiological perspective will be the main focus of this thesis.

1.3 Introduction to neuroplasticity, brain morphometry and measurement techniques

1.3.1 Neuroplasticity in health and disease

Neuroplasticity is a general term that subsumes different kinds of structural or functional neuronal reorganization in response to experience or sensory stimulation. It is an intrinsic feature of the human nervous system and a mechanism for development and learning as much as a cause of pathology (Pascual-Leone et al., 2005). In healthy people, learning new tasks or adhering to certain habits and life styles e.g. playing Tetris (Haier et al., 2009), juggling (Draganski et al., 2004), learning new language (Mechelli et al., 2004), alcohol drinking (Momenan et al., 2012, Pfefferbaum et al., 1992), opioids consumption (Younger et al., 2011), or practicing meditation (Grant et al., 2010), can induce significant neuroplastic changes. Performing music, for instance requires intensive integration of sensory and motor information in addition to precise monitoring of the performance. Therefore, musicians have offered an ideal model (Munte et al., 2002) to investigate both functional (Elbert et al., 1995) and structural (Gaser and Schlaug, 2003) neuroplasticity under normal circumstances. The study of neuroplasticity in humans is a fairly recent development and has been massively facilitated by the advances in neuroimaging that allow neuroplastic changes to be studied in vivo.

Different types of disease and damage to the nervous system can also trigger functional and structural reorganization of the brain. Neuroplastic changes have been described in a wide range of conditions including psychiatric disorders (Frodl et al., 2002, Pantelis et al., 2003, Radua et al., 2010, Shenton et al., 2001, Thompson et al., 2001), Alzheimer's disease (Thompson et al., 2003), diabetes (Kumar et al., 2008), trigeminal neuralgia (DaSilva et al., 2008) and many other genetic and acquired conditions (Peper et al., 2007, Kolb et al., 2011).

While in some cases the structural changes are clearly indicative of neuro-degeneration or neuronal loss, the mechanism in most diseases remains largely unknown.

1.3.2 Structural plasticity in chronic pain

It is just recently that the study of structural brain changes associated with chronic pain has become a research interest. The advance in medical imaging techniques, in particular magnetic resonance imaging, and dedicated image processing software, has opened new exciting avenues to study the central processing of chronic pain at supra-spinal levels and also provided insights into the structural as well as functional and neurochemical brain changes associated with chronic pain.

The first in vivo findings of MRI-based grey matter changes in chronic pain were reported in 2004 in patients with chronic low back pain (Apkarian et al., 2004). Ever since the published articles on grey matter changes in chronic pain have risen dramatically from just (6) in 2005 to (114) in 2013, (based on PubMed search, www.ncbi.nlm.nih.gov/pubmed performed on 23 December 2013).

The commonly used study design to investigate structural differences in chronic pain is often a cross sectional case-control, where the averaged grey matter volume, density or cortical thickness in chronic pain patients is compared with that in age and sex matched healthy controls. Some studies may however correlate significant differences with the duration of the disease, pain severity and other aspects of pain. Longitudinal studies are scarce in the literature, as they require more time and resources, albeit they may prove more informative in elucidating the causal relationship between chronic pain and structural changes.

Previous neuroimaging studies have reported morphological cerebral changes in various chronic pain states (Kuchinad et al., 2007, Schmidt-Wilcke et al., 2006, Rodriguez-Raecke et al., 2009, Apkarian et al., 2004, Draganski et al., 2006, Burgmer et al., 2009, Buckalew et al., 2010b, Schmidt-Wilcke et al., 2005, Seminowicz et al., 2010, Schmidt-Wilcke et al., 2008, Gwilym et al., 2010b). Most

studies found a reduction in grey matter (GM) density, volume or cortical thickness. However, the distribution of brain structures, which show pain-related GM changes, is highly varied between and within different primary aetiologies of chronic pain disorders. A recent systematic review and meta-analysis of voxel-based morphometric studies in patients with chronic pain of any cause compared to healthy controls (Smallwood et al., 2013b) found several clusters of reduced grey matter volume in known pain processing areas and beyond, but also an enlarged hippocampus and parahippocampus cluster. A wide range of underlying disorders, disease duration and sample size (8-56; median 18 per group) were included. The mechanisms by which these changes occur and which factors may explain heterogeneity of results are largely unknown. A causative link to the pain experience can be inferred from a few longitudinal studies showing that these changes can be at least partially normalized upon pain relief (Rodriguez-Raecke et al., 2009, Gwilym et al., 2010b, Seminowicz et al., 2011).

There seems to be an overlap among studies regarding the involvement of certain areas in the brain including thalamus, DLPFC, cingulate cortex, and brain stem. Most of the regions found with grey matter changes are believed to be part of the central pain processing network or that has been referred to as '*The Pain*

Matrix' (Melzack, 1989).

1.3.3 Underpinnings and neuroanatomical basis of structural plasticity

Direct histological evidence for structural reorganization is available from animal studies. In support of the mechanism of synaptic plasticity and formation of new connections, animal studies have revealed that exposure to new environmental challenges such as spatial learning tasks (Holahan et al., 2006), or exposure to enriched environment (Galimberti et al., 2006) can remarkably induce axonal remodelling. Following experimentally induced lesions in monkeys, Jain Nerraj and others (Jain et al., 2000) found new axonal growth taking place from the trigeminal nucleus in the brainstem into the cuneate nucleus, after several months following transection of the dorsal column of the spinal cord, as confirmed by examination of autopsy slices. These findings provide evidence that structural plasticity in the form of new connections growing into regions in the brain that no longer receive primary afferent input as a result of damage, also has a subsequent impact on function i.e. functional plasticity as confirmed by microelectrode recordings in the aforementioned study. Axonal dynamics have also been shown in pathological conditions such as brain injury (Dancause et al., 2005) and focal retinal lesions (Darian-Smith and Gilbert, 1994).

Production of new neurons as a mechanism of plasticity in adult human brain (neurogenesis) was long considered as a non-existent event. However, using advanced techniques, several lines of evidence have relatively recently emerged revealing that neurogenesis can indeed occur in the sub-granular zone of hippocampal dentate gyrus (Eriksson et al., 1998, Roy et al., 2000) and sub-ventricular zone of lateral ventricle which contributes interneurons to the olfactory bulb (Bedard and Parent, 2004).

These mechanisms of generating new neurons or axonal connections may partially explain morphometric increases in volume or density as a directional pattern of structural plasticity. On the other hand, the mechanism of morphometric reductions reported in several specific and non-specific regions in association with a multitude of pathological conditions including chronic pain diseases, remains poorly understood. All up to date explanations available for the structural changes in humans are based on assumptions and have been open to speculations.

It has been proposed that these changes may represent neuroanatomical substrate for the disease, an epiphenomenon or even an artefact (Reiss et al., 2004b). Simple change in cell size,

shrinkage or atrophy of neurons or glia, changes in the intra-cortical axonal architecture (synaptic loss) have been suggested (Rodriguez-Raecke et al., 2009, May, 2008). A concept of changes in the extracellular matrix without substantial involvement of neurons or neuroglia was offered by Schmidt-Wilcke and colleagues (Schmidt-Wilcke et al., 2008), supporting their idea by a piece of growing evidence showing that extracellular matrix influences neural cell activity. These mechanisms are thought to be secondary to, or in conjunction with a reduction in the cerebral blood flow, as proposed by Gwilym and co-workers (Gwilym et al., 2010b), and upheld by studies reporting reduced cerebral blood flow to the contralateral thalamus during pain that reverts to normal flow after analgesia is achieved (Di Piero et al., 1991, Garcia-Larrea et al., 1999, Hsieh et al., 1995, Peyron et al., 1995). However, all cited studies had recruited very low sample sizes n= 5, 10, 8, and 2 patients respectively, rendering generalizability of results questionable.

The gold standard method to explain the mechanism of structural changes would be histopathology. However, histological data are lacking, and there are not known studies that provide direct histological evidence for global or regional brain atrophy in humans with chronic pain (Schmidt-Wilcke et al., 2008). It should be noted

that the evidence of reversibility of GM volume loss provided by some longitudinal studies (Rodriguez-Raecke et al., 2009, Gwilym et al., 2010b, Seminowicz et al., 2011), implies that an overt neurodegenerative process with irreversible damage is unlikely.

1.3.4 Dynamic changes and reversibility of structural changes in chronic pain

It is not clearly understood whether chronic pain is precedent to or a consequence of structural brain changes, i.e. regional brain variations due to factors other than chronic pain (e.g. genetic) predispose to pain chronicity. In the literature of chronic pain imaging, the vast majority of studies are of cross-sectional nature leaving the temporal relationship between chronic pain and brain morphology intriguingly uncertain. Longitudinal studies at various stages of chronic pain with same patients as their own controls would allow better understanding the causal relationships between structural brain changes and chronic pain. Findings that support the hypothesis that morphological brain changes are secondary to persistent pain include:

- The reversibility of structural brain changes following therapeutic pain relief (Gwilym et al., 2010b, Rodriguez-Raecke et al., 2009, Seminowicz et al., 2011),

- The changes in grey matter due to repetitive painful stimulation (Teutsch et al., 2008), and
- The correlation between duration of pain and the degree of grey matter shrinkage i.e. GM decrease with longer pain (Apkarian et al., 2004, Schmidt-Wilcke et al., 2006, Kuchinad et al., 2007, Buckalew et al., 2010a, Baliki et al., 2011, Wartolowska et al., 2012).

Chronic pain of osteoarthritic origin offers an ideal model to study the reversibility of pain-related GM changes. The pathological problem in OA can be treated with joint replacement yielding highly satisfactory rates in different aspects of life quality including pain relief and improved daily activity in as high as 85% of cases (Rodriguez-Raecke et al., 2009, Wylde et al., 2011).

Two studies have investigated the dynamic grey matter changes in OA before and following hip replacement surgery; (Gwilym et al., 2010b, Rodriguez-Raecke et al., 2009). It was found that structures that exhibited reductions in GM before surgery as compared to healthy controls were largely reversible to normal post-operatively. The time span for reversibility however took several months to show significant normalization. Strikingly, the regional changes in these two studies were dissimilar despite being associated with the same clinical condition i.e. hip OA. In the first study (Rodriguez-Raecke et al., 2009), authors found a significant

decrease of the GM in the anterior cingulate cortex, right insular cortex and operculum, DLPFC, amygdala, and brain stem, which all except the right insular cortex and operculum showed a significant increase (reversibility) post operatively. Whereas, in the second study (Gwilym et al., 2010b), pre and post surgical changes were limited to the thalamus (although postoperative analysis was restricted to thalamus as it was the only ROI that showed reduction pre-operatively). Patients however in both studies were similar in age, and the volumetric techniques were largely identical. This discrepancy is not easy to interpret and it could be attributed to other co-morbidities beyond pain such as medications, lack of mobility or other psychosocial and genetic factors (Nikolajsen et al., 2006). Some methodological differences in image processing and/or scanning protocol or time of scanning which was 4 and 9 months postoperatively respectively, could also contribute to the inconsistency.

1.3.5 Imaging-based measurement techniques

1.3.5.1 Manual morphometry

Manual morphometry is considered as the gold standard technique for region of interest (ROI) volumetric measurements (Cherbuin et al., 2009, Morey et al., 2009, Shen et al., 2010). However it requires good understanding of anatomy and well-established protocol with clearly defined landmarks, and therefore it can be largely labour intensive as well as subjected to human error. The agreement among multiple measures and reliability of manual volumetric technique can be tested within and between observers.

Intraclass correlation is recommended for such testing (Rousson et al., 2002), though there is no consensus as to what constitutes poor, good, or excellent ICC-based reliability (Shrout, 1998). However low ICCs indicate that larger study sample is needed to detect a statistically significant effect (Perkins et al., 2000).

In my experimental studies where manual morphometry was the technique of choice I have used 3D slicer version 4.1 (Pieper et al., 2006) to obtain quantifiable measures. The software allows for exchangeable visualization and tracing of the ROI in three different planes as well as an automated calculation of ROI volumes.

1.3.5.2 Voxel-based morphometry

Voxel based morphometry (VBM) is a voxel-wise automated technique to assessing MRI-based grey matter changes in the brain (Ashburner and Friston, 2000b, Wright et al., 1995).

Using SPM8 (Statistical Parametric Mapping8, available from http://www.fil.ion.ucl.ac.uk) implemented in MATLAB (The Math Works Inc., Natick, MA, USA, http://www.mathworks.co.uk/), the processing steps briefly begin with the use of T1 structural brain MRI scans which are then spatially normalized to the same stereotactic space. Next step involves segmentation of the three major components of the brain; grey matter, white matter and cerebrospinal fluid followed by smoothing of the grey matter and finally performing statistical analysis to localize, and make inferences about within (correlation) or between group differences.

The output is a statistical parametric map indicating significant differences of grey matter concentration within or between groups (Ashburner and Friston, 2000b). In the modulation step, 'preserve volume' can be chosen to yield GM volume rather than concentration. DARTEL (Diffeomorphic Anatomical Registration using Exponentiated Lie algebra) (Ashburner, 2007), implemented in SPM8 can be used, which offers improvement in spatial normalization and more reliable analysis outcome (Mak et al., 2011) largely comparable with manual morphometry.

As a fully and unbiased automated technique as well as relatively easy to use, VBM has grown widely in popularity since its development yielding biologically plausible results (Whitwell, 2009).

1.3.5.3 Surface-based morphometry

Surface based morphometric changes can be measured following reconstruction of the cortical surface from high-resolution brain imaging data. For cortical reconstruction and thickness estimation in this thesis studies I have used the Freesurfer software package (Mac version 5.1.0 available online http://surfer.nmr.mgh.harvard.edu/). Freesurfer allows for analysis and visualization of structural brain MRI data using semi automated processing steps. Briefly the processing steps involve removal of non-brain tissue (Segonne et al., 2004), transformation into Talairach space, intensity normalization (Sled et al., 1998), tessellation of the grey matter white matter boundary, automated topology correction (Segonne et al., 2007, Fischl et al., 2001), and surface deformation is then performed to indicate the grey/white and grey/cerebrospinal fluid borders based on detection of greatest shift in intensity (Fischl and Dale, 2000, Dale and Sereno, 1993, Dale et al., 1999). The method uses both intensity and continuity information from the entire three dimensional MR volume in

deformation procedures to produce representations of cortical thickness, calculated as the closest distance from the grey/white boundary to the grey/CSF boundary at each vertex on the tessellated surface. The reconstruction output is then visually inspected and any parcellation inaccuracies are manually corrected. Free-surfer brain construction output yields massive results of statistical data with regard to different aspects of cortical and subcortical morphometric measures. The output can be used to derive individual ROI morphometric statistics, or cortical data can be used for group-level statistical analysis by co-registration of the cortical surface into a common spherical space prior to vertex-wise statistical analysis.

The maps produced are not restricted to the voxel resolution of the original data thus are capable of detecting changes at sub-millimetre levels. Procedures for the measurement of cortical thickness have been validated against histological analysis (Rosas et al., 2002) and manual measurements (Salat et al., 2004).

1.3.6 Mechanistic imaging of structural plasticity

Mechanistic imaging, which is relatively a new field, refers to the use of advanced imaging with a focus on specific pathophysiologic questions and pathways (Sorensen, 2013). For mechanistic neuroimaging, advanced molecular, structural, or functional techniques can be implemented. In this section I will introduce the use of diffusion tensor imaging-derived mean diffusivity (MD) measures to studying the microstructural changes in structural plasticity. This approach will be used chapter 9 to investigate the potential microstructural correlates that may elucidate the histopathological mechanisms of grey matter changes seen in patients with chronic knee OA pain.

The cellular microstructure, within the parenchyma of brain tissue influences the overall mobility of diffusing molecules via numerous barriers and compartments formed by e.g. nerve cells, glial cells and axons within the tissue (Beaulieu, 2002). MD, which can be quantified from diffusion tensor imaging (DTI) (Pierpaoli et al., 1996) provides as an index of the magnitude of water diffusion (Schmierer et al., 2007), and hence reflects the microstructural elements that effect water diffusion (particularly cellularity) of the brain tissue. DTI-derived measures e.g. MD and diffusion anisotropy can be altered by several brain microstructural

environmental factors such as inflammation, intra- or extra-cellular swelling, cell death, membrane damage, demyelination, cell growth, axonal loss or reorganization, or gliosis (Wheeler-Kingshott et al., 2003). For example, clinical examples of higher MD values can be seen in e.g. chronic demyelinating lesions (Larsson et al., 1992) and Alzheimer's disease (Kantarci et al., 2005). Underpinnings of reductions in MD may include any process that leads to hindered or restricted diffusion (Le Bihan, 1995), such as acute pathological reactions with subsequent putative cell swelling (Kumar et al., 2012), acute ischemia (Ahlhelm et al., 2002), and generally in cases of non-necrotic neoplasia (Alexander et al., 2007).

Literature regarding changes in MD of the cortex is sparse, although studies have shown changes in cortical MD in multiple sclerosis (MS), Alzheimer's disease and healthy ageing. Cortical MD increase has been proposed to reflect putative cortical grey matter integrity loss (Vrenken et al., 2006), but without the availability of histological data, the importance of its microstructural basis in disease states (including chronic pain) is unclear.

In summary, MD is primarily affected by microstructural parenchymal components, and hence provides a potential means to

study the mechanism of alterations to cortical volume in both health and disease states. However, inferences from the correlates of MD changes alone are less specific unless combined with findings from other imaging metrics such as cortical thickness, MR spectroscopy, and fMRI, to provide more specific information about the neuro-histopathological substrates of grey matter changes in chronic pain.

1.4 Systematic review of the literature

1.4.1 Overview and aims

Chronic musculoskeletal (MSK) pain is a major health and societal burden affecting about 30% (13.5-47%) of the general population (Cimmino et al., 2011). There has been converging evidence in the literature that chronic MSK pain and morphological brain changes are associative. However, there is little consensus on correlation pattern or direction, temporal relation, and spatial location of these changes. In this review, I'll employ a systematic approach in searching the available literature for MRI studies that investigate grey matter changes in chronic pain in MSK disorders. The following objectives are set:

- To investigate the association of musculoskeletal chronic pain with structural brain changes
- To investigate the pattern of correlation if any, as well as evidence for whether chronic pain is an antecedent or a consequence of structural brain changes
- To review the evidence for reversibility of structural brain changes associated with chronic pain following therapeutic treatment

1.4.2 Methodology

1.4.2.1 Search strategy

A computerized search for articles published in the last 10 years up to early June 2013 was performed using PubMed search engine, for structural brain MRI studies in MSK chronic pain. Chronic pain imaging is a relatively new research topic; the sentinel paper studying brain morphology in chronic pain was that by Apkarian et al (Apkarian et al., 2004). Based on initial searches of publications prior to this, only a single paper, though with negative findings, investigating morphological correlates of pain in migraine was identified (Matharu et al., 2003). On this basis, the systematic review of electronic databases was limited to publications from 2004 onwards, although references of relevant articles including review articles were hand searched.

Search terms were derived from the key words (pain, brain, grey matter, musculoskeletal pain, and MRI). Possible derivatives of each key word were entered and complete search matrix was generated (Appendix 3, table1). PRISMA guidelines (Moher et al., 2009) were used to identify literature. Selection criteria were applied to the initially identified 1278 papers. Following cascading steps of screening and assessments (Fig 1, and appendix 3 table

1), based on information from titles and if needed from abstracts, and then a further assessment step was carried out based on information from abstract and if needed from full text, titles not pertaining to the research question or papers that deemed not relevant were accordingly removed.

1.4.2.2 Eligibility criteria

Studies were included if they were:

- Original human research (adults only)
- Using MRI anatomical scanning
- Of any chronic pain condition that can be categorized under MSK disorder
- Pain for ≥ 3 months
- Published in Peer-reviewed journal in or translated into English

Studies were excluded if they were if they were:

- Non original research e.g. reviews, letters, or case studies
- Preclinical
- Using imaging modalities other than structural MRI
- Pilot studies (in terms of being conducted to evaluate feasibility and predict requirements of mother study. Studies with small sample sizes are still considered)
- Chronic pain condition other than MSK
- Using acute (< 3 months) or experimental pain paradigm

A table by search terms and outcome records is shown in (appendix 3, table 1). Flow diagram is shown below (Fig 1).

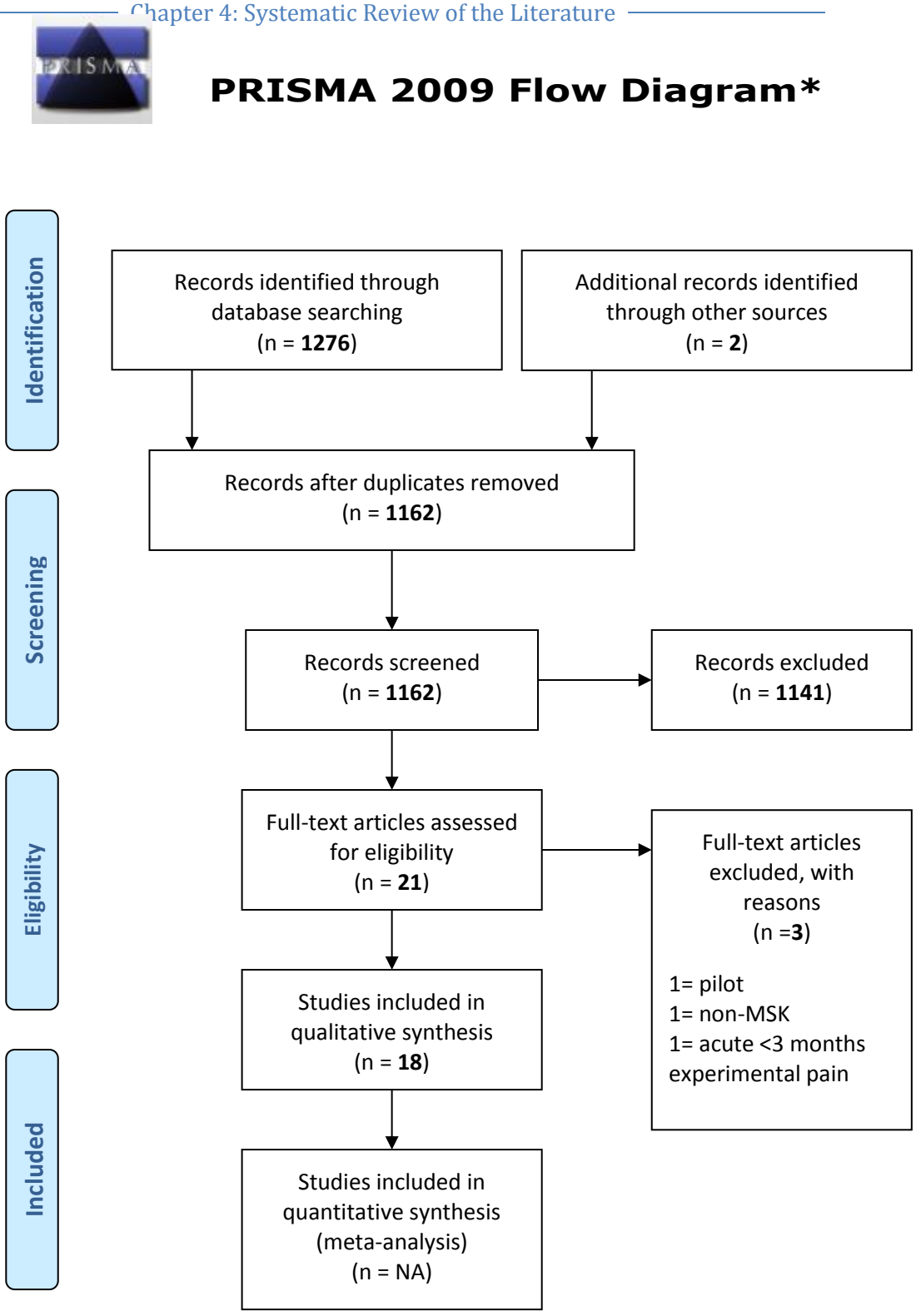


Figure 1. A flow chart of the process of identifying relevant literature

PRISMA chart from (Moher et al., 2009): The PRISMA Group (2009)* **www.prisma-statement.org

1.4.3 Results

1.4.3.1 Search results

The process of selection (Fig 1) resulted in 21 papers being considered for inclusion. Further detailed full-text read-through led to an exclusion of 3 papers (1 pilot, 1 non MSK, and 1 used experimental repetitive acute pain paradigm), and therefore the remained total number, which met eligibility criteria for final inclusion, was 18. Digital copies of all 18 studies were obtained. The earliest study was published in 2004 (Apkarian et al., 2004) and the most recent in June 2013 (Rodriguez-Raecke et al., 2013).

1.4.3.2 Quality assessment and characteristics of studies

Quality of studies was evaluated using the Critical Appraisal Skills Program (CASP) guidelines (Critical Appraisal Skills Programme CASP, 2004). The CASP guidelines for case control studies ask eleven questions to assess studies for clear and unambiguous focus, appropriate methodology, unbiased recruitment of cases and controls, unbiased measurements of exposure, control for confounders, and precision of results, trustworthy, generalizability and consistency with evidence from others. These questions can be summarized under three categories: validity of the study, evaluation of the results, and assessment of generalizability of

results. I have omitted one question (what are the results of this study?) as it will be stated and answered in the results section, and combined two questions into one (How precise are the results? And do you believe the results? Into: are the results precise and believable?).

Each paper included in the review underwent quality assessment. Findings are summarized in (Appendix 3, table 2). Overall, all studies addressed their aims and objectives clearly with not much difference in research approach. However, most studies appeared to be underpowered with small sample sizes included. No study provided a rationale for the investigated sample size or how it was calculated.

The focus of the included studies was mainly to address the question of whether chronic pain is associated with morphological brain changes. A range of MSK disorders were studied, including chronic back pain, fibromyalgia, osteoarthritis, temporo-mandibular disorders, phantom limb, and rheumatoid arthritis. In all studies, a cross sectional case control design was implemented where average GM and/or cortical thickness of patient was compared with that of matched healthy controls. Some studies (table 1) investigated the interrelation between the morphological changes and duration of chronic pain, pain severity or other pain-related characteristics such as psychometrics. In three studies (Rodriguez-

Raecke et al., 2009, Gwilym et al., 2010a, Seminowicz et al., 2011), patients were further followed-up in a longitudinal design after therapeutic treatments that involved joint replacement to treat OA disease or spinal surgery or facet injection in case of chronic low back pain. The morphometric technique used was mostly voxel-based morphometry (VBM) with some exceptions (Moayedi et al., 2011, Seminowicz et al., 2011, Wartolowska et al., 2012), where surface based morphometry (cortical thickness) was the used or additively used technique.

Buckalew et al (Buckalew et al., 2010a) used in-house software.

1.4.3.3 Participants

480 patients were included in all 18 studies with an average age of 52 years (based on mean of the mean ages in all groups). While sex distribution was well balanced in most studies some however had exclusively or mainly female patients due in part to the prevalence nature of the investigated disease e.g. fibromyalgia (Kuchinad et al., 2007, Schmidt-Wilcke et al., 2007, Hsu et al., 2009, Moayedi et al., 2011, Robinson et al., 2011). All patients had experienced persistent pain for at least three months.

1.4.3.4 MRI scanning

High resolution structural MR imaging was obtained by all studies. With the exception of (Apkarian et al., 2004, Draganski et al., 2006, Schmidt-Wilcke et al., 2006, Kuchinad et al., 2007, Schmidt-Wilcke et al., 2007), where 1.5 Tesla was used, in the rest of the studies scanning was performed with 3 Tesla scanners.

1.4.3.5 Outcomes

The studies' main findings are summarized in table 1. Most studies found a reduction in grey matter (GM) density, volume or cortical thickness. However, the distribution of brain structures, which show pain-related GM changes, was highly varied between and within different primary aetiologies of chronic pain disorders. Some studies reported negative correlations i.e. less GM volume with longer pain (Apkarian et al., 2004, Schmidt-Wilcke et al., 2006, Kuchinad et al., 2007, Buckalew et al., 2010a, Baliki et al., 2011, Wartolowska et al., 2012), while others found positive correlations(Younger et al., 2010, Moayedi et al., 2011) or no correlation(Draganski et al., 2006, Schmidt-Wilcke et al., 2007, Buckalew et al., 2008, Hsu et al., 2009, Gustin et al., 2011).

Only few studies investigated the reversibility of GM changes longitudinally when chronic pain was alleviated (Rodriguez-Raecke et al., 2009, Gwilym et al., 2010b, Seminowicz et al., 2011)

Table 1. Characteristics and main findings of the review studies

Study	Condition	Sample size and age* (Y)	Pain* duration (M)	Main findings/Correlation with pain duration/reversibility
1. (Apkarian et al., 2004)	CBP	n= 26 47.0± 12.6 G2/G1=non/ neuropathic	> 12 G1 135.6± 136.8 G2 62.4 ± 44.4	Patients had less volume in total GM, DLPF, and thalamus. Pain duration correlated negatively with total GM volume corrected for age. DLPF had negative correlation with pain characteristics including duration. Thalamus had negative correlation only with pain duration.
2. (Draganski et al., 2006)	Phantom limb	n= 28 41.9± 13.8	> 9 119.3± 140	Significant decrease in GM of thalamus in patients which positively correlated with time span after amputation but not with frequency or magnitude of phantom pain
3. (Schmidt-Wilcke et al., 2006)	CBP	n= 18 50.4± 6.8	> 36 176 ±87.60	Patients had larger GM in basal ganglia and thalamus but less GM in brainstem and S1, which -the latter- negatively correlated with pain intensity and unpleasantness but not with pain duration.
4. (Kuchinad et al., 2007)	FM	n= 10 52	> 3	Patients had less total GM, negatively correlated with pain duration corrected for age, also less GM in several clusters (uncorrected)
5. (Schmidt-Wilcke et al., 2007)	FM	n= 19 53.6± 7.7	> 3 173± 86.5	GM increase and decrease in several areas though with no significant correlation with pain duration (both un/corrected results)
6. (Buckalew et al., 2008)	CBP	n= 8 74.5± 4.2	> 3	Patients had less GM in the posterior parietal but no correlation between pain duration and total grey matter or ROI volume
7. (Hsu et al., 2009)	FM	n= 29 41.7	> 3 153.6± 99.6	Patients had less GM in Lt. anterior insula, which correlated negatively with trait anxiety, but not with pain duration
8. (Rodriguez-Raecke et al., 2009)	Primary hip OA	n= 32 66.8± 9	> 12 88.2	Significant GM changes with pain in several areas and **partial reversibility** after arthroplasty but correlation with pain duration was not investigated
9. (Buckalew et al., 2010a)	CBP	n= 16 74.6	≥ 3 93	Negative findings for GM, though a significant correlation between WM integrity and pain duration was found
10. (Gwilym et al., 2010a)	Primary hip OA	n= 16 68	NA¶	GM changes in patients mostly increase in several areas except thalamus, which showed a decrease that **reversed** to normal after arthroplasty. Correlation with pain duration was not investigated

*Expressed as (M± SD). CBP= chronic back pain, FM= fibromyalgia, OA= osteoarthritis, TM= temporo-mandibular, CRPS= chronic regional pain syndrome, RA= rheumatoid arthritis. ¶ Authors used a definition of chronicity as "pain that exceeded normal healing time", so > 3 months was assumed.

Continue Table 1.

Study	Condition	Sample size and age* (Y)	Pain* duration (M)	Main findings/Correlation with pain duration/reversibility
11. (Younger et al., 2010)	Myofascial TM pain	n= 15 38± 13.7	> 12 52.8± 34.8	GM changes in patients both increase e.g. S1 and decrease e.g. inferior frontal, anterior insula. ONLY positive correlation with pain duration in numerous regions

12. (Baliki et al., 2011)	CBP
	CRPS
	Knee OA
		n=36;
		48.2±11.4
		n=28; 40.6 ±7.4
		n=20;
		53.5±7.4
			147.9±137.9
			39.3± 40.8
			146.7± 114
				Condition-specific GM reductions in several areas. No significant correlation with pain duration, intensity or their interaction for any group. However by combining and median-split all groups, longer pain group showed a significant negative correlation with the insula GM density.

13. (Gustin et al., 2011)	TM disorders	n1= 20 n2=21 45.7± 2.9	> 18 138± 40.8	Significant GM reductions ONLY in the neuropathic group in several areas e.g. S1, insula. No significant correlation between GM volume and pain duration
14. (Moayedi et al., 2011)	TM disorders	n= 17 33.1± 11.9	> 3 117.7± 99	Patients had thicker cortex in S1, and PFC. Positive correlation with thalamus GM volume. Patients who had the disease for ≥7 years showed a progressive increase in thalamic GM
15. (Robinson et al., 2011)	FM	n= 14 43.1± 6.9	> 3	GM reductions in e.g. ACC, insula compared to HCs. No significant correlation between measures of negative affect and the grey matter volume. Pain duration was not investigated
16. (Seminowicz et al., 2011)	CBP	n= 18 46± 10.64	> 12 60± 57	Significant changes with pain and **reversibility** of DLPFC after treatment (surgical or medical). Correlation with pain duration was not investigated. Recovery of GM was significantly correlated with reduced pain intensity and improvement in physical activity
17. (Wartolowska et al., 2012)	RA	n= 31 Median= 57	> 18 Median= 180	Patients showed a GM increase in basal ganglia. Negative correlation between disease duration and gray matter density in the right thalamus, which abolished after controlling for age
18. (Rodriguez-Raecke et al., 2013)	Hip OA Coxarthro-sis	n= 20 63.3± 9.5	> 12 88.2	Extension of previous study (no. 8). Reversibility and significant changes after arthroplasty with progressive GM increase in frontal lobe areas. Correlation with pain duration was not investigated

1.4.4 Discussion

Association of chronic MSK pain with structural brain changes

Most studies included in the review have reported grey matter changes in association with chronic pain, though with striking inconsistency as to which areas are specifically affected. While predominantly reduction, GM enlargement in some areas has also been found. These discrepancies may reflect a random error due to small sample sizes, limitations of the grey matter volume estimation or true biological differences in underlying pain characteristics or predisposing factors. A spatial dissociation may be explained by the multidimensionality of chronic pain experience and related comorbidities. Chronic MSK pain can result in reduced quality of life (Reginster, 2002, Salaffi et al., 2005), reduced exercise, impaired sleep and long-term medication that may contribute to the pattern of progressive cortical thinning.

Importantly, most studies have low power that is limited by their inability to control for the multitude of factors that influence brain morphometry. As these factors are usually not well controlled between patients and controls, they may significantly confound between group analyses.

GM reductions have been found in several cortical and subcortical areas including the thalamus, primary somatosensory, insular cortex, DLPFC, cingulate cortex, amygdala, posterior parietal cortex, orbitofrontal cortex, opercula, and brainstem. There seems to be an overlap among these studies in the (thalamus, DLPFC, insula, cingulate cortex, and brainstem). Most of the regions which showed structural changes are considered part of the central pain processing network or what is known as 'the pain matrix' (Melzack, 1989).

The mechanisms by which these changes occur and which factors may explain heterogeneity of results are largely unknown. As detailed previously in (chapter 2), the exact histopathological substrates of these changes remain intriguingly unclear, especially in the absence of any confirmatory histological evidence. Imaging-based non-invasive techniques e.g. diffusion tensor imaging, and the derived mean diffusivity metrics (see chapter 2) along with other multimodal techniques such as MR spectroscopy and functional imaging may play a substantial role in elucidating the histopathological mechanisms of GM changes and provide unprecedented insights into the central mechanism of pain chronicity.

Most studies, with few exceptions, used voxel based morphometric technique in detecting differences between groups. Quantification of grey matter changes can be achieved using different image analysis techniques, however measuring the cortical thickness using a surface-based approach arguably improves robustness over standard volumetric methods: Cortical thickness assessment provides a directly interpretable metric, and is more sensitive allowing for detection of sub-voxel changes (Liu et al., 2012b, Pereira et al., 2012), less sensitive to inaccuracies of spatial normalization and smoothing (Bookstein, 2001, Augustinack et al., 2013). However, it remains limited by its inability to investigate subcortical structures. Manual morphometry in some cases of ROI volumetric measurement is still considered the gold standard (Cherbuin et al., 2009, Morey et al., 2009, Shen et al., 2010). However, it can be time consuming and liable to human errors.

The anatomical MRI and morphological brain measures, as biomarkers, have been already utilized to evaluate e.g. Alzheimer's disease (www.adni-info.org). The morphological brain signature in chronic pain can be an invaluable candidate as an objective biomarker for chronic pain to monitor pain therapy and responsiveness, determine eligibility for invasive treatment

(Gwilym et al., 2010a), and predict pre-existing vulnerability and disease-driven changes (Davis, 2011, Mansour et al., 2013).

In my research I'll combine findings from (cortical) vertex-based, (subcortical) voxel-based, and (ROI) manual morphometric techniques to overcome the limitations of using each approach individually and provide a framework to understand the basic mechanisms of structural brain changes in chronic pain.

1.4.4.1 Reversibility, and the temporal relationship between structural brain changes and chronic pain: Which comes first?

The dynamic influential relationship between chronic pain and brain morphology is poorly understood. There are no longitudinal studies to demonstrate the progression individually, and the slow progression would be challenging for such studies. However, there are few longitudinal studies before and after pain relief providing strong evidence for at least partial reversibility of morphometric findings (Rodriguez-Raecke et al., 2009, Gwilym et al., 2010b, Seminowicz et al., 2011).

The cumulative effect of morphometric changes over several years to decades of chronic pain indexed by the correlations shown between GM volume or density and pain duration in MSK disorders in e.g. chronic back pain (Apkarian et al., 2004, Schmidt-Wilcke et

al., 2006), rheumatoid arthritis (Wartolowska et al., 2012) and fibromyalgia (Kuchinad et al., 2007) argue strongly for but cannot prove them to result from the chronic pain experience.

In an experimental study (Teutsch et al., 2008), authors exposed healthy subjects to a long tem painful stimulation for reasonably long periods of time ~ 20 minutes, on a daily basis for 8 consecutive days. The researchers were able to detect a substantial increase of grey matter in brain areas involved in pain processing including mid-cingulate and S1. These changes were thought to have convincingly resulted from the painful stimulations as they receded when the regular nociceptive input was stopped. However, the study can be criticised for lacking controls.

In another more recent study (Stankewitz et al., 2013), authors were able to reproduce the aforementioned study findings yet with different results. A group of 27 healthy volunteers received repetitive painful stimuli for 11 consecutive days. They were compared to a control group, and behavioural data were analysed. The experimental group was divided in terms of behaviour into those who developed pain sensitization n= 14 and habituaters n=13. Only sensitizers developed grey matter reductions in pain centres (including ACC, insula, middle frontal cortex, and amygdala) as a result of the nociceptive stimulation.

Regardless the pattern of cerebral change, the above experiments provide some evidence that brain structural plasticity can occur secondary to pain. However, the complexity of pain experience arising not only from the multidimensionality nature of pain itself but also from the association of multiple co-morbidities, overall change in quality of life (Tuzun, 2007) and pain-related medications, all these factors make it difficult to infer a cause and effect relationship.

Austin Bradford Hill (Hill, 1965) proposed criteria for assessing causal relationship, which were further summarized by (Petrie and Sabin, 2005) under the following points (with modification by myself to fit the pain and brain plasticity model):

- The cause must precede the effect
- The association should be plausible
- Results should be consistent among studies
- The association between the cause and the effect should be strong
- Higher levels of the effect should lead to more severe disease or faster onset
- Removing the nociceptive stimulus should reduce the pain-related brain changes

Some information can be obtained from cross sectional studies however to provide strong evidence for the casual role of chronic

pain in brain plasticity, longitudinal studies involving evaluation of the progressive brain changes with pain, and their reversibility upon pain relief are strongly required.

1.4.5 Conclusion

There is converging evidence that structural brain alterations are associated with persistent nociceptive stimulation. Theses changes seem to cumulate over the duration of the disease and may partially reverse to normal when the regular nociceptive input is stopped. Anatomical neuroimaging along with other neuroimaging modalities may play an important role in better understanding the central mechanism of pain chronification, monitor pain therapy and responsiveness, and provide a promising biomarker tool for evaluating chronic pain.

1.5 Aims and scope of the thesis:

In spite of the literature-based evidence presented above, that chronic musculoskeletal pain is associated with grey matter changes, the direction of such changes, spatial location, and correlation with pain characteristics and interlinked behaviours are still poorly understood and furthermore inconsistent between studies. The ultrastructural mechanism of such cerebral changes is largely unknown and has not been attempted previously in MSK pain disorders. Previous studies have mostly used individual modalities of image analysis, often implementing VBM approach, which can provide only partial information on the structural brain plasticity in association with chronic pain. Moreover, small samples, technical limitations of GM volume estimation may largely contribute to discrepancies between studies.

In this research I choose to combine advanced imaging and morphometric techniques to investigate and mechanistically assess structural brain correlates of chronic pain due to knee osteoarthritis. By doing so, the researcher is aiming to provide a broad and more conclusive morphological profile of brain in chronic pain that can constitute a contribution to knowledge on the central processing of pain and pain chronification, as well as provide novelty and potential to understand pain phenotypes for stratified pain management.

The experimental part 2 will be concerned with a series of studies that I have conducted during my PhD registration period, and will be introduced here in brief:

In chapter 5, a full description of the participants of the study (knee OA patients and HC) will be provided including their recruitment and scanning procedures. The demographic data, between-group differences in cognition, depressive disorders, pain catastrophizing, state and trait anxiety, as well as characteristics of pain dimensions in patient group will be detailed. This chapter will be referred to in the methods section of subsequent experimental chapters.

Chapter 6 investigates the hippocampal volume and cognitive function in chronic knee OA patients using up-to-date manual morphometric techniques and tightly defined volumetric measurement protocol.

In chapter 7, combined manual morphometric and cortical thickness techniques will be used to investigate the ACC (a pivotal limbic structure involved in central pain processing) and its sub divisional morphology in relation to cognitive and emotional aspects of pain as well as its potential role in the process of central pain sensitization.

Improved technique of voxel based morphometry using DARTEL (Diffeomorphic Anatomical Registration using Exponentiated Lie algebra) (Ashburner, 2007), will be presented in chapter 8 to investigate cortical and subcortical grey matter correlates of chronic pain and chronic pain severity.

The effect of pain duration on cortical thickness will be the main focus of the first half of chapter 9, with stratification by sex to investigate its moderating effect between chronic pain and neocortical changes.

The second half of chapter 9 will be dealing mainly with mechanistic explorations by means of investigating cortical mean diffusivity to inform on ultrastructural status of cortical tissue in association with cortical remodelling in chronic pain, and analyses will be performed to compute gyrification index to investigate the gyrification changes that may relate to the overall plasticity of brain in chronic pain.

2 PART TWO: EXPERIEMTNS

2.1 Generic methods and characterisation of study participants

2.1.1 Introduction

The studied participants, both knee OA patients and healthy controls, will be described with regard to their recruitment pathways, data acquisition, and demographic, psychological and pain-related characteristics. This chapter will be referred to, in the methods sections in the subsequent chapters of experimental studies. Written informed consent was obtained from all participants, and Nottingham Research Ethics Committee2 approved the study (10/H0408/115).

2.1.2 Methods

2.1.2.1 Recruitment pathways

The recruitment process* for studies included in this thesis was facilitated by a research coordinator (Maggie wheeler) with my input and directions. I was responsible for confirming eligibility prior to inclusion.

*My study was part of a bigger project run by the Arthritis Research UK pain centre, which is funded by a substantial award from the Arthritis Research UK (Grant number 18769), and operating as an umbrella under which several studies were conducted. Along with our neuroimaging studies, preclinical, pharmacological, questionnaires-based and other studies were also similarly performed. A research coordinator (Maggie Wheeler) was employed by the centre to help with participants' recruitment, quantitative sensory testing, and other administrative issues. The research coordinator relied on my input for my study recruitment via phone conversations, email exchange, and through several direct meetings regarding eligibility of participants and age and sex matching before liaising further or confirming bookings with potential participants. Once eligibility criteria were met, The research coordinator would confirm bookings, arrange transportation and send questionnaires to the

eligible participant by post. On scanning day, The research coordinator would meet (on few occasions I did) the participant at the main entrance of Queens Medical Centre and walk them through to our division, where I meet the participant, greet them and introduce myself. I would then receive questionnaires and make sure all sections were answered in the right way and no question was missed and/or un-answered. I would then start by explaining the study to the participant and answer their questions if any, consent them, and then I provisionally check their safety and eligibility for MR scanning before final approval is obtained by the radiographer (Anita French).

The research coordinator would then take measures of pressure pain thresholds at the painful knee as well as at remote sites. Once finished, the research coordinator would hand over to me where I would conduct a face-to-face interview to assess the cognitive ability of the participant using the Montreal Cognitive Assessment test (MoCA) (Nasreddine et al., 2005), and also record educational level, pain duration and severity, and take measures of body weight and height.

Next, I would escort the participant down to the scanning room where I would introduce the participant and the radiographer to each other and help with getting the participant to the scanning table. The radiographer would start scanning using the pre-defined protocol, which involved localization, T1 scanning, DTI, spectroscopy, resting state fMRI.

As soon as the anatomical T1 scans became available I would visually inspect the images on the scanner console, check quality and request repetition if e.g. motion artefact or other correctable factors emerged. The radiographer, a co-worker, or I would then request a taxi for the participant and escort them to the main entrance of the medical school of University of Nottingham.

- ❑ Participants with chronic knee OA pain

The aim was to recruit 25 Patients with radiologically defined knee OA if they had chronic pain defined as ‘pain most of the day on most days of the week’ for at least the last month (Peat et al., 2006a). Patients were identified and contacted by mail from 15 general practices in Nottinghamshire and Derbyshire (supported and organized by primary care research network, PCRN), orthopaedic outpatient clinics of Nottingham University Hospitals, and from previous questionnaire and trial studies that were carried out in the Rheumatology Department at the University of Nottingham.

Sample size was increased to (n= 31), by including eligible and quality assured cases from pilot study, powered to detect 0.25mm cortical thickness changes (Pardoe et al., 2013).

❑ Inclusion Criteria:

- Radiologically defined OA knee changes
- Pre-operative status
- Chronic knee pain 'according to the definition above'
- Un-medicated (with the exception of traditional pain killers or antihypertensive drugs)
- Able to give informed consent

❑ Exclusion criteria:

- Under 18 years
- Pregnancy
- MRI contraindications
- Major medical or neuro-psychiatric comorbidities such as:
  - Diabetes
  - Strokes
  - All types of cancer
  - Epilepsy
  - Alzheimer's disease
  - Chronic hepatitis
  - Chronic renal failure

These criteria were selected based on my literature search during the first few months of my PhD studies. Having had read several structural neuroimaging studies on chronic pain during the period of my literature review, I came to know factors and confounders that need to be considered when selecting patients. This knowledge was further enhanced by discussions with my supervisors and

colleagues in the pain centre during our internal scientific meetings.

Due to some recruitment difficulties (acknowledged in the limitation section - general discussion part 3) it was quite challenging to avoid recruiting elderly patients who have been on antihypertensive or diuretic medications. The impact of such drugs on brain volumetric measurements cannot be underrated. However, having searched the literature, the exact effect is not clearly understood and no studies on such influential relationship could be found.

❑ Healthy control participants

The aim was to recruit 25 pain-free healthy controls age- and sex matched to the knee OA patients. HCs were recruited via posters advertised in general public places¶ including libraries, community centres and hospitals waiting areas and also through invitations to patients' partners. (Recruitment poster can be found in appendix 4)

❑ Inclusion criteria:

- Pain-free and generally fit and healthy
- Un-medicated (with the exception of occasional acute states medication)
- Age and sex matched to the included knee OA participants
- Able to give informed consent

❑ Exclusion criteria

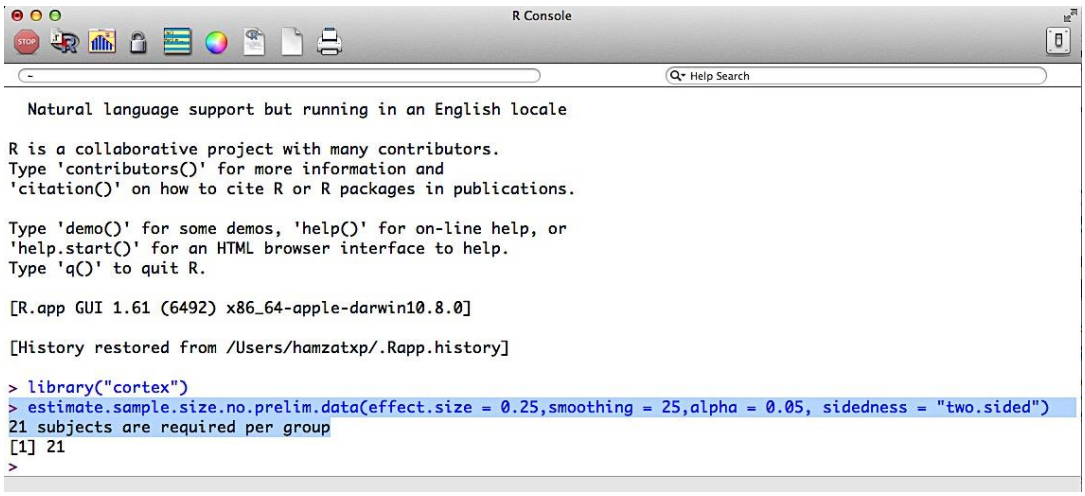
- Under 18 years
- Pregnancy
- MRI contraindications
- Major medical or neuro-psychiatric comorbidities such as:

- Diabetes
- Strokes
- All types of cancer
- Epilepsy
- Alzheimer's disease
- Chronic hepatitis
- Chronic renal failure

¶The healthy control recruitment poster was printed out by myself and distributed locally in around 2 miles radius where it was advertised in public places such as libraries, community centres and churches, as well as locally around the Queens Medical Centre, and University of Nottingham campus- all with permissions from people in charge whenever required. I also sensibly approached patients to invite their partners, if they were interested, to take part as healthy volunteers. I acknowledge help and assistance from Maggie Wheeler in speeding up the HC recruitment process by advertising the posters in the city hospital and taking a number of posters to public places in her local district.

2.1.2.2 Power calculation

The statistical model provided by Pardoe and colleagues (Pardoe et al., 2013) that has been published in the "Human Brain Mapping" journal, was used to calculate how many subjects are required for a well-powered cortical thickness analysis. The model relies basically on pre-determined parameters including the cortical thickness differences to be detected, smoothing factor, and type I error rate. Using these parameters an equation is developed that can be run using "R" software available at (http://www.r-project.org/). R is an open access software environment for statistical computing and graphics that runs on UNIX platforms. R was downloaded on (MacBook Pro 17-inch, Late 2011) for personal use by the author.



"R" software package used on UNIX platform for power calculations

The sample size was powered to detect 0.25mm cortical thickness between-group differences, using a smoothing factor of 25mm and type one error rate was accepted at 0.05. Therefore, 21 subjects were required per group. As there were some cases scanned during the pilot study that met the eligibility criteria for the main study, these cases were added for added high power at no extra human or financial cost.

2.1.2.3 Protocol for MRI scanning

Prior to scanning, every participant was checked for safety to undergo MRI. Safety questionnaire is provided in the appendix 4. High reliability of brain morphometric measurements requires excellent image quality in terms of spatial and contrast resolutions. Freesurfer software package (http://surfer.nmr.mgh.harvard.edu/)

used to estimate cerebral cortical thickness, for instance, relies mainly on a good contrast between grey matter and white matter in one end and grey matter and CSF in the other from the entire three dimensional MR volume to produce representations of cortical thickness, calculated as the closest distance from the grey/white boundary to the grey/CSF boundary in an un-intervened fully automated process. Same requirements of good image quality apply to the manual and voxel-based morphometric measurement techniques.

It is conceivable that higher field strength scanners (e.g. 3T vs. 1.5T) provide significantly improved anatomical images in terms of better signal to noise ratio resulting in improved detailed spatial resolution, and better validity and sensitivity to morphometric changes as well as a faster scanning time (Schmitt et al., 2004, Stankiewicz et al., 2011, Stehling et al., 2007). For all anatomical scanning (T1 weighted imaging) of studies included in this thesis, MRI was performed at 3T (Discovery 750, GE Medical Healthcare, Milwaukee, US) using a 32-channel head coil.

3D anatomical brain scans were acquired using axial T1 FSPGR-BRAVO sequence (FOV= 256x256mm, voxel size= $1mm^3$, TE=3.3ms, TR=8.5ms, TI=450, FA=12, Acceleration factor= 2, acquisition time= 4min 10s).

2.1.2.3.1. Steps used to optimize MRI scans for morphometric analysis

The anatomical scanning protocol was developed empirically where the same subject (myself) underwent anatomical MR scanning as part of an on going REC-approved MRI protocol development project. Three candidate protocols with different imaging parameters were tested (Fig 1). Each volume of images underwent a fully automated step of analysis using Freesurfer (Mac version 5.1.0 available online http://surfer.nmr.mgh.harvard.edu/). The protocol with which best analysis outcome could be achieved (i.e. the least segmentation errors) was selected (by agreement from two consultant neuroradiologists and one medical physicist and my self) as a standard for all-subsequent scanning of experimental studies.

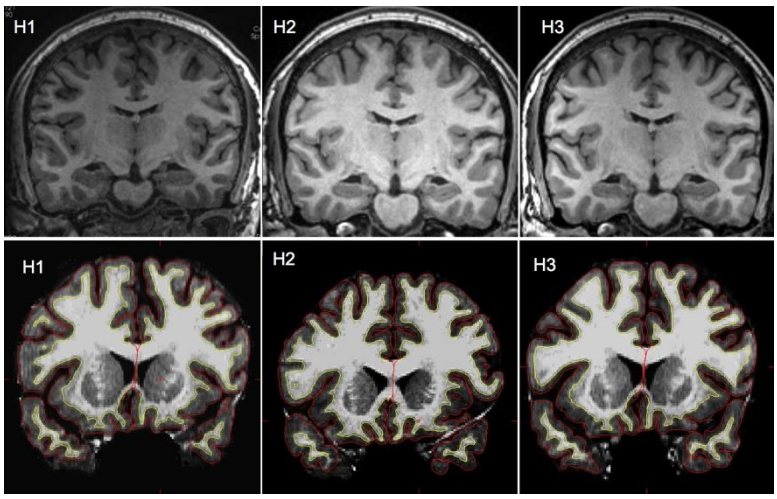


Figure 1: Three T1 scans (top) with slightly different scanning parameters were used to select a protocol with best achievable image quality. Corresponding Freesurfer analysis outputs are shown in the bottom row

2.1.2.3.2 Diffusion weighted MRI

Diffusion weighted magnetic resonance imaging (DWI) provides image contrast based on the random Brownian motion of water molecules in a given voxel. Normally with time there will be a chance for an individual molecule to be found at a distance from its original location. However, the cellular microstructure, within the parenchyma of brain tissue, influences the Brownian molecular motion via barriers and compartments formed by tissue components such as nerve and glia cells, axons, intracellular organelles, myelin sheaths and intra- and extracellular matrix (Beaulieu, 2002).

Using DWI, water motion can be quantified in vivo using T2 weighted spin-echo sequence and bipolar gradients (Apparent diffusion) (Stejskal and Tanner, 1965). As the image volume is formed of a number of voxels, each voxel intensity indicates the highest estimate of water molecular diffusion at that particular voxel. The motion is detected as attenuation of the signal intensity, and the degree of attenuation has been found to proportionate with motion of water, for which sensitivity detection can be varied by changing the gradient amplitude (known as "b" value), the duration of the applied gradient, and the time interval between the paired gradients. (Koh and Collins, 2007). In vivo, large diffusion will show signal attenuation at small b values whereas restricted

diffusion will require larger b values to be detected (Koh and Collins, 2007).

Mean diffusivity (MD) measures the magnitude of water diffusion (Schmierer et al., 2007), and is sensitive to pathological disruption of brain tissue integrity (Pierpaoli et al., 1996), and to adaptive histomorphologial changes following learning tasks as recently shown in an animal model (Blumenfeld-Katzir et al., 2011).

Diffusion weighted images were acquired with the same MR scanner using spin-echo echo-planar imaging (EPI) with b=1000 s/mm^2 along 30 evenly spaced and non-collinear directions, plus one b=0 volume, TR=5600ms, TE=89.0ms. Voxel sizes were 1.75 x 1.75 x 2.2 mm^3 or 2.2 x 2.2 x 2.2 mm^3. A medical physicist PhD student Diane Reckziegel checked quality assurance of DTI data.

2.1.2.4 Participant characterization by questionnaires and face-to-face interview

To carefully phenotype participants and account for potential confounders, a set of questionnaires was used to record personal, psychological, and pain related characteristics of participants. These questionnaires were used based on a number of considerations including mainly the ease of administration and interpretation, self-reporting whenever suitable, and importantly adoption by arthritic pain literature. All questionnaires were

checked for completeness on the day of scanning and scored exclusively by myself, and will be individually and briefly explained. Copies of questionnaires used are included in appendix 4. Face to face completion of MoCA, educational level and pain scores was performed by myself on the day of scanning.

1. Edinburgh handedness inventory (EHI)

EHI (Oldfield, 1971), which is a self-administered questionnaire, was used to classify hand dominance of participants. Ten questions are asked to assess the hand dominance that relate to preferences of hand use in normal daily activities. The score for handedness ranges from -100 for strong left-handed to +100 for strong right-handed.

Handedness can be assessed by one of two main ways, either by asking a number of questions to determine *preference* or by physically testing for *performance* (Rigal, 1992). However, studies have shown that inventory-based preference is a reliable laterality indicator relative to the performance test (Raczkows.D et al., 1974, Coren and Porac, 1978), and furthermore it is much quicker and more convenient to administer without the need for the presence of an examiner (Bishop et al., 1996). Various types of criteria from different questionnaires are used to determine handedness dominance in everyday activities (Bryden, 1977, Chapman and Chapman, 1987). However, the advantage of EHI over other

available questionnaires is that it is brief and the items of the inventory are simple and self-explanatory especially when considering use with elderly people, as is the case in the type of cohort required for the studies in this thesis. Moreover EHI has been widely used in the literature (Veale, 2014), and a simple search using web of knowledge (www.webofknowledge.com) revealed that the original publication (Oldfield, 1971) has been cited more than sixteen thousand times as per 23 December 2014, making any results emerging from this work comparable with the literature when considering publication. EHI was received by each participant either in hand or by post to be filled in and brought by the participant on scanning day.

2. Educational level

Education scores were derived based on eight ordinal categories represent the British education system levels where 1= higher degrees and 8= none [modified from (Egerton and Mullan, 2006)].

The educational attainment (nature and quality of learning) rather than the number of years spent in schooling is believed to imply on health status (Feinstein et al., 2006), hence the highest educational achievement was recorded by myself for each subject during an interview prior to MRI scanning.

3. Visual analogue scale (VAS)

VAS is a 10-cm line starting with zero, which indicates no pain, and 10 at the end, representing the worst pain imaginable. It is used to translate the subjective feeling of pain sensitivity into a quantifiable measure. It is a one-dimensional measure of pain intensity and it has been widely and reliably used in research and clinical practice (McCormack et al., 1988). It is very convenient and almost instant to administer and quite easily appreciated by elderly participants and furthermore it is recommended by the European Federation of Neurological Societies (EFNS) (Cruccu et al., 2004).

Pain catastrophizing scale (PCS)

PCS (Sullivan et al., 1995) consists mainly of 13 statements regarding various thoughts and feelings while in pain. The scores can range between 0 and 52, and subscales for rumination, magnification and helplessness levels can be derived. PCS has been widely used and validated in different age groups and different cultures (Garcia Campayo et al., 2008, Fernandes et al., 2012, Sehn et al., 2012, Cho et al., 2013). There are a few questionnaires available to measure pain catastrophizing e.g. the Catastrophizing scale by Rosenstiel and Keefe (Rosenstiel and Keefe, 1983), which is derived as a sub-scale from the Coping Strategies Questionnaire (CSQ) by the same researchers with only 5 questions used to assess individual's catastrophizing tendency in

pain states. The PCS has the advantage of being easily and conveniently self-administered and more comprehensive in assessing the different components of pain catastrophizing. PCS questionnaire was received by each participant either in hand or by post to be filled in and brought by the participant on the scanning day.

4. McGill pain questionnaire

Two indices can be derived from McGill pain questionnaire (Melzack, 1975); the pain rating index (PRI), and present pain index (PPI). The PRI is scored from a set of descriptive words included within 20 questions and ranked in ascending order of intensity. Each patient has to select the best word that describes his or her pain. Sub-scores for sensory, affective and evaluative aspects of pain can be acquired From PRI. PPI is a 5-point (1=mild, 2=discomforting, 3=distressing, 4=horrible, 5=excruciating) pain intensity scale that evaluates the severity of pain as a general experience including the present pain in question. Scores for PRI can range from 0-78 and for PPI 0-30.

Several tools are available as measures of pain in adults particularly in arthritic diseases. The advantage of using McGill pain questionnaire is that it is a multidimensional (sensory, affective, evaluative and intensity) measuring tool that gives broad information about the various aspects of pain impact upon

individuals. Furthermore, McGill pain questionnaire has been validated and has shown high reliability (Burckhardt and Jones, 2003) since its publication in 1975. However, the disadvantage of the questionnaire is that it uses a sophisticated wording for pain description that may be difficult to understand by non-English native speakers and moreover its translation into other languages may not always match the exact original meaning (Burckhardt and Jones, 2003), which requires more caution when including people from different cultures. McGill pain questionnaire was received by each participant either in hand or by post to be filled in and brought by the participant on the scanning day.

5. PainDETECT

The PainDETECT (Freynhagen et al., 2006) questionnaire records the nociceptive and neuropathic components of pain. The scores range 0-38 with 0-12 indicating a likelihood of nociceptive, 13-18 indeterminate, and 19-38 indicating neuropathic-like pain components. There are several questionnaires available for distinguishing nociceptive from neuropathic pain. For instance, the DN4 "Douleur Neuropathique en 4 Questions" (Bouhassira et al., 2005), which combines self-report and physical tests, is another useful screening tool, but however it has been criticised for its limited sensitivity (Spallone et al., 2012). Other tools are also available such as the Leeds Assessment of Neuropathic Symptoms

and Signs (LANSS) (Bennett, 2001), The Neuropathic Pain Questionnaire (NPQ) (Krause and Backonja, 2003) and others. The European Federation of Neurological Societies (Cruccu et al., 2004) recommends that most screening tools have been sensitive in randomised controlled trials. In addition to its simplicity and self-administration features, PainDETECT was found among the highest tools in terms of sensitivity and specificity, in distinguishing neuropathic from non-neuropathic pain components, which was recorded at 85% and 80% respectively (Cruccu et al., 2004). PainDETECT questionnaire was received by each participant either in hand or by post to be filled in and brought by the participant on the scanning day.

6. Beck's depression inventory (BDI)

Beck's Depression Inventory BDI-II (Beck et al., 1996) consists of 21 multiple choice questions that record the severity of depression in participants, and it is widely used in research as well as in clinical practice. Scores can range from 0 to 63, though in practice the scores threshold for different categories may be lowered to increase the sensitivity of detecting symptoms of depression (Beck et al., 1996). In research the following scores and corresponding indicative categories can be used 0-9; normal, 10-15: minimal depression, 16-19; mild to moderate depression, 20-29; moderate to severe depression, 30-63 severe depression (Spreen, 1998).

The most relevant self-report depression and depressive symptoms measuring tools in the research context of musculoskeletal disorders according to the review published in the arthritis care and research (the official journal of the America College of Rheumatology) (Smarr and Keefer, 2011) include along with Beck's Depression Inventory; the Hospital Anxiety and Depression Scale (HADS) (Zigmond and Snaith, 1983), the Patient Health Questionnaire-9 (Löwe et al., 2004), the Centre for Epidemiologic Studies Depression Scale (CES-D) (Radloff, 1977), and the Geriatric Depression Scale (GDS) (Yesavage et al., 1983). BDI has been of high research usability making results emerging from this thesis comparable with the literature, and furthermore it has recorded high sensitivity and responsiveness to change (Smarr and Keefer, 2011), rendering it ideal for use in longitudinal studies. A concern has been raised that symptoms may overlap between BDI and medical conditions (Norris et al., 2004), which requires more caution when interpreting any evolving findings. Evidence for Reliability (internal consistency; α= 0.94) (Joe et al., 2008), and validity (Grothe et al., 2005), has been independently assessed.

BDI was received by each participant either in hand or by post to be filled in and brought by the participant on the scanning day.

7. Spielberger, State-Trait Anxiety Inventory (STAI)

(STAI) (Spielberger et al., 1983), consists of two questionnaires each of 20 items with a 4-point likert scale. The questionnaire measures two types of anxiety; state (feelings at present), and trait (feelings overall or generally) anxiety, with higher scores indicating greater anxiety. Other anxiety screening tools are available such as the "Diagnostic and statistical manual of mental disorders" (Spitzer et al., 1980), the "Hospital and Anxiety Depression Scale (HADS)" (Zigmond and Snaith, 1983), the "Beck Anxiety Inventory" (Beck and Steer, 1988), and the "Anxiety Sensitivity Index (ASI)" (Reiss et al., 1986). However, the ability of distinguishing a temporary condition and general tendency of anxiety makes STAI a perfect choice to use in assessing both short-term and long-term neurological impacts particularly when considering the use of functional and structural MRI techniques respectively as it is the case in our divisional neuroimaging studies. Since its development in 1983, STAI has been widely used as a self-report measure of anxiety in research studies and has proven high degree of reliability and validity (Quek et al., 2004). It uses non-sophisticated wording that can be conveniently self-administered specially by elderly. STAI was received by each participant either in hand or by post to be filled in and brought by the participant on the scanning day.

8. Montreal cognitive assessment (MoCA)

MoCA (Nasreddine et al., 2005), is a 30-point test to assess the different cognitive aspects of participants including visuospatial/ executive, naming, memory, attention, language, abstraction, delayed recall, and orientation. The test has been validated to detecting a mild cognitive impairment. Scores range from 0-30 and a cut-off score of ≥ 26 are considered normal. It is also available in several different languages.

"Mini mental state" questionnaire (Folstein et al., 1975) is also a well-known cognitive impairment measuring tool that has been widely used in clinical and research settings (Pangman et al., 2000). However, studies have shown superiority of MoCA over "Mini mental state" in detection of age- (Gluhm et al., 2013), and neurological disorders- (Oudman et al., 2014) related decrements of cognitive functions.

While the use of MoCA has the advantage of being relatively easy to administer (in around 12 minutes on average), I however acknowledge that it provides a summary of cognitive performance, but does not provide detailed assessment of individual cognitive domains. MoCA was administered by my self during a face-to-face interview that involved explanation and giving instruction to perform the test prior to MRI scanning.

2.1.2.5 Quantitative sensory testing

Quantitative sensory testing has been widely used to characterize sensory and pain perception in both normal and chronic pain conditions (Suokas et al., 2012, Pavlakovic and Petzke, 2010). Pressure pain thresholds (PPTs) were recorded by a trained single rater (MW)※ by applying three individual repetitions at the index finger, sternum, medial tibia, and at the medial and lateral joint lines of the painful knee. Each participant was instructed to press a hand-held button (Fig. 2A) as soon as they feel the gradually applied pressure is perceived as pain. Measurements (kPa) were obtained using a digital algometer (Somedic AB, Sweden), (Fig.2B). The average of the last two readings for the three non-local sites was used to indicate non-knee PPTs, and those from knee joint lines to indicate knee PPTs.

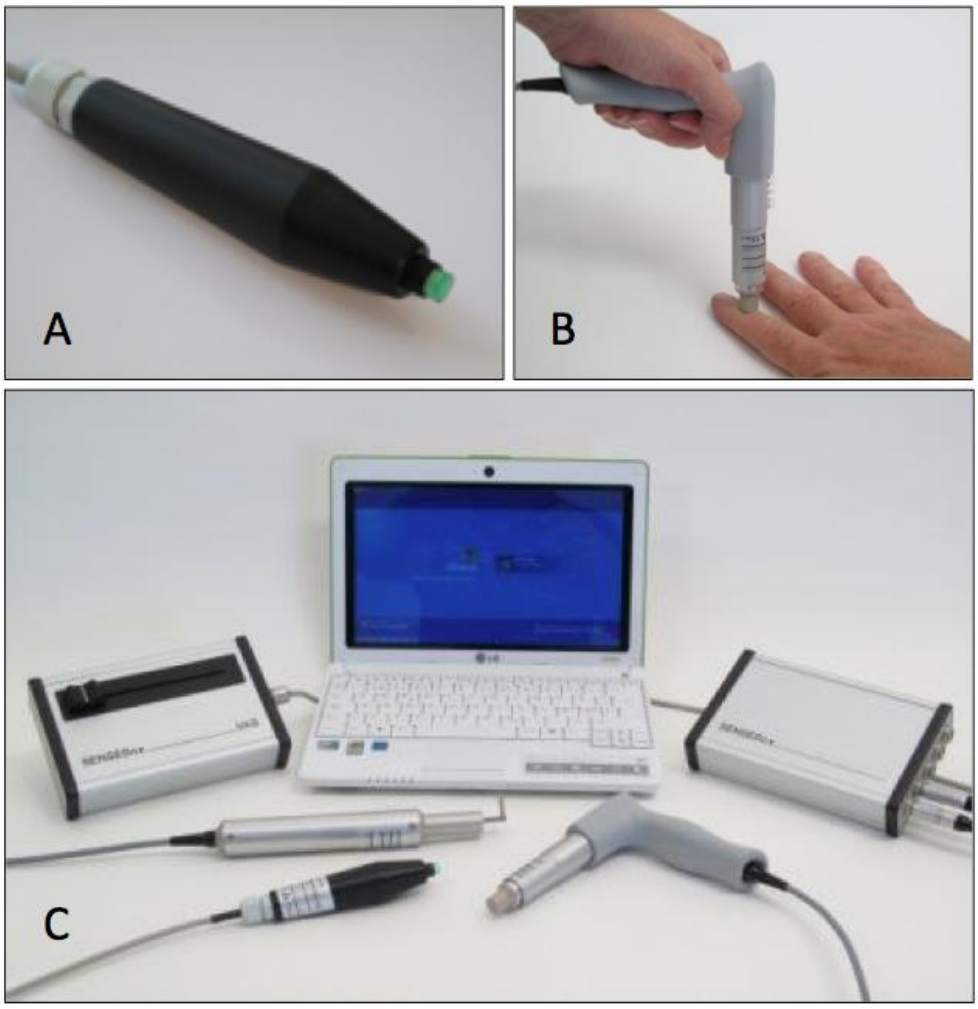


Figure 2: Mechanical pain threshold examination kit, A: patient response unit, B: algometer deep tissue transducer, C: the complete examination kit. Images taken from SOMEDIC (www.somedic.com)

※All QST data were acquired by Maggie Wheeler (except 2 cases because of exceptional incident, I had to acquire data for the subjects under direct guidance and supervision by MW). However I carried out the final calculations and data input and analysis.

2.1.2.6 Image archiving and initial processing steps

❑ Anatomical (T1) data

Data access and protection was in compliance with study protocol approved by the local ethics committee. The raw images were received in the scanner native format (DICOM), and uploaded from DVDs onto the locally protected server in our division (***PNJPUCK***). Using in-house ***dtoa***[◇] Unix based programme, DICOM data were converted into ANALYZE format which then could be used and accepted by 1) 3D Slicer software (Pieper et al., 2006) for manual morphometry, 2) SPM8 VBM (Ashburner and Friston, 2000a) upon conversion to NIfTI data format using ***MRIcro***[¥] for voxel-based morphometry, and by 3) Freesurfer (http://surfer.nmr.mgh.harvard.edu/) following conversion by Freesurfer (mri_convert) into (.mgz) data (the native Freesurfer format), for surface-based (vertex wise) cortical morphometry.

◇ In-house software written by Prof. Paul Morgan, University of Nottingham (http://www.nottingham.ac.uk/~msapm1/software/dtoa.html)

¥ Software developed by Dr Chris Rorden (http://www.mccauslandcenter.sc.edu/mricro)

- DTI and cortical MD analysis

Diffusion tensor imaging data were analysed[ϟ] using FSL version 5.0 (Smith et al., 2004). Following eddy correction, brain mask was created and non-brain voxels were removed using the FSL brain extraction tool (BET) (Smith, 2002). MD maps were derived using FSL diffusion toolbox (FDL), and subsequently underwent partial volume (PV) correction[ϟ] using in-house software to account for CSF partial volume effects (Jeon et al., 2012, Koo et al., 2009). Regional and whole-brain (global) cortical MDs were calculated using Freesurfer software package (Unix-based calculation tools), as described more fully in chapter X.

[ϟ]The preliminary DTI processing analysis was carried out by Diane Reckziegel (PhD student) as part of her studies,. I used the output of DTI processing including mean diffusivity data for further cortical MD analysis. Calculations and analyses of cortical MD metrics were carried out by myself

2.1.3 Results

2.1.3.1 Recruitment of study participants

The recruitment process resulted in 34 knee OA patients and 22 HC being identified as eligible for scanning. Three datasets from knee OA patients were excluded due to poor image quality. The final imaging data, which passed quality assurance assessment and hence were analysed were (*n*= 53: 31 knee OA patients vs. 22 HCs). In addition, five subjects were recruited but not scanned due to the following reasons; claustrophobia n=2, metal in the eye n=1, voluntarily withdraw n=1, and MRI un-safe n=1 (potential vascular clips around the heart that was inserted decades ago according to the participant's description, however neither existence nor absence of risk could be verified, so it was decided not to scan).

2.1.3.2 Demographic data

Knee OA and HC did not differ in age or sex, (Knee OA patients, *n*=31, age= 64.61± 8.36; 15 females) and (Healthy controls, *n*=22, age= 61.30± 7.46; 13 females). Detailed participants' characteristics are shown in table 1. Patients suffered from chronic knee pain as their main complaint (17 with dominant right, and 14 with dominant left knee pain), and had not undergone knee or

other joint replacement. Fourteen out of 31 patients were on regular medication mostly anti-hypertensive drugs (*n*=8) or pain-killers (Paracetamol; *n*=5, see supplementary data, table1 appendix X). Healthy controls were pain-free and non-medicated except for three subjects. *n*=1 on Zopiclone for insomnia, *n*=1 on eye drops for glaucoma and *n*=1 on oestrogens (post-menopausal), respectively.

2.1.3.3 Pain characteristics

Most patients described nociceptive or unclear pain symptoms according to painDETECT scores (0-12: 'nociceptive', *n*=19, 61%, 13-18 'indeterminate', *n*=9, 29%), with only three (<10%) considered to have neuropathic-like symptoms (painDETECT scores 19-38). For further details on pain-related characteristics see Table. 1, and supplementary table 1 appendix X.

2.1.3.4 Psychological and pain-related characteristics

Knee OA patients and HC showed significant differences only in depression and state anxiety scores, where patients exhibited higher rates in both scales (p=0.001 and p=0.04 respectively) Table 1.

Table 1: characteristics of study participants

		Age (Y)	Sex (M: F)	BMI	H (R: L)	Educ. level 1-8	MoCA Max. 30	BDI 0-63	PCS	S/ Anx.	T/ Anx.	Pain duration (Months)	Ch. Pain severity 0-10	VAS 0-10	Sen-sory 0-42	Affect-ive 0-14	Pain-DETECT 0-38
OA	Mean	64.61		28.7		*4.2*	*27.4*	*6.3*	11.8	14.3	18.5	*114.6*	4.8	2.4	11.6	*1.2*	11.5
	SD	8.36		4.9									2.4		7.5		6
	Median					4	28	7	10	14	17.5	96		2.2		0	
	Range	45.4-81.0		20-39		1-8	20-30	0-19	0-41	10-26	11-37	12-456	1-10	0-9	0-29	0-11	0-25
	Ratio		16:15		28:2 (1NA)												
HC	Mean	61.30		26.7		*5.7*	*27.2*	*2.2*	12.1	12	15.6						
	SD	7.46		5.4													
	Median					6	27	1	11	10	16						
	Range	46.8-72.9		18.9-41.4		1-8	23-30	0-9	0-29	10-23	11-23						
	Ratio		9:13		19:3												
P-Value		***.14§***	***.44Φ***	***.16§***	***.6Φ***	***.15Φ***	***.6¶***	***.001¶***	***.7¶***	***.04¶***	***.16¶***						

BMI= Body Mass Index, Education scores based on 8 categories represent the British education system levels where 1= higher degrees and 8= none [modified from (Egerton and Mullan, 2006)], MoCA= Montreal Cognitive Assessment. BDI= Beck's Depression Inventory. PCS= pain catastrophizing scale, S/T anx. = State-trait anxiety. Ch. Pain severity= chronic pain severity; average pain intensity over the past four weeks on a scale from 0 to10. VAS= visual analogue scale. ***§****= Independent t-test.* ***¶****= Independent samples Mann-Whitney U Test.* ***Φ****= Chi-squared Tests or Fisher's Exact Test when sample size is <5 in any cell.*

2.1.3.5 Quantitative Sensory Testing (Pressure pain)

One KOA dataset was discarded due to a technical error. Knee PPTs were significantly lower in KOA vs. HC (p=0.03, table 2), but no differences were noted in PPT at remote sites.

Table 2: Pressure pain threshold measurements for participants' subgroups

	Average knee PPT KPa (M± SD)	**Average non-knee PPT KPa (M± SD)**
Knee OA (*n*=30)	281.4± 152.4	227.3± 106.5
HC (*n*=22)	385.1± 182.9	253.3± 107.2
***P* value**	**0.03**	**0.39**
SPD (*n*= 12)	260.2± 126.1	253.2± 100.6
LPD (*n*=18)	295.5± 169.6	210.1± 109.6
***P* value**	**0.54**	**0.28**

SPD= short pain duration, LPD= Long pain duration, PPT= pressure pain threshold

2.1.4 Summary

This chapter explained and summarized the cohort, which will be included in the experimental part of this thesis. Some data may be quoted in the subsequent chapters; however most information in this chapter will be rather referred to from other chapters particularly in the methods section of subsequent studies.

2.1.4.1 Study participants

The recruitment process overall was efficient and the target number of knee OA patients was satisfactory achieved. The number of HC participants was slightly below target, however this was already accounted for in the study design as it was expected, based on previous experience, that 20% of data may be non-usable due to patient and/ or technical factors. Nevertheless the sample size was powered enough to detect 0.25mm grey matter differences/ changes (Pardoe et al., 2013), and compares favourably with most previous morphometric studies.

2.1.4.2 Demographics

Patients and healthy controls were age and sex matched and further more similar in body mass index, handedness preferences, and educational levels. However it was difficult in the beginning to

matching both groups as responders from healthy volunteers tended to be from an age range far younger from patients and predominantly from females. Therefore we (I and colleagues, as a team studying same participants from different perspectives) had to write an amendment to limit the minimum age of healthy volunteer to 45 years, which then worked more efficiently.

2.1.4.3 Psychological and pain-related characteristics

Compared to healthy controls, KOA presented with similar levels of cognitive ability, pain catastrophizing, and trait anxiety. However, patients had significantly higher scores of state anxiety ($p=0.04$) and greater depressive symptoms ($p=0.001$, table 1), ranging from minimal to upper limit of mild depression (Spreen, 1998). Pain related depression has been consistently reported in epidemiological studies (Munce and Stewart, 2007), however the temporal relationship between chronic pain and depression is poorly understood (Banks and Kerns, 1996), with some emerging evidence that pain is precedential (Hilderink et al., 2012).

Mechanical pain hypersensitivity recorded locally at the painful joint was significantly higher in knee OA patient. No difference could be detected for remote sites.

2.2 Hippocampal volume in health and disease: characteristics in healthy population and correlates of chronic pain

2.2.1 Study I: Hippocampal volume and asymmetry in healthy population

2.2.1.1 Introduction

Several studies have investigated the hippocampal asymmetry in healthy subjects and provided converging evidence that the right larger than left asymmetry is present in normal people. A systematic review and meta-analysis (Pedraza et al., 2004) of 82 studies that involved 3564 participants, revealed that there exists a significant right larger than left hippocampal asymmetry in a wide range of population. However, variations in scanning factors and acquisition techniques had a major impact on the outcome of volumetric measurements. Studies that used a magnetic field strength of less than 1.5 Tesla and/or slice thickness of more than 2mm were less likely to find asymmetry compared to those of higher magnetic field strength and less slice thickness.

It has been consistently reported that different asymmetry patterns in HV occur in patients with certain clinical disorders when compared to healthy controls. The direction of the asymmetry has been linked to a variety of clinical observations; (1) right larger than left, as found in semantic dementia (Barnes et al., 2006),

depression (Mervaala et al., 2000), and unsuccessful psychopaths (Raine et al., 2004), (2) left larger than right as found in mood disorder (Boccardi et al., 2010) and marijuana abuse (Medina et al., 2007). (3) The normal asymmetry can be lost in schizophrenic patients (Fukuzako et al., 1997).

An understanding of the normal characteristics of hippocampal volumes is essentially required prior to assessing any disorder-related changes. Hippocampal volume characterization in healthy subjects has been widely attempted in a substantial number of studies (Woolard and Heckers, 2012b, Watson et al., 1992, Tae et al., 2008, Pruessner et al., 2000, Li et al., 2007a, Jeukens et al., 2009, Jack et al., 1989a, Honeycutt and Smith, 1995). However, studies were mostly limited by low magnetic field strength, image resolution and slice thickness, which imply that the normative ranges provided are not necessarily relevant to current high-resolution HV studies at 3T and higher (Levy-Reis I, 2000). Furthermore, the extent to which HV asymmetry is determined by lobar or hemispheric asymmetry is unclear.

We* conducted a high-resolution and high field strength volumetric study with a primary aim of providing characterisation of normal hippocampal volume and asymmetry in healthy adults with stratification for age and gender. Given that the asymmetry may be a consequence of inter-temporal or –hemispheric volumetric

variations, the secondary aim is to attempt an explanatory investigation as to whether HV asymmetry can be explained by global or lobar inter-hemispheric volumetric variations.

2.2.1.2 Material and Methods

Subjects

Fifty-three healthy adults who were free of any neurological, psychiatric and major medical conditions volunteered to undergo 3-Tesla structural brain MRI scanning as healthy controls in local Research Ethic Committee approved neuroimaging studies conducted at the Radiological Sciences Research Group of our institution. Informed consent was obtained from all participants prior to scanning. The mean age was 35.6 years ± 11.8 SD (range 18-65 years), 31 males and 22 females. Subjects were further subdivided into three equal-range age groups 18-33y (n=25; 21 males), 34-49y (n= 21; 7 males) and 50-65y (n= 7; 3 males).

*Data for this study (ONLY) were collected by previous PhD students (Robert Dineen, Antonio Napolitano, Maryam Abaei) as part of their research. However, all manual volumetry work and data analysis were carried out solely by myself

Data Acquisition and analysis

MRI scanning was performed using 3 Tesla Philips Achieva Scanner and 8-channel head coil. Three-dimensional magnetization-prepared rapid gradient-echo (3D MP RAGE) T1-weighted images were obtained using the following parameters: TE= 3.7ms, TR= 8.1ms, 1-mm contiguous slices, TI=960ms, FA= 8° and FOV= 25.6x25.6x16cm. 3D-Slicer, open source software version 2.5 alpha (Pieper et al., 2006) was used for manual delineation of individual hippocampal and intracranial volumes. The software allows for exchangeable visualization and tracing of the region of interest (ROI) in three different planes as well as an automated calculation of ROI volumes. Hemispheric and lobar volumetric segmentations were carried out using FreeSurfer image analysis package (Jack et al., 1989a, Fischl et al., 1999) version 5.1 available at http://surfer.nmr.mgh.harvard.edu/. The volumetric measurements of different brain structures are obtained following a fully automated parcellation process. The technique involves assigning a neuroanatomical label to each voxel using probabilistic information based on a manually labelled tainting set (Fischl et al., 2001, Fischl et al., 2004), (Fischl et al., 2002). The segmentation technique has been reliably used to detect significant brain volume changes (Cerasa et al., 2009, Lehmann et al., 2010, Insausti et al., 2011).

Hippocampal morphometry, boundary definition, and protocol development

3D-Slicer, open source software Mac version 4.1 (Pieper et al., 2006) was used for manual delineation of individual hippocampal and intracranial volumes. The software allows for exchangeable visualization and tracing of the region of interest (ROI) in three different planes as well as an automated calculation of ROI volumes. HV was traced and measured on coronal images with the use of sagittal and axial views to aid defining borders and complement accurate delineation. To reduce observer bias images were duplicated and flipped (Fig. 1), so that all hippocampi were outlined on the left side of the image, and the observer blinded to image laterality. I carried out all manual segmentations, and high intra-rater, and inter-rater reliability against a second experienced observer (Dr. R Dineen) using 20% of original data, was achieved; intra- and inter-class correlations recorded 92% and 80%; 95% CI= 0.73-0.97 $p<0.001$ and 0.42-0.95 $p=0.001$, respectively.

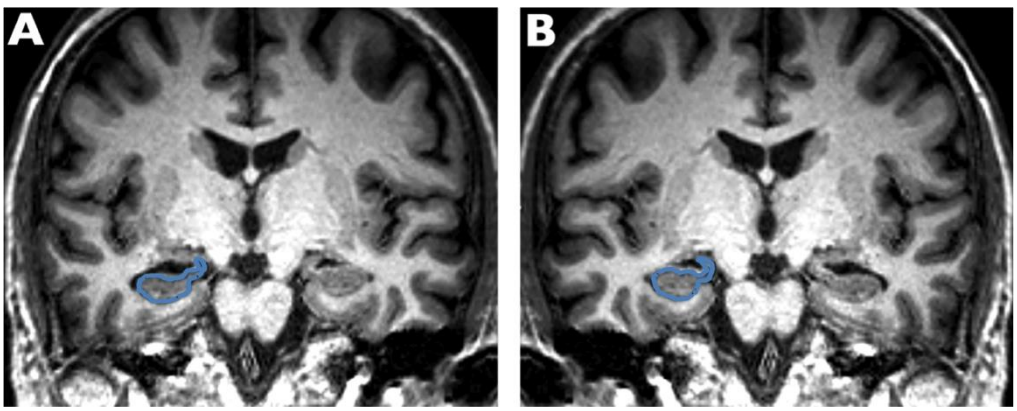


Figure 1: Right and left hippocampi segmented on the left side of the image. (A) un-flipped right and, (B) left hippocampus subsequent to flipping

The hippocampal grey and white matters can be differentially influenced by gender, disease condition or their interactions (Konrad et al., 2009). Therefore, grey matter only was segmented, which included (Cornu Ammonis CA, Gyrus dentatus, and hippocampal part of subiculum) (Fig. 2). The alveus and fimbria are white matter structures in the hippocampal formation that carry the hippocampal efferent axons along through the fornix to the mammillary bodies or directly to the anterior thalamus respectively (Duvernoy, 2005). Both structures were excluded. Anatomical boundaries were traced following a tightly defined protocol derived from previously published studies (Jack, 1994, Pantel et al., 2000, Pruessner et al., 2000, Watson et al., 1992).

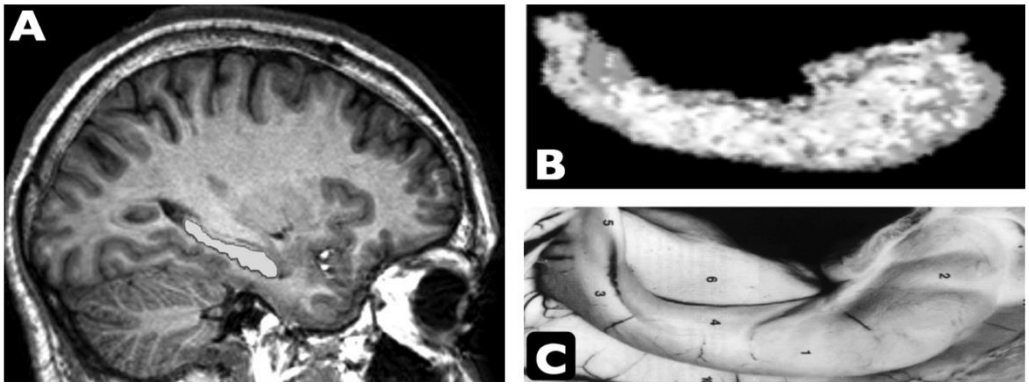


Figure 2: Entire length of segmented hippocampus shown on sagittal view (A), and 3D-Slicer segmented and volume-rendered hippocampus (B), compared to gross anatomy (C). Gross anatomy image from (Duvernoy 2005)

Hippocampal boundary definition

1- *Rostral and caudal parts:* Prior to manual outlining of hippocampus in the coronal view, auxiliary guidelines drawn on the sagittal plane were used to provide tangible separation between

anterior-most and posterior-most parts of the hippocampus and the surrounding non-hippocampal structures. A number of delineations were drawn on sagittal slices then switching to coronal and scrolling through the slices until the most anterior and posterior markers for the hippocampal boundaries are identified. A hippocampal anatomy text book (Duvernoy, 2005) was used as an external guideline. At the middle segment of the hippocampal tail structural modifications appear caudally where margo denticulatus extends to form fasciola cinerea, the fimbria ascends to join the crus fornix, Cornu Ammonis part CA3 covered with the alveus forms gyrus fasciolaris and the CA1 part bulges to form gyrus of Andreas Retzius (Duvernoy, 2005). The very terminal parts of the tail (Gyrus fasciolaris, Fasciola cinerea, and Gyrus of Andreas Retzius) were included. The posterior-most end is defined when the crura of the fornix are seen in full profile.

2- *Head of Hippocampus*: Superiorly the hippocampal head is defined by the alveus and/or cerebrospinal fluid (CSF) of inferior horn of lateral ventricle and/or auxiliary sagittal outline. Lateral border is delineated by the inferior horn of lateral ventricle. The grey/white matter junction between subiculum and parahippocampal gyrus demarks its inferior border. The medial border is defined superiorly by the CSF in the ambient cistern and inferiorly by a straight imaginary line with an angle of ~45°

connecting the most inferior part of the hippocampal head medially to the ambient cistern (Pruessner et al., 2000).

3- *Body of Hippocampus*: Superiorly, the alveus and/or CSF and/or choroidal fissure delineate the hippocampal body. The temporal stem and inferior horn of lateral ventricle define the lateral border. The grey/white matter junction between subiculum and parahippocampal gyrus labels its inferior border. The medial border is superiorly defined by the CSF in the ambient cistern and inferiorly by a line representing the semi-straight line of letter 'S' formed by the body together with the entorhinal cortex with an angle of ~45° connecting the most inferior part of the hippocampal body medially to the ambient cistern (Pruessner et al., 2000).

4- Tail of Hippocampus: Superiorly the tail is defined by the quadrigeminal cistern and/or crus fornices. Lateral border is determined by the temporal horn of the lateral ventricle. Further caudally, crus fornices and CSF of the atrium of the lateral ventricle define the tail. The inferior border is demarked by the white matter of the parahippocampal gyrus and the CSF of the quadrigeminal cistern defines its medial border.

Intracranial volume

Intracranial volume (ICV) was measured following the method of Eritaia et al (Eritaia et al., 2000), which involves ICV segmentations at a rate of one every ten slices. The reliability

exceeds 0.999 compared to the strategy of ICV measurement by segmenting every slice and, as per the study of Eritaia et al, this approach allows a 12-fold time reduction in measurement time. Results were used to correct HV for differences in head size following the adjustment method by Jack et al (Jack et al., 1989b). For volumetric normalization of HV the following equation was used:

$$Normalized\ HV = raw\ HV - [b\ (ICV - group\ mean\ ICV)],$$

where b is the slope of regression of HV on ICV across the study population.

Asymmetry index

Asymmetry index (AI) expressed as a R-L percentage difference was calculated for each subject as [(R-L)/0.5*(R+L)]*100 (Galaburda et al., 1990). To further explore the nature and possible ontogeny of any asymmetry identified, we tested for the correlation between total raw hippocampal volume (THV) comprising the sum of bilateral volumes, and the magnitude of the asymmetry index. As argued by Galaburda et al., a negative correlation between the two variables indicate that asymmetry results from reduction in size of one hippocampus compared to the symmetrical condition, a positive correlation indicates that asymmetry results from enlargement of one hippocampus compared to the symmetrical condition and no correlation indicates

that asymmetry results from an increase in volume of one hippocampus and a reduction in the other compared to the symmetrical condition (Galaburda et al., 1990).

Statistical Analysis

Analyses were performed using IBM-SPSS v20. Data were tested for normality. Paired *t*-test was used to determine if there was a significant difference between right and left hippocampal volumes within the study group, and independent *t*-test (2-tailed) was used to compare corrected mean differences between sexes. ANOVA tests were performed to detect any hippocampal volume or asymmetry differences between age groups. Correlation analysis was performed using Pearson correlation.

2.2.1.3 Results

Volumetric and asymmetry findings

Volumetric data for both right and left hippocampi were normally distributed. ICV-Normalized measurements in cm^3 were as follow: Right HV =2.51± 0.28; Mean± SD with a range of (1.94–3.07). Left HV= 2.45±0.29; Mean± SD, with a range of (1.96–2.93). Right larger than left HV (Fig. 1) was found significant (p=0.028), and the significant right larger than left relationship was maintained, though the mean hippocampal asymmetry index was reduced by 11%, when HV was normalized to ipsilateral temporal

lobe (p=0.045) (Fig. 2b). A reduction by 15% in the mean hippocampal asymmetry index, only suggestive a trend for rightward asymmetry, was achieved when controlled for ipsilateral hemisphere volumes (p=0.06) (Fig. 2c). Asymmetry index ranged from -15.2 to +19.7 (+2.5± 8.1, mean± SD) with right dominance was prevalent (Fig 2a). Table 1 summarizes the volumetric and asymmetry results.

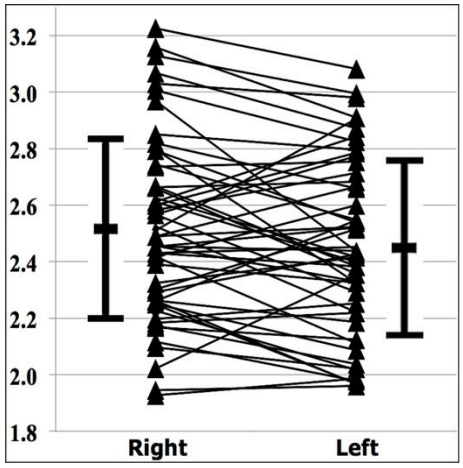


Figure 1: Raw right and left HV in all participants. Error bars represent mean±1SD

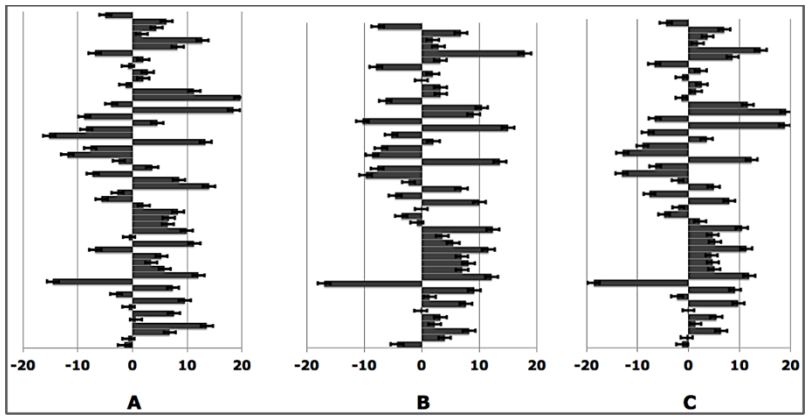


Figure 2: Diagrams of asymmetry index (Mean and SE for all subjects). A: Hippocampal raw volumes, B: Hippocampal volume normalized to ipsilateral temporal lobe, C: Hippocampal volume normalized to ipsilateral hemisphere

Table 1: Hippocampal, lobar and hemispheric volumes and asymmetry

	Volume (cm^3) Mean± SD	**p Value**	**AI** Mean± SD
Raw Rt. HV **Raw-Lt. HV**	2.51± 0.32 2.45± 0.31	**0.028**	(+)2.51±8
Rt. HV-N-ICV **Lt. HV-N-ICV**	2.51± 0.28 2.45± 0.29	**0.027**	(+)2.54±8
Rt. HV-N-Rt. Temp. **Lt. HV-N-Lt. Temp.**	2.52± 0.25 2.47± 0.25	**0.045**	(+)2.25±7.6
Rt. HV-N-Rt. Hem. **Lt. HV-N-Lt. Hem.**	2.52±0.26 2.47± 0.24	**0.06**	(+)2.15±8.1
Rt. Temporal Volume **Lt. Temporal Volune**	54.32± 6.73 56.10± 7.76	**0.0001**	(-)3.03±5.3
Rt. Hemisphere Volume **Lt. Hemisphere Volume**	511.27± 50.58 511.58± 52.10	0.74	(-)0.03±1.2

HV= hippocampal volume, **Rt**.= right, **Lt**.= left, **ICV**= intracranial volume, **N**= normalized to, **Temp**= temporal lobe, **Hem**= hemisphere. Paired t-test was used to test for significant difference between right and left HV.

Effect of age and gender

HV decreased bilaterally and significantly with increasing age (right -0.0137cm^3/year; left -0.0130cm^3/year ($p < 0.02$). There was a significant difference ($p < 0.05$) in total hippocampal volume between age groups, but not between sexes ($p > 0.6$) men= 4.99± 0.51 and women= 4.93± 0.54 (mean± SD). Age did not show any significant correlation with any asymmetry. No significant differences were found between age groups for either raw or normalized asymmetry data $p>0.4$ ANOVA, nor between sexes $p>0.09$ independent t-test.

Correlation of raw total hippocampal volume with asymmetry index

There was no significant correlation between raw total HV and raw asymmetry index scores (r=-0.01; p>0.9) (Fig. 3).

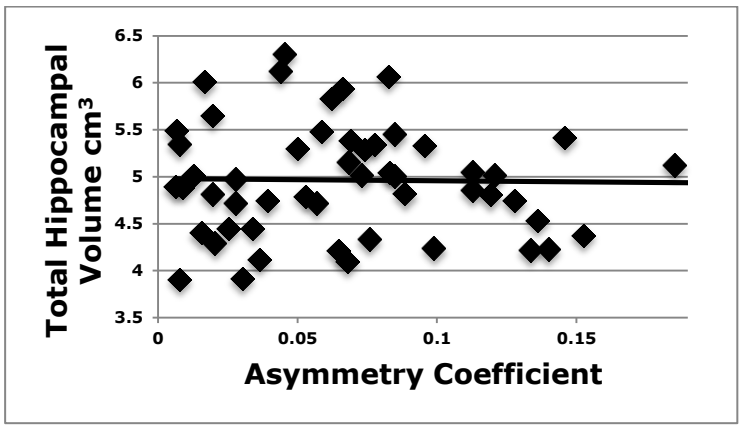


Figure 3: Scatterplot of total raw hippocampal volume and asymmetry index scores, with regression line showing absence of correlation (r=-0.01; p> 0.9)

2.2.1.4 Discussion

We provide hippocampal volumetric measurements in normal adults using high resolution and high magnetic field strength data. In our segmentation protocol we endeavoured to minimize any anatomical inaccuracy using a tightly defined protocol aided by conjoint reference of classic anatomy books and modern software techniques. We attempted to reduce any rater-dependent laterality bias in segmentation by using the flipping technique, which has shown to fundamentally eliminate putative right-left asymmetry observer bias (Maltbie et al., 2012). Our results are largely consistent with previously published studies and meta-analysis (Honeycutt and Smith, 1995, Li et al., 2007b, Pedraza et al., 2004,

Shi et al., 2009). However, there has been marked heterogeneity in the values reported among different studies with a mean hippocampal volume ranging from 1.73 to 5.68 cm^3 (Honeycutt and Smith, 1995). Sample heterogeneity, methodological issues such as image acquisition and processing, anatomical boundary definition, and structural inclusion/exclusion criteria may account for this variation. Konrad et al., (Konrad et al., 2009) reviewed 71 different published anatomical protocols and ascribed major points of discrepancy to 1) inclusion/ exclusion of while matter 2) the definition of the anterior-superior border of the head 3) the terminal part of the tail 4) the inferior-medial border and 5) the use of varying arbitrary lines. To date, there has been no consensual hippocampal volumetry protocol to adhere to, though an attempt by a multicentre group is under-development to harmonize a standard and shared protocol (www.hippocampal-protocol.net/).

Significant although modest right-dominant HV asymmetry was found in the studied cohort, although left-dominant asymmetry was present in a number of individuals (Fig 1). The asymmetry was found across all age and gender groups without significant differences between groups. This is consistent with the outcome of hippocampal asymmetry meta-analyses by (Pedraza et al., 2004) and (Shi et al., 2009).

Our data suggest that a relatively small part of hippocampal asymmetry is explained by lobar and hemispheric volume differences. By accounting for temporal lobe asymmetry, the mean hippocampal asymmetry index was reduced by 11% but remained significantly right dominant. A similar magnitude of reduction by 15% in hippocampal asymmetry was achieved when ipsilateral hemispheric volume variation was accounted for, and the right dominance was no longer significant.

We explored the possible ontogenic basis for hippocampal asymmetry by testing for correlation between total raw hippocampal volume and magnitude of asymmetry index, and the lack of correlation identified suggests that the asymmetry is likely to be due to a conjoint bilateral change involving an increase in the right hippocampal volume and a reduction in the left hippocampal volume relative to the symmetrical condition. It is however conceivable that the right ward hippocampal asymmetry could exist in early developmental stages at neonatal age (Thompson et al., 2009). This may suggest that genetic, developmental and environmental factors are influential. On the other hand molecular and functional asymmetry may as well be predictive of the structural asymmetry (Hou et al., 2013). The right hippocampus has been strongly linked to the spatial memory and navigation (Smith and Milner, 1981, Kessels et al., 2001) and further more

neurogenesis in the sub-granular zone of the dentate gyrus is now a well established fact that can be activity dependent (Ma et al., 2009, Ming and Song, 2005).

In a relatively larger sample size study of 110 healthy adults (Woolard and Heckers, 2012a), it was found that the raw right ward asymmetry is limited to the anterior part of hippocampus. This would probably highlight the need for further evaluation of sub-divisional hippocampal volumetric in future studies.

Although our study is large compared to many published studies, it remains limited by the overall small sample size and in particular of elderly subjects. We were unable to investigate the effect of handedness as the study was limited by very few number of left handed participants compared to right handed.

2.2.1.5 Conclusion

In conclusion, we confirm the right ward hippocampal asymmetry in healthy adults, which is neither age, nor sex dependent. The findings show that hippocampal asymmetry is in part explained by hemispheric asymmetry. Accounting for hemispheric volume reduced hippocampal asymmetry by 15% such that the significant hippocampal asymmetry was no longer present. Researchers who relate hippocampal asymmetry to disease states need to be aware

that associations with hippocampal asymmetry may in part reflect hemispheric asymmetry as opposed to specific asymmetry of the hippocampi.

2.2.2 Study II: Hippocampal volume and cognitive function in chronic pain due to knee osteoarthritis

2.2.2.1 Introduction

The hippocampus, a crucial limbic area best known for its role in cognition (particularly learning and memory), is one of the most widely investigated regions of the brain. Advances in neuroimaging and volumetric techniques have exquisitely enabled in-vivo quantification of magnetic resonance-based hippocampal volumetry. Relatively recently, hippocampal volume (HV) has been a research target for studies concerned with imaging biomarkers of neurological and neuropsychiatric diseases. Significant changes in the HV have been described in a number of clinical conditions including Alzheimer's disease (Convit et al., 1997, Kramer et al., 2004, Prestia et al., 2011), depression (Baare et al., 2010, Beyer et al., 2004, Bremner et al., 2000, Javadapour et al., 2010, Lloyd et al., 2004, MacMaster and Kusumakar, 2004), epilepsy (Briellmann et al., 2002, Marsh et al., 1997), schizophrenia (Koolschijn et al., 2010, Seidman et al., 2002), post-traumatic stress disorder (Wignall et al., 2004), and lately in association with chronic pain (Mutso et al., 2012).

The impact of chronic pain on hippocampal volume (HV) however has been minimally explored and remained largely controversial.

HV reduction was reported in some chronic pain states (Zimmerman et al., 2009, Mutso et al., 2012), with some evidence that stress (Vachon-Presseau et al., 2013) or depression (Brown et al., 2002) may be mediating factors. Conversely, a meta-analysis of morphometric studies of wide chronic pain aetiologies (Smallwood et al., 2013a) reported an enlargement of HV in association with chronic pain. The published studies, were however limited by the use of automated volumetric techniques (Mutso et al., 2012) and studies reviewed by Smallwood et al (Smallwood et al., 2013a), which is arguably considered less advantageous over manual volumetry (Cherbuin et al., 2009, Morey et al., 2009, Shen et al., 2010), or further limited by small and heterogeneous sample sizes (Zimmerman et al., 2009).

The impact of chronic pain on hippocampal function has been intensely investigated in animal models (Mutso et al., 2012). Mutso and co-workers found that persistent neuropathic pain significantly affected the neurogenesis in the sub-granular zone of hippocampus. Moreover, when compared with pain-free controls, mice with neuropathic-like persistent pain exhibited behavioural abnormalities and showed less cellular quantity per hippocampal slice. The investigators emphasized 0that this was indicative of a

decrease in neurogenesis rather than degeneration, as revealed by bromodeoxyuridine (BrdU) labelling.

In the same aforementioned study, hippocampal volume was also assessed in three different chronic pain conditions in humans (chronic back pain CBP, complex regional pain syndrome CRPS, and knee OA) in reference to healthy controls, n=15 each. It was found that bilateral hippocampal volumes were significantly smaller in CBP and CRPS but not in OA patients relative to healthy controls, suggesting that hippocampal volume may be differentially affected by distinct chronic pain syndromes. In another study by Zimmerman et al (Zimmerman et al., 2009), a trend for smaller hippocampal volume ($p=0.06$) was found in a 20 non-demented elderly patients with chronic pain. However the study can be criticized for the heterogeneity of the studied cohort, which collectively included patients with OA, peripheral neuropathy and spinal stenosis.

Chronic pain related cognitive changes were not evaluated in either of the aforementioned studies. It is conceivable that pain experience involves cognitive-evaluative as well as sensory-discriminative and motivational-affective dimensions (Melzack R. and Casey K., 1968). Previous work suggests that chronic pain is associated with cognitive dysfunction. There have been several studies, reviewed by Moriarty and others (Moriarty et al., 2011),

reporting that chronic pain patients evidence more cognitive dysfunction compared to pain-free matched controls. However, in the same review, authors also presented a percentage of studies 5/30 that reported no association between chronic pain and impaired cognitive function. Moreover, the affected cognitive domains may vary between different chronic pain aetiologies. For example, in chronic fatigue syndrome (involves widespread muscle and joint pain) and fibromyalgia, impairments in learning and working memory were noted, but processing speed (impaired in chronic fatigue syndrome) and attention (impaired in fibromyalgia) were differentially affected (Glass, 2006). In a study of musculoskeletal pain, 32% of patients (n=73) had cognitive impairment in at least one cognitive domain (Kewman et al., 1991).

The pain influenced cognition is further evidenced from studies e.g. reporting incrementally reduced cognitive performance (working memory) with increasing levels of experimental painful stimulation (Buhle and Wager, 2010), and others reporting cognitive improvements upon pain relief (Jamison et al., 2003, Tassain et al., 2003). It's been suggested that pain affects cognitive function particularly on tasks that require attention that is already being diverted to coping with pain (Park et al., 2001).

The mechanistic basis of pain related cognitive impairment is however poorly understood and remains open to speculations, with some researchers proposed that persistent pain emerge over other demands for attention (Eccleston and Crombez, 1999), while others suggested that chronic-pain related neuroplastic changes impact upon brain functions including cognition via e.g. connectivity reorganizations (Hart et al., 2000). However, the exact neurobiological mechanism remains largely unknown. Moriarty and colleagues (Moriarty et al., 2011) proposed a model based on three potential mechanistic theories (though not tested) that may explain the pain-related cognitive impairment namely; 1) limited resources, 2) altered neuroplasticity, and 3) dysregulated neurochemistry. Theories 1 and 3 are beyond the scope of this thesis, therefore the focus of the current study will be directed to the pain-related structural neuroplasticity; namely the hippocampal volume correlates of chronic pain and its putative linking role between chronic pain and impaired cognition.

Hippocampal volume measured by magnetic resonance imaging has been consistently shown as a strong predictor of cognitive decline in non-painful conditions (den Heijer et al., 2010, Grundman et al., 2002, Wolf et al., 2001), and depression is consistently known to impact on hippocampal volume (Campbell et

al., 2004, Videbech and Ravnkilde, 2004). It is not clear whether persistent arthritic pain, a primarily nociceptive disorder, is associated with hippocampal volume change, and putatively related cognitive impairment and low mood.

2.2.2.2 Aims and hypothesis

The aim of this study is to investigate the HV, cognitive function and mood in patients with chronic painful knee OA. I hypothesize that: Chronic knee OA pain is associated with a reduction in hippocampal volume and patients will show global cognitive and delayed recall memory dysfunction, and these two; HV loss and cognitive decline are associated, with the former also linked with low mood.

2.2.2.3 Methods

Subjects

A detailed description of participants recruited for this study is provided in chapter 5. In brief, all patients (n=31, age= 64.61± 8.36; 15 females) and all pain-free healthy controls (n=22, age= 61.30± 7.46; 13 females) were included.

Data acquisition

Brain magnetic resonance imaging

Brain MRI has bee described in details in chapter 5. In summary, data acquisition involved the used of 3T scanner (Discovery 750, GE Medical Healthcare, Milwaukee, US) with a 32-channel head coil, to acquire 3D structural brain scans at a native resolution of $1mm^3$.

Global cognition and delayed recall

Cognitive ability was assessed during a face-to-face interview using MoCA test (described previously in chapter 5), and a delayed recall data was derived from a sub-scale in the MoCA test defined as the ability to remember a list of 5 words following two trials of immediate recall and a third, for which the score is appointed, after a short period of time occupied by some other questions in the assessment. To investigate the HV correlates of depression and to control for its confounding effect, Beck's Depression Inventory (BDI) (Beck et al., 1996) was used to assess depressive symptoms in all participants.

Volumetric methodology

Hippocampal volumetry and intracranial volume measurement

Measurements were performed using same protocol as in study I.

Statistical analysis

Analyses were performed using IBM-SPSS v20. Data were tested for normality. Independent *t*-test (2-tailed) was used to compare corrected mean differences between groups for volumetric data, and independent samples Mann-Whitney U test was used for between group comparisons of depression and cognitive data. ANCOVA tests were performed to account for age and sex, and correlation analysis was performed using Pearson correlation.

2.2.2.4 Results

Cognition and depression levels in all participants

General cognition, delayed-recall memory, and depression results in KOA patients and controls are shown in table 1.

Similar cognition and delayed recall scores were noted in KOA patients relative to HCs. KOA patients however, recorded significantly higher levels of depression, however still subclinical in the range of minimal to moderate depression (Spreen, 1998).

(As described previously in chapter 5, educational status, handedness, and body mass index were similar in both groups).

Table 1. Cognition and depression data of KOA patients vs. HC

	MoCA 0-30	**Delayed recall (0-5)**	**BDI (0-63)**
KOA:			
Mean	27.4	3.77	6.32
Median	28	4	7
Range	20-30	1-5	0-19
HC:			
Mean	27.2	3.41	2.19
Median	27	3.5	1
Range	23-30	0-5	0-9
p-value*	=0.63	0.34	**=0.001**

KOA= knee osteoarthritis, **HC=** healthy control, **MoCA=** Montreal cognitive assessment, **BDI=** Beck's depression inventory. ***Based on Independent samples Mann-Whitney U test.

Volumetric results

Hippocampal and intra-cranial volumes were normally distributed. Results are summarized in table 2. On average, KOA patients had significantly larger HV bilaterally Fig. 3, however ICV was similar among groups.

Table 2. Volumetric data (cm^3) for ICV and normalized (right and left) hippocampal volumes represented as mean (SD)

	N-Rt. HV	**N-Lt. HV**	**ICV**
KOA	2.24 (0.31)	2.16 (0.26)	1498.6 (157.4)
HC	2.06 (0.26)	1.92 (0.28)	1489.5 (155.4)
p-value*	**=0.031**	**=0.003**	=0.84

N-Rt. and **N-Lt HV=** normalized left and right hippocampal volumes. **ICV=** intracranial volume. ***Based on independent samples t-test.

By accounting for age and sex (ANCOVA), differences remained significant for Rt. HV (2.26± 0.05 vs. 2.03± 0.06, adjusted mean± std. error; F=8.5 p=0.005), and for Lt. HV (2.18± 0.04 vs. 1.89± 0.05, adjusted mean± std. error; F=16.2 p<0.001), and continued non-significant for ICV (F= 0.5 p=0.49).

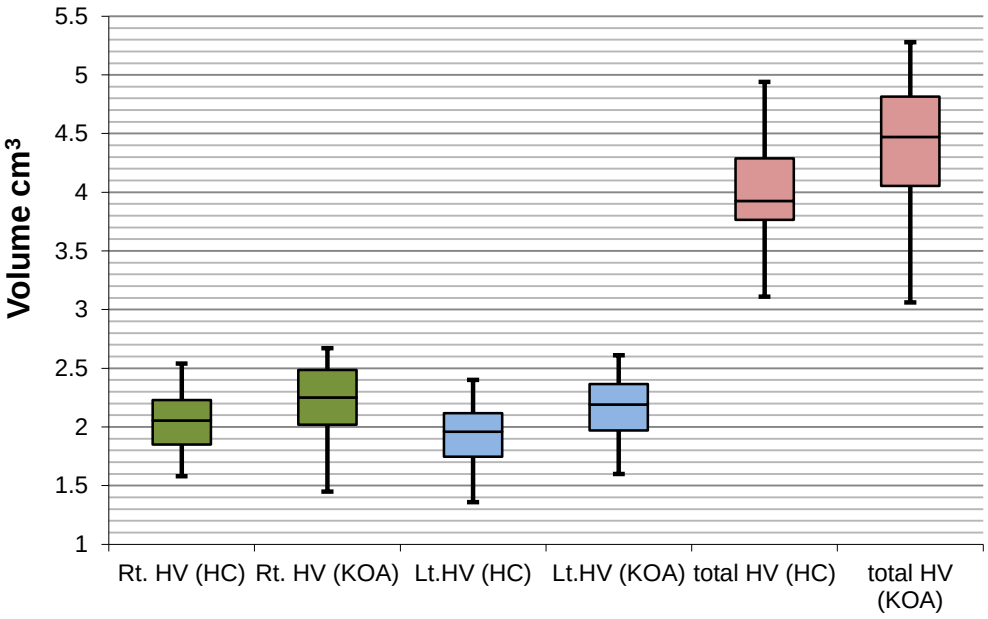


Figure 3. Box-plots showing normalized hippocampal volumes in KOA patients compared to HCs

Hippocampal volume correlates of pain-related behaviours and pain characteristics

No significant correlation was found, in KOA group, between right or left HV and cognitive ability, delayed recall or depression, controlled for the partial effects of age and sex.

Partial correlation analysis (controlled for age and sex) between HV and pain duration, chronic pain severity, and affective and sensory components of pain, showed only a significant moderate positive

correlation between pain duration and left HV (r=0.4 p=0.03). Cognition in HC correlated significantly with left HV (r=0.52 p=0.02 controlled for age and sex).

2.2.2.5 Discussion

The main aim of this study was to investigate the putative impact of chronic pain due to knee OA on hippocampal volume and cognitive function. However, the null hypothesis that 'there is no pain-related cognition dysfunction' could not be rejected in this cohort. Interestingly, the hippocampal volume in knee OA patients was on average significantly larger than that in age- and sex-matched healthy controls. The next intriguing question that naturally follows is that why hippocampal volume would not only be preserved but also showing significant enlargement in this cohort? This is contradicting and inconsistent with what has been reported by others (Mutso et al., 2012, Zimmerman et al., 2009), i.e. smaller HV associated with chronic pain. However, Mutso et al studied three chronic pain condition including knee OA, but interestingly HV was found reduced in all but the knee OA group, which authors attributed to the uniqueness of brain profiles (and I presume, also behavioural changes such as mood and cognition as a consequence) in different chronic pain states.

Mutso and colleagues highlighted that the variance in neuropathic component of chronic pain among different conditions may be accountable for such neurobiological differences. The two groups studied by Mutso et al, where patients had smaller HV namely; chronic back pain and chronic regional pain syndrome both were potentially having larger neuropathic component (Mutso et al., 2012). The neuropathic-like component in this studied cohort was investigated previously (by myself) using the PainDETECT questionnaire (Freynhagen et al., 2006), and it was found that only less than 10% of patients were considered to have neuropathic-like symptoms which was also consistent with independent knee OA study (Ohtori et al., 2012), involved 92 patients where only 5.4% and 15.2% were found to 'likely' and 'possibly' have neuropathic component respectively. The hypothesis of neuropathic influential role on HV is supported by preclinical studies of neuropathic models that showed reduced neurogenesis rate in rat's hippocampus (Mutso et al., 2012), and a functional disturbance in the fronto-hippocampal circuits that resulted in neuropathic pain-related working memory deficits in rats as recently shown by Cardoso-Cruz and co-workers (Cardoso-Cruz et al., 2013).

Very few medications are know to induce HV enlargement such as Lithium (Yucel et al., 2008), which enhances neurogenesis as found

in preclinical studies (Chen et al., 2000), but none of the studied patients were taking Lithium for medical purposes. Medications taken for pain and disease-related comorbidities may on the other hand induce significant volumetric reduction. Opioid analgesics, for instance, are known to rapidly alter grey matter volume in several brain regions in as short as one month of regular administration as shown by Younger and other researchers in a study where hippocampus was among three regions which had shown volumetric decreases (Younger et al., 2011).

Several studies have documented opiates chronic use-related inhibition of neurogenesis and apoptosis both in animals (Hauser et al., 2000, Mao et al., 2002) and in human (Hu et al., 2002) with particular impact on cognition and memory. A recent review on the impact of psychotropic drugs on hippocampal neurogenesis in adult animal models was conducted by Kubesova et al, (Kubesova et al., 2012), and authors presented compelling evidence that high doses of opiates can have a negative impact on neurogenesis via different molecular mechanisms, and consequently may result in cognitive impairment during drug withdrawal as well as induction of anxiety and depression upon chronic use. Medication intake for this studied cohort was documented (see appendix 4 supplementary table 1), and only five patients were on regular, but simple analgesics namely; Paracetamol alone, except one patient was regularly

taking Paracetamol along with Aspirin. As 5 patients only out of 31 were on regular, yet non-opioid painkillers, one may hypothesise that the non-negative impact of chronic pain on either HV or cognition seen in this cohort may be attributed to the lack of effect from strong medications taken for pain. However, the moderating effect of medication on cognition in chronic pain need to be confirmed and it warrants further investigation.

Depression status was evaluated in the studied participants, and chronic painful KOA was found associative with more depressive disorders in sufferers relative to matched controls. This is plausible in the light of large body of evidence from large-scale surveys and chronic pain reviews, which showed that 21% of patients with chronic pain had been diagnosed with depression because of their pain (Breivik et al., 2006b). Chronic pain and depression may exacerbate one another (Morley, 2008), and furthermore may share a similar neurotransmitters and mechanism of action of therapeutic pharmacological drugs (Goesling et al., 2013).

Brown and co-workers found in a structural equation modelling analysis that depression significantly mediated the relationship between chronic pain and cognitive functioning in one hundred and twenty one rheumatoid arthritis patients (Brown et al., 2002). Although the hippocampal volume was not investigated, their findings may suggest a hypothetical bio-psychological model in

which chronic pain leads to depression, which results in reduced HV with subsequent impact on cognition. Similar mediating model was presented with stress a potential factor mediating chronic pain and reduced HV (Vachon-Presseau et al., 2013). However, the outcomes of this study argue against this model, as both cognition and HV were preserved, and no link could be found between depression and HV, although only few of the patients had mild to moderate, but no one had clinical depression.

The finding of larger HV found in the studied population is well in line with the outcome of a meta-analysis involved 23 chronic pain imaging studies (Smallwood et al., 2013a). However, the mechanism by which HV changes in either direction remains intriguingly unknown.

2.2.2.6 Conclusion

In this study, sufferers of chronic pain due to knee osteoarthritis presented with larger hippocampal volumes, normal cognition but lower mood relative to matched controls. It can be concluded that persistent arthritic pain, a model of chronic nociceptive pain does not reduce hippocampal volume nor impair cognition, and stress related HV loss does not explain the observed mildly lower mood. In contrast, the chronic pain experience seems to increase HV perhaps due to an unknown mechanism warranted further studies

to characterize the complex role of the hippocampus in pain progression.

2.3 Anterior cingulate cortex in chronic pain: The relationship between sub-regional volumetric and cortical thickness and the behavioural and phenotypic features of chronic OA knee pain

The anterior cingulate cortex (ACC) is one of the largest limbic regions (MacLean, 1990), and anatomically considered part of the ventromedial frontal cortex encompassing Broadmann's area 24 and adjacent regions (Gasquoine, 2013). It is a key structure involved in emotion, cognition, and executive functions (Carter et al., 1999, Stevens et al., 2011). ACC is often subdivided into two major parts; dorsal ACC (dACC) (comprises BA 24b'-c' and 32'; Fig 1) and is thought to be associated with attention and cognitive functions (Bush et al., 2000, MacDonald et al., 2000), whereas the rostral-ventral part (includes BA 24a-c and 32 and ventral areas 25 and 33; Fig 1) is engaged in emotional and affective behaviours (Devinsky et al., 1995, Vogt et al., 1995).

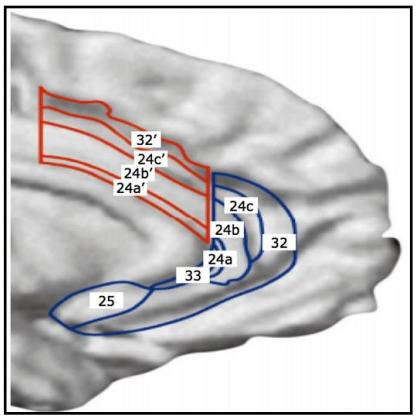


Figure. 1: A Schematic representation of cytoarchitectural areas of dorsal (red) and rostral-ventral (blue) parts of ACC [modified from (Bush et al., 2000)]

With recent advances in structural and functional neuroimaging techniques, a better and more specific localization of functional characterizations of ACC sub-divisions has been achieved. For example, a four-region model of the ACC has been defined on structural imaging based on individual microscopic and functional properties (McCormick et al., 2006) to allow investigation of specific volumetric correlates of various brain related disorders. McCormick and colleagues developed an MRI-based segmentation protocol that enabled a reliable investigation of the volumetric features of ACC sub-regions namely the dorsal, rostral, subcallosal, and subgenual parts Fig 2.

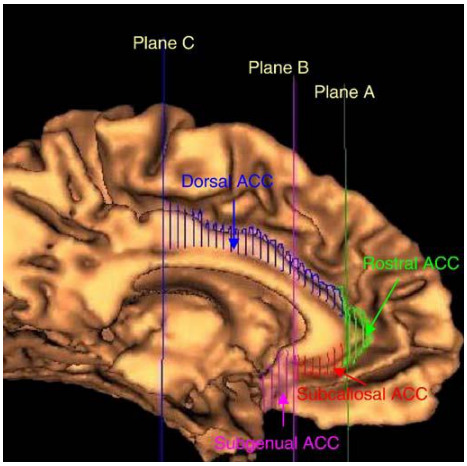


Figure 2: the ACC, divided into 4 distinct regions (dorsal, rostral, subcallosal and subgenual) using landmarks-based planes A, B and C. From (McCormick et al., 2006)

The ACC plays a major role in chronic pain processing (Jones and Derbyshire, 1996), and is considered a pivotal region in the medial pain system (Vogt et al., 1993), which is concerned with the affective-motivational and cognitive-evaluative dimensions of pain

response (see chapter 1). It is now recognized that individual parts of ACC are differentially engaged in mediating distinctive aspects of pain and pain-related emotions (Vogt, 2005). In this chapter, which will be centred primarily on the morphological changes of ACC in chronic pain, the structural correlates of ACC sub-divisions of chronic pain in knee OA will be investigated in two separate experiments, in accordance with the functional characterization of each sub-region.

2.3.1 Study I: The anterior cingulate sub-regional volumetric correlates of behavioural features in chronic painful knee OA

2.3.2 Introduction

Recently, functional magnetic resonance imaging has provided an outstanding advance in localizing different aspects of pain with their neural substrates. The brain regions that are involved in processing sensory and affective components of pain, for example, can be disentangled in terms of spatial localization, as shown in an empathy-for-pain study which revealed involvement of affective (mainly activation of anterior insula and rostral ACC) but not sensory aspects of pain (Singer et al., 2004).

In this study, the volumetric changes of ACC parts will be studied in accordance with the following rationales:

1- Cognition and attention: Dorsal ACC is implicated in cognition and attention, and pain seems to impose a negative impact on these functions and interrupt cognitive processing (Buffington et al., 2005), or divert attention towards pain (Eccleston and Crombez, 1999). dACC appears to play a mediating role between pain and cognitive disruption (Davis et al., 1997). Buffington et al investigated the activation patterns in the ACC during cognitive task performance using fMRI in patients with persistent pain due

to knee OA as well as in healthy controls, and found that the ACC activation patterns were different between the two groups such that healthy subjects showed more diffuse activations compared to knee OA patients (Buffington et al., 2005).

2- Affection and emotions: The ACC has been consistently shown to code for affective dimensions of pain (Rainville et al., 1997, Sawamoto et al., 2000), including unpleasantness and suffering (Vogt et al., 1996). Spatial dissociation studies of anterior cingulate function revealed evident recruitment of rostral part (Fig 3) in affective-emotional processing (Whalen et al., 1998), and deactivation during cognitive task performance.

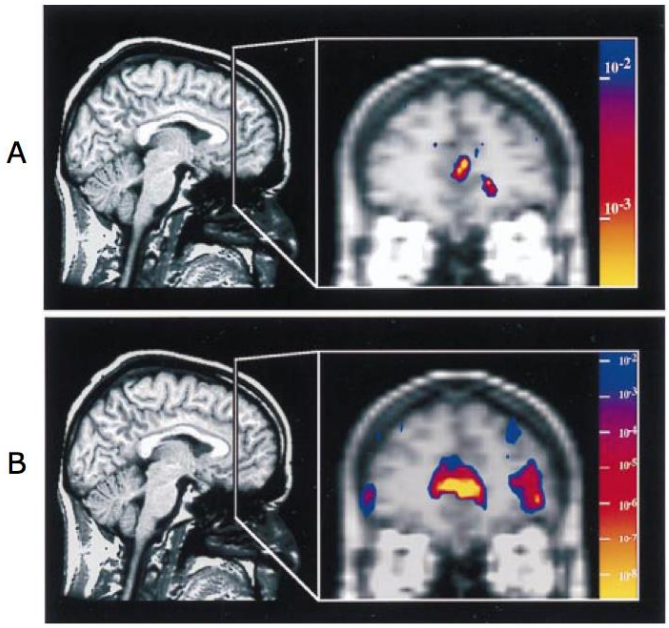


Figure 3: (A) Significant activation of rostral ACC during negative- compared to neutral word presentation block. (B) Decreased fMRI signal during task performance. [Adapted from (Whalen et al., 1998)]

Although pain is consciously perceived as a uniform experience, it is not uniformly engaged throughout the brain or the cingulate (Vogt, 2005). A meta-analysis (Vogt et al., 2003) of PET or fMRI studies of healthy subjects that monitored recognition, recall, and retrieval of emotional material revealed 45 cingulate activations, (plotted on the medial surface, Fig 4). Sites activated by sadness or happiness were distinct from those activated by fear or anger Fig 4.

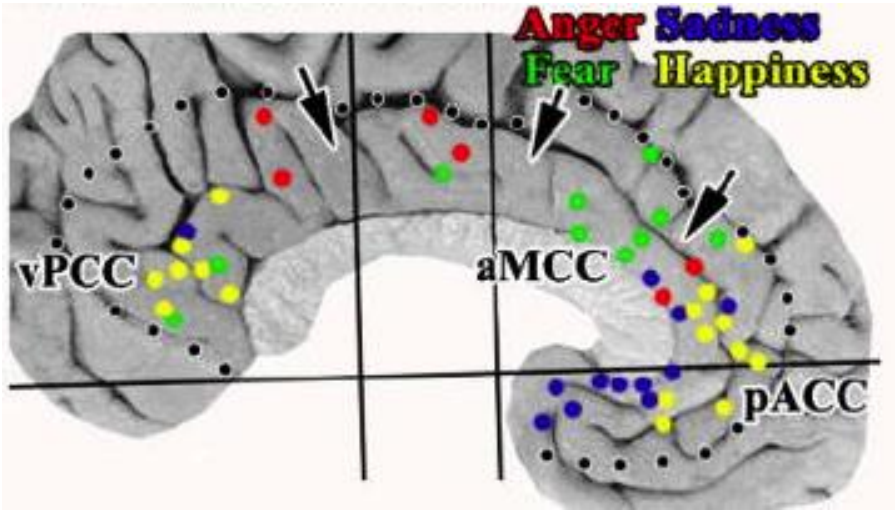


Figure 4: Activation sites in ACC in association with specific emotion: happiness (yellow), sadness (blue), anger (red), and fear (green). Courtesy (Vogt et al., 2003)

It has been postulated that the subgenual ACC (sgACC) (BA 25) shows sad events-related activation that is associated with personal events and not necessarily with the noxious stimulation (Vogt, 2005). Vogt argues that the sadness paradigms used to provoke subgenual activity may not be relevant to the individual pain-induced internal state. The

affective-emotional correlates of ACC volume in chronic knee OA pain that will be investigated in this study will be spatially limited to the rostral parts which comprise rostral and sub-callosal (BA 24a-c, 32 and 33), but not the sub-genual ACC. The sub-genual part is however largely involved in depressive and mood disorders (Drevets et al., 2002), that was further reflected by an abnormal reduction in subgenual GM volume in association with major depressive disorder and bipolar disorder (Drevets et al., 2008). Interestingly, post-mortem neuro-pathological assessment of subgenual ACC confirmed GM reduction and revealed a decrease in glial cells but not neurons as per Drevets et al.

(The sub-divisional definitions used in this chapter are in accordance with the terminology used by McCormick et al in their volumetry protocol, which was adapted for the ACC volumetric measurements. The rostral part is also known as pre-genual in the ACC literature).

3- Pain catastrophizing: (PC) is defined as "an exaggerated negative mental set brought to bear during actual or anticipated painful experience" (Sullivan et al., 2001). PC can be associated with more emotional distress and intensified pain experience (George et al., 2011, Martin et

al., 2011, Campbell et al., 2012) including in knee OA pain (Forsythe et al., 2008). Furthermore, the tendency to catastrophize may contribute to pain chronicity even upon curative treatment of the cause (Forsythe et al., 2008, Martin et al., 2011).

PC has been found significantly associative with increased activity in the ACC (Gracely et al., 2004, Seminowicz and Davis, 2006). While catastrophizing as a psychological predictor of pain experience has been widely studied, little is known about its neural correlates.

The ACC has been investigated in pain imaging literature mainly as a key region of the pain matrix that is elicited during experimental nociceptive stimulation, and largely involved in central pain processing. However, the role of ACC in pain related cognitive and emotional behaviours has remained minimally explored, and moreover the volumetric correlates of ACC parts as sub-regions of interest with such behaviours in chronic pain have not been studied.

2.3.3 Aims and hypothesis

The aim of this study is to investigate the volumetric correlates of individual ACC sub-divisions with the specific behavioural function (based on fMRI findings- explained above) in chronic knee OA pain. The following hypotheses will be independently tested:

1) Dorsal ACC is involved in cognitive function (which is allegedly impaired in chronic pain) and hence its volume will co-vary with the cognitive performance in KOA patients
2) Rostral ACC volume will correlate with the affective components of chronic pain in KOA patients, and volumetric changes in subgenual ACC will correlate with variation in depressive symptoms among patients
3) Individual tendency to pain catastrophizing is associated with morphometric variations in emotional pain processing centres namely the rostral ACC.

2.3.4 Methods

2.3.4.1 Subjects

A detailed description of participants recruited for this study is provided in chapter 5. In short, all patients (n=31, age= 64.61± 8.36; 15 females) and all age- and sex-matched pain-free healthy controls (n=22, age= 61.30± 7.46; 13 females) were included.

2.3.4.2 Data acquisition

Brain magnetic resonance imaging

The brain MRI protocol has been described in detail in chapter 5. In summary, data acquisition involved the used of 3T scanner (Discovery 750, GE Medical Healthcare, Milwaukee, US) with a 32-channel head coil, to acquire 3D T1-weighted structural brain scans at a native resolution of $1mm^3$.

Affective dimension of pain, cognitive ability, depression, and pain catastrophizing

Patients' data of affective components of pain were derived from the affective descriptors of pain in the self-administered McGill pain questionnaire (Melzack, 1975), (a copy of the questionnaire is included in appendix 4). In brief, the affective qualities are derived from a group of descriptive words in terms of tension, fear, and autonomic properties that are part of the pain experience (Melzack and Katz, 2013).

General cognition was assessed during a face-to-face interview using the Montreal cognitive assessment test (Nasreddine et al., 2005), previously described in chapters 5 and 6.

Depression levels in all participants were assessed using the self-administered Beck's Depression Inventory (Beck et al., 1996).

Pain catastrophizing was evaluated using the self-administered pain catastrophizing scale (PCS) (Sullivan et al., 1995), which consists mainly of 13 statements regarding various thoughts and feelings while in pain. Detailed description of the used questionnaires is provided in chapter 5.

Volumetric measurement techniques

Anterior cingulate volumetry and protocol development

3D-Slicer, open source software Mac version 4.1 (Pieper et al., 2006) was used for manual delineation of individual anterior cingulate and intracranial volumes. The software allows for exchangeable visualization and tracing of the region of interest (ROI) in three different planes as well as an automated calculation of ROI volume. Anterior cingulate sub-regions were traced and measured on coronal with the use of sagittal and axial views to aid defining borders and complement accurate delineation, following the protocol by McCormick et al (McCormick et al., 2006). Although these four regions were separately segmented, the rostral and subcallosal parts were combined to form one ROI indicating the pre-genual or rostral ACC, as explained in the introduction. To reduce observer bias images were duplicated and flipped (using the same technique implemented previously for hippocampal manual volumetry), so that all ACC ROIs were outlined on the left side of

the image, and the observer blinded to image laterality. I carried out all manual segmentations, and high intra-rater, and inter-rater reliability using 10 cases of original data were achieved against a second observer (Kurdistan Abdulla, a MSc student in our division who was trained to use 3D slicer for manual volumetry in her MSc project). Intra- and inter-class correlations recorded 92% and 86%; 95% CI= 0.72-0.98 $p<0.001$ and 0.53-0.96 $p<0.001$, respectively for dorsal ACC, and 94% and 88%; 95% CI= 0.78-0.98 $p<0.001$ and 0.60-0.97 $p<0.001$, respectively for rostral-ventral ACC.

Definition of anterior cingulate boundaries

General rules: Three vertical planes A, B and C were used to divide the ACC into its 4 sub-regions (Figure 1).

Plane A: defines the caudal borders of rostral ACC and separates it from the dorsal ACC (superiorly) and from the subcallosal ACC (inferiorly). Plane A is drawn on the coronal view one slice forward to the slice in which the two sides of the anterior corpus callosum are no longer connected through the genu (Fig. 5).

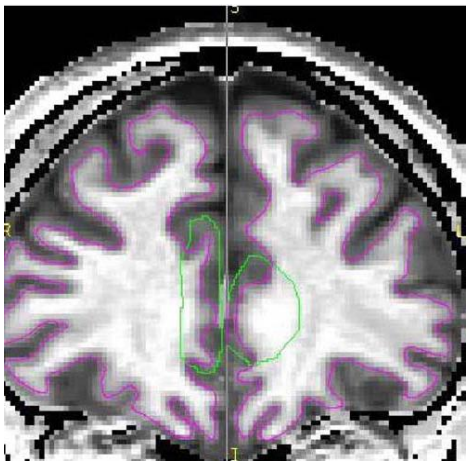


Figure 5: Plane A is drawn one slice forward past where the two sides of the corpus callosum are no longer connected through the genu. From (McCormick et al., 2006)

Plane B: defines the posterior boundary of the subcallosal ACC. (the anterior boundary has been already defined by plane A). Plane B is drawn one coronal slice before the putamen is seen within the basal ganglia (Fig. 6)

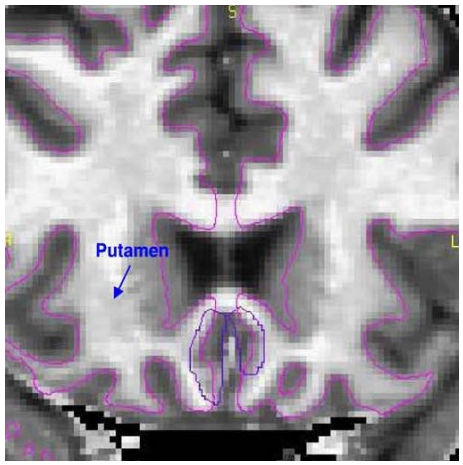


Figure 6: plane B is drawn one slice anterior to when the putamen is first visualized within the basal ganglia. From (McCormick et al., 2006)

Plane C: defines the posterior boundary of the dorsal ACC. Plane C is drawn first on the sagittal view as a vertical line through the middle (or tip) of the first gyrus anterior to where the ascending marginal sulcus joins the prominent cingulate sulcus horizontally (Fig. 7)

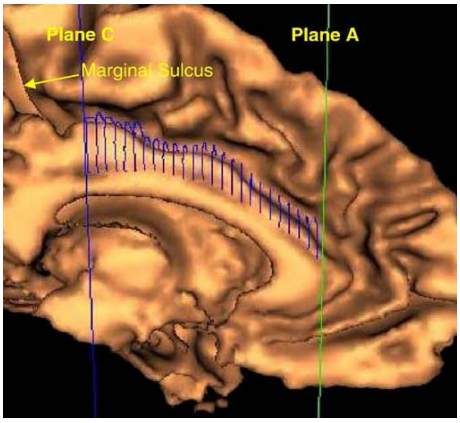


Figure 7: Plane C is drawn in the sagittal plane as a vertical line through the tip of the first gyrus anterior to where the ascending ramus becomes horizontal. From (McCormick et al., 2006)

Grey matter of ACC parts was segmented every second slice as per McCormick's protocol for all regions except for the subgenual which was segemeted on every slice. The four segmented parts are shown in Fig. 8. Althought, shown on sagittal views for better outlook, practically, the segmetation was carried out on coronal planes.

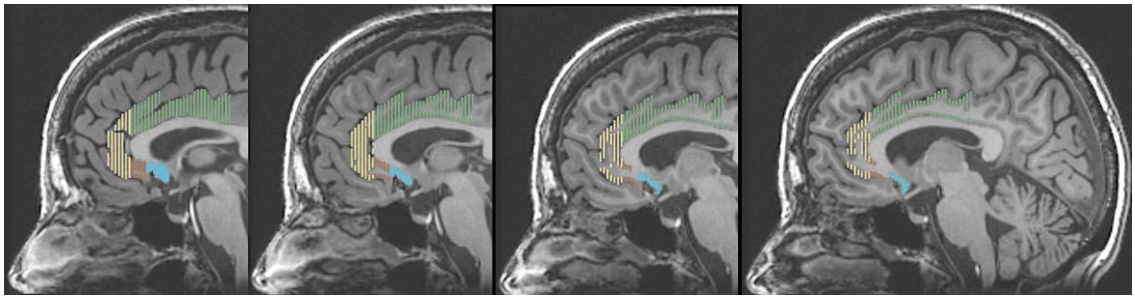


Figure 8: Manual segmetation of ACC 4 ROIs using 3D Slicer sofware; green= dACC, yellow= rACC, brown= scACC, and blue= sgACC.

Intracranial volume measurement

Intracranial volume (ICV) was measured as described previously in chapter 6. In brief, ICV was measured following the method of Eritaia et al (Eritaia et al., 2000), which involves ICV segmentations at a rate of one every ten slices. Results were used to correct ROI volumes for differences in head size following the adjustment method by Jack et al (Jack et al., 1989b). For volumetric normalization of an ROI the following equation was used:

$$\textit{Normalized ROI volume} =$$
$$\textit{Raw ROI volume} - [B\ (ICV - \textit{group mean ICV})],$$

"B" is the slope of regression of ROI volume on ICV across the study population.

2.3.4.3 Statistical analysis

Analyses were performed using IBM-SPSS v20. Data were tested for normality. Independent *t*-test (2-tailed) was used to compare normalized mean differences between groups for volumetric data, and independent samples Mann-Whitney U test was used for between group comparisons of pain related behavioural data. Correlation analysis was performed using Pearson correlation controlled for the partial effects of age and sex.

2.3.5 Results

2.3.5.1 Demographic data

Demographic data were described previously in chapter 5. Knee OA patients and healthy controls were matched in age and sex. Educational status, handedness, and body mass index were similar between both groups.

2.3.5.2 Pain-related behavioural features

Cognitive data for both groups was described in chapters 5 and 6. In brief, similar scores of general cognition were recorded in both groups: range= 20-30, median=28 for knee OA vs. range= 23-30, median=27 for HC; p=0.63; independent samples Mann-Whitney U test.

Affective component of pain in knee OA: The affective ratings, as derived from McGill pain questionnaire, can range from 0-14 with the higher being worse. Knee OA patients had the following statistics: mean= 1.2, SD=2.4, median=0, and a range of 0-11.

Knee OA patients presented with higher levels of depression: range=0-19, median=7 vs. range=0-9, median= 1; p=0.001; independent samples Mann-Whitney U test.

Pain catastrophizing scale has a range between 0 and 52, with the higher being more catastrophizing. Patients and HCs recorded similar scores (p=0.69; independent samples Mann-Whitney U test). Knee OA patients scored 11.8± (9.97) with a range of 0-41 and HCs=12.05± (8.15) and a range of 0-29.

2.3.5.3 Volumetric measures

The normalized data of dorsal, rostral, and subgenual ACC, as well as ICV measures in knee OA and HC groups are shown in table 1. All data of ACC sub-divisions (except for subgenual parts p<0.03) as well as ICV were normally distributed in both groups, p>0.06, (Shapiro-Wilk test). There was no significant difference in any of the studied volumetric data between knee OA patients and HCs.

Table 1. Volumetric data (cm^3) for normalized (Rt. And Lt.) dorsal and rostral ACC, and ICV volumes in knee OA and HC groups represented as mean (SD)

	N-Rt. dACC Volume	**N-Lt. dACC Volume**	**N-Rt. rACC Volume**	**N-Lt. rACC Volume**	**N-Rt. sgACC Volume**	**N-Lt. sgACC Volume**	**ICV**
KOA	4.3 (0.81)	4.04 (0.76)	2.19 (0.45)	2.66 (0.71)	0.34 (Median=0.35, range= 0.19-0.78)	0.40 (Median=0.38, range= 0.08-0.70)	1498.6 (157.4)
HC	4.2 (0.63)	3.76 (0.72)	2.3 (0.57)	2.89 (0.62)	0.33 (Median=0.32, range= 0.11-0.50)	0.42 (Median=0.39, range= 0.17-0.87)	1489.5 (155.4)
P-value*	=0.54	=0.19	0.43	0.22	0.64	0.95	=0.87

N-Rt. and **N-Lt (dACC, rACC, sgACC)=** normalized right and left dorsal, rostral, and subgenual ACC volumes. **ICV=** intracranial volume. ***Based on independent samples t-test, except for sgACC where independent samples Mann-Whitney U test was used.

2.3.5.4 Correlation analysis

1- General cognitive performance and dorsal ACC volume in knee OA patients

No significant correlation was found between scores of general cognition and either side of dorsal ACC volume ($p>0.6$ controlled for age and sex).

2- Affective dimension of chronic pain in knee OA and rostral ACC volume, and depression levels with subgenual ACC volume

No significant correlation was found between scores of pain affective ratings and either side of rostral ACC volume ($p>0.3$ controlled for age and sex).

No significant correlation was found for depressive symptoms with sgACC volume ($p>0.7$, Spearman's rho correlation).

3- Pain catastrophizing and rostral ACC volume

A modest but significant correlation was found between pain catastrophizing scores and left rostral ACC, i.e. higher PC scores with larger left rACC volume $r=0.37$, ($p=0.047$ controlled for age and sex), Fig 9.

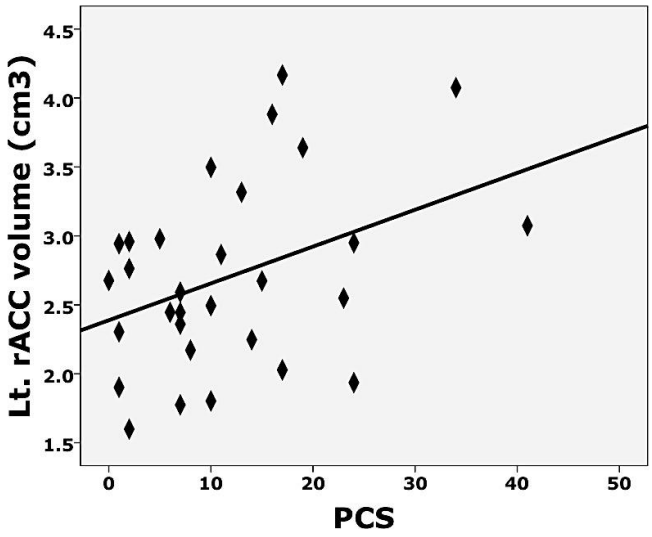


Figure 9: Scatter plots and regression line showing a positive relationship between PCS and left rostral ACC volume

2.3.6 Discussion

The grey matter volumetric correlates of two functionally distinct parts of ACC namely the rostral-ventral part, which is involved in pain related emotional processing and the dorsal part, associated with cognitive performance, were independently investigated in relation to pain related behaviours specifically; cognition, affection, depression, and pain catastrophizing. There was no significant covariance in general cognition, depression, or affective dimension of pain with volumetric changes in the functionally corresponding parts. Of note, cognition in patients did not differ from that in HCs, and furthermore, chronic pain in knee OA seemed not very influential with regard to its impact on negative affective feelings, as data of the latter did not vary much and furthermore was low in

the studied group, with a mean of 1.2, median=0, and a variance of 5.8 (squared SD of 2.41). While inferences will be limited by the cross sectional design of the study, it can be argued that the normal findings of ACC volume may explain such stability in pain-related behaviours in the studied population.

Contrariwise, left rostral ACC volume increased with higher pain catastrophizing scores. This suggests that structural alterations in this area, which is involved in emotional pain processing and interoception, may mediate emotional negative bias towards pain experience. This accords well with the concept that PC reflects increased attention and emotional response to pain processing as shown in patients with fibromyalgia (Gracely et al., 2004). The study findings are also consistent with and may explain reported increased activities within the rostral ACC, insula, and dorsolateral prefrontal cortex in healthy subjects with higher PC scores (Seminowicz and Davis, 2006). The fact that the grey matter volume was not abnormal in this area provides preliminary evidence for a dispositional rather than acquired nature.

The preserved cognitive function in the studied cohort has been discussed previously in the hippocampal volume correlates, chapter

6, and will be reiterated briefly in here. There is a large body of evidence to suggest that chronic pain is associative with cognitive impairment, for review see (Moriarty et al., 2011), and in musculoskeletal pain 32% of patients may present with cognitive dysfunction in at least one cognitive domain (Kewman et al., 1991). Two main intriguing disputes naturally emerge which remain poorly understood in this context 1) what is the neural substrate of pain-cognition relationship? And 2) why is cognition impaired in some but preserved in other chronic pain states? The answer to these questions is beyond the scope of this thesis, and indeed these queries may be considered as highlights for future directions. Putative neural links between pain and cognition have been investigated here using unprecedented rigorous morphometric techniques both in this and the hippocampal chapters, however in the absence of cognitive impairment in this cohort, further investigations of neural mechanisms may deem purposeless.

My hypotheses for such variability between chronic pain diseases in relation to cognitive impairment was discussed in chapter 6, and briefly, it could lie in other moderating factors that are not present in the studied cohort or in the investigated clinical condition. Medication effect e.g. use of opioids (Hu et al., 2002) is expected

to impact upon GM volume, and since such and other medication intake is likely to be a disease specific, consequent effects may hypothetically vary accordingly. Sleep disturbance (Jongsma et al., 2011), or neuropathic component of pain which can vary according to different chronic pain aetiologies, as recently shown in preclinical models of neuropathic-like pain with subsequent cognitive deficits Mutso et al., 2012), have been highlighted in the literature and may hold accountable as potential moderating factors.

Robert et al (Hart et al., 2003) underlined the significance of stress as a plausible contributing factor in the pain-impaired cognition relationship. The role of stress was also emphasized by Vachon-Presseau (Vachon-Presseau et al., 2013) as a mediating factor between chronic pain and hippocampal volume reduction. Stress was not assessed in this studied cohort, nonetheless stress and also depression (Brown et al., 2002), as putative pain-cognition mediators would hoverer be expected to influence upon hippocampal volume (Duric and McCarson, 2006, McEwen, 2001) which, the latter was found preserved and furthermore significantly larger in the studied knee OA patients when compared to matched healthy controls. These findings provide evidence that depression may not explain cognitive disorder in chronic pain due to knee OA, and argue for the complexity of the mechanism of chronic pain

related abnormal behaviours warranted further studies to investigate this complex relationship.

The low ratings of affective component of knee OA pain reported by the studied patients may sound counterintuitive at first, as knee OA pain is a leading cause of disability (Murray and D., 1997). However, it is predictable that the qualities of pain experience may largely vary among different chronic pain diseases. In as study of three chronic pain conditions (fibromyalgia, OA, and low back pain), it was found that patients with OA presented comparatively with the lowest ratings of affective pain descriptors, followed by low back pain, and the highest scoring was recorded in fibromyalgia patients (Marques et al., 2001). It has been argued that pain does not necessarily have to have an affective component (Horn et al., 2012) in certain circumstance; Horn et al called it the "safe situation" when pain is predictable in terms of intensity and course, and hence it would likely elicit very low degrees of pain unpleasantness. Although, this hypothesis was confirmed by Horn and co-workers in an acute state of painful experience, it emphasizes that affective component of chronic pain may be modulated by context and cognitive appraisal of pain (Horn et al., 2012).

2.3.7 Conclusion

Dorsal and rostral parts of ACC did not show volumetric abnormalities in association with chronic painful knee OA, nor had they any volumetric covariance with the cognitive performance or pain affective component respectively. However, higher scores of pain catastrophizing correlated significantly and positively with increases in left rostral ACC sizes suggesting that it may underlie the pain catastrophizing tendency in patients with chronic knee pain.

2.4 Study II: Anterior cingulate and central pain sensitisation: Cortical thickness correlates in chronic pain in knee OA

2.4.1 Introduction

Central sensitization is defined by the International Association for the Study of Pain as ”an increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input‟ (Merskey and Bogduk, 1994). The increase in dorsal horn neural responses is a well-understood mechanism of pain sensitisation in rodent models (Li et al., 2013, Wu et al., 2005b) including MIA models (Sagar et al., 2011). Direct measurement of CNS neuronal activity in humans cannot be made and therefore direct evidence of neuronal hyper-excitability may not be provided (Curatolo et al., 2006). However, central sensitisation in humans can be indexed by, for instance, secondary punctate or pressure hyperalgesia (Woolf, 2011).

Mechanical hyperalgesia at the painful knee in OA is thought to result from peripheral deep tissue sensitisation, however widespread hyperalgesia at remote sites (Lee et al., 2011a), and for review (Suokas et al., 2012), is likely to be explained by one possible mechanism that is central sensitisation (Pavlakovic and Petzke, 2010). Interindividual differences in pain sensitivity may

however also reflect a number of predisposing factors notably, female sex: women show consistently lower mechanical and thermal pain thresholds (Racine et al., 2012, Riley et al., 1998), and are at greater risk of chronic pain disorders including OA (Mogil, 2012). Female knee OA patients report higher levels of pain and higher physical and pain related disability (Hame and Alexander, 2013, Keefe et al., 2000).

As discussed in chapter 1, the pain intensity in knee osteoarthritis can be quite variable ranging from hardly perceptible to immobilizing, and the severity of pain in knee osteoarthritis cannot often be predicted from the degree of structural local damage. Several studies (Kornaat et al., 2006, Link, 2009, Phan et al., 2006) have reported no significant correlation between the severity of pain and structural knee osteoarthritic lesions. Therefore, the degree of pain perceived is unlikely to be due to peripheral sensitization alone (Lee et al., 2011b). These findings along with other outcomes such as secondary hyperalgesia (Imamura et al., 2008) and pain at rest (Schaible et al., 2002), suggest a central mechanism of pain sensitization in knee OA (Jones et al., 2012).

The contribution of supraspinal structures to central sensitization is evidenced from brain functional imaging like functional MRI and magnetoencephalography (MEG). Studies have reported increased

activities in structures including the brainstem, thalamus, cingulate, insula, middle frontal, posterior parietal, and primary and secondary somatosensory cortices, in association with experimentally induced mechanical hyperalgesia (Lee et al., 2008, Maihofner et al., 2010, Mainero et al., 2007, Zambreanu et al., 2005). Gwilym and co-researchers (Gwilym et al., 2009) investigated the supraspinal (brainstem) correlates of punctate stimuli in areas of referred pain (indicative of possible central sensitization) in chronic hip OA using fMRI. Authors found significantly higher activity in the periaqueductal grey in response to punctate stimulation suggesting its role in central sensitization. The structural correlates, on the other hand, have received less focus from researchers particularly in chronic pain disorders, though attempts have been recently made to investigate the cortical thickness correlates of thermal thresholds in healthy young subjects (Erpelding et al., 2012).

The role of ACC in central sensitization is of particular interest; preclinical studies have shown molecular and microstructural changes in the ACC in association with synaptic potentiation (Chen et al., 2012, Li et al., 2010, Wu et al., 2005a). The long term potentiation of synaptic transmission in the ACC is likely to impose a contribution towards sustainability of chronic pain (Zhuo, 2014).

Interestingly, in humans, those who regularly practice meditation, known for its endogenous analgesic effect, the ACC was found thicker with longer periods of practicing, and meditators had significantly higher pain thresholds (Grant et al., 2010).

The exact role of ACC in central sensitization however remains poorly understood in sufferers of chronic pain, and the extent by which ACC contributes to sustainability and hence chronification of pain is unclear.

2.4.2 Aim and hypothesis

The aim of this study is to investigate the anterior cingulate cortical thickness correlates of pain hyper-sensitization in patients with chronic painful knee OA, and weather sex is a moderating factor of such relation. I hypothesize that cortical thickness changes in ACC, will associate with reduced pain thresholds due to central sensitization, and these changes will be more pronounced in females.

2.4.3 Methods

2.4.3.1 Subjects

A detailed description of participants recruited for this study has been provided in chapter 5. In brief, patients (n=31, age= 64.61±

8.36; 15 females) and age- and sex-matched pain-free healthy controls (n=22, age= 61.30± 7.46; 13 females) were included.

2.4.3.2 MRI and cortical thickness analysis

Brain scanning and cortical thickness analysis have been described in details in chapters 5 and 9, respectively. Values of average cortical thickness of total ACC bilaterally were derived from freesurfer output data, for both knee OA patients and HCs. Total ACC comprised rostral, mid, and dorsal ACC as per the default atlas implemented by freesurfer. Further whole brain vertex-wise analysis was performed to localise significant correlates with pain sensitivity.

2.4.3.3 Quantitative Sensory Testing (pressure pain threshold PPT)

Pressure pain thresholds (PPTs) were recorded by a trained single rater (MW) using a digital algometer (Somedic AB, Sweden) with three repetitions at the index finger, sternum, medial tibia, and at the medial and lateral joint lines of the painful knee (Described previously in details in chapter 5). The average of the last two readings for the three non-local sites was used to indicate non-knee or remote PPTs, and those from knee joint lines to indicate knee or local PPTs.

2.4.3.4 Statistical analysis

Difference in anterior cingulate cortical thickness between knee OA and HC groups was tested using ANCOVA while controlling for age and sex. Further correlation analysis within knee OA group was performed using Pearson's correlation controlled for partial effects of age and sex.

QDEC (freesurfer statistical and visualization tool) was used to perform surface based correlation analysis, with significance accepted at $p<0.001$ uncorrected, controlled for age and sex. Independent samples t-test was used to compare means of PPTs between groups.

2.4.4 Results

One dataset from a Knee OA participant was discarded due to a technical error. Knee PPTs were significantly lower in Knee OA vs. HC participants ($p=0.03$, table 1), but no differences were noted in PPT at remote sites. Neither knee nor remote PPTs correlated with chronic pain severity or duration. In each subgroup, no difference was found between males and females for PPTs at the knee and remote sites (table 2).

Table 1: Pressure pain threshold measurements for participants

	Average knee PPT KPa (M± SD)	**Average non-knee PPT KPa (M± SD)**
Knee OA (*n*=30)	281.4± 152.4	227.3± 106.5
HC (*n*=22)	385.1± 182.9	253.3± 107.2
P value*	**0.03**	**0.39**

*Independent sample t-test

Table 2: Pressure pain threshold measurements for sexes in subgroups

	Average knee PPT KPa (M± SD)	**Average non-knee PPT KPa (M± SD)**
Male Knee OA (*n*= 16)	313.2± 172.5	243.2± 121.7
Female Knee OA (*n*=14)	245.1± 121.4	209.2± 86.9
***P* value**	**0.23**	**0.39**
Male HC (*n*= 9)	391± 175.9	271.8± 105.7
Female HC (*n*=13)	381± 194.6	240.5± 110.5
P value*	**0.9**	**0.51**

*Independent sample t-test

Data of average thickness of ACC in both groups (knee OA and HC) are summarized in table 3. No significant difference in ACC thickness was found between groups.

Table 3: Average AC cortical thickness in knee OA vs. HC participants

	Right ACC Average CT	**Left ACC Average CT**
Knee OA (*n*=30)	2.49± 0.11	2.54± 0.11
HC (*n*=22)	2.53± 0.13	2.56± 0.11
P value*	**0.27**	**0.53**

*Independent sample t-test

AC cortical thickness differences remained non-significant bilaterally after adjusting for age and sex p>0.3 ANCOVA.

Moderate but significant positive correlation was found between thickness of the left ACC and higher remote PPTs (r= 0.46 p=0.013), controlled for partial effects of age and sex, Fig. 1. No such relation was found in HCs.

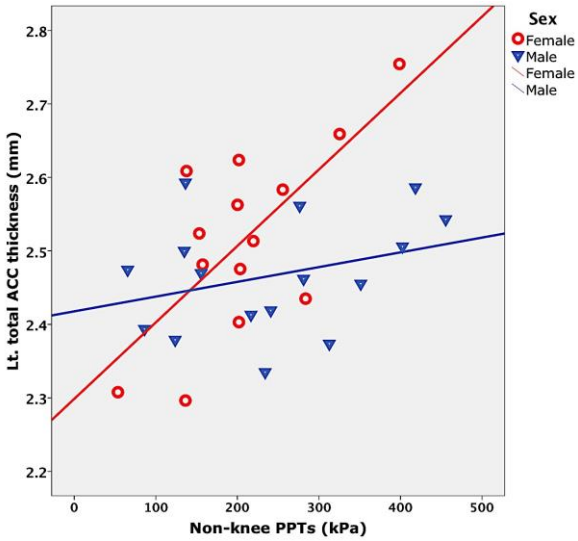


Figure 1: Scatter plots and regression lines marked by sex depicting relation between Lt. ACC thickness and remote pressure pain thresholds. (Female R^2= 0.49, Male R^2=0.01)

Vertex-wise correlation analysis in Knee OA patients revealed that thickness of the left anterior cingulate cortex correlated positively with remote PPTs. Namely, higher pain sensitivity (lower PPTs) was associated with cortical thinning in the left anterior cingulate ($p<0.001$ uncorrected, controlled for age and sex, Fig. 2A). A similar correlation was noted for the knee PPTs ($p<0.001$ uncorrected, controlled for age and sex, Fig. 2B). This relationship was significantly moderated by sex (scatter plots and correlation graph Fig. 3; $t=2.8$, $p=0.01$ and $t=2.6$, $p=0.02$, multiple regression tests for non-knee and knee sites respectively). No relationship emerged in healthy controls for both knee and non-knee PPTs.

Of note, focal ACC change was the only cluster that showed significant correlation in both hemispheres, though it did not survive multiple test correction. Anatomical localization revealed that the cluster spanned over anterior part, middle anterior part, and pericallosal sulcus, as per the latest Destrieux atlas a2009s implemented in freesurfer (Destrieux et al., 2010), Fig 3.

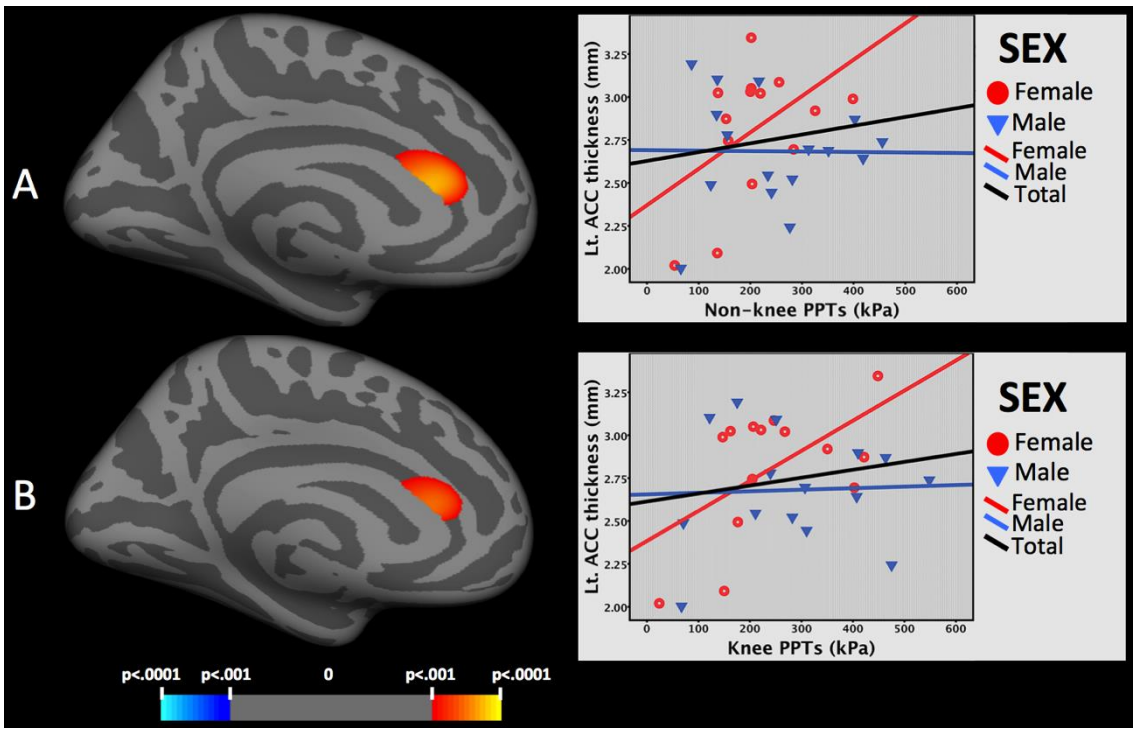


Figure 2: Correlation between cortical thickness and pressure pain thresholds. A: non-knee and B: knee- sites. Higher scores of pressure pain threshold significantly correlate with thickening in the ACC (r=0.72, p<0.001 and r=0.7 p<0.001 for A and B respectively, adjusted for age and sex). Scatter plot and correlation graph marked by sex showing a significant sex moderating effect p<0.02.

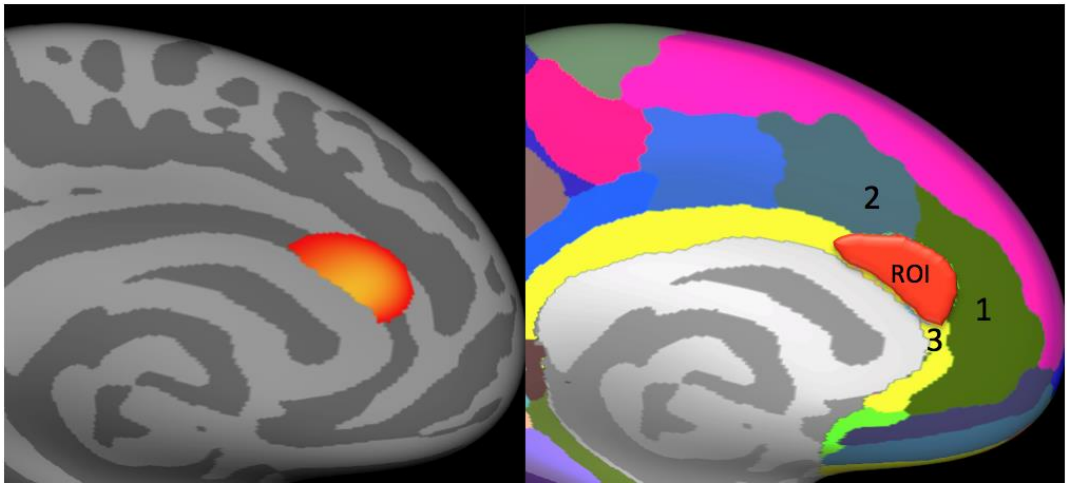


Figure 4: Inflated cortical surface to illustrate location of cluster that showed positive cortical thickness correlation with higher remote PPTs. 1=anterior part of ACC, 2= mid-anterior part of ACC, and 3=pericallosal sulcus. ROI=region of interest.

2.4.5 Discussion

In this study, it was shown that mechanical pain thresholds correlated with left anterior cingulate cortical thickness in patients suffering from knee OA, which was notably and significantly moderated by sex such that women exhibited more pronounced effect. Pressure pain thresholds only differed between knee OA patients and HCs at the affected joint and sex had no influential effect.

Quantitative sensory testing has been widely used to characterize sensory and pain perception in both normal and chronic pain conditions (Suokas et al., 2012, Pavlakovic and Petzke, 2010), yet little is known about its neural correlates especially in painful states. Here, it was found that higher pain sensitivity both at the affected joint and remote sites were associated with thinning in the anterior cingulate cortex. In human studies ACC has been consistently found to be involved in processing the emotional and affective components of experimental and clinical pain (Devinsky et al., 1995, Peyron et al., 2000, Tolle et al., 1999, Vogt et al., 1996). Interestingly, adults practicing Zen meditation (Grant et al., 2010) were found to be less sensitive to pain and to have thicker cortex in the anterior cingulate compared to non-meditating controls. Moreover cortical thickness in the ACC correlated with the number of years of meditation. In another longitudinal experimental pain

study by Stankewitz et al (Stankewitz et al., 2013), it was found that only a subgroup of sensitizers developed post-experimental GM density reduction in the ACC as well as other brain areas involved in pain processing and modulation. Furthermore, the sensitizers group exhibited less GM density in several areas including the ACC when compared to a control group and a subgroup of habituaters respectively.

The plasticity seen in the ACC in association with higher pain sensitivity is supported by cumulative evidence from preclinical studies (Chen et al., 2012, Li et al., 2010, Wu et al., 2005a) that show a synaptic potentiation enhancing microstructural and molecular changes in the ACC, suggesting that the ACC is a key structure in central sensitization. The concept of long term potentiation (LTP), a potential neurobiological substrate of learning and memory (Bliss and Collingridge, 1993), has led some researchers (Ji et al., 2003) to postulate that similar mechanisms are involved in central sensitization and hence persistency of pain (Zhuo, 2014), however, the cortical thinning seen in the ACC in sensitizers in this study argue for complex ultrastructural changes. It is not clear whether such plastic changes at the synaptic level are putatively of molecular and functional nature only or they are accompanied by microstructural plasticity e.g. synaptic re-

organization. However, the findings from this study provide some evidence that changes may involve structural reorganization. The fact that no abnormal difference was found in left ACC in patients when compared to HCs, and that the cortical thickness correlation with pain sensitivity was seen only in patients suggests that the changes could be of acquired nature that is related to chronic pain in the studied knee OA cohort.

2.4.6 Conclusion

In conclusion, the study findings demonstrate that higher sores of mechanical pain sensitivity are correlated with reduced cortical thickness in the anterior cingulate highlighting its potential key role in the process of central sensitization. The sex moderating effect may partially explain the higher prevalence and comorbidity rates of chronic knee OA pain in females.

2.5 Voxel-based morphometric correlates of chronic pain in knee OA patients

2.5.1 Introduction

Chronic pain, as a syndrome involving multiple dimensions, is expected to impact upon extensive brain networks concerned with different aspects of pain including sensory, affective, and cognitive domains. Voxel based morphometry (Ashburner and Friston, 2000b) has been the most popular morphometric tool of choice to investigate GM changes on a voxel by voxel levels. Several neuroimaging studies have reported morphological cerebral changes in various chronic pain states (Kuchinad et al., 2007, Schmidt-Wilcke et al., 2006, Rodriguez-Raecke et al., 2009, Apkarian et al., 2004, Draganski et al., 2006, Burgmer et al., 2009, Buckalew et al., 2010b, Schmidt-Wilcke et al., 2005, Seminowicz et al., 2010, Schmidt-Wilcke et al., 2008, Gwilym et al., 2010b). Most studies found reductions, yet of unknown nature, in GM density, volume or in fewer circumstances, where surface-based morphometry was implemented, decreases in cortical thickness were observed. However, the distribution of pain-related GM changes is highly varied between studies even when studying identical primary aetiologies of chronic pain disorders. The affected regions are also inconsistent between studies of musculoskeletal disorders (chapter 4), including chronic painful hip osteoarthritis

(Gwilym et al., 2010b, Rodriguez-Raecke et al., 2009), and knee OA (Baliki et al., 2011).

Brain regions that show higher activity during noxious stimulation include, by large convergence, the secondary somatosensory cortex, insula, anterior cingulate cortex, and with less consistency the thalamus and primary somatosensory cortex (Peyron et al., 2000), and frontal and parietal lobes (Peyron et al., 2000). By contrast, structural brain changes associated with different chronic pain conditions, as reviewed and meta-analysed by Smallwood et al (Smallwood et al., 2013b), were consistently found, as voxel-based morphometric reductions, in several clusters spanning over areas considered as part of the known pain matrix but also in areas that are largely outside the pain matrix. The largest and most significant cluster of reduced grey matter volume in patients with chronic pain as per Smallwood et al was noted covering regions including right putamen and claustrum, insula, and right posterior inferior frontal gyrus. Other areas of reduced GM involved ACC, medial frontal gyrus, insula, superior temporal gyrus, thalamus, paracentral lobule, and superior, middle and inferior frontal gyri.

The correlation of chronic pain-related GM changes with the intensity of perceived pain is not clearly elucidated. Functional MRI studies found that experimental pain intensity to correlate

positively with activity in the primary somatosensory, cingulate, motor and premotor cortex, and negatively in medial parietal, perigenual cingulate and medial prefrontal regions (Porro et al., 1998). In chronic pain conditions, higher intensity of spontaneous pain in fibromyalgia was found to correlate with greater intrinsic connectivity between insula and default mode network and executive attention networks (Napadow et al., 2010).

The structural brain correlates on the other hand have not received as much attention from researchers particularly in MSK disorders, and findings to date seem controversial. Some studies reported positive correlation between average (normally over past 4 weeks), or acute (on day of scanning) pain severity and GM volume (Gwilym et al., 2010a), negative correlation (Schmidt-Wilcke et al., 2006, Younger et al., 2010), or no correlation (Buckalew et al., 2008, Baliki et al., 2011). Of Note, Baliki and co-workers studied three chronic pain conditions (chronic back pain, complex regional pain syndrome, and knee OA) and found no relation with GM volume in any group.

Literature of MSK pain imaging is scarce and in particular of studies of pain in osteoarthritis. Furthermore, studies to date have been limited by sample size, heterogeneity of studied cohort and less rigorous morphometric techniques. In this study, up-to-date voxel-

based morphometric technique using DARTEL (Diffeomorphic Anatomical Registration using Exponentiated Lie algebra) (Ashburner, 2007), implemented in SPM8 (Statistical Parametric Mapping8: http://www.fil.ion.ucl.ac.uk/spm) was used, which evidently offers improvement in spatial normalization and more reliable analysis outcome (Mak et al., 2011) comparable with manual morphometry, which is considered as a gold standard in volumetric measurements (Cherbuin et al., 2009, Morey et al., 2009, Shen et al., 2010).

2.5.2 Aims and hypothesis

The aim of this study is to investigate whether chronic pain due to primary nociceptive knee OA is associated with abnormal grey matter structural changes as compared to age and sex matched controls, and to determine whether GM changes are related chronic pain severity in sufferers of knee OA. I hypothesize that knee OA patients will show grey matter reduction in areas involved in pain processing, and that chronic average pain intensity will correlate with volumetric changes in areas concerned with the sensory aspects of pain namely in the lateral thalamus and somatosensory cortex

2.5.3 Methods

2.5.3.1 Subjects

Patients (n=31, age= 64.61± 8.36; 15 females) and age- and sex-matched pain-free healthy controls (n=22, age= 61.30± 7.46; 13 females) were included. A detailed description of participants recruited for this study is provided in chapter 5.

2.5.3.2 Data acquisition

Brain MR imaging involved the used of 3T scanner (Discovery 750, GE Medical Healthcare, Milwaukee, US) with a 32-channel head coil, to acquire 3D structural brain scans at a native resolution of $1mm^3$. Full description is provided in chapter 5.

2.5.3.3 Chronic pain severity

Average pain severity over four weeks prior to scanning was derived from a response to a question in the self-administered PainDETECT questionnaire (Freynhagen et al., 2006), which used the following statement: "How strong was the pain during the past 4 weeks?" and patient could choose their response based on a scale from 0 to 10, which represent none and maximum respectively.

2.5.3.4 Voxel based morphometry

VBM-DARTEL was implemented using standard procedure and default parameters except where stated below, to investigate GMV differences between knee OA patients and healthy controls, and to investigate the GMV correlates with pain severity, using SPM8 implemented in Matlab R2012a (The Math Works Inc., Natick, MA, USA). DARTEL was used to create study-specific templates for segmented tissues and to enhance accuracy of inter-subject alignment. Pre-processing steps included manual intervention to later improve automated image registration, involved adjusting orientation and setting origin to anterior commissure for all images using the 'Display' and 'Check Registration' features in SPM8. Segmented GM images were then normalized to MNI space and smoothed using isotropic Gaussian kernel of 8mm full-width at half-maximum (FWHM). In the modulation step, preserve was changed to 'preserve volume' to yield GM volume rather than concentration. Smoothed images were used to test for focal differences in GMV between groups (knee OA vs. HC) using independent samples t test (implemented in the Factorial Design Specification SPM8) with intracranial volume as a global calculation to account for head size difference, (ICV was manually measured for each subject, as explained in chapter 6). Voxel-based correlation between GM volume and chronic pain severity was performed using multiple regression design (SPM8), with age and

sex treated as covariates of no interest. Significance was accepted at $p<0.001$ uncorrected.

2.5.4 Results

Demographic and behavioural data were presented in details in chapter 5.

2.5.4.1 Chronic pain severity

Data of pain severity, which could range 0-10, were normally distributed in patients ($p>0.06$, Shapiro-Wilk test), and recorded the following statistics: a mean of 4.77 and SD= 0.43 (median=5, range=1-10).

2.5.4.2 VBM results

Between-group differences

VBM comparisons revealed two clusters of significantly reduced grey matter volume in patients compared to HC ($p<0.001$, uncorrected), namely in the left dorsolateral prefrontal cortex (MNI -45, 12, 44) and right lower cerebellum (MNI 8, -56, -64) Fig. 1. However, these results did not survive multiple test corrections.

There were no regions of larger GMV in patients relative to HC.

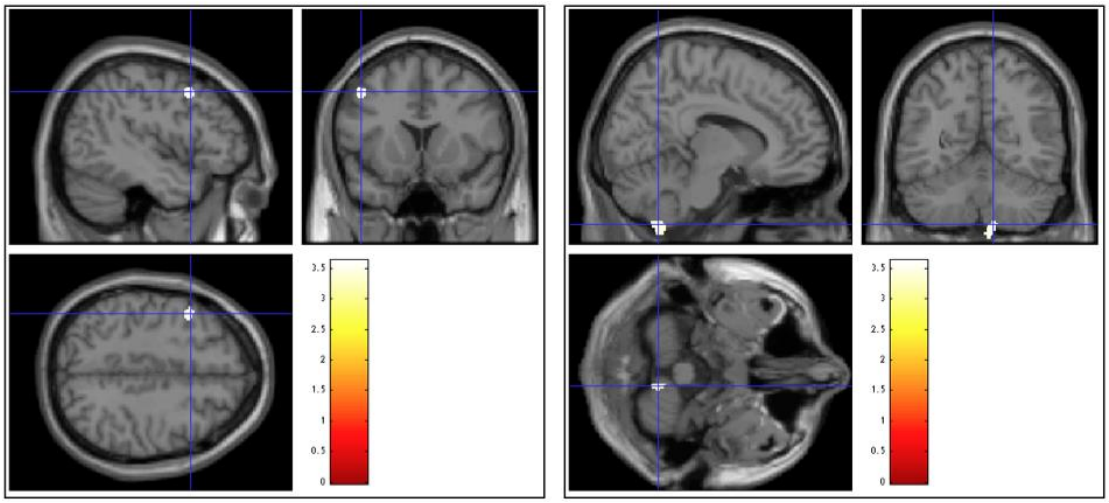


Figure 1: Regions of less grey matter volume in patients compared to healthy controls are presented in left DLPFC (set of images on the left), and right lower cerebellum (VIIIb) (images on the right), ($p<0.001$ uncorrected).

Correlation analysis

Chronic pain severity did not correlate with any grey matter volume changes.

2.5.5 Discussion

Voxel-based morphometric analysis revealed that patients with chronic pain due to knee OA had less grey matter volume in the left dorsolateral prefrontal cortex and right lower cerebellum (VIIIb), but no correlation was found between GM changes and average pain severity. The lack of correlation with pain severity is consistent with study of chronic pain conditions and in particular in knee OA patients (Baliki et al., 2011). There is good evidence to

suggest that DLPFC (Graff-Guerrero et al., 2005, Veldhuijzen et al., 2009) and cerebellum (Casey et al., 1994, Iadarola et al., 1998), along with other structures, participate in a neural network involved in pain processing.

The lack of consistency in GM findings among similar studies raises an intriguing question: why do findings vary between and even within identical chronic pain conditions? I have carried out a systematic review of MSK chronic pain neuroimaging studies (chapter4), and some selected studies of the review (based on comparability with the current study in terms of e.g. sample size, scanning and morphometric techniques, chronic pain condition) will be contrasted here to investigate the potential underlying causes of inconsistency (table 1). As can be seen from the table, several confounders may contribute to the discordance observed between chronic pain imaging studies. First, demographic factors such as age and sex are likely to vary with subsequently different results. Some specific disorders e.g. fibromyalgia are more prevalent and hence expected to be dominated by females, and age on the other hand can interact with the disease to change the brain (Ceko et al., 2013). Second, heterogeneity in scanning and methodological procedures including scanning parameters, analysis approach and statistical method and significance threshold, may also influence the outcome. For instance, field strength can impose considerable

regional impact on VBM results (Tardif et al., 2010). Third, the multitude of pain related factors with regard to duration, severity, emotional and cognitive aspects as well as pain comorbidities e.g. psychological changes, lack of mobility and proper sleep, as well as medications may impact upon the findings. For example, Hsu and co-workers (Hsu et al., 2009) studied two groups of chronic fibromyalgia patients with and without affective disorders compared to matched healthy controls (n=29 each), and found GM deficits in the group with, but not in the group without affective disorders, when post-hoc analysed. Although some factors seem un-modifiable, most can be addressed especially those related to methodological issues where international and harmonized protocol can be developed and implemented, see for example, the ADNI project (Alzheimer's Disease Neuroimaging Initiative; http://www.adni-info.org/).

Table 1: Sample of comparable MSK studies including current study, showing main characteristics and outcomes

Study	Condition, Sample size	Age Mean (SD)	Sex (M: F)	Pain duration Mean (SD)	Average Pain severity mean (SD) and range	Matched group	Tesla/ voxel size/ smoothing (FWHM)	P value	Findings Total/ROI analysis
Present study	Knee OA n=31	64.6 (8.4)	(16: 15)	114.6 (96)	4.8 (2.4) 1-10	n=21 age and sex matched HCs	3T/ 1mm^3/ 8 mm	<0.001 un-corrected	**P<HC:** Lt. ***DLPFC***, and Rt. Lower cerebellum **P>HC:** none
(Apkarian et al., 2004)	CBP n=26	47 (12.6)	NA	135.6± (136.8)	NA Pain severity on scanning day= 6(2.3)	n=26 age and sex matched	1.5T/ 2 protocols/ 12 mm	<0.05 corrected	**P<HC:** bilateral ***DLPFC*** (Total analysis), and Rt. Thalamus (mask) **P>HC:** none
(Hsu et al., 2009)	Fibromyalgia n=29	Median= 41.7	Female only	153.6± (99.6)	NA	n=29 age and sex matched	3T/ 0.9x0.9x1.5 mm/ 10mm	<0.001 un-corr. For whole brain, <.05 corr. (ROI)	**P<HC:** none (Total analysis), and Lt. ant. insula (mask) **P>HC:** none
(Rodriguez-Raecke et al., 2009)	1ry hip OA n=32	66.8 (9)	(13: 19)	88.2	NA	n=32 age and sex matched	3T/ FOV=256x256,1mm of 240 slices/ 10mm	<0.001 un-corrected	**P<HC:** ACC, Rt. Insula, Lt. ***DLPFC***, amygdala, brain stem **P>HC:** left middle frontal gyrus
(Baliki et al., 2011)	CBP n=36 CRPS n=28 Knee OA n=20	48.2(11.4) 40.6(7.4) 53.5(7.4)	(23:13) (4:28) (16:4)	147.9(137.9) 39.3(40.8) 146.7(114)	NA Pain severity on scanning day was available	Total of n=46,26F Age=38.8(12.5)	3T/ 1mm^3/ 8 mm	<0.01 corrected	**CBP<HC:** insula, S2, pre/post central, temp. lobe **CRPS<HC:** insula, orbitofrontal **OA<HC:** insula, mid ACC **No increases** in any patient group vs. HC
(Wartolowska et al., 2012)	Rheumatoid arthritis n=31	Median= 57	(9:22)	Median= 180	NA Pain severity on scanning day, median=6.5	n=28 age and sex matched HCs	3T/ 1mm^3/ ~7 mm	<0.05 corrected	**P<HC:** intracranial volume **P>HC:** nucleus accumbens, caudate nucleus

OA= osteoarthritis, CBP= chronic back pain, CRPS= chronic regional pain syndrome, P= patients, HC= healthy control, DLPFC= dorso-lateral prefrontal cortex, ACC= anterior cingulate cortex, NA= not available

Despite inconsistent findings, there seems to be an overlap between studies in the DLPFC. The DLPFC (found reduced in patients) is of particular importance because of its modulatory role in nociceptive transmission (Boggio et al., 2008, Brighina et al., 2011). Repetitive transcranial magnetic stimulation of left prefrontal cortex in healthy subjects showed a significant increase in thermal pain thresholds (Borckardt et al., 2007), supporting its proposed inhibitory role in pain perception. The results of this study are compatible with a key finding of reduced bilateral DLPFC (however, reduction was larger and more significant on left side) in chronic back pain patients (Apkarian et al., 2004), and a reduced left DLPFC in another surface-based morphometric study of low back pain patients (Seminowicz et al., 2011), and importantly in chronic pain due to hip OA (Rodriguez-Raecke et al., 2009). Notably, a significant thinning of left DLPFC with longer pain was found in the surface-based correlation analysis performed on the same cohort (cluster 12 in Fig. 2, chapter 9).

The nature of such reduction in DLPFC is not clear, and whether such a change would lead to functional disturbance of its inhibitory role and hence a putative contribution to pain persistency remains intriguingly and mechanistically unknown. Interestingly, a case-control study (Grachev et al., 2000) of proton magnetic resonance spectroscopy revealed reductions of N-acetyl aspartate (NAA) and glucose (relative to creatine/phosphocreatine complex) in the left

DLPFC in patients with chronic back pain, but not in the cingulate, insula, thalamus or sensorimotor regions. NAA is known to be localized primarily within neurons and is widely used as a marker for functional and structural neuronal integrity (Tsai and Coyle, 1995). NAA levels can exhibit relative decreases in neurodegenerative diseases such as Alzheimer's (Adalsteinsson et al., 2000, Schuff et al., 2002). Its relative reduction in DLPFC found in chronic pain patients (Grachev et al., 2000) may suggest atrophic changes. However, longitudinal studies have shown reversibility of chronic-pain related reductions in DLPFC following therapeutic treatment that was also accompanied by a significant pain relief as found in chronic back pain (Seminowicz et al., 2011), and in chronic pain due to hip OA (Rodriguez-Raecke et al., 2009).

These findings argue for non-degenerative plastic change, however it remains unproven and of unclear mechanisms. As discussed previously (chapter 3) it has been proposed that these changes may represent neuroanatomical substrate for the disease, an epiphenomenon or even an artefact (Reiss et al., 2004b). Simple change in cell size, shrinkage or atrophy of neurons or glia, changes in the intra-cortical axonal architecture (synaptic loss) have been suggested (Rodriguez-Raecke et al., 2009, May, 2008). A concept of changes in the extracellular matrix without substantial involvement of neurons or neuroglia was offered by Schmidt-Wilcke and colleagues

(Schmidt-Wilcke et al., 2008), supporting their idea by a piece of growing evidence showing that extracellular matrix influences neural cell activity. These mechanisms are thought to be secondary to, or in conjunction with a reduction in the cerebral blood flow, as proposed by Gwilym and co-workers (Gwilym et al., 2010a).

The finding of GM changes in cerebellum is less consistent among chronic pain states, however in MSK disorders, GM changes in cerebellum are likely to be attributed to a combined effect of pain and gait modification i.e. altered gate (Gwilym et al., 2010a), which is evident in lower limb OA disorders. Little is known about the specific role of VIIIb part of cerebellum in nociception, however preclinical studies showed its activation during visceral noxious stimulation (Saab and Willis, 2003).

2.5.6 Conclusion

Patients with chronic painful knee OA showed reduced grey matter volume in left DLPFC and right lower cerebellum, but no correlations were noted with average pain severity. The role of DLPFC in chronic pain perception is noteworthy and warrants further multimodal investigations to understand the mechanism of its involvement in pain chronification and the possibility of its therapeutic targeting.

2.6 Surface-based morphometric changes in chronic pain due to knee OA

2.6.1 Study I: Cerebral cortical remodelling in chronic knee osteoarthritis pain: Effects of pain duration and female sex

2.6.1.1 Introduction

Several neuroimaging studies have reported morphological cerebral changes in various chronic pain states (Kuchinad et al., 2007, Schmidt-Wilcke et al., 2006, Rodriguez-Raecke et al., 2009, Apkarian et al., 2004, Draganski et al., 2006, Burgmer et al., 2009, Buckalew et al., 2010b, Schmidt-Wilcke et al., 2005, Seminowicz et al., 2010, Schmidt-Wilcke et al., 2008, Gwilym et al., 2010b). Most studies found a reduction in GM density, volume or cortical thickness, but also increases in some areas such as hippocampus and parahippocampal (Smallwood et al., 2013b). The mechanism by which these changes take place in either direction is largely unknown and furthermore the distribution of pain-related GM changes is highly varied between studies also when studying identical primary aetiologies of chronic pain disorders.

The correlations between the duration of pain and the extent of structural brain changes appear inconsistent. Some studies reported negative correlations i.e. less GM volume or white matter integrity with longer pain (Apkarian et al., 2004, Schmidt-Wilcke et al., 2006, Kuchinad et al., 2007, Buckalew et al., 2010a, Baliki et al., 2011,

Wartolowska et al., 2012), while others found positive correlations (Younger et al., 2010, Moayedi et al., 2011) or no correlation (Draganski et al., 2006, Schmidt-Wilcke et al., 2007, Buckalew et al., 2008, Hsu et al., 2009, Gustin et al., 2011). These discrepancies may reflect a random error due to small sample sizes, limitations of the grey matter volume estimation or true biological differences in underlying pain characteristics or predisposing factors. As mentioned in previous chapters, the female sex is a known risk factor for OA and related pain. Women are at greater risk of chronic pain disorders including OA (Mogil, 2012). Female knee OA patients report higher levels of pain and higher physical and pain related disability (Hame and Alexander, 2013, Keefe et al., 2000). There are some reports suggesting that people with smaller brains, which may be linked to developmental or genetic differences, are more liable to chronic pain disorders (Wartolowska et al., 2012)

Importantly, most pain neuroimaging studies have low power not allowing controlling for the multitude of factors that influence cortical morphometry that may not be well controlled between groups. Apart from relevant comorbidities such as depression and anxiety, simple training tasks such as a few weeks of juggling (Draganski et al., 2004), common lifestyle factors such as alcohol consumption (Momenan et al., 2012, Pfefferbaum et al., 1992) and genetic factors

are known to affect brain morphometry (Peper et al., 2007, Kolb et al., 2011).

Quantification of grey matter changes can be achieved using different image analysis techniques, however measuring the cortical thickness using a surface-based approach arguably improves robustness over standard volumetric methods; cortical thickness assessment provides a directly interpretable metric, and is more sensitive allowing for detection of sub-voxel changes (Liu et al., 2012b, Pereira et al., 2012), less sensitive to inaccuracies of spatial normalization and smoothing (Bookstein, 2001, Augustinack et al., 2013), and has been well validated (Lee et al., 2006, Rosas et al., 2002, Salat et al., 2004). Only recently, the use of surface based morphometric approach to investigate cerebral correlates of chronic pain has gained considerable research attention. However, the directional and spatial patterns are still inconsistent among different chronic pain conditions using this approach (Frokjaer et al., 2012, Jensen et al., 2013, Kong et al., 2013, Messina et al., 2013, Seifert et al., 2012, Seminowicz et al., 2011). To the best of my knowledge and based on literature search (performed 2013 and updated March 2014) using combined terms (cortical thickness AND pain AND osteoarthritis) entered into web of knowledge (www. webofknowledge.com) and PubMed (http://www.ncbi.nlm.nih.gov/) search engines, cortical thickness

investigation has not been done previously in chronic pain due to OA disorders

2.6.1.2 Aims and hypothesis

To overcome some of the main limitations of previous studies, I chose to characterize the interrelations of cortical thickness and duration of persistent pain in a homogenous group of patients with painful knee OA. This approach is more powerful than voxel-based morphometric assessment of cortical grey matter (Pereira et al., 2012), and allows a more meaningful interpretation of results than those obtained from between group comparisons.

In this study I investigate the neocortical changes in chronic knee OA pain with a primary aim to evaluate the cortical thickness relation to pain duration rectified by sex. I hypothesise that pain-related reductions in cortical thickness:

- Are more pronounced in neocortical areas related to pain processing in patients as compared to matched healthy controls;
- Progress over the duration of chronic pain; and
- Are more pronounced in women

2.6.1.3 Methods

Subjects

A detailed description of participants recruited for this study has been provided in chapter 5. In brief, patients (*n*=31, age= 64.61± 8.36; 15 females) and age- and sex-matched pain-free healthy controls (*n*=22, age= 61.30± 7.46; 13 females) were included. Participants were further divided, in line with previous reports (Baliki et al., 2011), into those with pain duration of 5 years or less (short pain duration- SPD group, *n*=12) or more than 5 years (long pain duration- LPD group, *n*=19). Although the division by time was based on median-split of pain duration in the aforementioned study, it was chosen here to investigate whether changes after several years of persistent pain would be comparatively more pronounced

Brain Magnetic resonance Imaging

MR scanning has bee described in details in chapter 4. Briefly data acquisition involved the used of 3T scanner (Discovery 750, GE Medical Healthcare, Milwaukee, US) with a 32-channel head coil, to acquire 3D structural brain scans at a native resolution of $1mm^3$.

Cortical thickness analysis

Cortical reconstruction and thickness estimation was performed using the Freesurfer software package (Mac version 5.1.0 available online http://surfer.nmr.mgh.harvard.edu/). Briefly the processing involves removal of non-brain tissue(Segonne et al., 2004), transformation into Talairach space, intensity normalization(Sled et al., 1998), tessellation of the grey matter white matter boundary, automated topology correction(Segonne et al., 2007, Fischl et al., 2001), and surface deformation is then performed to indicate the grey/white and grey/cerebrospinal fluid borders based on detection of greatest shift in intensity(Fischl and Dale, 2000, Dale and Sereno, 1993, Dale et al., 1999). The method uses both intensity and continuity information from the entire three dimensional MR volume in deformation procedures to produce representations of cortical thickness, calculated as the closest distance from the grey/white boundary to the grey/CSF boundary at each vertex on the tessellated surface. The reconstruction output is then visually inspected and any parcellation inaccuracies are manually corrected. The maps produced are not restricted to the voxel resolution of the original data thus are capable of detecting changes at sub-millimetre levels. Procedures for the measurement of cortical thickness have been validated against histological analysis(Rosas et al., 2002) and manual measurements(Salat et al., 2004).

Statistical analysis

Based on the distribution of data independent *t*-test or independent samples Mann-Whitney U tests (or Fisher's Exact test) were used to examine group differences for age, BMI, pain severity, sensory, affective (parametric), MoCA, BDI, pain duration and painDECTECT scores (non-parametric). Between group comparisons of sex, handedness and educational status were assessed using Chi-Squared tests.

QDEC (freesurfer statistical and visualization tool) was used to perform whole-brain surface based correlation and between group analysis controlled for age. Significance was set at $p<0.025$ (corrected for multiple comparisons using false discovery rate FDR) to account for repeated testing of both hemispheres.

To further characterize putative sex effects, subgroup analysis in male and female patients were performed.

Results of within group whole cortical analysis are reported as mean± SD with $p<0.05$ FDR corrected and at uncorrected $p<0.001$ for between group comparisons. Global and region-of-interest analyses are reported at the $p<0.05$.

2.6.1.4 Results

Demographic data and pain characteristics

Demographic data and pain characteristics were described in details in chapter 5. SPD and LPD groups did not differ in age, sex distribution or pain characteristics (McGill and painDETECT pain scores, acute or chronic pain severity). Only depression scores were higher in the LPD group (table. 1). However depression symptoms ranged from minimal to upper limit of mild depression (Spreen, 1998).

Table 1: Demographic data and pain characteristics of short vs. long pain groups

		Age (Y)	**Sex** (M: F)	**Pain duration** (Months)	**C. Pain severity** 0-10	**VAS** 0-10	**Sensory** 0-42	**Affective** 0-14	**Pain-DETECT** 0-38	**BDI** 0-63
SPD n=12	*Mean*	61.7		39.5	5.6	2.7	12.6	1.5	10.3	3.75
	SD	7			2.8	2.8	6.4		6.5	
	Median			36				0.5		2
	Range	45.4-72		12-60	2-10	0-9	4-28	0-11	0-23	0-14
	Ratio		5:7							
LPD n=19	*Mean*	66.4		162	4.3	2.3	11	1	12.2	8
	SD	8.8			2.1	1.8	8.2		5.7	
	Median			120				0		7
	Range	48.3-81		72-456	1-8	0-5.5	0-29	0-7	1-25	0-19
	Ratio		11:8							
P-value		**0.13§**	**0.4Φ**	**< 0.001¶**	**0.14§**	**0.64§**	**0.56§**	**0.57¶**	**0.4§**	**0.02¶**

SPD= Short Pain Duration, **LPD=** Long Pain Duration. **§=** Independent t-test. **¶=** Independent samples Mann-Whitney U Test. **Φ=** Fisher's Exact Test.

Cortical thickness findings in chronic painful knee OA participants

The average cortical thickness was less in KOA compared to HC for the left hemisphere (2.36± 0.1 vs. 2.42± 0.09 p< 0.03), total brain (2.36± 2.42 vs. 2.42± 0.09 p<0.04) and borderline reduced for the right hemisphere (2.37± 0.09 vs. 2.42± 0.08 p=0.05) using

independent samples *t*-test. However after accounting for age and sex, similar trends did not reach significance (left hemisphere, p=0.07 and whole brain, p=0.087 ANCOVA).

Whole brain vertex-based analysis showed that thinner cortex in the right posterior inferior frontal (rPIF) gyrus and sulcus (Brodmann area 44), and right pre-central and sub-central gyri (Brodmann areas 4 and 43) in KOA, p<0.001 uncorrected, controlled for age and sex (Fig. 1). However, findings did not survive multiple test correction. No differences were seen for the left hemisphere.

For the between-group differences the analysis was re-run after exclusion of one HC (was on Zopiclone) to explore if this can have a confounding effect on the results. This revealed no change in the results significance.

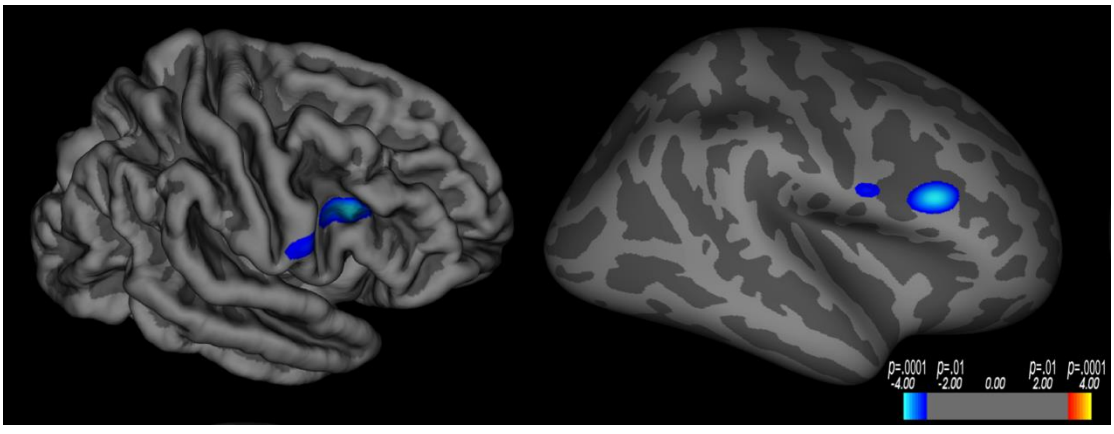


Figure 3: Cortical thickness differences between knee OA participants and healthy controls rendered on the Freesurfer reconstructed average brain (left) and the inflated cortical surface (right). Blue/cyan indicated knee OA<HCs (p<0.001 uncorrected)

Correlations of cortical thickness with pain duration in chronic knee OA patients

Average CT of left and right hemispheres and total brain, correlated moderately negatively with the duration of pain r=-0.50 (p=0.005), r=-0.58 (p=0.001), and r=-0.54 (p=0.002) respectively; controlled for age. Stratified for sex, correlation of pain duration was strongly negatively correlated in women, but not in men for the left (r=-0.75; p=0.002), right hemisphere (r=-0.81; p=0.0004), and total CT (r=-0.78; p=0.001) all controlled for the effect of age.

Detailed vertex-based whole brain revealed extended bilateral areas with 13 clusters (right 1-9, and left 10-13 ranked according to Z-score, Fig. 2 and table 2) where pain duration correlated negatively with cortical thickness i.e. thinner cortex with longer pain (p<0.025, FDR corrected). However, clusters appear located largely outside the known cortical pain centres e.g. the somatosensory cortex, insula, anterior cingulate cortex (Peyron et al., 2000), they are largely extended over frontal and parietal lobes, which the latter show higher activities during noxious stimulation (Peyron et al., 2000). As some clusters spanned over several regions (e.g. cluster 1 with a surface area of 12382.5mm^2 included 15 regions), sub-clusters were anatomically detailed and description can be found in supplementary data, (appendix 5, table 1). No positive correlations were seen at uncorrected p<0.001 levels.

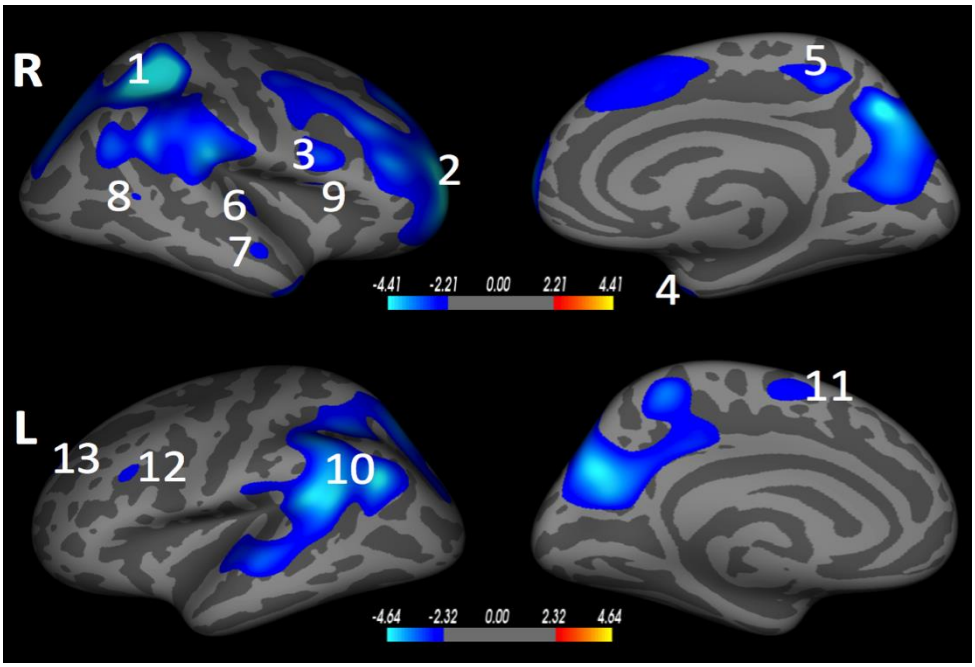


Figure 4: Inflated cortical surface maps showing progressive cortical thinning with longer pain duration (right hemisphere; top, and left hemisphere; bottom). Significance at $p<0.025$ FDR-corrected for multiple comparisons and controlled for age.

Table 2: Clusters of significant negative correlation with pain duration (Rt. & Lt. hemispheres)

Cluster		Max	Size (mm2)	Tal X	Tal Y	Tal Z	Brodmann Area	Anatomy
R	**1**	-5.6	12382.5	30.7	-41.9	49.1	2	**Intraparietal sulcus**
	2	-5.2	9714.8	24.2	56.3	7.3	10	**Transverse frontopolar gyri and sulci**
	3	-3.1	556.9	43.9	8	18.9	44	**Inferior part of the precentral**
	4	-3	568.5	38.3	9.1	-37	38	**Temporal pole**
	5	-2.9	364.7	15.1	-46.2	49.4	5	**Marginal branch of the cingulate sulcus**
	6	-2.5	121.1	47.5	-17.7	2.6	22&42	**Anterior transverse temporal gyrus (of Heschl)**
	7	-2.3	97.6	48.3	-8.8	-18.2	22	**Superior temporal sulcus (parallel sulcus)**
	8	-2.2	23.4	58.2	-56.7	5.3	39	**Middle temporal gyrus**
	9	-2.2	27.3	43.8	9.6	6.9	44	**Opercular part of the inferior frontal gyrus**
L	**10**	-4.7	11949.8	-38	-61.5	27.	39	**Superior temporal sulcus (parallel sulcus)**
	11	-2.8	254.6	-6.6	-4.5	57.9	6	**Superior frontal gyrus**
	12	-2.5	88	-39.3	23.8	26.9	46	**Inferior frontal sulcus**
	13	-2.5	49.8	-15.4	50.1	31.1	9	**Superior frontal gyrus**

Female and male Knee OA subgroup analysis

Female KOA (*n*=15) showed strong negative correlation of cortical thickness with pain duration similar to the main group effect ($p<0.025$ FDR corrected, controlled for age) appendix 5, supplementary Fig 1. Areas of >100 mm^2 included right precuneus, fronto-marginal gyrus and sulcus, middle frontal gyrus, middle temporal gyrus, superior frontal gyrus, temporal pole, and left superior temporal sulcus, posterior transverse collateral sulcus, superior and middle frontal gyri, fronto-marginal gyrus, short insular gyrus, and precuneus. For all clusters and detailed sub-clusters see appendix 5, supplementary data, table 2. However, comparing female OA with female HC (n=13) did not show any differences on global levels at $p<0.001$ uncorrected, controlled for age.

Conversely, male KOA (*n*=16) did not show significant correlations between cortical thickness and pain duration.

Long vs. short duration of chronic OA pain

Vertex-based analysis showed that LPD, but not SPD had thinner cortices in the left post-central sulcus and right inferior frontal and precentral gyri, when compared to HC ($p<0.001$ uncorrected, controlled for age and sex). There was no difference between short and long pain groups, controlled for age and sex.

To determine the quantitative cortical thickness effect of pain duration, further illustrative analysis by averaging cortical thickness over the four regions showing the strongest association between CT and pain duration (Fig. 3) revealed significantly reduced thickness in KOA vs. HC in the left mesial parieto-occipital cluster (Fig. 3C: Knee OA 2.2mm± 0.02 vs. HC 2.3 mm± 0.02 adjusted means± SE, F=6.83, p=0.012) and in the left temporo-parietal cluster (Fig. 3D: Knee OA 2.4mm± 0.02 vs. HC 2.5mm± 0.03 F=5.14, p=0.028, controlled for age and sex).

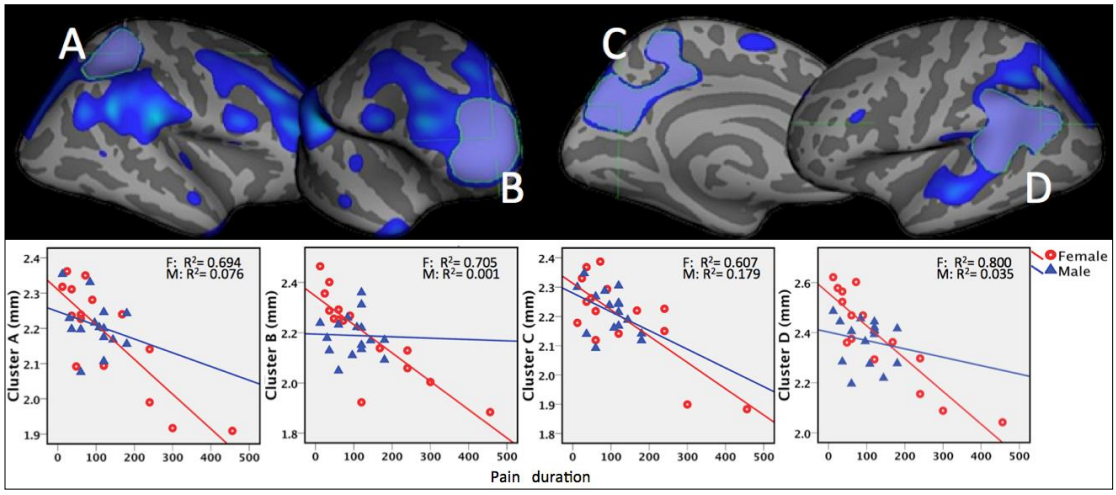


Figure 5: Four regions (A-D; Rt. Superior parietal and frontal pole, and left mesial parieto-occipital and temporo-parietal, respectively) labelled for region of interest analysis (purple) defined from clusters with strongest negative correlation with pain duration in knee OA group. Scatter plots and correlations marked by sex for pain duration and ROIs A-C, indicating cortical thickness decreases over the duration of pain

Subgroup comparisons (HC, SPD and LPD) (Fig. 4) showed similar results: significant differences in cluster (C) F=4.2, p=0.02 and cluster (D) F=5.8, p=0.006. Further post-hoc analysis (Tukey HSD), located the differences in clusters (C and D) between LPD vs. HC (p=0.015 and p=0.005 respectively). In addition, LPD was found to

have thinner cerebral cortex in cluster (B) compared to SPD (p=0.042). There were no differences seen between SPD and HC.

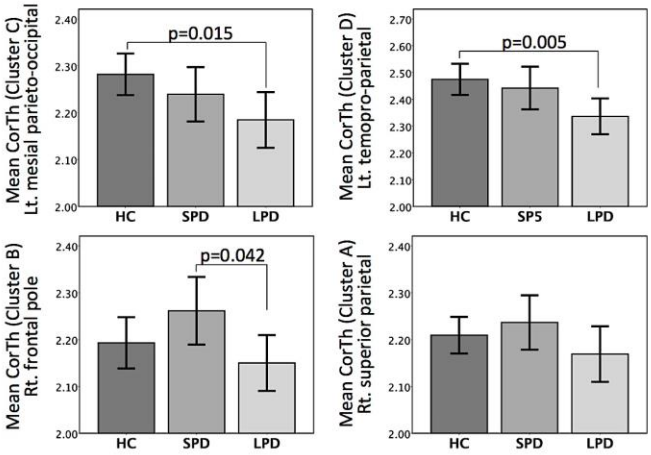


Figure 6: Bar charts of regions of interest mean age and mean adjusted cortical thickness and 95% CI for short and longer pain groups in reference to healthy controls

Surface-based correlation analysis with pain duration was repeated after removing one dataset with very long pain duration (38y) that may have driven the results and behaved as an outlier. Similar, however less in size and number, clusters were found that survived multiple test correction $p<0.05$, controlled for age (Fig 5). Clusters were predominantly spanning over bilateral parietal, right frontal, and bilateral precuneus areas

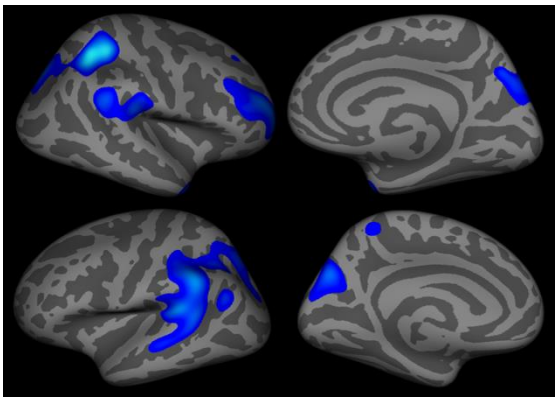


Fig 5: Clusters of significant thinning with longer pain in knee OA patients after removal of one outlier. $p<0.05$ corrected, controlled for age.

2.6.1.5 Discussion

Progressive and widespread cortical thinning was found in chronic knee OA pain preferentially in female patients, and largely located outside of known pain processing areas. The right inferior posterior gyrus in patients showed a tendency reduced cortical thickness.

Patients with longer pain duration showed extended and bilateral cortical thinning after controlling for age effects using whole brain analysis. There was also a significant negative correlation of moderate strength between pain duration and global and hemispheric cortical thickness. This confirms a link between cerebral morphometric changes and chronicity of pain symptoms. The notion that chronic pain may lead to neocortical atrophy was first proposed by Apkarian and co-workers (Apkarian et al., 2004). Interestingly, while several papers report on grey matter density or volume loss assumed to accumulate with pain chronification, the association with pain duration is controversial. In a volumetric study of chronic back pain (Apkarian et al., 2004), grey matter was found to shrink by about 1.3cm^3 per year. However, after controlling for age and sex, the variance in grey matter explained by pain duration was largely abolished. No previous study in OA established significant correlation between pain duration and grey matter density controlling for age (Rodriguez-Raecke et al., 2009, Gwilym et al., 2010a, Baliki et al., 2011, Rodriguez-Raecke et al., 2013), however a subgroup analysis (Baliki et al., 2011) suggested that negative correlations with pain

duration and grey matter density only emerged after several years of persistent pain in multiple brain areas.

The findings of progressive neocortical thinning is furthermore well in line with negative correlations shown between GM volume or density and pain duration in other musculoskeletal disorders including chronic back pain (Apkarian et al., 2004, Schmidt-Wilcke et al., 2006), rheumatoid arthritis (Wartolowska et al., 2012). Fibromyalgia patients were also found to show an overall negative association between pain duration and total grey matter (Kuchinad et al., 2007) and cortical thickness (Jensen et al., 2013). However, the nature of these progressive morphometric changes in chronic pain is largely unknown. The cumulative effect of morphometric changes over several years to decades of chronic pain shown in this study and by others argues strongly for but cannot prove them to result from the chronic pain experience. There are no longitudinal studies to demonstrate the progression individually, and the slow progression would be challenging for designing such studies.

However, there are some longitudinal studies before and after pain relief providing good evidence for at least partial reversibility of morphometric findings (Rodriguez-Raecke et al., 2009, Gwilym et al., 2010b, Seminowicz et al., 2011). There is partial overlap of regions showing increase in GM volume/density after pain relief between

these studies, and only little overlap with the regions identified here to show the most pronounced linear cortical thinning over the course of chronic pain. While at first counter-intuitive, a spatial dissociation may be explained by the multidimensional experience of chronic pain and related comorbidities. Chronic OA pain may result in reduced quality of life (Reginster, 2002, Salaffi et al., 2005), reduced exercise, impaired sleep and long-term medication that may contribute to the pattern of progressive cortical thinning.

The observed pattern of cortical thinning with increasing duration of chronic OA pain in this study showed substantive overlap with the default mode network (DMN). The DMN is increasingly recognized as a core brain network related to homeostasis and introspection as opposed to task-oriented brain functions (Raichle et al., 2001, Buckner et al., 2008). The most striking overlap we found was in the inferior parietal, retrosplenial and posterior cingulate but with lesser extent to the medial frontal and lateral temporal. The DMN shows reduced brain activity during experimental pain conditions as during most other tasks (Baliki et al., 2008a, Buckner et al., 2008). Moreover during rest DMN functional connectivity appears to be disrupted in a number of chronic pain conditions including musculoskeletal (Napadow et al., 2010, Kornelsen et al., 2013, Bolwerk et al., 2013, Baliki et al., 2008a). This may reflect an abnormal state of self-referential thought that is directed towards pain (Smallwood et al., 2013b). Our findings of cortical thinning with

increased chronic pain duration in the DMN may explain the functional disruption seen in resting state fMRI studies.

There is a large body of evidence on individual predisposing factors predicting chronification of pain after an acute tissue injury (McGreevy et al., 2011). Female gender appears to predispose to chronic knee OA pain: In an extensive review by Fillingim et al (Fillingim et al., 2009) chronic pain prevalence was found higher in women including in knee OA pain. Furthermore, females were consistently reported (Fillingim et al., 2009) to show higher sensitivity than males to different experimental pain modalities (except ischemic pain). Importantly, in this study pain duration affected cortical thickness mainly in female knee OA participants. Some of the study participants had experienced chronic pain for several decades with a maximum of 38 years. Nevertheless, even when excluding this subject, similar results were found (Fig 5).

Also pain related depression is reported in epidemiological studies as twice as prevalent in women compared to men (Munce and Stewart, 2007), but I did not find a clear differences in depression scores between the studied male and female knee OA patients, although power was limited by sample size and furthermore, none of the patients presented with more than mild-moderate symptoms. Sex differences in pain processing have also been widely reported in fMRI studies in experimental visceral pain (Berman et al., 2006), heat pain (Derbyshire et al., 2002, Henderson et al., 2008), muscle and

cutaneous pain (Henderson et al., 2008). There are a number of possible causative factors explaining the sex differences including psychosocial (Derbyshire, 1997) genetic (Wang et al., 2008) or hormonal (Kowalczyk et al., 2010) predispositions. In this study only postmenopausal women were included which limits hormonal heterogeneity.

This study revealed only minor changes in cortical thickness with global reduction in knee OA patients with chronic pain that failed significance after controlling for age and sex. Whole brain analysis found reduced cortical thickness in the right posterior inferior frontal (BA 44), and precentral and sub-central (BA 4 and 43) ($p<0.001$ uncorrected controlled for age and sex), which did not survive FDR correction. The findings accord partially with the neuroimaging literature of chronic pain in that chronic pain patients exhibit grey matter reductions in association with pain when compared to age and sex matched pain-free controls, yet with less agreement as to which areas are involved. Findings in the studied patient cohort were exclusively right hemispheric supporting the hypothesis that pain processing may be dominantly right lateralized (Symonds et al., 2006).

The finding of reduced cortical thickness in the right posterior inferior frontal (PIF) cortex is furthermore well in line with a recent meta-analysis involving 23 volumetric studies of chronic pain highlighting right PIF as one of the regions with most significant and consistent grey matter reduction in chronic pain patients. In several functional MRI studies the inferior frontal gyrus has consistently shown activity during nociceptive stimulation (Tolle et al., 1999, Brooks et al., 2002, Moulton et al., 2005, Ochsner et al., 2006, Kong et al., 2010), and during empathy for pain or vicarious pain (Lamm et al., 2011, Vachon-Presseau et al., 2012). Indeed the right inferior frontal has been found to relate to emotion and pain experience among the range of other functions it is involved in e.g. cognition and gustation, as revealed by functional characterization (Smallwood et al., 2013b). Taken together these data support the notion that chronic arthritis pain related activation of the right PIF induces structural cortical remodelling.

2.6.1.6 Conclusion

In summary, cortical thickness analyses in chronic painful knee OA have shown that chronic pain is associated with widespread slowly progressive neocortical thinning preferentially in female patients exhibited in extended areas largely outside the known pain processing area.

2.6.2 Study II: surface-based exploratory investigations: Preliminary results from cortical mean diffusivity, gyrification, and longitudinal analyses

2.6.2.1 Cortical mean diffusivity mapping to study cortical alterations in chronic pain

2.6.2.1.1. Introduction

The cellular microstructure, within the parenchyma of brain tissue, influences the Brownian molecular motion via barriers and compartments formed by tissue components such as nerve and glia cells, axons, intracellular organelles, myelin sheaths and intra- and extracellular matrix (Beaulieu, 2002). Mean diffusivity (MD) can be readily quantified from diffusion tensor imaging data. It allows quantifying the magnitude of water diffusion (Schmierer et al., 2007), and hence is sensitive to disruption of brain tissue integrity resulting in any change of diffusion barriers (Pierpaoli et al., 1996).

DTI-derived MD can be altered by several brain microstructural factors associated with neurodegeneration such as cell loss, membrane or cell organelle damage, altered extracellular matrix demyelination, axonal loss or gliosis (Wheeler-Kingshott et al., 2003). These changes will lead to higher MD values that are consistently seen in diseases such as Alzheimer's disease (Kantarci et al., 2005). However, cortical MD may appear elevated in brain atrophy if partial volume (PV) effects are not accounted for, hence the CSF PV effects need to be supressed in order to achieve reliable results. Assessment of PV-corrected MD across the cerebral cortex thus has the potential

to provide information regarding the ultrastructural status of the cortex, which in turn may help to elucidate the mechanisms underlying the cortical morphometric changes, reported in study I. For example, if cortical thinning is a predominantly neurodegenerative process, MD would be expected to increase in line with the loss of integrity of the ultrastructural barriers that accompanies neurodegeneration; conversely, a stable or reduced MD values will emphasise that neurodegenerative processes are unlikely and other mechanisms such as ultrastructural synaptic reorganization are possible which warrant further investigations.

2.6.2.1.2. Aims and hypothesis

To investigate the ultrastructural mechanisms that may underlie the cortical morphometric changes observed in association with chronic knee OA pain. The cortical microstructural tissue properties (as measured by MD) will be evaluated in KOA patients and matched HCs both globally and in areas that had shown pain-related changes in the morphometry studies presented previously. I hypothesize that the cortical thickness changes reflect brain plasticity rather than atrophic changes and will not be associated with mean diffusivity increases.

2.6.2.1.3. Methods

Subjects

A detailed description of participants is provided in chapter 5, patients (n=31, age= 64.61± 8.36; 15 females) and age- and sex-matched pain-free healthy controls (n=22, age= 61.30± 7.46; 13 females) were recruited and scanned.

Magnetic resonance imaging and data analysis

MRI was performed at 3T (Discovery 750, GE Medical Healthcare, Milwaukee, US) using a 32-channel head coil, and DTI was acquired during the same scanning session in which the anatomical MRI was performed. Diffusion tensor images were acquired using spin-echo echo-planar imaging (EPI) with b=1000 s/mm2 along 30 non-collinear directions, plus one b=0 volume, TR=5600ms, TE=89.0ms. Voxel sizes were 1.75 x 1.75 x 2.2 mm^3 for 12 patients and 5 controls or 2.2 x 2.2 x 2.2 mm^3 for the remaining participants. As mentioned in chapter 5, quality assessment of DTI data and some pre-processing steps were carried out by Diane Reckziegel (a PhD student, medical physics, University of Nottingham)

Diffusion tensor imaging data were analysed using FSL version 5.0 (Smith et al., 2004). Following eddy correction, brain mask was

created and non-brain voxels were removed using the FSL brain extraction tool (BET) (Smith, 2002). MD maps were derived using FSL diffusion toolbox (FDT), and subsequently underwent PV correction using the method described by Koo et al (Jeon et al., 2012, Koo et al., 2009) implemented in Matlab 2010 (The Math Works Inc., Natick, MA, USA). To further investigate any group differences and perform within-group correlation analyses, both predefined regional and whole-brain (global) cortical MDs were calculated. To derive the global MD values, all PV-corrected MD maps were firstly transferred and projected onto the cortical surface of both hemispheres for each subject. The average MDs were extracted in all the ROIs based on the same parcellation algorithm to the measurement of cortical thickness. These values were then summed up with weights of their corresponding surface areas, respectively, to obtain the overall MD in each hemisphere. In addition, the MD values in the exact ROIs defined by the significant findings from the cortical thickness analyses were also extracted. All of these processes were performed with Freesurfer software package (Unix-based calculation tools).

Statistical analysis

For derived global or selected regions of interest metrics, independent t-test and ANCOVA (with age and sex as covariates) were used to compare the chronic painful knee OA (KOA) group, its

subgroups (SPD, LPD) and HC. Results are reported as mean± SD at the significance threshold of $p<0.05$.

2.6.2.1.4. Results

A subgroup from the main study involved 15 chronic knee OA patients (65± 7.5y; 5 females), and 21 age- and sex matched HCs (61± 7.4y; 12 females).

There was no significant difference in average global cortical MD between KOA patients and HC, nor was there any significant correlation of MD values globally with the pain duration in KOA group (controlled for the partial effects of age and sex).

Cortical MD analysis in areas which had shown cortical thinning in KOA patients relative to HCs (illustrated in Fig. 1) revealed significantly lower MD in KOA vs. HC in the right posterior inferior frontal rPIF (0.95± 0.11 x10-3mm2/s vs. 1.07± 0.22 x10-3mm2/s; F= 6.9, p=0.013), but with no difference in the right sub-central (F=0.2, p=0.62, ANCOVA with age and sex as covariates).

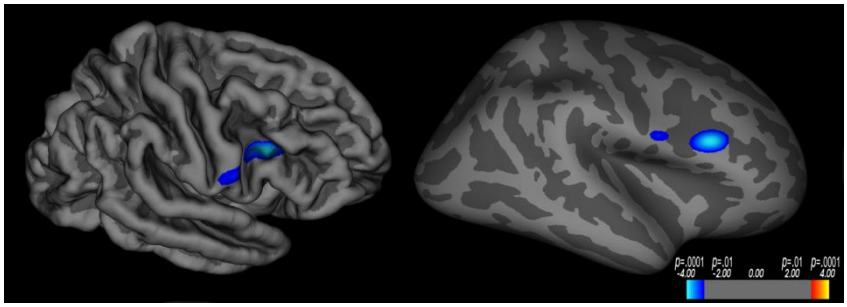


Figure 1: Areas of cortical thickness differences between knee OA participants and healthy controls for which mean diffusivity scores were derived.

By comparing the MD values with those in HCs, no significant differences were seen in Knee OA patients for any of the 4 ROIs, (which had shown strongest cortical thinning with longer pain duration, illustrated in Fig. 2), (controlled for age and sex), nor was there any significant correlations in these ROIs MD with either pain duration or corresponding CorTh ($p>0.2$ and $p>0.12$, respectively).

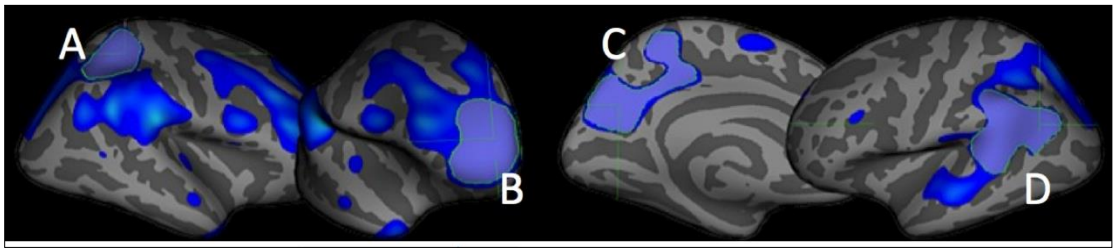


Figure 2: Four regions with strongest negative correlation with pain duration in knee OA group for which mean diffusivity scores were derived.

MD in the left anterior cingulate cortex of KOA participants (which had shown cortical thinning with higher local and remote pain sensitivity; illustrated in Fig. 3, below- quantitative sensory testing study) did not correlate with local or remote PPTs. However, a tendency was found for a negative correlation between left ACC MD and ACC CorTh ($r=-0.52$ $p=0.07$).

And lastly, cortical mean diffusivity analysis for right sub-central, (which was thinner in KOA patients with higher remote mechanical pain sensitivity compared to patients with lower pain sensitivity, illustrated in Fig. 4, below: quantitative sensory testing study) revealed no differences between patients/ or more sensitive patients

and HCs p>0.7, and there were no significant correlations between right sub-central MD values and regional CorTh, local or non-local PPTs.

2.6.2.1.5. Discussion

As hypothesised, there were no increases in cortical mean diffusivity in association with chronic pain, and the MD of cortical parenchyma did not change with pain duration. There are no comparable studies published in musculoskeletal or other chronic pain disorders investigating the interrelation between pain duration and cortical MD.

Decreased MD reflects an ultrastructural alteration with less freely diffusion water protons due to more diffusion barriers or more tortuous diffusion paths. In fact, such changes were described in brain plasticity induced by a learning/memory task in rodent (Blumenfeld-Katzir et al., 2011). The authors further studied the histological correlates of these activity-dependent reductions in diffusivity showing that MR diffusion can index structural remodelling mainly of dendritic splines and glial processes.

To my best knowledge this is the first study to investigate the cortical ultra-structural correlates of chronic pain in musculoskeletal disorders. The results are however generally well in line with previous studies: Gustin and colleagues (Gustin et al., 2010) in a voxel-based study found predominantly lower MD in patients with persistent

neuropathic pain compared with matched controls in numerous GM areas. In a study of chronic irritable bowel syndrome (Ellingson et al., 2013), MD was found reduced within the globus pallidus but also increased values were found within the thalamus in patients. In a study of migraine patients (Liu et al., 2013), authors failed to detect any significant MD differences after one year of patients follow-up. This is intriguing as I found no MD changes in areas of progressive cortical thinning with pain duration whereas MD was reduced in right PIF that showed no association with pain duration but cortical thinning compared with controls. This may indicate differential local microstructural alterations with different pain phenotypes and pain progression despite uniform cortical thinning.

2.6.2.1.6Conclusion

In this sub-study, I have shown that the progressive cortical thinning exhibited in patients with knee osteoarthritis is not accompanied by microstructural tissue damage, favouring an ultrastructural re-organizational rather than neurodegenerative changes.

2.6.2.2 Cortical folding morphology: Gyrification index and its correlates of chronic pain in patients with knee OA

2.6.2.2.1. Introduction

Gyrification index (GI) is a measure of the cortical folding morphology that is calculated as a ratio of the whole length of the inner folded (buried within the sulcal folds) cortex to the length of outer exposed contour (Zilles et al., 1988). GI increases proportionally to the number of gyri (Harris et al., 2004), and it can provide a novel biomarker into the mechanisms involved in developmental diseases, aberrant development or abnormal aging (Mangin et al., 2010). GI can be obtained to characterize the cortical folding both for global brain and specific region of interest, and is sensitive to the magnitude (depths of sulci or heights of gyri) as well as to the frequency of folding (number of sulci/gyri) (Luders et al., 2012).

Cortical gyrification patterns have been studied in patients with neurodegenerative and neuropsychiatric diseases (Debbane et al., 2009, Liu et al., 2012a, Nesvag et al., 2014, Zhang et al., 2009, Zhang et al., 2014), as well as in some healthy subjects e.g. those who practice certain physical and spiritual exercises known to promote endogenous analgesia such as meditation (Luders et al., 2012). However, investigations of GI in chronic pain states have not been established previously. GI has been found to vary in, and hence a strong predictor of e.g. psychiatric syndromes and neurologic

disorders and therefore it may provide insight into the underlying neuro-anatomical correlates of chronic pain.

The widespread cortical thinning observed with on-going pain shown previously in study I, suggests that the neocortex is structurally altered by processes related to or moderated by pain. GI variations in pain could be affected by such acquired plastic changes, but it could also be due to neurodevelopmental or genetic factors that could influence development of chronic pain and manifest as altered GI. There is some evidence for a relation between the surface morphology of the brain indexed by GI metrics and underlying connections and hence functional connectivity (Wallace et al., 2013, White and Hilgetag, 2011). While grey matter volume, density or cortical thickness, have been widely investigated in a range of chronic pain states, gyrification as an additional morphological and brain geometric feature has not been studied, to the best of my knowledge, in chronic pain.

2.6.2.2.2. Hypothesis and aims

The aim of this sub-study is to evaluate the gyrification patterns in chronic pain due to knee OA, and to investigate the gyrification correlates of chronic pain in areas that had shown strongest relation of thinning with longer pain. I hypothesize that:

- Patients with chronic knee OA pain will show pain related global GI differences relative to that in matched controls, and variations will be pronounced in areas that had shown strongest cortical thinning with longer pain, as shown in study I
- Changes in GI will correlate with pain duration, indicating a possible acquired maladaptive nature.

2.6.2.2.3. Methods

Subjects, data acquisition, and image processing

Description of participants included and image acquisition and processing have been provided above in Study I. All participants of the main research were included here: patients (n=31, age= 64.61± 8.36; 15 females) and age- and sex-matched pain-free healthy controls (n=22, age= 61.30± 7.46; 13 females).

Cortical gyrification index calculation

Imaging-based three-dimensional metrics of gyrification index were calculated according to the freesurfer-based method developed by Schaer and co-workers (Schaer et al., 2008). Basically, Schaer's method, which is a 3-dimensional implementation of the standard 2-dimensional method proposed by Zilles and co-worker (Zilles et al., 1988), relies on calculating the GI as a ratio of the whole length of

the inner folded cortex to the length of outer visible contour. The computation involves Freesurfer-based 3D cortical outer surface recreation that will serve as a basis for GI calculation. The latter is calculated at each vertex as a ratio of cortical areas of circular region centred on the vertex divided by the area of a disc with the same radius (Schaer et al., 2012). For comparisons, individual reconstructed cortical surfaces were averaged and registered to Talairach template, and GI maps were projected on the surface for further surface-based statistical analysis using QDEC.

Statistical analyses

Vertex-wise between group comparisons (controlled for age and sex) and within group correlations (controlled for age) of GI were performed using QDEC- the Freesurfer statistical and visualization tool. GI values for ROIs were extracted individually based on findings in study I (Fig 3). ANCOVA (with age and sex as covariates) were used to compare values between Knee OA and HC. Correlation analysis for ROI GI with pain duration was performed using Pearson's correlation while controlling for the partial effects of age and sex.

Given that no GI data have been previously established in chronic pain imaging, results here are considered preliminary and significance was accepted at uncorrected threshold of $p<0.01$ for total brain and

$p<0.05$ for ROI analyses. This allows for an exploratory characterization that can provide a framework for future studies.

2.6.2.2.4. Results

Group differences in GI

Whole brain analysis revealed that KOA patients had larger gyrification index bilaterally in the superior parietal, and smaller gyrification index in the right temporal pole; $p<0.01$ uncorrected, controlled for age and sex (Fig. 1 and 2)

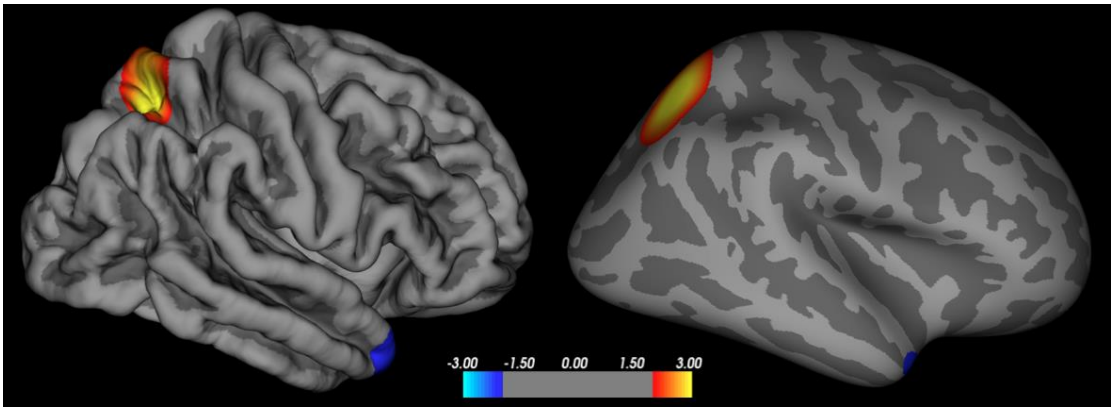


Figure 1: Group difference in right hemispheric gyrification. Areas with larger gyrification index in KOA patients (superior parietal) are depicted in yellow/ orange whereas areas with smaller gyrification index in KOA patients (temporal pole) are depicted in cyan/ blue. $p<0.01$ uncorrected, controlled for age and sex.

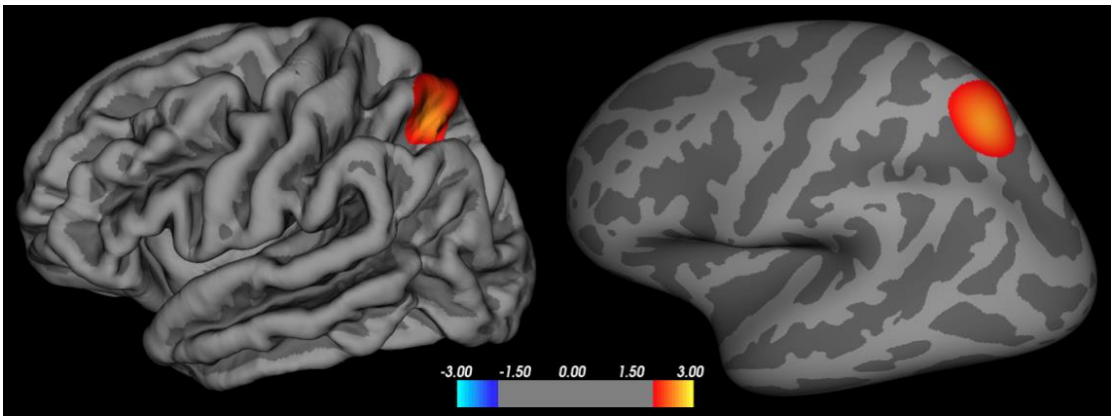


Figure 2: Group difference in left hemispheric gyrification. Areas with larger gyrification index in KOA patients (superior parietal) are depicted in yellow/ orange, p<0.01 uncorrected, controlled for age and sex.

ROI analysis

The local GI for areas that had shown most significant cortical thinning with longer pain was individually derived and between group comparisons revealed that: knee OA patients had significantly higher local GI values in the right superior parietal, 3.19±0.25 vs. 3.07±0.30 (adjusted mean± std. error), F=8.2; p=0.006, controlled for age and sex, Fig 3.

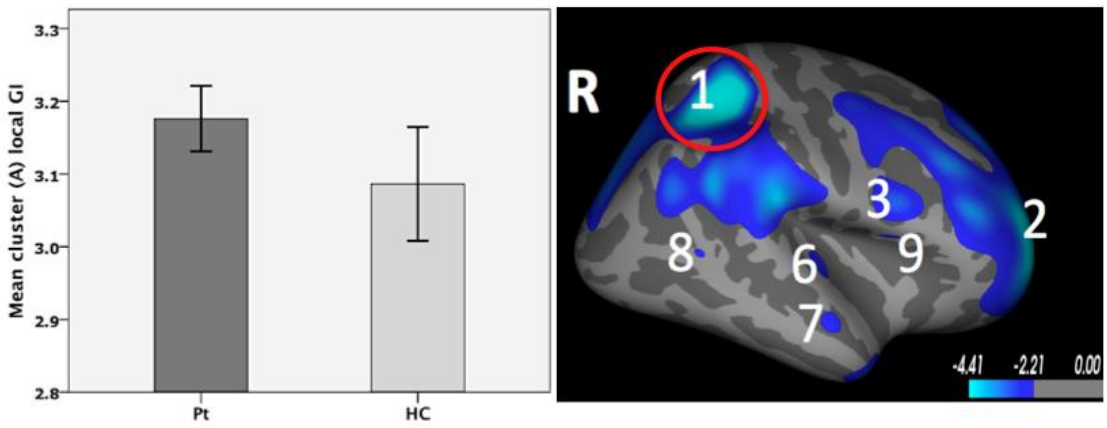


Fig 3: Bar graphs showing mean local GI and 95% CI for right superior parietal cortex (cluster 1, circled in the illustrative image), patients showed higher local GI; p=0.006 adjusted for age and sex

Correlation analysis

Whole KOA group

Correlation analysis between global GI and pain duration for all KOA patients revealed a significant positive correlation in the bilateral anterior insula ($p<0.01$ uncorrected controlled for age and sex; Fig. 4). No negative correlations were found in either hemisphere.

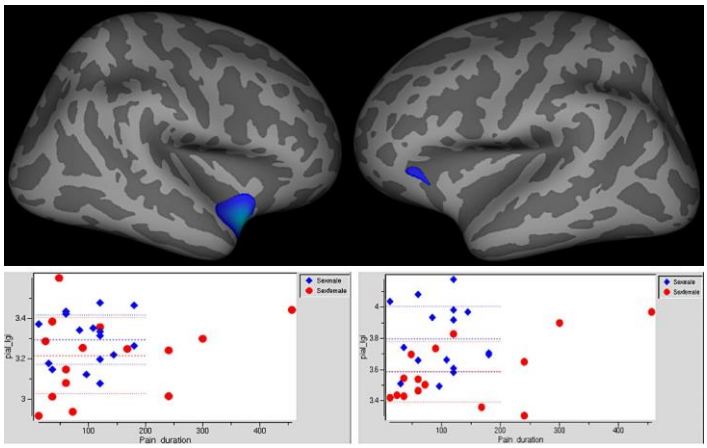


Figure 4: Cortical areas, which showed positive correlation in whole KOA group between GI and pain duration namely; bilateral anterior insula) and scatter plots of corresponding ROI, $p<0.01$ uncorrected, controlled for age and sex.

Correlation analysis for ROIs

Moderate but significant positive correlation was found between pain duration and local GI in cluster C (left mesial parieto-occipital), and cluster D (left temporo-parietal), $p=0.03$ and $p=0.001$ respectively, controlled for partial effects of age and sex, Fig 5.

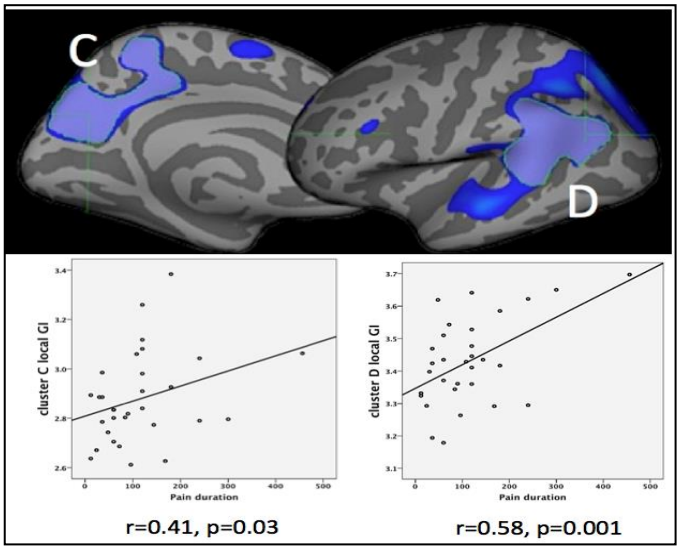


Fig 5: Scatter plots and regression lines showing correlation between pain duration and local GI in the left mesial parieto-occipital (cluster C), and left temporo-parietal (cluster D). p=0.03 and p=0.001 respectively controlled for age and sex.

2.6.2.2.5. Discussion

Group differences in gyrification index revealed that KOA patients exhibited predominantly larger GI bilaterally mainly in the superior parietal cortex. These areas do strikingly overlap with areas that showed most significant cortical thinning with longer pain. This was further confirmed by ROI analysis where comparison of mean local GI of right superior parietal between knee OA and HC groups revealed that knee OA patients had significantly higher local GI. Whole brain correlation analysis between changes in gyrification index and pain duration revealed only positive correlations (no negative correlations) in bilateral anterior insula, and ROI analysis showed significant positive correlation between pain duration and local GI in the left mesial parieto-occipital (cluster C), and left temporo-parietal (cluster D). By controlling for the effect of age I aimed to disentangle GI changes related to chronic pain from changes as a factor of aging.

This study is the first to address cortical gyrification correlates of chronic pain. Generally, the 3D imaging-based gyrification index computation has been relatively recently developed. The search terms "gyrification index" gave 143 records; as a topic search in web of knowledge (www.webofknowledge.com) and 80 records in PubMed (http://www.ncbi.nlm.nih.gov/pubmed), (as per 14 Feb. 2014).

Gyrification index as an additional brain morphology biomarker can provide valuable information associated with the disease development and progression. The genetic impact on cortical morphogenesis cannot be ignored; however, GI can be influenced by both genetic and non-genetic factors. The genetic impact nonetheless can be less influential than environmental effects as shown in a mono (MZ) and di-zygotic (DZ) twin study (Hasan et al., 2011), where it was found that the GI in the prefrontal cortex did not show more similarity in MZ when compared to DZ twins. Furthermore, it is estimated that only 30% of gyrification phenotypic variation can be explained by genetic variation (Rogers et al., 2010). The genetic predisposing factors (to brain morphogenesis and hence hypothetically to chronic pain) versus environmentally acquired changes cannot be disentangled in this study, which is further hampered by the cross-sectional design that limited any causal inferences.

The cortical gyrification pattern and degree are thought to reflect attempts of optimal intra-cortical organization (Van Essen, 1997, Zhang et al., 2014), with maximum axonal connections within the

smallest possible volume to optimize interregional connections (Klyachko and Stevens, 2003). The change in gyrification index could be influenced by tensions in the underlying white matter connections (Herculano-Houzel et al., 2010), and therefore these changes may indicate underlying white matter re-organizational or disruption changes.

The increases in GI seen in the studied cohort along with evidence of their positive correlations with pain duration may indicate re-organizational processes associated with or moderated by the on-going chronic knee OA pain process. The spatial overlap between findings of higher GI changes and cortical thinning with longer pain suggest a putative compensational structural changes by which increases in the surface area (yet to be confirmed) accompany the observed cortical thinning, in which case the overall volume will be theoretically preserved which again supports re-organizational non-degenerative changes.

2.6.2.2.6. Summary

The results of gyrification index correlates of chronic knee OA pain presented here are considered preliminary. I highlight the importance of investigating GI in chronic pain as an additional and complemental tool to understand the neuro-anatomical predispositions or

consequences of chronic pain. There are no comparable studies published in musculoskeletal or other chronic pain disorders investigating the interrelation between pain duration and cortical GI. In this study, an exploratory characterization was presented that can provide a framework for future studies.

2.6.2.3 Longitudinal investigations and future directions

2.6.2.3.1. Introduction

Most studies on the morphological brain changes in chronic pain have been cross sectional leaving the question of temporal relationship between chronic pain and brain morphology intriguingly unanswered. Longitudinal studies at various stages of chronic pain with same patients as their own controls would allow better understanding the causal relationships between structural brain changes and chronic pain. Findings that support the hypothesis that morphological brain changes are secondary to persistent pain include:

- The reversibility of structural brain changes following therapeutic pain relief (Gwilym et al., 2010b, Rodriguez-Raecke et al., 2009, Seminowicz et al., 2011),
- The changes in grey matter due to repetitive painful stimulation (Teutsch et al., 2008), and
- The correlation between duration of pain and the degree of grey matter shrinkage i.e. GM decrease with longer pain (Apkarian et

al., 2004, Schmidt-Wilcke et al., 2006, Kuchinad et al., 2007, Buckalew et al., 2010a, Baliki et al., 2011, Wartolowska et al., 2012).

The dynamic influential relationship between chronic pain and brain morphology is poorly understood. There are no longitudinal studies to demonstrate the progression individually, and the slow progression would be challenging for such studies.

In this pilot study I assess the progressive effect of chronic pain on brain cortical thickness while controlling for the confounding effect of age.

2.6.2.3.2. Methods

Subjects, data acquisition, and image processing

Seven patients (from the main study) with chronic knee OA pain were scanned twice, 12 months apart in a sub-study that was initially designed to investigate the temporal relationship between chronic pain and brain changes. However, due to time constraints this study could not be accomplished hence only pilot results will be presented.

Data acquisition and image processing have been described previously in chapter 5. Demographics at first scan involved the recruitment of six males and one female (age= 65.1± 7.4). Patients suffered from chronic knee OA pain for 7.1± 3.3 years at first scan.

Cortical thickness analysis

Steps for cortical thickness analysis have been described in details previously.

Statistical analyses

Vertex-wise between first and second scan comparisons (controlled for age) was performed using QDEC (the statistical and display tool of freesurfer). Ideally, results would be accepted at $p< 0.05$ corrected for multiple comparisons, however for the purpose of pilot evaluation, results will be presented at $p<0.05$ uncorrected, adjusted for age. This would allow for an exploratory characterization that can provide a framework for future studies.

2.6.2.3.3. Results

Average group differences between first and 12-month later scans revealed a progressive cortical thinning in several areas bilaterally including the precentral fig. 1, superior parietal, and postcentral. However, cortical thickness increases were also detected bilaterally in areas included parsopercularis and lateral-orbito-frontal $p<0.05$ uncorrected, adjusted for age.

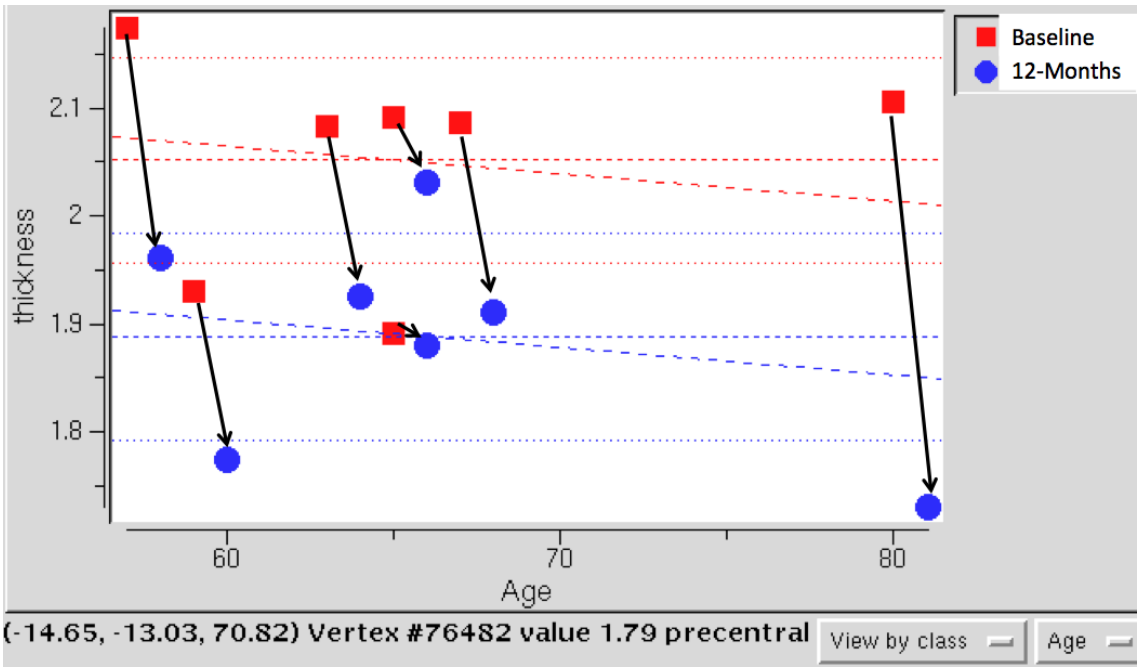


Fig. 1: scatter graph shows the dynamic change of precentral cortical thickness in patients with chronic painful knee OA over a period of 12 months controlled for age.

2.6.2.3.4. Discussion

The aim of this pilot study was to explore the dynamic cortical thickness changes in chronic painful knee OA over a period of one year. Although no conclusion can be derived from this study as it is a pilot one with only seven subjects included and furthermore liberal p value was adopted, findings highlight the importance of following up the temporal relationship between chronic pain and brain morphology. Both decreases and increases in cortical thickness were observed, however and regardless the pattern of cerebral change, this experiment provides some framework for further studies to dynamically evaluate the brain structural plasticity secondary to pain.

The 12-month period was chosen arbitrary due to time restrictions, however, one would expect cumulative effect of morphometric changes over several years to decades of chronic pain as it has been so far indexed by the correlations between grey matter volume or density and pain duration in musculoskeletal disorders as in e.g. chronic back pain (Apkarian et al., 2004, Schmidt-Wilcke et al., 2006), rheumatoid arthritis (Wartolowska et al., 2012) and fibromyalgia (Kuchinad et al., 2007). This argues for but cannot prove them to result from the chronic pain experience.

The complexity of pain experience arising not only from the multidimensionality nature of pain itself but also from the association of multiple co-morbidities, overall change in quality of life (Tuzun, 2007) and pain-related medications, for which future studies need to account for in order to figure out any dynamic relationship. Moreover, such changes are expected to be subtle which requires highly sensitive techniques in order to be detected.

3 PART THREE: GENERAL DISCUSSIONS

3.1 Summary of the results

The overall purpose of this area of research was to use advanced structural neuroimaging measures and image analysis techniques to quantify and investigate morphological brain correlates of chronic pain in a primarily nociceptive disorder due to knee osteoarthritis. An imaging-based attempt was made to explore, in part, the mechanism of such changes in chronic pain in humans.

The demographics of the selected patients represent a sector of chronic pain sufferers with a broad range of pain characteristics and diverse associative behaviours. Chronic knee OA pain patients were recruited resulting in a cohort with a mean age of 65 years (standard deviation 8.4 years) and balanced sex distribution (16M: 15F). Pain duration ranged between 12-456 months with a median of 96 months, and average pain severity over four weeks prior to scanning extended between 1 and 10 out of 10 with a mean of 4.8. The sensory component of chronic pain recorded values between 0-29 /42 with a mean of 11.6, whereas the affective dimension was in the range of 0-11 /14 with a mean of 1.2. The investigation of neuropathic component of chronic pain in the studied cohort revealed less than 10% of neuropathic-like symptoms. An effort was made to age and sex match patients with pain free controls for further case-control comparisons. Overall, patients and healthy controls were similar in age, sex, BMI, handedness, educational levels, and furthermore patients showed no significant distinction from controls

in general cognitive ability, pain catastrophizing tendency, or trait anxiety. However, significant differences were found between groups such that patients exhibited higher but subclinical and below moderate rates of depression, as well as higher levels of state anxiety.

The main brain morphological and putatively inter-linked behavioural findings in chronic pain patients can be summarised as follow:

1) Patients with chronic knee OA pain had larger hippocampal volume and preserved general cognitive function. Contrary to the belief that chronic pain leads to a reduction in hippocampal volume and allegedly to cognitive deficits, the results of chapter 6, study 1, contradicts previous studies showing such a relation, in the studied patients with chronic pain due to knee OA. Moreover, findings from this study also suggest that the model of stress or low mood mediating between chronic pain and hippocampal shrinkage may not always explain this relation.
2) Manual volumetric studies of ACC subdivision (chapter 7, study 1), revealed that larger volumes of left rostral ACC (concerned with emotional aspects of pain processing) correlated significantly and positively with higher scores of pain catastrophizing suggesting a tight coupling between rostral ACC structure and the catastrophizing tendency in chronic knee pain. Such negative behaviour of catastrophizing may largely

contribute to chronification of pain, more emotional distress, intensified pain experience, and moreover catastrophizers are more likely to develop intractable pain even upon removal of causative agent.

3) The cortical thickness correlation analysis (chapter 6, study 2), demonstrated that higher sores of remote mechanical pain sensitivity were correlated with reduced cortical thickness solely in the left anterior cingulate. Although this result did pass multiple test correction for the whole brain analysis, the finding was confirmed by ROI analysis, highlighting the potential key role of the left ACC in the process of central sensitization

4) Voxel-based morphometric analysis (chapter 7) showed that patients had less grey matter volume in left DLPFC ($p<0.001$, uncorrected). This tendency of reduction in volume may reflect a functional disturbance in the DLPFC role of chronic pain inhibition. Although consistent with findings from other studies of chronic pain particularly of MSK origin, it is noteworthy that results need to be confirmed with more robust corrected analysis. The importance of DLPFC in pain modulation warrants further multimodal investigations to understand the mechanism of its involvement in pain chronification and the possibility of therapeutic targeting.

5) In chapter 8, study 1, a vertex-wise cortical thickness analysis was performed and results provided provisional evidence for extensive cortical thinning with progressive pain irrespective of aging, and preferentially in females with chronic knee OA.
6) Preliminary results from partially investigating the mechanism of cortical thinning in patients were presented in study 2, chapter 8. Results suggest a non-degenerative cortical remodelling reflecting potential morphological maladaptive plasticity in association with chronic pain due to knee OA.

3.2 Limitations and methodological problems

There are a few limitations in this research that need further consideration. First, the sample size of the study was powered to detect 0.25mm cortical thickness changes (Pardoe et al., 2013), and compares favourably with most previous morphometric studies. I however acknowledge limited power for the subgroup analysis that may be responsible for results that did not survive the multiple test correction. Second, the study was limited by not performing small volume corrections so that analysis could be restricted to a mask of interest to increase the statistical power. Third, female patients suffered from pain for longer time (range= 12-450 Months) than males did (12-180), therefore the sex differences have to be

interpreted with caution, and further well-matched studies are required to confirm the study findings.

The cross-sectional design of the study limits from describing dynamic and temporal associations and not allowing inferring on causal relationships. The study participants underwent careful phenotyping to investigate interrelations with pain duration, severity and pain sensitization and to account for depression and cognitive performance, but detailed questionnaires on insomnia or quality of life were not included.

A number of methodological issues could potentially impact on the validity of the results, and therefore need to be considered

1) While the rationale for using different significance thresholds (corrected / uncorrected) throughout the research was explained individually in corresponding studies, for overall conclusion it is necessary to consider uncorrected results as preliminary requiring confirmation by further studies.
2) An attempt was made to carefully record and account for potential confounders, however still there are some factors that may have the potential to affect or mediate effects of brain changes were not considered in the study design such as activity monitoring and pain related sleep disturbance.
3) Some limitations are related to the inherent features of image analysis software; for example, VBM requires proper image

registration (Bookstein, 2001), which can be problematic with atypical brains, and in order to achieve reliable parcellation by freesurfer with least manual intervention, excellent image contrast resolution is mandatory, which I acknowledge that was not the case with the empirically obtained scanning sequence, and as a consequence moderate to major manual corrections were needed in most analysis outcomes. However, results were carefully inspected and manually corrected whenever needed, and a random sample comprised 25% of data analysed by freesurfer were further inspected and scored for quality by a researcher with freesurfer expertise (Dr R Dineen), see appendix 6, table1.

4) Analysis with images flipped to account for differences in contra-laterality between somatic source of nociception (knee) and corresponding brain hemisphere could have been performed. The reason that I did not do so is that there is no strong evidence in pain neuroimaging to suggest laterality effect in pain-related grey matter changes. This was tested by Rodriguez-Raecke et al (Rodriguez-Raecke et al., 2009) in patients with chronic pain due hip OA and no subsequent change in results was found.
5) While every effort was taken to carefully match cases and controls leaving chronic OA pain as the only difference whenever feasibly possible, I confess that it was very difficult

to recruit elderly patients who are medically well apart from being unfortunate to suffer from chronic knee osteoarthritic pain. Therefore, the use of antihypertensive and diuretic drugs was allowed in the selection criteria, and I acknowledge their impact could potentially have had influential effects on the results obtained.

6) I acknowledge the problem of testing multiple hypotheses on the same group of patients. However, the way these hypotheses were formulated may be considered independent. The chance of having type I error may increase when trying to detect a significant finding between two variables in different locations. For instance, if testing the relationship between chronic pain duration and cognitive function reveals no change in, for example, the hippocampus, the attempt of trying to find such an effect in another location e.g. the anterior cingulate, then the frontal lobe and so on, will result in some positive findings found just by chance alone (type I error or false positive result). This is pretty much applicable to the surface based analysis where significant findings are tested using a vertex-based (several thousands of vertices per hemisphere) approach, for which correction for multiple testing is essential.

The hypotheses presented in this thesis were framed independently, with each test being performed on a relevant brain structure e.g. chronic pain and cognition with regard to

the hippocampus, affective component of pain and the anterior cingulate cortex and so on. I'll just quote here what Perneger (Perneger, 1998) has written about what might be wrong about adjustments for multiple comparisons, in his paper that was published in the British Medical Journal (http://www.bmj.com/) and cited more than 2800 times as per December 2014 (www.scholar.google.co.uk); "Such information is usually of no interest to the researcher, who wants to assess each variable in its own right. A clinical equivalent would be the case of a doctor who orders 20 different laboratory tests for a patient, only to be told that some are abnormal" (Perneger, 1998). Furthermore, although adjustments for multiple testing will decrease the likelihood of type I error, the chance for type II error will increase resulting in true important findings being deemed as non-significant. Perneger adds that "Simply describing what tests of significance have been performed, and why, is generally the best way of dealing with multiple comparisons" (Perneger, 1998).

3.3 Implications

The research presented in this thesis has used advanced neuroimaging and image analysis techniques to principally investigate grey matter changes in chronic pain due to knee OA. Previous studies have mostly used individual modalities of image analysis often implementing VBM approach. Manual volumetry (with meticulous technique) is considered the gold standard for volumetric assessment of region of interest (Cherbuin et al., 2009, Morey et al., 2009, Shen et al., 2010). Furthermore, for whole brain cortical grey matter measurement surface based analysis is preferred over voxel based for a number of reasons including higher sensitivity to subtle changes, less vulnerability to problems due to registration and smoothing such as spatial mis-localization, well validated, and importantly, quantitative measures can be derived for further more specific analysis and direct interpretation. However, a combination of these modalities may offer more robustness over single approach and provide broad and more conclusive morphological profile of brain in chronic pain.

The importance of this research from a translational perspective lies mainly in the fact that demonstration of in vivo changes of grey matter in association with chronic pain could potentially:

- Provide neuroanatomical substrates for the disease (Reiss et al., 2004a), which consequently may offer a framework for

further investigational and therapeutic trials. For example, repetitive transcranial magnetic stimulation of left prefrontal cortex (which has shown reduction in patients in the studied cohort), showed a significant increase in thermal pain thresholds in healthy subjects (Borckardt et al., 2007), supporting its role in pain modulation namely; inhibition of nociceptive perception

- Signify a specific morphological brain signature that can be used as a candidate biomarker in chronic pain to monitor pain therapy and responsiveness, determine eligibility for invasive treatment (Gwilym et al., 2010a), and predict pre-existing vulnerability and disease-driven changes (Davis, 2011, Mansour et al., 2013).
- May provide an objective and questionnaire-alternative tool for patient's phenotypic classification such as characterisation of pain catastrophizing and pain sensitization, and hence may help determine type of treatment for effective therapy. For instance, reduction of pain catastrophizing can reduce pain intensity, disability and main complaints including pain related depression as shown in chronic back pain patients (Smeets et al., 2006)
- Highlight the need for a complementary tool to other imaging modalities e.g. fMRI, DTI, and MR spectroscopy for better understanding the role of the brain in pain processing and development of intractable pain chronicity.

- Although it may be better achieved with longitudinal studies and combined modalities, cross sectional findings may however help distinguishing pre-existing (e.g. genetic) from acquired (e.g. environmental) factors in the development of chronic pain. For example, it has been proposed that people with smaller brain volume, which may be linked to developmental or genetic differences, are more susceptible to developing chronic pain disorders (Wartolowska et al., 2012)

However, larger and longitudinal studies are required to confirm structural changes in chronic pain and evaluate sensitivity and specificity of quantitative neuroimaging findings as an objective biomarker of the disease.

3.4 Future directions

Structural neuroimaging in chronic pain is a relatively recent field of research that has developed remarkably over the past decade. The first study to report morphological brain changes in chronic pain was conducted by Apkarian and co-workers about 10 years ago (Apkarian et al., 2004). Ever since the published articles on grey matter changes in chronic pain have risen dramatically from just 6 in 2005 to 114 in 2013, (based on PubMed search, www.ncbi.nlm.nih.gov/pubmed performed on 23 December 2013). Our understanding of the central changes associated with chronic

pain has increased remarkably. However, there have been several shortcomings and limitations from previous studies, which I attempted to overcome in this thesis. The strength of the presented research is that it combines advanced imaging and more rigorous image analysis techniques to provide wide-ranging structural brain profile in chronic pain in carefully selected and well-characterized patients. The sample size was reasonably larger compared with previous studies and additionally based on power calculation to detect subtle changes at sub-millimetric levels. Manual morphometry of hippocampal volume in chronic pain and sub-divisional studies of anterior cingulate correlates were not investigated previously. The putative moderating effect of sex, which received less focus from researchers in previous investigations, was considered in this research. With-in group analysis that was carried out in study 1, chapter 8, offers more statistical power and direct interpretable results by eliminating confounding factors that cannot be easily controlled between groups. Last, but not least, the attempt of mechanistic investigations, using mean diffusivity and gyrification index analyses in chronic musculoskeletal pain, although in part considered preliminary, is a novel method that can provide a framework for future investigational studies. The preliminary analyses presented in the thesis can be tested in larger follow-on studies, with sample size informed by the preliminary results.

Nonetheless, several problems remain for future studies to resolve. Firstly, it is not known whether the findings reported are specific to the studied group or to the type of clinical disorder patients presented with. Secondly, the ambiguity between studies in the direction of grey matter changes and the inconsistency in the spatial locations need to be carefully considered and explained, probably better-tackled by exploiting multi-centre facilities using harmonized and tightly defined protocols.

Thirdly, it would be more informative to investigate the dynamic correlates and temporal patterns of brain changes over time prior to treatment and after therapeutic intervention. However the slowly progressing changes would be challenging for designing studies investigating such changes.

With the continuous advent of neuroimaging and image analysis techniques, it would probably be feasible and more revealing to investigate sub-divisional correlates of chronic pain e.g. different parts of hippocampus, sub-nuclear divisions of thalamus, so that results can be specific and more useful for further investigational studies or interventional trials.

Finally, conjoint multimodal imaging research involving structural, functional, and chemical investigation of brain in chronic pain can provide invaluable insights into the central mechanisms of pain. Nevertheless, these investigations may contribute only to the

understanding of the '*bio*' part of the biopsychosocial model of pain, which ultimately calls for further and more comprehensive research involving psychosocial investigations in order to effectively tackle the problem of chronic pain.

3.5 Conclusion

Complementary methods were used to investigate structural brain correlates of chronic pain in knee OA and putatively related psycho-cognitive and sensory aspects. It can be concluded that persistent knee arthritic pain, a model of chronic nociceptive pain does not reduce hippocampal volume nor impair cognition, and stress related hippocampal volume loss does not explain the observed low mood. In contrast, the chronic pain experience seems to increase hippocampal volume perhaps due to an unknown mechanism warranted further studies to characterize the complex role of the hippocampus in pain progression. Morphometric correlates of left anterior cingulate cortex with pain catastrophizing and remote pain hypersensitivity emphasise the pivotal role of this limbic structure in emotional aspects of pain processing and central pain sensitization respectively.

Chronification of pain was associated with progressive neocortical thinning preferentially in female patients in extended areas largely outside the know pain processing areas and without associated microstructural tissue damage.

4 PART FOUR: REFERENCES AND APPENDICES

4.1 References:

ADALSTEINSSON, E., SULLIVAN, E. V., KLEINHANS, N., SPIELMAN, D. M. & PFEFFERBAUM, A. 2000. Longitudinal decline of the neuronal marker N-acetyl aspartate in Alzheimer's disease. *Lancet,* 355**,** 1696-1697.

AHLHELM, F., SCHNEIDER, G., BACKENS, M., REITH, W. & HAGEN, T. 2002. Time course of the apparent diffusion coefficient after cerebral infarction. *Eur Radiol,* 12**,** 2322-9.

ALBE-FESSARD, D., BERKLEY, K. J., KRUGER, L., RALSTON, H. J., 3RD & WILLIS, W. D., JR. 1985. Diencephalic mechanisms of pain sensation. *Brain Res,* 356**,** 217-96.

ALEXANDER, A. L., LEE, J. E., LAZAR, M. & FIELD, A. S. 2007. Diffusion Tensor Imaging of the Brain. *Neurotherapeutics,* 4**,** 316-29.

ALTMAN, R., ALARCON, G., APPELROUTH, D., BLOCH, D., BORENSTEIN, D., BRANDT, K., BROWN, C., COOKE, T. D., DANIEL, W., FELDMAN, D. & ET AL. 1991. The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hip. *Arthritis Rheum,* 34**,** 505-14.

ALTMAN, R., ASCH, E., BLOCH, D., BOLE, G., BORENSTEIN, D., BRANDT, K., CHRISTY, W., COOKE, T. D., GREENWALD, R., HOCHBERG, M., HOWELL, D., KAPLAN, D., KOOPMAN, W., LONGLEY, S., MANKIN, H., MCSHANE, D. J., MEDSGER, T., MEENAN, R., MIKKELSEN, W., MOSKOWITZ, R., MURPHY, W., ROTHSCHILD, B., SEGAL, M., SOKOLOFF, L. & WOLFE, F. 1986. Development of criteria for the classification and reporting of osteoarthritis: Classification of osteoarthritis of the knee. *Arthritis & Rheumatism,* 29**,** 1039-1049.

APKARIAN, A. V., BALIKI, M. N. & GEHA, P. Y. 2009. Towards a theory of chronic pain. *Prog Neurobiol,* 87**,** 81-97.

APKARIAN, A. V., BUSHNELL, M. C., TREEDE, R. D. & ZUBIETA, J. K. 2005. Human brain mechanisms of pain perception and regulation in health and disease. *Eur J Pain,* 9**,** 463-84.

APKARIAN, A. V., SOSA, Y., SONTY, S., LEVY, R. M., HARDEN, R. N., PARRISH, T. B. & GITELMAN, D. R. 2004. Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. *J Neurosci,* 24**,** 10410-5.

ARDEN, N. K. & LEYLAND, K. M. 2013. Osteoarthritis year 2013 in review: clinical. *Osteoarthritis Cartilage,* 21**,** 1409-13.

ASHBURNER, J. 2007. A fast diffeomorphic image registration algorithm. *Neuroimage,* 38**,** 95-113.

ASHBURNER, J. & FRISTON, K. J. 2000a. Voxel-based morphometry - The methods. *Neuroimage,* 11**,** 805-821.

ASHBURNER, J. & FRISTON, K. J. 2000b. Voxel-based morphometry--the methods. *Neuroimage,* 11**,** 805-21.

AUGUSTINACK, J. C., HUBER, K. E., STEVENS, A. A., ROY, M., FROSCH, M. P., VAN DER KOUWE, A. J., WALD, L. L., VAN LEEMPUT, K., MCKEE, A. C. & FISCHL, B. 2013. Predicting the location of human perirhinal cortex, Brodmann's area 35, from MRI. *Neuroimage,* 64**,** 32-42.

BAARE, W. F. C., VINBERG, M., KNUDSEN, G. M., PAULSON, O. B., LANGKILDE, A. R., JERNIGAN, T. L. & KESSING, L. V. 2010. Hippocampal volume changes in healthy subjects at risk of unipolar depression. *Journal of Psychiatric Research,* 44**,** 655-662.

BALIKI, M. N., GEHA, P. Y., APKARIAN, A. V. & CHIALVO, D. R. 2008a. Beyond feeling: Chronic pain hurts the brain, disrupting the default-mode network dynamics. *Journal of Neuroscience,* 28**,** 1398-1403.

BALIKI, M. N., GEHA, P. Y., JABAKHANJI, R., HARDEN, N., SCHNITZER, T. J. & APKARIAN, A. V. 2008b. A preliminary fMRI study of analgesic treatment in chronic back pain and knee osteoarthritis. *Mol Pain,* 4**,** 47.

BALIKI, M. N., SCHNITZER, T. J., BAUER, W. R. & APKARIAN, A. V. 2011. Brain morphological signatures for chronic pain. *PLoS One,* 6**,** e26010.

BANIC, B., PETERSEN-FELIX, S., ANDERSEN, O. K., RADANOV, B. P., VILLIGER, P. M., ARENDT-NIELSEN, L. & CURATOLO, M. 2004. Evidence for spinal cord hypersensitivity in chronic pain after whiplash injury and in fibromyalgia. *Pain,* 107**,** 7-15.

BANKS, S. M. & KERNS, R. D. 1996. Explaining high rates of depression in chronic pain: A diathesis-stress framework. *Psychological Bulletin,* 119**,** 95-110.

BARNES, J., WHITWELL, J. L., FROST, C., JOSEPHS, K. A., ROSSOR, M. & FOX, N. C. 2006. Measurements of the amygdala and hippocampus in pathologically confirmed Alzheimer disease and frontotemporal lobar degeneration. *Archives of Neurology,* 63**,** 1434-1439.

BEAULIEU, C. 2002. The basis of anisotropic water diffusion in the nervous system - a technical review. *NMR Biomed,* 15**,** 435-55.

BECK, A. T. & STEER, R. 1988. Beck anxiety inventory (BAI). *BiB 2010***,** 54.

BECK, A. T., STEER, R. A. & BROWN, G. 1996. Manual for the Beck Depression Inventory-II. San Antonio. *TX: Psychological Corporation*.

BEDARD, A. & PARENT, A. 2004. Evidence of newly generated neurons in the human olfactory bulb. *Developmental Brain Research,* 151**,** 159-168.

BEECHER, H. K. 1946. Pain in men wounded in battle. *Bull U S Army Med Dep,* 5**,** 445-54.

BENDTSEN, L., JENSEN, R. & OLESEN, J. 1996. Decreased pain detection and tolerance thresholds in chronic tension-type headache. *Arch Neurol,* 53**,** 373-6.

BENNETT, M. 2001. The LANSS Pain Scale: the Leeds assessment of neuropathic symptoms and signs. *Pain,* 92**,** 147-57.

BERMAN, S. M., NALIBOFF, B. D., SUYENOBU, B., LABUS, J. S., STAINS, J., BUELLER, J. A., RUBY, K. & MAYER, E. A. 2006. Sex differences in regional brain response to aversive pelvic visceral stimuli. *Am J Physiol Regul Integr Comp Physiol,* 291**,** R268-76.

BESWICK, A. D., WYLDE, V., GOOBERMAN-HILL, R., BLOM, A. & DIEPPE, P. 2012. What proportion of patients report long-term pain after total hip or knee replacement for osteoarthritis? A systematic review of prospective studies in unselected patients. *Bmj Open,* 2.

BEYER, J. L., KUCHIBBATLA, M., PAYNE, M. E., MOO-YOUNG, M., CASSIDY, F., MACFALL, J. & KRISHNAN, K. R. R. 2004. Hippocampal volume measurement in older adults with bipolar disorder. *American Journal of Geriatric Psychiatry,* 12**,** 613-620.

BINGEL, U., LORENZ, J., SCHOELL, E., WEILLER, C. & BUCHEL, C. 2006a. Mechanisms of placebo analgesia: rACC recruitment of a subcortical antinociceptive network. *Pain,* 120**,** 8-15.

BINGEL, U., LORENZ, J., SCHOELL, E., WEILLER, C. & BÜCHEL, C. 2006b. Mechanisms of placebo analgesia: rACC recruitment of a subcortical antinociceptive network. *Pain,* 120**,** 8-15.

BINGEL, U. & TRACEY, I. 2008. Imaging CNS modulation of pain in humans. *Physiology (Bethesda),* 23**,** 371-80.

BISHOP, D. V. M., ROSS, V. A., DANIELS, M. S. & BRIGHT, P. 1996. The measurement of hand preference: A validation study comparing three groups of right-handers. *British Journal of Psychology,* 87**,** 269-285.

BITTON, R. 2009. The economic burden of osteoarthritis. *Am J Manag Care,* 15**,** S230-5.

BJORDAL, J. M., JOHNSON, M. I., LOPES-MARTINS, R. A., BOGEN, B., CHOW, R. & LJUNGGREN, A. E. 2007. Short-term efficacy of physical interventions in osteoarthritic knee pain. A systematic review and meta-analysis of randomised placebo-controlled trials. *BMC Musculoskelet Disord,* 8**,** 51.

BLAGOJEVIC, M., JINKS, C., JEFFERY, A. & JORDAN, K. P. 2010. Risk factors for onset of osteoarthritis of the knee in older adults: a systematic review and meta-analysis. *Osteoarthritis and Cartilage,* 18**,** 24-33.

BLISS, T. V. P. & COLLINGRIDGE, G. L. 1993. A SYNAPTIC MODEL OF MEMORY - LONG-TERM POTENTIATION IN THE HIPPOCAMPUS. *Nature,* 361**,** 31-39.

BLUMENFELD-KATZIR, T., PASTERNAK, O., DAGAN, M. & ASSAF, Y. 2011. Diffusion MRI of structural brain plasticity induced by a learning and memory task. *PLoS One,* 6**,** e20678.

BOCCARDI, M., ALMICI, M., BRESCIANI, L., CAROLI, A., BONETTI, M., MONCHIERI, S., GENNARELLI, M. & FRISONI, G. B. 2010. Clinical and medial temporal features in a family with mood disorders. *Neuroscience Letters,* 468**,** 93-97.

BOGGIO, P. S., ZAGHI, S., LOPES, M. & FREGNI, F. 2008. Modulatory effects of anodal transcranial direct current stimulation on perception and pain thresholds in healthy volunteers. *European Journal of Neurology,* 15**,** 1124-1130.

BOIVIE, J., LEIJON, G. & JOHANSSON, I. 1989. Central post-stroke pain — a study of the mechanisms through analyses of the sensory abnormalities. *Pain,* 37**,** 173-185.

BOLWERK, A., SEIFERT, F. & MAIHOFNER, C. 2013. Altered Resting-State Functional Connectivity in Complex Regional Pain Syndrome. *Journal of Pain,* 14**,** 1107-1115.

BONICA, J. 1953. *The management of pain*, Phildelphia: Lea & Febiger.

BOOKSTEIN, F. L. 2001. "Voxel-based morphometry" should not be used with imperfectly registered images. *Neuroimage,* 14**,** 1454-62.

BORCKARDT, J. J., SMITH, A. R., REEVES, S. T., WEINSTEIN, M., KOZEL, F. A., NAHAS, Z., SHELLEY, N., BRANHAM, R. K., THOMAS, K. J. & GEORGE, M. S. 2007. Fifteen minutes of left prefrontal repetitive transcranial magnetic stimulation acutely increases thermal pain thresholds in healthy adults. *Pain Res Manag,* 12**,** 287-90.

BOUHASSIRA, D., ATTAL, N., ALCHAAR, H., BOUREAU, F., BROCHET, B., BRUXELLE, J., CUNIN, G., FERMANIAN, J., GINIES, P., GRUN-OVERDYKING, A., JAFARI-SCHLUEP, H., LANTERI-MINET, M., LAURENT, B., MICK, G., SERRIE, A., VALADE, D. & VICAUT, E. 2005. Comparison of pain syndromes associated with nervous or somatic lesions and development of a new neuropathic pain diagnostic questionnaire (DN4). *Pain,* 114**,** 29-36.

BPS. 2009. *Pain News* [Online]. London: The British Pain Society Available: http://www.britishpainsociety.org/bps_nl_sum_2009.pdf [Accessed 05 August 2010].

BREIVIK, H., COLLETT, B., VENTAFRIDDA, V., COHEN, R. & GALLACHER, D. 2006a. Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. *Eur J Pain,* 10**,** 287-333.

BREIVIK, H., COLLETT, B., VENTAFRIDDA, V., COHEN, R. & GALLACHER, D. 2006b. Survey of chronic pain in Europe: Prevalence, impact on daily life, and treatment. *European Journal of Pain,* 10**,** 287-333.

BREMNER, J. D., NARAYAN, M., ANDERSON, E. R., STAIB, L. H., MILLER, H. L. & CHARNEY, D. S. 2000. Hippocampal volume reduction in major depression. *American Journal of Psychiatry,* 157**,** 115-117.

BRIELLMANN, R. S., BERKOVIC, S. F., SYNGENIOTIS, A., KING, M. A. & JACKSON, G. D. 2002. Seizure-associated hippocampal volume loss: A longitudinal magnetic resonance study of temporal lobe epilepsy. *Annals of Neurology,* 51**,** 641-644.

BRIGHINA, F., DE TOMMASO, M., GIGLIA, F., SCALIA, S., COSENTINO, G., PUMA, A., PANETTA, M., GIGLIA, G. & FIERRO, B. 2011. Modulation of pain perception by transcranial magnetic stimulation of left prefrontal cortex. *J Headache Pain,* 12**,** 185-91.

BROOKS, J. C., NURMIKKO, T. J., BIMSON, W. E., SINGH, K. D. & ROBERTS, N. 2002. fMRI of thermal pain: effects of stimulus laterality and attention. *Neuroimage,* 15**,** 293-301.

BROWN, S. C., GLASS, J. M. & PARK, D. C. 2002. The relationship of pain and depression to cognitive function in rheumatoid arthritis patients. *Pain,* 96**,** 279-84.

BRYDEN, M. P. 1977. MEASURING HANDEDNESS WITH QUESTIONNAIRES. *Neuropsychologia,* 15**,** 617-624.

BUCKALEW, N., HAUT, M. W., AIZENSTEIN, H., MORROW, L., PERERA, S., KUWABARA, H. & WEINER, D. K. 2010a. Differences in brain structure and function in older adults with self-reported disabling and nondisabling chronic low back pain. *Pain Med,* 11**,** 1183-97.

BUCKALEW, N., HAUT, M. W., AIZENSTEIN, H., MORROW, L., PERERA, S., KUWABARA, H. & WEINER, D. K. 2010b. Differences in Brain Structure and Function in Older Adults with Self-Reported Disabling and Nondisabling Chronic Low Back Pain. *Pain Med*.

BUCKALEW, N., HAUT, M. W., MORROW, L. & WEINER, D. 2008. Chronic pain is associated with brain volume loss in older adults: preliminary evidence. *Pain Med,* 9**,** 240-8.

BUCKNER, R. L., ANDREWS-HANNA, J. R. & SCHACTER, D. L. 2008. The brain's default network: anatomy, function, and relevance to disease. *Ann N Y Acad Sci,* 1124**,** 1-38.

BUFFINGTON, A. L. H., HANLON, C. A. & MCKEOWN, M. J. 2005. Acute and persistent pain modulation of attention-related anterior cingulate fMRI activations. *Pain,* 113**,** 172-184.

BUHLE, J. & WAGER, T. D. 2010. Performance-dependent inhibition of pain by an executive working memory task. *Pain,* 149**,** 19-26.

BURCKHARDT, C. S. & JONES, K. D. 2003. Adult measures of pain: The McGill Pain Questionnaire (MPQ), Rheumatoid Arthritis Pain Scale (RAPS), Short- Form McGill Pain Questionnaire (SF- MPQ), Verbal Descriptive Scale (VDS), Visual Analog Scale (VAS), and West Haven- Yale Multidisciplinary Pain Inventory (WHYMPI). *Arthritis Care & Research,* 49**,** S96-S104.

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**,** 566-73.

BUSH, G., LUU, P. & POSNER, M. I. 2000. Cognitive and emotional influences in anterior cingulate cortex. *Trends Cogn Sci,* 4**,** 215-222.

BUTLER, R. K. & FINN, D. P. 2009. Stress-induced analgesia. *Progress in Neurobiology,* 88**,** 184-202.

CAMPBELL, C. M., MCCAULEY, L., BOUNDS, S. C., MATHUR, V. A., CONN, L., SIMANGO, M., EDWARDS, R. R. & FONTAINE, K. R. 2012. Changes in pain catastrophizing predict later changes in fibromyalgia clinical and experimental pain report: cross-lagged panel analyses of dispositional and situational catastrophizing. *Arthritis Res Ther,* 14**,** R231.

CAMPBELL, S., MARRIOTT, M., NAHMIAS, C. & MACQUEEN, G. M. 2004. Lower hippocampal volume in patients suffering from depression: A meta-analysis. *American Journal of Psychiatry,* 161**,** 598-607.

CARDOSO-CRUZ, H., LIMA, D. & GALHARDO, V. 2013. Impaired spatial memory performance in a rat model of neuropathic pain is associated with reduced hippocampus-prefrontal cortex connectivity. *J Neurosci,* 33**,** 2465-80.

CARTER, C. S., BOTVINICK, M. M. & COHEN, J. D. 1999. The contribution of the anterior cingulate cortex to executive processes in cognition. *Rev Neurosci,* 10**,** 49-57.

CASEY, K. L., MINOSHIMA, S., BERGER, K. L., KOEPPE, R. A., MORROW, T. J. & FREY, K. A. 1994. Positron emission tomographic analysis of cerebral structures activated specifically by repetitive noxious heat stimuli. *J Neurophysiol,* 71**,** 802-7.

CEKO, M., BUSHNELL, M. C., FITZCHARLES, M. A. & SCHWEINHARDT, P. 2013. Fibromyalgia interacts with age to change the brain. *Neuroimage Clin,* 3**,** 249-60.

CERASA, A., MESSINA, D., NICOLETTI, G., NOVELLINO, F., LANZA, P., CONDINO, F., ARABIA, G., SALSONE, M. & QUATTRONE, A. 2009. Cerebellar atrophy in essential tremor using an automated segmentation method. *AJNR Am J Neuroradiol,* 30**,** 1240-3.

CHAPMAN, L. J. & CHAPMAN, J. P. 1987. THE MEASUREMENT OF HANDEDNESS. *Brain and Cognition,* 6**,** 175-183.

CHEN, F. L., DONG, Y. L., ZHANG, Z. J., CAO, D. L., XU, J., HUI, J., ZHU, L. & GAO, Y. J. 2012. Activation of astrocytes in the anterior cingulate cortex contributes to the affective component of pain in an inflammatory pain model. *Brain Res Bull,* 87**,** 60-6.

CHEN, G., RAJKOWSKA, G., DU, F., SERAJI-BOZORGZAD, N. & MANJI, H. K. 2000. Enhancement of hippocampal neurogenesis by lithium. *Journal of Neurochemistry,* 75**,** 1729-1734.

CHERBUIN, N., ANSTEY, K. J., REGLADE-MESLIN, C. & SACHDEV, P. S. 2009. In vivo hippocampal measurement and memory: a comparison of manual tracing and automated segmentation in a large community-based sample. *PLoS One,* 4**,** e5265.

CHO, S., KIM, H.-Y. & LEE, J.-H. 2013. Validation of the Korean version of the Pain Catastrophizing Scale in patients with chronic non-cancer pain. *Quality of Life Research,* 22**,** 1767-1772.

CIMMINO, M. A., FERRONE, C. & CUTOLO, M. 2011. Epidemiology of chronic musculoskeletal pain. *Best Pract Res Clin Rheumatol,* 25**,** 173-83.

CODERRE, T. J., KATZ, J., VACCARINO, A. L. & MELZACK, R. 1993. Contribution of central neuroplasticity to pathological pain: review of clinical and experimental evidence. *Pain,* 52**,** 259-85.

CONVIT, A., DELEON, M. J., TARSHISH, C., DESANTI, S., TSUI, W., RUSINEK, H. & GEORGE, A. 1997. Specific hippocampal volume reductions in individuals at risk for Alzheimer's disease. *Neurobiology of Aging,* 18**,** 131-138.

COOPER, C., MCALINDON, T., COGGON, D., EGGER, P. & DIEPPE, P. 1994. OCCUPATIONAL ACTIVITY AND OSTEOARTHRITIS OF THE KNEE. *Annals of the Rheumatic Diseases,* 53**,** 90-93.

COREN, S. & PORAC, C. 1978. VALIDITY AND RELIABILITY OF SELF-REPORT ITEMS FOR MEASUREMENT OF LATERAL PREFERENCE. *British Journal of Psychology,* 69**,** 207-211.

COX, J. J., REIMANN, F., NICHOLAS, A. K., THORNTON, G., ROBERTS, E., SPRINGELL, K., KARBANI, G., JAFRI, H., MANNAN, J., RAASHID, Y., AL-GAZALI, L., HAMAMY, H., VALENTE, E. M., GORMAN, S., WILLIAMS, R., MCHALE, D. P., WOOD, J. N., GRIBBLE, F. M. & WOODS, C. G. 2006. An SCN9A channelopathy causes congenital inability to experience pain. *Nature,* 444**,** 894-8.

CREAMER, P., LETHBRIDGE-CEJKU, M., COSTA, P., TOBIN, J. D., HERBST, J. H. & HOCHBERG, M. C. 1999. The relationship of anxiety and depression with self-reported knee pain in the community: data from the Baltimore Longitudinal Study of Aging. *Arthritis Care Res,* 12**,** 3-7.

CRITICAL APPRAISAL SKILLS PROGRAMME CASP. 2004. Public Health Resource Unit, Oxford. Available: http://www.casp-uk.net/wp-content/uploads/2011/11/CASP-Case-Control-Study-Checklist-31.05.13.pdf [Accessed 22/07/2013.

CRUCCU, G., ANAND, P., ATTAL, N., GARCIA-LARREA, L., HAANPAA, M., JORUM, E., SERRA, J. & JENSEN, T. S. 2004. EFNS guidelines on neuropathic pain assessment. *Eur J Neurol,* 11**,** 153-62.

CURATOLO, M., ARENDT-NIELSEN, L. & PETERSEN-FELIX, S. 2006. Central hypersensitivity in chronic pain: mechanisms and clinical implications. *Phys Med Rehabil Clin N Am,* 17**,** 287-302.

DALE, A. M., FISCHL, B. & SERENO, M. I. 1999. Cortical surface-based analysis. I. Segmentation and surface reconstruction. *Neuroimage,* 9**,** 179-94.

DALE, A. M. & SERENO, M. I. 1993. Improved localizadon of cortical activity by combining eeg and meg with mri cortical surface reconstruction: A linear approach. *J. Cognitive Neuroscience,* 5**,** 162-176.

DANCAUSE, N., BARBAY, S., FROST, S. B., PLAUTZ, E. J., CHEN, D., ZOUBINA, E. V., STOWE, A. M. & NUDO, R. J. 2005. Extensive cortical rewiring after brain injury. *J Neurosci,* 25**,** 10167-79.

DANZIGER, N., FAILLENOT, I. & PEYRON, R. 2009. Can we share a pain we never felt? Neural correlates of empathy in patients with congenital insensitivity to pain. *Neuron,* 61**,** 203-12.

DARIAN-SMITH, C. & GILBERT, C. D. 1994. Axonal sprouting accompanies functional reorganization in adult cat striate cortex. *Nature,* 368**,** 737-40.

DASILVA, A. F., BECERRA, L., PENDSE, G., CHIZH, B., TULLY, S. & BORSOOK, D. 2008. Colocalized Structural and Functional Changes in the Cortex of Patients with Trigeminal Neuropathic Pain. *Plos One,* 3.

DAVIS, K. D. 2011. Neuroimaging of pain: what does it tell us? *Curr Opin Support Palliat Care,* 5**,** 116-21.

DAVIS, K. D., TAYLOR, S. J., CRAWLEY, A. P., WOOD, M. L. & MIKULIS, D. J. 1997. Functional MRI of pain- and attention-related activations in the human cingulate cortex. *Journal of Neurophysiology,* 77**,** 3370-3380.

DAVIS, M. A., ETTINGER, W. H., NEUHAUS, J. M., BARCLAY, J. D. & SEGAL, M. R. 1992. Correlates of knee pain among US adults with and without radiographic knee osteoarthritis. *J Rheumatol,* 19**,** 1943-9.

DEBBANE, M., SCHAER, M., GLASER, B. & ELIEZ, S. 2009. Gyrification Index and Cortical Thickness Maturation Associated with Positive Symptoms of Psychosis in Adolescents with 22q11DS. *Biological Psychiatry,* 65**,** 86S-87S.

DEN HEIJER, T., VAN DER LIJN, F., KOUDSTAAL, P. J., HOFMAN, A., VAN DER LUGT, A., KRESTIN, G. P., NIESSEN, W. J. & BRETELER, M. M. B. 2010. A 10-year follow-up of hippocampal volume on magnetic resonance imaging in early dementia and cognitive decline. *Brain,* 133**,** 1163-1172.

DERBYSHIRE, S. W., NICHOLS, T. E., FIRESTONE, L., TOWNSEND, D. W. & JONES, A. K. 2002. Gender differences in patterns of cerebral activation during equal experience of painful laser stimulation. *J Pain,* 3**,** 401-11.

DERBYSHIRE, S. W. G. 1997. Sources of variation in assessing male and female responses to pain. *New Ideas in Psychology,* 15**,** 83-95.

DERBYSHIRE, S. W. G., JONES, A. K. P., GYULAI, F., CLARK, S., TOWNSEND, D. & FIRESTONE, L. L. 1997. Pain processing during three levels of noxious stimulation produces differential patterns of central activity. *Pain,* 73**,** 431-445.

DESTRIEUX, C., FISCHL, B., DALE, A. & HALGREN, E. 2010. Automatic parcellation of human cortical gyri and sulci using standard anatomical nomenclature. *Neuroimage,* 53**,** 1-15.

DEVINSKY, O., MORRELL, M. J. & VOGT, B. A. 1995. Contributions of anterior cingulate cortex to behaviour. *Brain,* 118 (Pt 1)**,** 279-306.

DI PIERO, V., JONES, A. K., IANNOTTI, F., POWELL, M., PERANI, D., LENZI, G. L. & FRACKOWIAK, R. S. 1991. Chronic pain: a PET study of the central effects of percutaneous high cervical cordotomy. *Pain,* 46**,** 9-12.

DIEPPE, P., CUSHNAGHAN, J., TUCKER, M., BROWNING, S. & SHEPSTONE, L. 2000. The Bristol 'OA500 study': progression and impact of the disease after 8 years. *Osteoarthritis and Cartilage,* 8**,** 63-68.

DRAGANSKI, B., GASER, C., BUSCH, V., SCHUIERER, G., BOGDAHN, U. & MAY, A. 2004. Neuroplasticity: changes in grey matter induced by training. *Nature,* 427**,** 311-2.

DRAGANSKI, B., MOSER, T., LUMMEL, N., GANSSBAUER, S., BOGDAHN, U., HAAS, F. & MAY, A. 2006. Decrease of thalamic gray matter following limb amputation. *Neuroimage,* 31**,** 951-7.

DREVETS, W. C., BOGERS, W. & RAICHLE, M. E. 2002. Functional anatomical correlates of antidepressant drug treatment assessed using PET measures of regional glucose metabolism. *European Neuropsychopharmacology,* 12**,** 527-544.

DREVETS, W. C., SAVITZ, J. & TRIMBLE, M. 2008. The subgenual anterior cingulate cortex in mood disorders. *CNS Spectr,* 13**,** 663-81.

DURIC, V. & MCCARSON, K. E. 2006. Persistent pain produces stress-like alterations in hippocampal neurogenesis and gene expression. *Journal of Pain,* 7**,** 544-555.

DUVERNOY, H. M. 2005. *The Human Hippocampus: Functional Anatomy, Vascularization and Serial Sections with MRI* Verlag Berlin Heidelberg:Germany

, Springer.

ECCLESTON, C. & CROMBEZ, G. 1999. Pain demands attention: a cognitive-affective model of the interruptive function of pain. *Psychol Bull,* 125**,** 356-66.

EGERTON, M. & MULLAN, K. 2006. An analysis and monetary valuation of formal and informal voluntary work by gender and educational attainment. *ISER Working Paper 2006-22*.

ELBERT, T., PANTEV, C., WIENBRUCH, C., ROCKSTROH, B. & TAUB, E. 1995. Increased cortical representation of the fingers of the left hand in string players. *Science,* 270**,** 305-7.

ELLINGSON, B. M., MAYER, E., HARRIS, R. J., ASHE-MCNALLY, C., NALIBOFF, B. D., LABUS, J. S. & TILLISCH, K. 2013. Diffusion tensor imaging detects microstructural reorganization in the brain associated with chronic irritable bowel syndrome. *Pain,* 154**,** 1528-41.

ERIKSSON, P. S., PERFILIEVA, E., BJORK-ERIKSSON, T., ALBORN, A. M., NORDBORG, C., PETERSON, D. A. & GAGE, F. H. 1998. Neurogenesis in the adult human hippocampus. *Nat Med,* 4**,** 1313-7.

ERITAIA, J., WOOD, S. J., STUART, G. W., BRIDLE, N., DUDGEON, P., MARUFF, P., VELAKOULIS, D. & PANTELIS, C. 2000. An optimized method for estimating intracranial volume from magnetic resonance images. *Magnetic Resonance in Medicine,* 44**,** 973-977.

ERPELDING, N., MOAYEDI, M. & DAVIS, K. D. 2012. Cortical thickness correlates of pain and temperature sensitivity. *Pain,* 153**,** 1602-9.

FEINSTEIN, L., SABATES, R., ANDERSON, T. M., SORHAINDO, A. & HAMMOND, C. 2006. What are the Effects of Education on Health?

FELSON, D. T. 2005. The sources of pain in knee osteoarthritis. *Curr Opin Rheumatol,* 17**,** 624-8.

FELSON, D. T. 2006. Clinical practice. Osteoarthritis of the knee. *N Engl J Med,* 354**,** 841-8.

FELSON, D. T. 2009. Developments in the clinical understanding of osteoarthritis. *Arthritis Res Ther,* 11**,** 203.

FELSON, D. T., CHAISSON, C. E., HILL, C. L., TOTTERMAN, S. M., GALE, M. E., SKINNER, K. M., KAZIS, L. & GALE, D. R. 2001. The association of bone marrow lesions with pain in knee osteoarthritis. *Ann Intern Med,* 134**,** 541-9.

FELSON, D. T., LAWRENCE, R. C., DIEPPE, P. A., HIRSCH, R., HELMICK, C. G., JORDAN, J. M., KINGTON, R. S., LANE, N. E., NEVITT, M. C. & ZHANG, Y. 2000. Osteoarthritis: new insights. Part 1: the disease and its risk factors. *Annals of internal medicine,* 133**,** 635-646.

FELSON, D. T., NAIMARK, A., ANDERSON, J., KAZIS, L., CASTELLI, W. & MEENAN, R. F. 1987. The prevalence of knee osteoarthritis in the elderly. The Framingham Osteoarthritis Study. *Arthritis Rheum,* 30**,** 914-8.

FELSON, D. T. & SCHAIBLE, H. G. 2009. *Pain in osteoarthritis,* Oxford, Wiley-Blackwell.

FELSON, D. T., ZHANG, Y., HANNAN, M. T., NAIMARK, A., WEISSMAN, B. N., ALIABADI, P. & LEVY, D. 1995. The incidence and natural history of knee osteoarthritis in the elderly. The Framingham Osteoarthritis Study. *Arthritis Rheum,* 38**,** 1500-5.

FERNANDES, L., STORHEIM, K., LOCHTING, I. & GROTLE, M. 2012. Cross-cultural adaptation and validation of the Norwegian pain catastrophizing scale in patients with low back pain. *Bmc Musculoskeletal Disorders,* 13.

FIELDS, H. L. 1992. Is there a facilitating component to central pain modulation? *APS Journal,* 1**,** 71-78.

FILLINGIM, R. B., KING, C. D., RIBEIRO-DASILVA, M. C., RAHIM-WILLIAMS, B. & RILEY, J. L., 3RD 2009. Sex, gender, and pain: a review of recent clinical and experimental findings. *J Pain,* 10**,** 447-85.

FISCHL, B. & DALE, A. M. 2000. Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc Natl Acad Sci U S A,* 97**,** 11050-5.

FISCHL, B., LIU, A. & DALE, A. M. 2001. Automated manifold surgery: constructing geometrically accurate and topologically correct models of the human cerebral cortex. *IEEE Trans Med Imaging,* 20**,** 70-80.

FISCHL, B., SALAT, D. H., BUSA, E., ALBERT, M., DIETERICH, M., HASELGROVE, C., VAN DER KOUWE, A., KILLIANY, R., KENNEDY, D., KLAVENESS, S., MONTILLO, A., MAKRIS, N., ROSEN, B. & DALE, A. M. 2002. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron,* 33**,** 341-55.

FISCHL, B., SALAT, D. H., VAN DER KOUWE, A. J., MAKRIS, N., SEGONNE, F., QUINN, B. T. & DALE, A. M. 2004. Sequence-independent segmentation of magnetic resonance images. *Neuroimage,* 23 Suppl 1**,** S69-84.

FISCHL, B., SERENO, M. I. & DALE, A. M. 1999. Cortical surface-based analysis. II: Inflation, flattening, and a surface-based coordinate system. *Neuroimage,* 9**,** 195-207.

FLOR, H., TURK, D. C. & BERNDT SCHOLZ, O. 1987. Impact of chronic pain on the spouse: Marital, emotional and physical consequences. *Journal of Psychosomatic Research,* 31**,** 63-71.

FOLSTEIN, M. F., FOLSTEIN, S. E. & MCHUGH, P. R. 1975. "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician. *Journal of psychiatric research,* 12**,** 189-198.

FORSYTHE, M. E., DUNBAR, M. J., HENNIGAR, A. W., SULLIVAN, M. J. & GROSS, M. 2008. Prospective relation between catastrophizing and residual pain following knee arthroplasty: two-year follow-up. *Pain Res Manag,* 13**,** 335-41.

FREYNHAGEN, R., BARON, R., GOCKEL, U. & TOLLE, T. R. 2006. painDETECT: a new screening questionnaire to identify neuropathic components in patients with back pain. *Current Medical Research and Opinion,* 22**,** 1911-1920.

FRODL, T., MEISENZAHL, E. M., ZETZSCHE, T., BORN, C., GROLL, C., JAGER, M., LEINSINGER, G., BOTTLENDER, R., HAHN, K. & MOLLER, H. J. 2002. Hippocampal changes in patients with a first episode of major depression. *American Journal of Psychiatry,* 159**,** 1112-1118.

FROKJAER, J. B., BOUWENSE, S. A., OLESEN, S. S., LUNDAGER, F. H., ESKILDSEN, S. F., VAN GOOR, H., WILDER-SMITH, O. H. & DREWES, A. M. 2012. Reduced cortical thickness of brain areas involved in pain processing in patients with chronic pancreatitis. *Clin Gastroenterol Hepatol,* 10**,** 434-8 e1.

FUKUZAKO, H., YAMADA, K., KODAMA, S., YONEZAWA, T., FUKUZAKO, T., TAKENOUCHI, K., KAJIYA, Y., NAKAJO, M. & TAKIGAWA, M. 1997. Hippocampal volume asymmetry and age at illness onset in males with schizophrenia. *European Archives of Psychiatry and Clinical Neuroscience,* 247**,** 248-251.

GALABURDA, A. M., ROSEN, G. D. & SHERMAN, G. F. 1990. Individual variability in cortical organization: its relationship to brain laterality and implications to function. *Neuropsychologia,* 28**,** 529-46.

GALIMBERTI, I., GOGOLLA, N., ALBERI, S., SANTOS, A. F., MULLER, D. & CARONI, P. 2006. Long-term rearrangements of hippocampal mossy fiber terminal connectivity in the adult regulated by experience. *Neuron,* 50**,** 749-63.

GARCIA CAMPAYO, J., RODERO, B., ALDA, M., SOBRADIEL, N., MONTERO, J. & MORENO, S. 2008. Validation of the Spanish version of the Pain Catastrophizing Scale in fibromyalgia. *Medicina Clinica,* 131**,** 487-492.

GARCIA-LARREA, L., PEYRON, R., MERTENS, P., GREGOIRE, M. C., LAVENNE, F., LE BARS, D., CONVERS, P., MAUGUIERE, F., SINDOU, M. & LAURENT, B. 1999. Electrical stimulation of motor cortex for pain control: a combined PET-scan and electrophysiological study. *Pain,* 83**,** 259-73.

GASER, C. & SCHLAUG, G. 2003. Brain structures differ between musicians and non-musicians. *Journal of Neuroscience,* 23**,** 9240-9245.

GASQUOINE, P. G. 2013. Localization of function in anterior cingulate cortex: from psychosurgery to functional neuroimaging. *Neurosci Biobehav Rev,* 37**,** 340-8.

GEORGE, S. Z., CALLEY, D., VALENCIA, C. & BENECIUK, J. M. 2011. Clinical Investigation of Pain-related Fear and Pain Catastrophizing for Patients With Low Back Pain. *Clin J Pain,* 27**,** 108-15.

GEVA, N. & DEFRIN, R. 2013. Enhanced pain modulation among triathletes: a possible explanation for their exceptional capabilities. *Pain,* 154**,** 2317-23.

GLASS, J. M. 2006. Cognitive dysfunction in fibromyalgia and chronic fatigue syndrome: new trends and future directions. *Curr Rheumatol Rep,* 8**,** 425-9.

GLUHM, S., GOLDSTEIN, J., LOC, K., COLT, A., LIEW, C. V. & COREY-BLOOM, J. 2013. Cognitive performance on the mini-mental state examination and the montreal cognitive assessment across the healthy adult lifespan. *Cogn Behav Neurol,* 26**,** 1-5.

GOESLING, J., CLAUW, D. & HASSETT, A. 2013. Pain and Depression: An Integrative Review of Neurobiological and Psychological Factors. *Current Psychiatry Reports,* 15**,** 1-8.

GRACELY, R. H., GEISSER, M. E., GIESECKE, T., GRANT, M. A., PETZKE, F., WILLIAMS, D. A. & CLAUW, D. J. 2004. Pain catastrophizing and neural responses to pain among persons with fibromyalgia. *Brain,* 127**,** 835-43.

GRACHEV, I. D., FREDRICKSON, B. E. & APKARIAN, A. V. 2000. Abnormal brain chemistry in chronic back pain: an in vivo proton magnetic resonance spectroscopy study. *Pain,* 89**,** 7-18.

GRAFF-GUERRERO, A., GONZALEZ-OLVERA, J., FRESAN, A., GOMEZ-MARTIN, D., MENDEZ-NUNEZ, J. C. & PELLICER, F. 2005. Repetitive transcranial magnetic stimulation of dorsolateral prefrontal cortex increases tolerance to human experimental pain. *Cognitive Brain Research,* 25**,** 153-160.

GRANT, J. A., COURTEMANCHE, J., DUERDEN, E. G., DUNCAN, G. H. & RAINVILLE, P. 2010. Cortical thickness and pain sensitivity in zen meditators. *Emotion,* 10**,** 43-53.

GROTHE, K. B., DUTTON, G. R., JONES, G. N., BODENLOS, J., ANCONA, M. & BRANTLEY, P. J. 2005. Validation of the Beck Depression Inventory-II in a low-income African American sample of medical outpatients. *Psychol Assess,* 17**,** 110-4.

GRUNDMAN, M., SENCAKOVA, D., JACK, C. R., PETERSEN, R. C., KIM, H. T., SCHULTZ, A., WEINER, M. F., DECARLI, C., DEKOSKY, S. T., VAN DYCK, C., THOMAS, R. G., THOMAS, R. G., THAL, L. J. & ALZHEIMERS DIS COOPERATIVE, S. 2002. Brain MRI

hippocampal of clinical status in a mild volume and prediction cognitive impairment trial. *Journal of Molecular Neuroscience,* 19**,** 23-27.

GUCCIONE, A. A., FELSON, D. T., ANDERSON, J. J., ANTHONY, J. M., ZHANG, Y., WILSON, P. W., KELLY-HAYES, M., WOLF, P. A., KREGER, B. E. & KANNEL, W. B. 1994. The effects of specific medical conditions on the functional limitations of elders in the Framingham Study. *Am J Public Health,* 84**,** 351-8.

GUSTIN, S. M., PECK, C. C., WILCOX, S. L., NASH, P. G., MURRAY, G. M. & HENDERSON, L. A. 2011. Different pain, different brain: thalamic anatomy in neuropathic and non-neuropathic chronic pain syndromes. *J Neurosci,* 31**,** 5956-64.

GUSTIN, S. M., WRIGLEY, P. J., SIDDALL, P. J. & HENDERSON, L. A. 2010. Brain anatomy changes associated with persistent neuropathic pain following spinal cord injury. *Cereb Cortex,* 20**,** 1409-19.

GWILYM, S. E., FILIPPINI, N., DOUAUD, G., CARR, A. J. & TRACEY, I. 2010a. Thalamic atrophy associated with painful osteoarthritis of the hip is reversible after arthroplasty: a longitudinal voxel-based morphometric study. *Arthritis Rheum,* 62**,** 2930-40.

GWILYM, S. E., FILLIPINI, N., DOUAUD, G., CARR, A. J. & TRACEY, I. 2010b. Thalamic atrophy associated with painful osteoarthritis of the hip is reversible after arthroplasty; a longitudinal voxel-based-morphometric study. *Arthritis Rheum*.

GWILYM, S. E., KELTNER, J. R., WARNABY, C. E., CARR, A. J., CHIZH, B., CHESSELL, I. & TRACEY, I. 2009. Psychophysical and functional imaging evidence supporting the presence of central sensitization in a cohort of osteoarthritis patients. *Arthritis Rheum,* 61**,** 1226-34.

HAIER, R. J., KARAMA, S., LEYBA, L. & JUNG, R. E. 2009. MRI assessment of cortical thickness and functional activity changes in adolescent girls following three months of practice on a visual-spatial task. *BMC Res Notes,* 2**,** 174.

HAME, S. L. & ALEXANDER, R. A. 2013. Knee osteoarthritis in women. *Curr Rev Musculoskelet Med,* 6**,** 182-7.

HAMILTON, D., HENDERSON, G. R., GASTON, P., MACDONALD, D., HOWIE, C. & SIMPSON, A. H. 2012. Comparative outcomes of total hip and knee arthroplasty: a prospective cohort study. *Postgrad Med J,* 88**,** 627-31.

HANNAN, M. T., ANDERSON, J. J., PINCUS, T. & FELSON, D. T. 1992. Educational attainment and osteoarthritis: differential associations with radiographic changes and symptom reporting. *J Clin Epidemiol,* 45**,** 139-47.

HARRIS, J. M., YATES, S., MILLER, P., BEST, J. J., JOHNSTONE, E. C. & LAWRIE, S. M. 2004. Gyrification in first-episode schizophrenia: a morphometric study. *Biol Psychiatry,* 55**,** 141-7.

HART, D. J. & SPECTOR, T. D. 1993. THE RELATIONSHIP OF OBESITY, FAT DISTRIBUTION AND OSTEOARTHRITIS IN WOMEN IN THE GENERAL-POPULATION - THE CHINGFORD STUDY. *Journal of Rheumatology,* 20**,** 331-335.

HART, R. P., MARTELLI, M. F. & ZASLER, N. D. 2000. Chronic pain and neuropsychological functioning. *Neuropsychol Rev,* 10**,** 131-49.

HART, R. P., WADE, J. B. & MARTELLI, M. F. 2003. Cognitive impairment in patients with chronic pain: the significance of stress. *Curr Pain Headache Rep,* 7**,** 116-26.

HASAN, A., MCINTOSH, A. M., DROESE, U. A., SCHNEIDER-AXMANN, T., LAWRIE, S. M., MOORHEAD, T. W., TEPEST, R., MAIER, W., FALKAI, P. & WOBROCK, T. 2011. Prefrontal cortex gyrification index in twins: an MRI study. *Eur Arch Psychiatry Clin Neurosci,* 261**,** 459-65.

HAUSER, K. F., HOUDI, A. A., TURBEK, C. S., ELDE, R. P. & MAXSON, W., 3RD 2000. Opioids intrinsically inhibit the genesis of mouse cerebellar granule neuron precursors in vitro: differential impact of mu and delta receptor activation on proliferation and neurite elongation. *Eur J Neurosci,* 12**,** 1281-93.

HAWKER, G. A., STEWART, L., FRENCH, M. R., CIBERE, J., JORDAN, J. M., MARCH, L., SUAREZ-ALMAZOR, M. & GOOBERMAN-HILL, R. 2008. Understanding the pain experience in hip and knee osteoarthritis--an OARSI/OMERACT initiative. *Osteoarthritis Cartilage,* 16**,** 415-22.

HEIBERG, T. & KVIEN, T. K. 2002. Preferences for improved health examined in 1,024 patients with rheumatoid arthritis: pain has highest priority. *Arthritis Rheum,* 47**,** 391-7.

HENDERSON, L. A., GANDEVIA, S. C. & MACEFIELD, V. G. 2008. Gender differences in brain activity evoked by muscle and cutaneous pain: a retrospective study of single-trial fMRI data. *Neuroimage,* 39**,** 1867-76.

HERCULANO-HOUZEL, S., MOTA, B., WONG, P. & KAAS, J. H. 2010. Connectivity-driven white matter scaling and folding in primate cerebral cortex. *Proc Natl Acad Sci U S A,* 107**,** 19008-13.

HILDERINK, P. H., BURGER, H., DEEG, D. J., BEEKMAN, A. T. & OUDE VOSHAAR, R. C. 2012. The temporal relation between pain and depression: results from the longitudinal aging study Amsterdam. *Psychosom Med,* 74**,** 945-51.

HILL, A. B. 1965. THE ENVIRONMENT AND DISEASE: ASSOCIATION OR CAUSATION? *Proc R Soc Med,* 58**,** 295-300.

HOLAHAN, M. R., REKART, J. L., SANDOVAL, J. & ROUTTENBERG, A. 2006. Spatial learning induces presynaptic structural remodeling in the hippocampal mossy fiber system of two rat strains. *Hippocampus,* 16**,** 560-70.

HONEYCUTT, N. A. & SMITH, C. D. 1995. Hippocampal volume measurements using magnetic resonance imaging in normal young adults. *J Neuroimaging,* 5**,** 95-100.

HORN, C., BLISCHKE, Y., KUNZ, M. & LAUTENBACHER, S. 2012. Does pain necessarily have an affective component? Negative evidence from blink reflex experiments. *Pain Res Manag,* 17**,** 15-24.

HOU, G., YANG, X. & YUAN, T. F. 2013. Hippocampal asymmetry: differences in structures and functions. *Neurochem Res,* 38**,** 453-60.

HSIEH, J. C., BELFRAGE, M., STONE-ELANDER, S., HANSSON, P. & INGVAR, M. 1995. Central representation of chronic ongoing neuropathic pain studied by positron emission tomography. *Pain,* 63**,** 225-36.

HSU, M. C., HARRIS, R. E., SUNDGREN, P. C., WELSH, R. C., FERNANDES, C. R., CLAUW, D. J. & WILLIAMS, D. A. 2009. No consistent difference in gray matter volume between individuals with fibromyalgia and age-matched healthy subjects when controlling for affective disorder. *Pain,* 143**,** 262-7.

HU, S., SHENG, W. S., LOKENSGARD, J. R. & PETERSON, P. K. 2002. Morphine induces apoptosis of human microglia and neurons. *Neuropharmacology,* 42**,** 829-36.

IADAROLA, M. J., BERMAN, K. F., ZEFFIRO, T. A., BYAS-SMITH, M. G., GRACELY, R. H., MAX, M. B. & BENNETT, G. J. 1998. Neural activation during acute capsaicin-evoked pain and allodynia assessed with PET. *Brain,* 121 (Pt 5)**,** 931-47.

IMAMURA, M., IMAMURA, S. T., KAZIYAMA, H. H., TARGINO, R. A., HSING, W. T., DE SOUZA, L. P., CUTAIT, M. M., FREGNI, F. & CAMANHO, G. L. 2008. Impact of nervous system hyperalgesia on pain, disability, and quality of life in patients with knee osteoarthritis: a controlled analysis. *Arthritis Rheum,* 59**,** 1424-31.

INSAUSTI, R., RINCÓN, M., DÍAZ-LÓPEZ, E., ARTACHO-PÉRULA, E., MANSILLA, F., FLORENSA, J., GONZÁLEZ-MORENO, C., ÁLVAREZ-LINERA, J., GARCÍA, S., PERAITA, H., PAIS, E. & INSAUSTI, A. M. 2011. FreeSurfer Automatic Brain Segmentation Adaptation to Medial Temporal Lobe Structures: Volumetric Assessment and Diagnosis of Mild Cognitive Impairment. *In:* FERRÁNDEZ, J., ÁLVAREZ SÁNCHEZ, J., PAZ, F. & TOLEDO, F. J. (eds.) *New Challenges on Bioinspired Applications.* Springer Berlin Heidelberg.

JACK, C. R. 1994. MRI-BASED HIPPOCAMPAL VOLUME MEASUREMENTS IN EPILEPSY. *Epilepsia,* 35**,** S21-S29.

JACK, C. R., JR., TWOMEY, C. K., ZINSMEISTER, A. R., SHARBROUGH, F. W., PETERSEN, R. C. & CASCINO, G. D. 1989a. Anterior temporal lobes and hippocampal formations: normative volumetric measurements from MR images in young adults. *Radiology,* 172**,** 549-54.

JACK, C. R., TWOMEY, C. K., ZINSMEISTER, A. R., SHARBROUGH, F. W., PETERSEN, R. C. & CASCINO, G. D. 1989b. ANTERIOR TEMPORAL LOBES AND HIPPOCAMPAL FORMATIONS - NORMATIVE VOLUMETRIC MEASUREMENTS FROM MR IMAGES IN YOUNG-ADULTS. *Radiology,* 172**,** 549-554.

JAIN, N., FLORENCE, S. L., QI, H. X. & KAAS, J. H. 2000. Growth of new brainstem connections in adult monkeys with massive sensory loss. *Proc Natl Acad Sci U S A,* 97**,** 5546-50.

JAMISON, R. N., SCHEIN, J. R., VALLOW, S., ASCHER, S., VORSANGER, G. J. & KATZ, N. P. 2003. Neuropsychological effects of long-term opioid use in chronic pain patients. *J Pain Symptom Manage,* 26**,** 913-21.

JAVADAPOUR, A., MALHI, G. S., IVANOVSKI, B., CHEN, X., WEN, W. & SACHDEV, P. 2010. Hippocampal Volumes in Adults With Bipolar Disorder. *Journal of Neuropsychiatry and Clinical Neurosciences,* 22**,** 55-62.

JENSEN, K. B., LOITOILE, R., KOSEK, E., PETZKE, F., CARVILLE, S., FRANSSON, P., MARCUS, H., WILLIAMS, S. C. R., CHOY, E., MAINGUY, Y., VITTON, O., GRACELY, R. H., GOLLUB, R., INGVAR, M. & KONG, J. 2012. Patients with fibromyalgia display less functional connectivity in the brain's pain inhibitory network. *Molecular Pain,* 8.

JENSEN, K. B., SRINIVASAN, P., SPAETH, R., TAN, Y., KOSEK, E., PETZKE, F., CARVILLE, S., FRANSSON, P., MARCUS, H., WILLIAMS, S. C., CHOY, E., VITTON, O., GRACELY, R., INGVAR, M. & KONG, J. 2013. Overlapping structural and functional brain changes in patients with long-term exposure to fibromyalgia pain. *Arthritis Rheum,* 65**,** 3293-303.

JEON, T., MISHRA, V., UH, J., WEINER, M., HATANPAA, K. J., WHITE, C. L., 3RD, ZHAO, Y. D., LU, H., DIAZ-ARRASTIA, R. & HUANG, H. 2012. Regional changes of cortical mean diffusivities with aging after correction of partial volume effects. *Neuroimage,* 62**,** 1705-16.

JEUKENS, C. R. L. P. N., VLOOSWIJK, M. C. G., MAJOIE, H. J. M., DE KROM, M. C. T. F. M., ALDENKAMP, A. P., HOFMAN, P. A. M., JANSEN, J. F. A. & BACKES, W. H. 2009. Hippocampal MRI Volumetry at 3 Tesla Reliability and Practical Guidance. *Investigative Radiology,* 44**,** 509-517.

JI, R. R., KOHNO, T., MOORE, K. A. & WOOLF, C. J. 2003. Central sensitization and LTP: do pain and memory share similar mechanisms? *Trends Neurosci,* 26**,** 696-705.

JOE, S., WOOLLEY, M. E., BROWN, G. K., GHAHRAMANLOU-HOLLOWAY, M. & BECK, A. T. 2008. Psychometric properties of the Beck Depression Inventory-II in low-income, African American suicide attempters. *J Pers Assess,* 90**,** 521-3.

JONES, A. K. & DERBYSHIRE, S. W. 1996. Cerebral mechanisms operating in the presence and absence of inflammatory pain. *Ann Rheum Dis,* 55**,** 411-20.

JONES, A. K., HUNEKE, N. T., LLOYD, D. M., BROWN, C. A. & WATSON, A. 2012. Role of functional brain imaging in understanding rheumatic pain. *Curr Rheumatol Rep,* 14**,** 557-67.

JONES, G., GLISSON, M., HYNES, K. & CICUTTINI, F. 2000. Sex and site differences in cartilage development - A possible explanation for variations in knee osteoarthritis in later life. *Arthritis and Rheumatism,* 43**,** 2543-2549.

JONGSMA, M. L., POSTMA, S. A., SOUREN, P., ARNS, M., GORDON, E., VISSERS, K., WILDER-SMITH, O., VAN RIJN, C. M. & VAN GOOR, H. 2011. Neurodegenerative properties of chronic pain: cognitive decline in patients with chronic pancreatitis. *PloS one,* 6**,** e23363.

JORDAN, K. M., ARDEN, N. K., DOHERTY, M., BANNWARTH, B., BIJLSMA, J. W., DIEPPE, P., GUNTHER, K., HAUSELMANN, H., HERRERO-BEAUMONT, G., KAKLAMANIS, P., LOHMANDER, S., LEEB, B., LEQUESNE, M., MAZIERES, B., MARTIN-MOLA, E., PAVELKA, K., PENDLETON, A., PUNZI, L., SERNI, U., SWOBODA, B., VERBRUGGEN, G., ZIMMERMAN-GORSKA, I. & DOUGADOS, M. 2003. EULAR Recommendations 2003: an evidence based approach to the management of knee osteoarthritis: Report of a Task Force of the Standing Committee for International Clinical Studies Including Therapeutic Trials (ESCISIT). *Ann Rheum Dis,* 62**,** 1145-55.

JUNI, P., REICHENBACH, S. & DIEPPE, P. 2006. Osteoarthritis: rational approach to treating the individual. *Best Pract Res Clin Rheumatol,* 20**,** 721-40.

KANTARCI, K., PETERSEN, R. C., BOEVE, B. F., KNOPMAN, D. S., WEIGAND, S. D., O'BRIEN, P. C., SHIUNG, M. M., SMITH, G. E., IVNIK, R. J., TANGALOS, E. G. & JACK, C. R., JR. 2005. DWI predicts future progression to Alzheimer disease in amnestic mild cognitive impairment. *Neurology,* 64**,** 902-4.

KEEFE, F. J., LEFEBVRE, J. C., EGERT, J. R., AFFLECK, G., SULLIVAN, M. J. & CALDWELL, D. S. 2000. The relationship of gender to pain, pain behavior, and disability in osteoarthritis patients: the role of catastrophizing. *Pain,* 87**,** 325-34.

KESSELS, R. P., DE HAAN, E. H., KAPPELLE, L. J. & POSTMA, A. 2001. Varieties of human spatial memory: a meta-analysis on the effects of hippocampal lesions. *Brain Res Brain Res Rev,* 35**,** 295-303.

KEWMAN, D. G., VAISHAMPAYAN, N., ZALD, D. & HAN, B. 1991. COGNITIVE IMPAIRMENT IN MUSCULOSKELETAL PAIN PATIENTS. *International Journal of Psychiatry in Medicine,* 21**,** 253-262.

KIDD, B. L. 2006. Osteoarthritis and joint pain. *Pain,* 123**,** 6-9.

KLYACHKO, V. A. & STEVENS, C. F. 2003. Connectivity optimization and the positioning of cortical areas. *Proc Natl Acad Sci U S A,* 100**,** 7937-41.

KOH, D.-M. & COLLINS, D. J. 2007. Diffusion-weighted MRI in the body: applications and challenges in oncology. *American Journal of Roentgenology,* 188**,** 1622-1635.

KOLB, B., MUHAMMAD, A. & GIBB, R. 2011. Searching for factors underlying cerebral plasticity in the normal and injured brain. *Journal of Communication Disorders,* 44**,** 503-514.

KONG, J., LOGGIA, M. L., ZYLONEY, C., TU, P., LAVIOLETTE, P. & GOLLUB, R. L. 2010. Exploring the brain in pain: activations, deactivations and their relation. *Pain,* 148**,** 257-67.

KONG, J., SPAETH, R. B., WEY, H. Y., CHEETHAM, A., COOK, A. H., JENSEN, K., TAN, Y., LIU, H., WANG, D., LOGGIA, M. L., NAPADOW, V., SMOLLER, J. W., WASAN, A. D. & GOLLUB, R. L. 2013. S1 is associated with chronic low back pain: a functional and structural MRI study. *Mol Pain,* 9**,** 43.

KONRAD, C., UKAS, T., NEBEL, C., AROLT, V., TOGA, A. W. & NARR, K. L. 2009. Defining the human hippocampus in cerebral magnetic resonance images-An overview of current segmentation protocols. *Neuroimage,* 47**,** 1185-1195.

KOO, B. B., HUA, N., CHOI, C. H., RONEN, I., LEE, J. M. & KIM, D. S. 2009. A framework to analyze partial volume effect on gray matter mean diffusivity measurements. *Neuroimage,* 44**,** 136-44.

KOOLSCHIJN, P. C. M. P., VAN HAREN, N. E. M., CAHN, W., SCHNACK, H. G., JANSSEN, J., KLUMPERS, F., HULSHOFF, H. E. & KAHN, R. S. 2010. Hippocampal Volume Change in Schizophrenia. *Journal of Clinical Psychiatry,* 71**,** 737-744.

KORNAAT, P. R., BLOEM, J. L., CEULEMANS, R. Y., RIYAZI, N., ROSENDAAL, F. R., NELISSEN, R. G., CARTER, W. O., HELLIO LE GRAVERAND, M. P. & KLOPPENBURG, M. 2006. Osteoarthritis of the knee: association between clinical features and MR imaging findings. *Radiology,* 239**,** 811-7.

KORNELSEN, J., SBOTO-FRANKENSTEIN, U., MCIVER, T., GERVAI, P., WACNIK, P., BERRINGTON, N. & TOMANEK, B. 2013. Default Mode Network Functional Connectivity Altered in Failed Back Surgery Syndrome. *Journal of Pain,* 14**,** 483-491.

KOWALCZYK, W. J., SULLIVAN, M. A., EVANS, S. M., BISAGA, A. M., VOSBURG, S. K. & COMER, S. D. 2010. Sex Differences and Hormonal Influences on Response to Mechanical Pressure Pain in Humans. *Journal of Pain,* 11**,** 330-342.

KRAMER, J. H., SCHUFF, N., REED, B. R., MUNGAS, D., DU, A. T., ROSEN, H. J., JAGUST, W. J., MILLER, B. L., WEINER, M. W. & CHUI, H. C. 2004. Hippocampal volume and retention in Alzheimer's disease. *Journal of the International Neuropsychological Society,* 10**,** 639-643.

KRAUSE, S. J. & BACKONJA, M. M. 2003. Development of a neuropathic pain questionnaire. *Clin J Pain,* 19**,** 306-14.

KUBESOVA, A., BUBENIKOVA-VALESOVA, V., MERTLOVA, M., PALENICEK, T. & HORACEK, J. 2012. Impact of psychotropic drugs on adult hippocampal neurogenesis. *Neuroscience Research,* 73**,** 93-98.

KUCHINAD, A., SCHWEINHARDT, P., SEMINOWICZ, D. A., WOOD, P. B., CHIZH, B. A. & BUSHNELL, M. C. 2007. Accelerated brain gray matter loss in fibromyalgia patients: premature aging of the brain? *J Neurosci,* 27**,** 4004-7.

KULKARNI, B., BENTLEY, D. E., ELLIOTT, R., JULYAN, P. J., BOGER, E., WATSON, A., BOYLE, Y., EL-DEREDY, W. & JONES, A. K. 2007. Arthritic pain is processed in brain areas concerned with emotions and fear. *Arthritis Rheum,* 56**,** 1345-54.

KUMAR, A., HAROON, E., DARWIN, C., PHAM, D., AJILORE, O., RODRIGUEZ, G. & MINTZ, J. 2008. Gray matter prefrontal changes in type 2 diabetes detected using MRI. *Journal of Magnetic Resonance Imaging,* 27**,** 14-19.

KUMAR, R., CHAVEZ, A. S., MACEY, P. M., WOO, M. A., YAN-GO, F. L. & HARPER, R. M. 2012. Altered global and regional brain mean diffusivity in patients with obstructive sleep apnea. *J Neurosci Res,* 90**,** 2043-52.

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**,** 2492-502.

LAMOTTE, R. H., SHAIN, C. N., SIMONE, D. A. & TSAI, E. F. 1991. Neurogenic hyperalgesia: psychophysical studies of underlying mechanisms. *J Neurophysiol,* 66**,** 190-211.

LANDI, F., LIPEROTI, R., RUSSO, A., CAPOLUONGO, E., BARILLARO, C., PAHOR, M., BERNABEI, R. & ONDER, G. 2010. Disability, more than multimorbidity, was

predictive of mortality among older persons aged 80 years and older. *J Clin Epidemiol,* 63**,** 752-9.

LAPANE, K. L., SANDS, M. R., YANG, S., MCALINDON, T. E. & EATON, C. B. 2012. Use of complementary and alternative medicine among patients with radiographic-confirmed knee osteoarthritis. *Osteoarthritis and Cartilage,* 20**,** 22-28.

LARSSON, H. B., THOMSEN, C., FREDERIKSEN, J., STUBGAARD, M. & HENRIKSEN, O. 1992. In vivo magnetic resonance diffusion measurement in the brain of patients with multiple sclerosis. *Magn Reson Imaging,* 10**,** 7-12.

LAUCHE, R., CRAMER, H., LANGHORST, J. & DOBOS, G. 2014. A Systematic Review and Meta-Analysis of Medical Leech Therapy for Osteoarthritis of the Knee. *Clinical Journal of Pain,* 30**,** 63-72.

LAUCHE, R., LANGHORST, J., DOBOS, G. & CRAMER, H. 2013. A systematic review and meta-analysis of Tai Chi for osteoarthritis of the knee. *Complementary Therapies in Medicine,* 21**,** 396-406.

LAWRENCE, R. C., HELMICK, C. G., ARNETT, F. C., DEYO, R. A., FELSON, D. T., GIANNINI, E. H., HEYSE, S. P., HIRSCH, R., HOCHBERG, M. C., HUNDER, G. G., LIANG, M. H., PILLEMER, S. R., STEEN, V. D. & WOLFE, F. 1998. Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. *Arthritis & Rheumatism,* 41**,** 778-799.

LE BIHAN, D. 1995. Molecular diffusion, tissue microdynamics and microstructure. *NMR Biomed,* 8**,** 375-86.

LEE, A. S., ELLMAN, M. B., YAN, D., KROIN, J. S., COLE, B. J., VAN WIJNEN, A. J. & IM, H. J. 2013. A current review of molecular mechanisms regarding osteoarthritis and pain. *Gene,* 527**,** 440-7.

LEE, J. K., LEE, J. M., KIM, J. S., KIM, I. Y., EVANS, A. C. & KIM, S. I. 2006. A novel quantitative cross-validation of different cortical surface reconstruction algorithms using MRI phantom. *Neuroimage,* 31**,** 572-84.

LEE, M. C., ZAMBREANU, L., MENON, D. K. & TRACEY, I. 2008. Identifying brain activity specifically related to the maintenance and perceptual consequence of central sensitization in humans. *J Neurosci,* 28**,** 11642-9.

LEE, Y. C., LU, B., BATHON, J. M., HAYTHORNTHWAITE, J. A., SMITH, M. T., PAGE, G. G. & EDWARDS, R. R. 2011a. Pain sensitivity and pain reactivity in osteoarthritis. *Arthritis Care Res (Hoboken),* 63**,** 320-7.

LEE, Y. C., NASSIKAS, N. J. & CLAUW, D. J. 2011b. The role of the central nervous system in the generation and maintenance of chronic pain in rheumatoid arthritis, osteoarthritis and fibromyalgia. *Arthritis Res Ther,* 13**,** 211.

LEHMANN, M., DOUIRI, A., KIM, L. G., MODAT, M., CHAN, D., OURSELIN, S., BARNES, J. & FOX, N. C. 2010. Atrophy patterns in Alzheimer's disease and semantic dementia: A comparison of FreeSurfer and manual volumetric measurements. *NeuroImage,* 49**,** 2264-2274.

LEVY-REIS I, G.-A. J., KING DS, ET AL 2000. Hippocampal imaging and volumetry in temporal lobe epilepsy at 1.5 and 4 Tesla. *Proc Intl Soc Magn Reson Med 2000;8:15*.

LI, J., WU, M., ZHUO, M. & XU, Z. C. 2013. Alteration of neuronal activity after digit amputation in rat anterior cingulate cortex. *International journal of physiology, pathophysiology and pharmacology,* 5**,** 43-51.

LI, X. Y., KO, H. G., CHEN, T., DESCALZI, G., KOGA, K., WANG, H., KIM, S. S., SHANG, Y., KWAK, C., PARK, S. W., SHIM, J., LEE, K., COLLINGRIDGE, G. L., KAANG, B. K. &

ZHUO, M. 2010. Alleviating neuropathic pain hypersensitivity by inhibiting PKMzeta in the anterior cingulate cortex. *Science,* 330**,** 1400-4.

LI, Y.-J., GA, S.-N., HUO, Y., LI, S.-Y. & GAO, X.-G. 2007a. Characteristics of hippocampal volumes in healthy Chinese from MRI. *Neurological Research,* 29**,** 803-806.

LI, Y. J., GA, S. N., HUO, Y., LI, S. Y. & GAO, X. G. 2007b. Characteristics of hippocampal volumes in healthy Chinese from MRI. *Neurological Research,* 29**,** 803-806.

LICHTENBERG, P. A., SWENSEN, C. H. & SKEHAN, M. W. 1986. Further investigation of the role of personality, lifestyle and arthritic severity in predicting pain. *Journal of Psychosomatic Research,* 30**,** 327-337.

LINK, T. M. 2009. Correlations Between Joint Morphology and Pain and Between Magnetic Resonance Imaging, Histology, and Micro-Computed Tomography. *J Bone Joint Surg Am,* 91**,** 30-32.

LIU, J., LAN, L., LI, G., YAN, X., NAN, J., XIONG, S., YIN, Q., VON DENEEN, K. M., GONG, Q., LIANG, F., QIN, W. & TIAN, J. 2013. Migraine-Related Gray Matter and White Matter Changes at a 1-Year Follow-Up Evaluation. *J Pain,* 14**,** 1703-8.

LIU, T., LIPNICKI, D. M., ZHU, W., TAO, D., ZHANG, C., CUI, Y., JIN, J. S., SACHDEV, P. S. & WEN, W. 2012a. Cortical Gyrification and Sulcal Spans in Early Stage Alzheimer's Disease. *Plos One,* 7.

LIU, Y., LI, Y. J., LUO, E. P., LU, H. B. & YIN, H. 2012b. Cortical thinning in patients with recent onset post-traumatic stress disorder after a single prolonged trauma exposure. *PLoS One,* 7**,** e39025.

LLOYD, A. J., FERRIER, I. N., BARBER, R., GHOLKAR, A., YOUNG, A. H. & O'BRIEN, J. T. 2004. Hippocampal volume change in depression: late- and early-onset illness compared. *British Journal of Psychiatry,* 184**,** 488-495.

LOESER, J. D. & MELZACK, R. 1999. Pain: an overview. *The Lancet,* 353**,** 1607-1609.

LÖWE, B., UNÜTZER, J., CALLAHAN, C. M., PERKINS, A. J. & KROENKE, K. 2004. Monitoring depression treatment outcomes with the patient health questionnaire-9. *Medical care,* 42**,** 1194-1201.

LUDERS, E., KURTH, F., MAYER, E. A., TOGA, A. W., NARR, K. L. & GASER, C. 2012. The unique brain anatomy of meditation practitioners: alterations in cortical gyrification. *Front Hum Neurosci,* 6**,** 34.

LYNCH, N. T. & VASUDEVAN, S. V. 1988. *Persistent Pain: Psychosocial Assessment and Intervention*, Springer.

MA, D. K., KIM, W. R., MING, G. L. & SONG, H. 2009. Activity-dependent extrinsic regulation of adult olfactory bulb and hippocampal neurogenesis. *Ann N Y Acad Sci,* 1170**,** 664-73.

MACDONALD, A. W., 3RD, COHEN, J. D., STENGER, V. A. & CARTER, C. S. 2000. Dissociating the role of the dorsolateral prefrontal and anterior cingulate cortex in cognitive control. *Science,* 288**,** 1835-8.

MACLEAN, P. D. 1990. *The triune brain in evolution: Role in paleocerebral functions*, Springer.

MACMASTER, F. P. & KUSUMAKAR, V. 2004. Hippocampal volume in early onset depression. *Bmc Medicine,* 2.

MAETZEL, A., LI, L., PENCHARZ, J., TOMLINSON, G. & BOMBARDIER, C. 2004. The economic burden associated with osteoarthritis, rheumatoid arthritis, and hypertension: a comparative study. *Annals of the rheumatic diseases,* 63**,** 395-401.

MAIHOFNER, C., JESBERGER, F., SEIFERT, F. & KALTENHAUSER, M. 2010. Cortical processing of mechanical hyperalgesia: a MEG study. *Eur J Pain,* 14**,** 64-70.

MAINERO, C., ZHANG, W. T., KUMAR, A., ROSEN, B. R. & SORENSEN, A. G. 2007. Mapping the spinal and supraspinal pathways of dynamic mechanical allodynia in the human trigeminal system using cardiac-gated fMRI. *Neuroimage,* 35**,** 1201-10.

MAK, H. K. F., ZHANG, Z. P., YAU, K. K. W., ZHANG, L. D., CHAN, Q. N. & CHU, L. W. 2011. Efficacy of Voxel-Based Morphometry with DARTEL and Standard Registration as Imaging Biomarkers in Alzheimer's Disease Patients and Cognitively Normal Older Adults at 3.0 Tesla MR Imaging. *Journal of Alzheimers Disease,* 23**,** 655-664.

MALTBIE, E., BHATT, K., PANIAGUA, B., SMITH, R. G., GRAVES, M. M., MOSCONI, M. W., PETERSON, S., WHITE, S., BLOCHER, J., EL-SAYED, M., HAZLETT, H. C. & STYNER, M. A. 2012. Asymmetric bias in user guided segmentations of brain structures. *Neuroimage,* 59**,** 1315-23.

MANGIN, J. F., JOUVENT, E. & CACHIA, A. 2010. In-vivo measurement of cortical morphology: means and meanings. *Curr Opin Neurol,* 23**,** 359-67.

MANSOUR, A. R., BALIKI, M. N., HUANG, L., TORBEY, S., HERRMANN, K. M., SCHNITZER, T. J. & APKARIAN, A. V. 2013. Brain white matter structural properties predict transition to chronic pain. *Pain,* 154**,** 2160-8.

MAO, J., SUNG, B., JI, R. R. & LIM, G. 2002. Neuronal apoptosis associated with morphine tolerance: evidence for an opioid-induced neurotoxic mechanism. *J Neurosci,* 22**,** 7650-61.

MARCH, L. M. & BACHMEIER, C. J. 1997. Economics of osteoarthritis: a global perspective. *Baillieres Clin Rheumatol,* 11**,** 817-34.

MARCUS., D. A., COPE., D. K., DEODHAR., A. & PAYNE., R. 2009. *Chronic Pain: An Atlas of Investigation and Management,* Oxford:UK, an imprint of Atlas Medical Publishing Ltd.

MARQUES, A. P., RHODEN, L., SIQUEIRA, J. D. O. & JOÃO, S. M. A. 2001. Pain evaluation of patients with fibromyalgia, osteoarthritis, and low back pain. *Revista do Hospital das Clínicas,* 56**,** 5-10.

MARSH, L., MORRELL, M. J., SHEAR, P. K., SULLIVAN, E. V., FREEMAN, H., MARIE, A., LIM, K. O. & PFEFFERBAUM, A. 1997. Cortical and hippocampal volume deficits in temporal lobe epilepsy. *Epilepsia,* 38**,** 576-587.

MARTIN, C. E., JOHNSON, E., WECHTER, M. E., LESERMAN, J. & ZOLNOUN, D. A. 2011. Catastrophizing: a predictor of persistent pain among women with endometriosis at 1 year. *Hum Reprod,* 26**,** 3078-84.

MARTIRE, L. M., KEEFE, F. J., SCHULZ, R., PARRIS STEPHENS, M. A. & MOGLE, J. A. 2013. The impact of daily arthritis pain on spouse sleep. *PAIN,* 154**,** 1725-1731.

MATHARU, M. S., GOOD, C. D., MAY, A., BAHRA, A. & GOADSBY, P. J. 2003. No change in the structure of the brain in migraine: a voxel-based morphometric study. *Eur J Neurol,* 10**,** 53-7.

MAY, A. 2008. Chronic pain may change the structure of the brain. *Pain,* 137**,** 7-15.

MCBETH, J. & JONES, K. 2007. Epidemiology of chronic musculoskeletal pain. *Best Pract Res Clin Rheumatol,* 21**,** 403-25.

MCCAFFERY M & BEEBE A 1989. *Pain : clinical manual for nursing practice (UK edition)* Aylesbury, Mosby.

MCCORMACK, H. M., HORNE, D. J. & SHEATHER, S. 1988. Clinical applications of visual analogue scales: a critical review. *Psychol Med,* 18**,** 1007-19.

MCCORMICK, L. M., ZIEBELL, S., NOPOULOS, P., CASSELL, M., ANDREASEN, N. C. & BRUMM, M. 2006. Anterior cingulate cortex: an MRI-based parcellation method. *Neuroimage,* 32**,** 1167-75.

MCEWEN, B. S. 2001. Plasticity of the hippocampus: Adaptation to chronic stress and allostatic load. *In:* SORG, B. A. & BELL, I. R. (eds.) *Role of Neural Plasticity in Chemical Intolerance.*

MCGREEVY, K., BOTTROS, M. M. & RAJA, S. N. 2011. Preventing Chronic Pain following Acute Pain: Risk Factors, Preventive Strategies, and their Efficacy. *Eur J Pain Suppl,* 5**,** 365-72.

MECHELLI, A., CRINION, J. T., NOPPENEY, U., O'DOHERTY, J., ASHBURNER, J., FRACKOWIAK, R. S. & PRICE, C. J. 2004. Neurolinguistics: structural plasticity in the bilingual brain. *Nature,* 431**,** 757.

MEDINA, K. L., SCHWEINSBURG, A. D., COHEN-ZION, M., NAGEL, B. J. & TAPERT, S. F. 2007. Effects of alcohol and combined marijuana and alcohol use during adolescence on hippocampal volume and asymmetry. *Neurotoxicology and Teratology,* 29**,** 141-152.

MEEUS, M. & NIJS, J. 2007. Central sensitization: a biopsychosocial explanation for chronic widespread pain in patients with fibromyalgia and chronic fatigue syndrome. *Clin Rheumatol,* 26**,** 465-73.

MELZACK, R. 1975. MCGILL PAIN QUESTIONNAIRE - MAJOR PROPERTIES AND SCORING METHODS. *Pain,* 1**,** 277-299.

MELZACK, R. 1989. PHANTOM LIMBS. *Regional Anesthesia,* 14**,** 208-211.

MELZACK, R. & KATZ, J. 2013. McGill Pain Questionnaire. *Encyclopedia of Pain.* Springer.

MELZACK R. & CASEY K. 1968. Sensory, motivational and central control determinants of chronic pain: A new conceptual model. In: The Skin Senses, edited by D.L.Kenshalo. pp. 423 – 443 Springfield, Illinois. Thomas.

MERSKEY, H. & BOGDUK, N. 1994. Classification of chronic pain. Seattle:

International Association for the Study of Pain Press.

MERVAALA, E., FOHR, J., KONONEN, M., VALKONEN-KORHONEN, M., VAINIO, P., PARTANEN, K., PARTANEN, J., TIIHONEN, J., VIINAMAKI, H., KARJALAINEN, A. K. & LEHTONEN, J. 2000. Quantitative MRI of the hippocampus and amygdala in severe depression. *Psychological Medicine,* 30**,** 117-125.

MESSINA, R., ROCCA, M. A., COLOMBO, B., VALSASINA, P., HORSFIELD, M. A., COPETTI, M., FALINI, A., COMI, G. & FILIPPI, M. 2013. Cortical abnormalities in patients with migraine: a surface-based analysis. *Radiology,* 268**,** 170-80.

MING, G. L. & SONG, H. 2005. Adult neurogenesis in the mammalian central nervous system. *Annu Rev Neurosci,* 28**,** 223-50.

MOAYEDI, M., WEISSMAN-FOGEL, I., CRAWLEY, A. P., GOLDBERG, M. B., FREEMAN, B. V., TENENBAUM, H. C. & DAVIS, K. D. 2011. Contribution of chronic pain and neuroticism to abnormal forebrain gray matter in patients with temporomandibular disorder. *Neuroimage,* 55**,** 277-86.

MOGIL, J. S. 2012. Sex differences in pain and pain inhibition: multiple explanations of a controversial phenomenon. *Nat Rev Neurosci,* 13**,** 859-66.

MOHER, D., LIBERATI, A., TETZLAFF, J. & ALTMAN, D. G. 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med,* 6**,** e1000097.

MOMENAN, R., STECKLER, L. E., SAAD, Z. S., VAN RAFELGHEM, S., KERICH, M. J. & HOMMER, D. W. 2012. Effects of alcohol dependence on cortical thickness as determined by magnetic resonance imaging. *Psychiatry Research-Neuroimaging,* 204**,** 101-111.

MONT, M. A., SERNA, F. K., KRACKOW, K. A. & HUNGERFORD, D. S. 1996. Exploration of radiographically normal total knee replacements for unexplained pain. *Clin Orthop Relat Res***,** 216-20.

MOORE, A., DERRY, S., ECCLESTON, C. & KALSO, E. 2013a. Expect analgesic failure; pursue analgesic success. *BMJ,* 346**,** f2690.

MOORE, R. A., STRAUBE, S. & ALDINGTON, D. 2013b. Pain measures and cut-offs - 'no worse than mild pain' as a simple, universal outcome. *Anaesthesia,* 68**,** 400-12.

MOREY, R. A., PETTY, C. M., XU, Y., HAYES, J. P., WAGNER, H. R., 2ND, LEWIS, D. V., LABAR, K. S., STYNER, M. & MCCARTHY, G. 2009. A comparison of automated segmentation and manual tracing for quantifying hippocampal and amygdala volumes. *Neuroimage,* 45**,** 855-66.

MORIARTY, O., MCGUIRE, B. E. & FINN, D. P. 2011. The effect of pain on cognitive function: A review of clinical and preclinical research. *Progress in Neurobiology,* 93**,** 385-404.

MORLEY, S. 2008. Psychology of pain. *Br J Anaesth,* 101**,** 25-31.

MOSELEY, J. B., O'MALLEY, K., PETERSEN, N. J., MENKE, T. J., BRODY, B. A., KUYKENDALL, D. H., HOLLINGSWORTH, J. C., ASHTON, C. M. & WRAY, N. P. 2002. A controlled trial of arthroscopic surgery for osteoarthritis of the knee. *N Engl J Med,* 347**,** 81-8.

MOULTON, E. A., KEASER, M. L., GULLAPALLI, R. P. & GREENSPAN, J. D. 2005. Regional intensive and temporal patterns of functional MRI activation distinguishing noxious and innocuous contact heat. *J Neurophysiol,* 93**,** 2183-93.

MOUNTCASTLE, V. B. 1974. *Medical physiology,* Saint Louis,, C. V. Mosby Co.

MUNCE, S. E. & STEWART, D. E. 2007. Gender differences in depression and chronic pain conditions in a national epidemiologic survey. *Psychosomatics,* 48**,** 394-9.

MUNTE, T. F., ALTENMULLER, E. & JANCKE, L. 2002. The musician's brain as a model of neuroplasticity. *Nat Rev Neurosci,* 3**,** 473-8.

MURRAY, C. J. L. & D., L. A. 1997. The global burden of disease. Geneva: World Health Organisation,.

MUTSO, A. A., RADZICKI, D., BALIKI, M. N., HUANG, L., BANISADR, G., CENTENO, M. V., RADULOVIC, J., MARTINA, M., MILLER, R. J. & APKARIAN, A. V. 2012. Abnormalities in hippocampal functioning with persistent pain. *J Neurosci,* 32**,** 5747-56.

NAPADOW, V., LACOUNT, L., PARK, K., AS-SANIE, S., CLAUW, D. J. & HARRIS, R. E. 2010. Intrinsic Brain Connectivity in Fibromyalgia Is Associated With Chronic Pain Intensity. *Arthritis and Rheumatism,* 62**,** 2545-2555.

NASREDDINE, Z. S., PHILLIPS, N. A., BEDIRIAN, V., CHARBONNEAU, S., WHITEHEAD, V., COLLIN, I., CUMMINGS, J. L. & CHERTKOW, H. 2005. The montreal cognitive

assessment, MoCA: A brief screening tool for mild cognitive impairment. *Journal of the American Geriatrics Society,* 53**,** 695-699.

NATIONAL COLLABORATING CENTRE FOR CHRONIC CONDITIONS. 2008. *Osteoarthritis: National clinical guideline for care and management in adults* [Online]. Royal College of Physicians. Available: http://www.nice.org.uk/nicemedia/pdf/CG059FullGuideline.pdf [Accessed 28/11/2013.

NEOGI, T. 2013. The epidemiology and impact of pain in osteoarthritis. *Osteoarthritis and Cartilage,* 21**,** 1145-1153.

NESVAG, R., SCHAER, M., HAUKVIK, U. K., WESTLYE, L. T., RIMOL, L. M., LANGE, E. H., HARTBERG, C. B., OTTET, M.-C., MELLE, I., ANDREASSEN, O. A., JONSSON, E. G., AGARTZ, I. & ELIEZ, S. 2014. Reduced brain cortical folding in schizophrenia revealed in two independent samples. *Schizophrenia Research,* 152**,** 333-338.

NIJS, J., MEEUS, M., VAN OOSTERWIJCK, J., ROUSSEL, N., DE KOONING, M., ICKMANS, K. & MATIC, M. 2011a. Treatment of central sensitization in patients with 'unexplained' chronic pain: what options do we have? *Expert Opin Pharmacother,* 12**,** 1087-98.

NIJS, J., PAUL VAN WILGEN, C., VAN OOSTERWIJCK, J., VAN ITTERSUM, M. & MEEUS, M. 2011b. How to explain central sensitization to patients with 'unexplained' chronic musculoskeletal pain: practice guidelines. *Man Ther,* 16**,** 413-8.

NIKOLAJSEN, L., BRANDSBORG, B., LUCHT, U., JENSEN, T. S. & KEHLET, H. 2006. Chronic pain following total hip arthroplasty: a nationwide questionnaire study. *Acta Anaesthesiol Scand,* 50**,** 495-500.

NIV, D. & DEVOR, M. 2004. Chronic pain as a disease in its own right. *Pain Pract,* 4**,** 179-81.

NORRIS, M. P., ARNAU, R. C., BRAMSON, R. & MEAGHER, M. W. 2004. The efficacy of somatic symptoms in assessing depression in older primary care patients. *Clinical gerontologist,* 27**,** 43-57.

NÜESCH, E., DIEPPE, P., REICHENBACH, S., WILLIAMS, S., IFF, S. & JÜNI, P. 2011. All cause and disease specific mortality in patients with knee or hip osteoarthritis: population based cohort study. *BMJ,* 342.

OCHSNER, K. N., LUDLOW, D. H., KNIERIM, K., HANELIN, J., RAMACHANDRAN, T., GLOVER, G. C. & MACKEY, S. C. 2006. Neural correlates of individual differences in pain-related fear and anxiety. *Pain,* 120**,** 69-77.

OFFICE FOR NATIONAL STATISTICS. 2006. *National statistics: focus on health* [Online]. Available: http://www.ons.gov.uk/ons/about-ons/index.html [Accessed 24/11/2013.

OHTORI, S., ORITA, S., YAMASHITA, M., ISHIKAWA, T., ITO, T., SHIGEMURA, T., NISHIYAMA, H., KONNO, S., OHTA, H., TAKASO, M., INOUE, G., EGUCHI, Y., OCHIAI, N., KISHIDA, S., KUNIYOSHI, K., AOKI, Y., ARAI, G., MIYAGI, M., KAMODA, H., SUZKUKI, M., NAKAMURA, J., FURUYA, T., KUBOTA, G., SAKUMA, Y., OIKAWA, Y., SUZUKI, M., SASHO, T., NAKAGAWA, K., TOYONE, T. & TAKAHASHI, K. 2012. Existence of a neuropathic pain component in patients with osteoarthritis of the knee. *Yonsei Med J,* 53**,** 801-5.

OLDFIELD, R. C. 1971. THE ASSESSMENT AND ANALYSIS OF HANDEDNESS: THE EDINBURGH INVENTORY. *Neuropsychologia,* 9**,** 97-113.

OSSIPOV, M. H., DUSSOR, G. O. & PORRECA, F. 2010. Central modulation of pain. *J Clin Invest,* 120**,** 3779-87.

OUDMAN, E., POSTMA, A., VAN DER STIGCHEL, S., APPELHOF, B., WIJNIA, J. W. & NIJBOER, T. C. 2014. The Montreal Cognitive Assessment (MoCA) is superior to the Mini Mental State Examination (MMSE) in detection of Korsakoff's syndrome. *Clin Neuropsychol,* 28**,** 1123-32.

PANGMAN, V. C., SLOAN, J. & GUSE, L. 2000. An examination of psychometric properties of the mini-mental state examination and the standardized mini-mental state examination: implications for clinical practice. *Appl Nurs Res,* 13**,** 209-13.

PANTEL, J., O'LEARY, D. S., CRETSINGER, K., BOCKHOLT, H. J., KEEFE, H., MAGNOTTA, V. A. & ANDREASEN, N. C. 2000. A new method for the in vivo volumetric measurement of the human hippocampus with high neuroanatomical accuracy. *Hippocampus,* 10**,** 752-758.

PANTELIS, C., VELAKOULIS, D., MCGORRY, P. D., WOOD, S. J., SUCKLING, J., PHILLIPS, L. J., YUNG, A. R., BULLMORE, E. T., BREWER, W., SOULSBY, B., DESMOND, P. & MCGUIRE, P. K. 2003. Neuroanatomical abnormalities before and after onset of psychosis: a cross-sectional and longitudinal MRI comparison. *Lancet,* 361**,** 281-288.

PARDOE, H. R., ABBOTT, D. F. & JACKSON, G. D. 2013. Sample size estimates for well-powered cross-sectional cortical thickness studies. *Hum Brain Mapp,* 34**,** 3000-9.

PARK, D. C., GLASS, J. M., MINEAR, M. & CROFFORD, L. J. 2001. Cognitive function in fibromyalgia patients. *Arthritis Rheum,* 44**,** 2125-33.

PARKS, E. L., GEHA, P. Y., BALIKI, M. N., KATZ, J., SCHNITZER, T. J. & APKARIAN, A. V. 2011a. Brain activity for chronic knee osteoarthritis: dissociating evoked pain from spontaneous pain. *Eur J Pain,* 15**,** 843 e1-14.

PARKS, E. L., GEHA, P. Y., BALIKI, M. N., KATZ, J., SCHNITZER, T. J. & APKARIAN, A. V. 2011b. Brain activity for chronic knee osteoarthritis: Dissociating evoked pain from spontaneous pain. *European Journal of Pain,* 15.

PASCUAL-LEONE, A., AMEDI, A., FREGNI, F. & MERABET, L. B. 2005. The plastic human brain cortex. *Annu Rev Neurosci,* 28**,** 377-401.

PAVLAKOVIC, G. & PETZKE, F. 2010. The role of quantitative sensory testing in the evaluation of musculoskeletal pain conditions. *Curr Rheumatol Rep,* 12**,** 455-61.

PEAT, G., THOMAS, E., DUNCAN, R., WOOD, L., HAY, E. & CROFT, P. 2006a. Clinical classification criteria for knee osteoarthritis: performance in the general population and primary care. *Ann Rheum Dis,* 65**,** 1363-7.

PEAT, G., THOMAS, E., DUNCAN, R., WOOD, L., HAY, E. & CROFT, P. 2006b. Clinical classification criteria for knee osteoarthritis: performance in the general population and primary care. *Annals of the rheumatic diseases,* 65**,** 1363-1367.

PEDRAZA, O., BOWERS, D. & GILMORE, R. 2004. Asymmetry of the hippocampus and amygdala in MRI volumetric measurements of normal adults. *Journal of the International Neuropsychological Society,* 10**,** 664-678.

PEPER, J. S., BROUWER, R. M., BOOMSMA, D. I., KAHN, R. S. & POLL, H. E. H. 2007. Genetic influences on human brain structure: A review of brain imaging studies in twins. *Human Brain Mapping,* 28**,** 464-473.

PEREIRA, J. B., IBARRETXE-BILBAO, N., MARTI, M. J., COMPTA, Y., JUNQUE, C., BARGALLO, N. & TOLOSA, E. 2012. Assessment of cortical degeneration in patients with Parkinson's disease by voxel-based morphometry, cortical folding, and cortical thickness. *Hum Brain Mapp,* 33**,** 2521-34.

PERKINS, D. O., WYATT, R. J. & BARTKO, J. J. 2000. Penny-wise and pound-foolish: the impact of measurement error on sample size requirements in clinical trials. *Biol Psychiatry,* 47**,** 762-6.

PERNEGER, T. V. 1998. What's wrong with Bonferroni adjustments. *BMJ,* 316**,** 1236-8.

PETRIE, A. & SABIN, C. 2005. *Medical statistics at a glance,* Oxford, Blackwell.

PEYRON, R., GARCIA-LARREA, L., DEIBER, M. P., CINOTTI, L., CONVERS, P., SINDOU, M., MAUGUIERE, F. & LAURENT, B. 1995. Electrical stimulation of precentral cortical area in the treatment of central pain: electrophysiological and PET study. *Pain,* 62**,** 275-86.

PEYRON, R., LAURENT, B. & GARCIA-LARREA, L. 2000. Functional imaging of brain responses to pain. A review and meta-analysis (2000). *Neurophysiol Clin,* 30**,** 263-88.

PFEFFERBAUM, A., LIM, K. O., ZIPURSKY, R. B., MATHALON, D. H., ROSENBLOOM, M. J., LANE, B., CHUNG, N. H. & SULLIVAN, E. V. 1992. BRAIN GRAY AND WHITE MATTER VOLUME LOSS ACCELERATES WITH AGING IN CHRONIC-ALCOHOLICS - A QUANTITATIVE MRI STUDY. *Alcoholism-Clinical and Experimental Research,* 16**,** 1078-1089.

PHAN, C. M., LINK, T. M., BLUMENKRANTZ, G., DUNN, T. C., RIES, M. D., STEINBACH, L. S. & MAJUMDAR, S. 2006. MR imaging findings in the follow-up of patients with different stages of knee osteoarthritis and the correlation with clinical symptoms. *European Radiology,* 16**,** 608-18.

PHILBIN, E. F., RIES, M. D., GROFF, G. D., SHEESLEY, K. A., FRENCH, T. S. & PEARSON, T. A. 1996. Osteoarthritis as a determinant of an adverse coronary heart disease risk profile. *J Cardiovasc Risk,* 3**,** 529-33.

PIEPER, S., LORENSEN, W., SCHROEDER, W. & KIKINIS, R. 2006. The NA-MIC Kit: ITK, VTK, Pipelines, Grids and 3D Slicer as an Open Platform for the Medical Image Computing Community. *Proc IEEE Intl Symp on Biomedical Imaging ISBI***,** 698-701.

PIERPAOLI, C., JEZZARD, P., BASSER, P. J., BARNETT, A. & DI CHIRO, G. 1996. Diffusion tensor MR imaging of the human brain. *Radiology,* 201**,** 637-48.

PORRECA, F., OSSIPOV, M. H. & GEBHART, G. F. 2002. Chronic pain and medullary descending facilitation. *Trends in Neurosciences,* 25**,** 319-325.

PORRO, C. A., CETTOLO, V., FRANCESCATO, M. P. & BARALDI, P. 1998. Temporal and intensity coding of pain in human cortex. *J Neurophysiol,* 80**,** 3312-20.

PRESTIA, A., BOCCARDI, M., GALLUZZI, S., CAVEDO, E., ADORNI, A., SORICELLI, A., BONETTI, M., GEROLDI, C., GIANNAKOPOULOS, P., THOMPSON, P. & FRISONI, G. 2011. Hippocampal and amygdalar volume changes in elderly patients with Alzheimer's disease and schizophrenia. *Psychiatry Research-Neuroimaging,* 192**,** 77-83.

PRICE, D. D. & BARRELL, J. J. 2012. *Inner experience and neuroscience : merging both perspectives,* Cambridge, Mass., MIT Press.

PRUESSNER, J. C., LI, L. M., SERLES, W., PRUESSNER, M., COLLINS, D. L., KABANI, N., LUPIEN, S. & EVANS, A. C. 2000. Volumetry of hippocampus and amygdala with high-resolution MRI and three-dimensional analysis software: Minimizing the discrepancies between laboratories. *Cerebral Cortex,* 10**,** 433-442.

QUEK, K. F., LOW, W. Y., RAZACK, A. H., LOH, C. S. & CHUA, C. B. 2004. Reliability and validity of the Spielberger State-Trait Anxiety Inventory (STAI) among urological patients: a Malaysian study. *Med J Malaysia,* 59**,** 258-67.

RACINE, M., TOUSIGNANT-LAFLAMME, Y., KLODA, L. A., DION, D., DUPUIS, G. & CHOINIERE, M. 2012. A systematic literature review of 10 years of research on sex/gender and experimental pain perception - part 1: are there really differences between women and men? *Pain,* 153**,** 602-18.

RACZKOWS.D, KALAT, J. W. & NEBES, R. 1974. RELIABILITY AND VALIDITY OF SOME HANDEDNESS QUESTIONNAIRE ITEMS. *Neuropsychologia,* 12**,** 43-47.

RADLOFF, L. 1977. Center for epidemiological studies—Depression scale. *Actualisation de la base de données BeST & Inclusion de nouvelles échelles dans la base de données existante BeST*.

RADUA, J., VAN DEN HEUVEL, O. A., SURGULADZE, S. & MATAIX-COLS, D. 2010. Meta-analytical Comparison of Voxel-Based Morphometry Studies in Obsessive-Compulsive Disorder vs Other Anxiety Disorders. *Archives of General Psychiatry,* 67**,** 701-711.

RAICHLE, M. E., MACLEOD, A. M., SNYDER, A. Z., POWERS, W. J., GUSNARD, D. A. & SHULMAN, G. L. 2001. A default mode of brain function. *Proceedings of the National Academy of Sciences of the United States of America,* 98**,** 676-682.

RAINE, A., ISHIKAWA, S. S., ARCE, E., LENCZ, T., KNUTH, K. H., BIHRLE, S., LACASSE, L. & COLLETTI, P. 2004. Hippocampal structural asymmetry in unsuccessful psychopaths. *Biological Psychiatry,* 55**,** 185-191.

RAINVILLE, P., DUNCAN, G. H., PRICE, D. D., CARRIER, B. & BUSHNELL, M. C. 1997. Pain affect encoded in human anterior cingulate but not somatosensory cortex. *Science,* 277**,** 968-71.

RAJ, P. P. 1996. *Pain medicine : a comprehensive review,* St. Louis ; London, Mosby.

REGINSTER, J. Y. 2002. The prevalence and burden of arthritis. *Rheumatology,* 41**,** 3-6.

REISS, A. L., ECKERT, M. A., ROSE, F. E., KARCHEMSKIY, A., KESLER, S., CHANG, M., REYNOLDS, M. F., KWON, H. & GALABURDA, A. 2004a. An experiment of nature: brain anatomy parallels cognition and behavior in Williams syndrome. *J Neurosci,* 24**,** 5009-15.

REISS, A. L., ECKERT, M. A., ROSE, F. E., KARCHEMSKIY, A., KESLER, S., CHANG, M., REYNOLDS, M. F., KWON, H. & GALABURDA, A. 2004b. An experiment of nature: Brain anatomy parallels cognition and behavior in Williams syndrome. *Journal of Neuroscience,* 24**,** 5009-5015.

REISS, S., PETERSON, R. A., GURSKY, D. M. & MCNALLY, R. J. 1986. Anxiety sensitivity, anxiety frequency and the prediction of fearfulness. *Behaviour research and therapy,* 24**,** 1-8.

RIGAL, R. A. 1992. WHICH HANDEDNESS - PREFERENCE OR PERFORMANCE. *Perceptual and Motor Skills,* 75**,** 851-866.

RILEY, J. L., 3RD, ROBINSON, M. E., WISE, E. A., MYERS, C. D. & FILLINGIM, R. B. 1998. Sex differences in the perception of noxious experimental stimuli: a meta-analysis. *Pain,* 74**,** 181-7.

ROBINSON, M. E., CRAGGS, J. G., PRICE, D. D., PERLSTEIN, W. M. & STAUD, R. 2011. Gray matter volumes of pain-related brain areas are decreased in fibromyalgia syndrome. *J Pain,* 12**,** 436-43.

RODRIGUEZ-RAECKE, R., NIEMEIER, A., IHLE, K., RUETHER, W. & MAY, A. 2009. Brain gray matter decrease in chronic pain is the consequence and not the cause of pain. *J Neurosci,* 29**,** 13746-50.

RODRIGUEZ-RAECKE, R., NIEMEIER, A., IHLE, K., RUETHER, W. & MAY, A. 2013. Structural brain changes in chronic pain reflect probably neither damage nor atrophy. *PLoS One,* 8**,** e54475.

ROGERS, J., KOCHUNOV, P., ZILLES, K., SHELLEDY, W., LANCASTER, J., THOMPSON, P., DUGGIRALA, R., BLANGERO, J., FOX, P. T. & GLAHN, D. C. 2010. On the genetic

architecture of cortical folding and brain volume in primates. *Neuroimage,* 53**,** 1103-8.

ROSAS, H. D., LIU, A. K., HERSCH, S., GLESSNER, M., FERRANTE, R. J., SALAT, D. H., VAN DER KOUWE, A., JENKINS, B. G., DALE, A. M. & FISCHL, B. 2002. Regional and progressive thinning of the cortical ribbon in Huntington's disease. *Neurology,* 58**,** 695-701.

ROSENSTIEL, A. K. & KEEFE, F. J. 1983. The use of coping strategies in chronic low back pain patients: relationship to patient characteristics and current adjustment. *Pain,* 17**,** 33-44.

ROTH, R. S., GEISSER, M. E. & WILLIAMS, D. A. 2012. Interventional pain medicine: retreat from the biopsychosocial model of pain. *Translational behavioral medicine,* 2**,** 106-16.

ROUSSON, V., GASSER, T. & SEIFERT, B. 2002. Assessing intrarater, interrater and test-retest reliability of continuous measurements. *Stat Med,* 21**,** 3431-46.

ROY, N. S., WANG, S., JIANG, L., KANG, J., BENRAISS, A., HARRISON-RESTELLI, C., FRASER, R. A., COULDWELL, W. T., KAWAGUCHI, A., OKANO, H., NEDERGAARD, M. & GOLDMAN, S. A. 2000. In vitro neurogenesis by progenitor cells isolated from the adult human hippocampus. *Nat Med,* 6**,** 271-7.

SAAB, C. Y. & WILLIS, W. D. 2003. The cerebellum: organization, functions and its role in nociception. *Brain Res Brain Res Rev,* 42**,** 85-95.

SAGAR, D. R., BURSTON, J. J., HATHWAY, G. J., WOODHAMS, S. G., PEARSON, R. G., BENNETT, A. J., KENDALL, D. A., SCAMMELL, B. E. & CHAPMAN, V. 2011. The contribution of spinal glial cells to chronic pain behaviour in the monosodium iodoacetate model of osteoarthritic pain. *Mol Pain,* 7**,** 88.

SALAFFI, F., CAROTTI, M., STANCATI, A. & GRASSI, W. 2005. Health-related quality of life in older adults with symptomatic hip and knee osteoarthritis: a comparison with matched healthy controls. *Aging Clinical and Experimental Research,* 17**,** 255-263.

SALAT, D. H., BUCKNER, R. L., SNYDER, A. Z., GREVE, D. N., DESIKAN, R. S., BUSA, E., MORRIS, J. C., DALE, A. M. & FISCHL, B. 2004. Thinning of the cerebral cortex in aging. *Cereb Cortex,* 14**,** 721-30.

SAWAMOTO, N., HONDA, M., OKADA, T., HANAKAWA, T., KANDA, M., FUKUYAMA, H., KONISHI, J. & SHIBASAKI, H. 2000. Expectation of pain enhances responses to nonpainful somatosensory stimulation in the anterior cingulate cortex and parietal operculum/posterior insula: an event-related functional magnetic resonance imaging study. *J Neurosci,* 20**,** 7438-45.

SCHAER, M., CUADRA, M. B., SCHMANSKY, N., FISCHL, B., THIRAN, J. P. & ELIEZ, S. 2012. How to measure cortical folding from MR images: a step-by-step tutorial to compute local gyrification index. *J Vis Exp***,** e3417.

SCHAER, M., CUADRA, M. B., TAMARIT, L., LAZEYRAS, F., ELIEZ, S. & THIRAN, J. 2008. A surface-based approach to quantify local cortical gyrification. *Medical Imaging, IEEE Transactions on,* 27**,** 161-170.

SCHAIBLE, H.-G., EBERSBERGER, A. & NATURA, G. 2011. Update on peripheral mechanisms of pain: beyond prostaglandins and cytokines. *Arthritis Res Ther,* 13**,** 210.

SCHAIBLE, H. G. 2012. Mechanisms of chronic pain in osteoarthritis. *Curr Rheumatol Rep,* 14**,** 549-56.

SCHAIBLE, H. G., EBERSBERGER, A. & VON BANCHET, G. S. 2002. Mechanisms of pain in arthritis. *Ann N Y Acad Sci,* 966**,** 343-54.

SCHAIBLE, H. G. & SCHMIDT, R. F. 1988. TIME COURSE OF MECHANOSENSITIVITY CHANGES IN ARTICULAR AFFERENTS DURING A DEVELOPING EXPERIMENTAL ARTHRITIS. *Journal of Neurophysiology,* 60**,** 2180-2195.

SCHEEF, L., JANKOWSKI, J., DAAMEN, M., WEYER, G., KLINGENBERG, M., RENNER, J., MUECKTER, S., SCHURMANN, B., MUSSHOFF, F., WAGNER, M., SCHILD, H. H., ZIMMER, A. & BOECKER, H. 2012. An fMRI study on the acute effects of exercise on pain processing in trained athletes. *Pain,* 153**,** 1702-1714.

SCHMIDT-WILCKE, T., GANSSBAUER, S., NEUNER, T., BOGDAHN, U. & MAY, A. 2008. Subtle grey matter changes between migraine patients and healthy controls. *Cephalalgia,* 28**,** 1-4.

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., LEINISCH, E., STRAUBE, A., KAMPFE, N., DRAGANSKI, B., DIENER, H. C., BOGDAHN, U. & MAY, A. 2005. Gray matter decrease in patients with chronic tension type headache. *Neurology,* 65**,** 1483-6.

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**,** S109-16.

SCHMIERER, K., WHEELER-KINGSHOTT, C. A., BOULBY, P. A., SCARAVILLI, F., ALTMANN, D. R., BARKER, G. J., TOFTS, P. S. & MILLER, D. H. 2007. Diffusion tensor imaging of post mortem multiple sclerosis brain. *Neuroimage,* 35**,** 467-77.

SCHMITT, F., GROSU, D., MOHR, C., PURDY, D., SALEM, K., SCOTT, K. T. & STOECKEL, B. 2004. [3 Tesla MRI: successful results with higher field strengths]. *Radiologe,* 44**,** 31-47.

SCHUFF, N., CAPIZZANO, A. A., DU, A. T., AMEND, D. L., O'NEILL, J., NORMAN, D., KRAMER, J., JAGUST, W., MILLER, B., WOLKOWITZ, O. M., YAFFE, K. & WEINER, M. W. 2002. Selective reduction of N-acetylaspartate in medial temporal and parietal lobes in AD. *Neurology,* 58**,** 928-35.

SEGONNE, F., DALE, A. M., BUSA, E., GLESSNER, M., SALAT, D., HAHN, H. K. & FISCHL, B. 2004. A hybrid approach to the skull stripping problem in MRI. *Neuroimage,* 22**,** 1060-75.

SEGONNE, F., PACHECO, J. & FISCHL, B. 2007. Geometrically accurate topology-correction of cortical surfaces using nonseparating loops. *IEEE Trans Med Imaging,* 26**,** 518-29.

SEHN, F., CHACHAMOVICH, E., VIDOR, L. P., DALL-AGNOL, L., CUSTODIO DE SOUZA, I. C., TORRES, I. L. S., FREGNI, F. & CAUMO, W. 2012. Cross-Cultural Adaptation and Validation of the Brazilian Portuguese Version of the Pain Catastrophizing Scale. *Pain Medicine,* 13**,** 1425-1435.

SEIDMAN, L. J., FARAONE, S. V., GOLDSTEIN, J. M., KREMEN, W. S., HORTON, N. J., MAKRIS, N., TOOMEY, R., KENNEDY, D., CAVINESS, V. S. & TSUANG, M. T. 2002. Left hippocampal volume as a vulnerability indicator for schizophrenia - A magnetic resonance imaging morphometric study of nonpsychotic first-degree relatives. *Archives of General Psychiatry,* 59**,** 839-849.

SEIFERT, C. L., MAGON, S., STAEHLE, K., ZIMMER, C., FOERSCHLER, A., RADUE, E. W., PFAFFENRATH, V., TOLLE, T. R. & SPRENGER, T. 2012. A case-control study on cortical thickness in episodic cluster headache. *Headache,* 52**,** 1362-8.

SEMINOWICZ, D. A. & DAVIS, K. D. 2006. Cortical responses to pain in healthy individuals depends on pain catastrophizing. *Pain,* 120**,** 297-306.

SEMINOWICZ, D. A., LABUS, J. S., BUELLER, J. A., TILLISCH, K., NALIBOFF, B. D., BUSHNELL, M. C. & MAYER, E. A. 2010. Regional gray matter density changes in brains of patients with irritable bowel syndrome. *Gastroenterology,* 139**,** 48-57 e2.

SEMINOWICZ, D. A., WIDEMAN, T. H., NASO, L., HATAMI-KHOROUSHAHI, Z., FALLATAH, S., WARE, M. A., JARZEM, P., BUSHNELL, M. C., SHIR, Y., OUELLET, J. A. & STONE, L. S. 2011. Effective treatment of chronic low back pain in humans reverses abnormal brain anatomy and function. *J Neurosci,* 31**,** 7540-50.

SHEN, L., SAYKIN, A. J., KIM, S., FIRPI, H. A., WEST, J. D., RISACHER, S. L., MCDONALD, B. C., MCHUGH, T. L., WISHART, H. A. & FLASHMAN, L. A. 2010. Comparison of manual and automated determination of hippocampal volumes in MCI and early AD. *Brain Imaging Behav,* 4**,** 86-95.

SHENTON, M. E., DICKEY, C. C., FRUMIN, M. & MCCARLEY, R. W. 2001. A review of MRI findings in schizophrenia. *Schizophrenia Research,* 49**,** 1-52.

SHERRINGTON, C. S. 1906. *The integrative action of the nervous system,* New York,, C. Scribner's sons.

SHI, F., LIU, B., ZHOU, Y., YU, C. & JIANG, T. 2009. Hippocampal volume and asymmetry in mild cognitive impairment and Alzheimer's disease: Meta-analyses of MRI studies. *Hippocampus,* 19**,** 1055-64.

SHROUT, P. E. 1998. Measurement reliability and agreement in psychiatry. *Stat Methods Med Res,* 7**,** 301-17.

SINGER, T., SEYMOUR, B., O'DOHERTY, J., KAUBE, H., DOLAN, R. J. & FRITH, C. D. 2004. Empathy for pain involves the affective but not sensory components of pain. *Science,* 303**,** 1157-1162.

SLED, J. G., ZIJDENBOS, A. P. & EVANS, A. C. 1998. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Trans Med Imaging,* 17**,** 87-97.

SLEMENDA, C., HEILMAN, D. K., BRANDT, K. D., KATZ, B. P., MAZZUCA, S. A., BRAUNSTEIN, E. M. & BYRD, D. 1998. Reduced quadriceps strength relative to body weight - A risk factor for knee osteoarthritis in women? *Arthritis and Rheumatism,* 41**,** 1951-1959.

SMALLWOOD, R. F., LAIRD, A. R., RAMAGE, A. E., PARKINSON, A. L., LEWIS, J., CLAUW, D. J., WILLIAMS, D. A., SCHMIDT-WILCKE, T., FARRELL, M. J., EICKHOFF, S. B. & ROBIN, D. A. 2013a. Structural brain anomalies and chronic pain: a quantitative meta-analysis of gray matter volume. *J Pain,* 14**,** 663-75.

SMALLWOOD, R. F., LAIRD, A. R., RAMAGE, A. E., PARKINSON, A. L., LEWIS, J., CLAUW, D. J., WILLIAMS, D. A., SCHMIDT-WILCKE, T., FARRELL, M. J., EICKHOFF, S. B. & ROBIN, D. A. 2013b. Structural Brain Anomalies and Chronic Pain: A Quantitative Meta-Analysis of Gray Matter Volume. *J Pain*.

SMARR, K. L. & KEEFER, A. L. 2011. Measures of depression and depressive symptoms: Beck Depression Inventory-II (BDI-II), Center for Epidemiologic Studies Depression Scale (CES-D), Geriatric Depression Scale (GDS), Hospital Anxiety and Depression Scale (HADS), and Patient Health Questionnaire-9 (PHQ-9). *Arthritis Care Res (Hoboken),* 63 Suppl 11**,** S454-66.

SMEETS, R. J. E. M., VLAEYEN, J. W. S., KESTER, A. D. M. & KNOTTNERUS, J. A. 2006. Reduction of Pain Catastrophizing Mediates the Outcome of Both Physical and Cognitive-Behavioral Treatment in Chronic Low Back Pain. *The Journal of Pain,* 7**,** 261-271.

SMITH, M. L. & MILNER, B. 1981. The role of the right hippocampus in the recall of spatial location. *Neuropsychologia,* 19**,** 781-93.

SMITH, S. M. 2002. Fast robust automated brain extraction. *Hum Brain Mapp,* 17**,** 143-55.

SMITH, S. M., JENKINSON, M., WOOLRICH, M. W., BECKMANN, C. F., BEHRENS, T. E., JOHANSEN-BERG, H., BANNISTER, P. R., DE LUCA, M., DROBNJAK, I., FLITNEY, D. E., NIAZY, R. K., SAUNDERS, J., VICKERS, J., ZHANG, Y., DE STEFANO, N., BRADY, J. M. & MATTHEWS, P. M. 2004. Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage,* 23 Suppl 1**,** S208-19.

SOFAT, N., EJINDU, V. & KIELY, P. 2011. What makes osteoarthritis painful? The evidence for local and central pain processing. *Rheumatology (Oxford),* 50**,** 2157-65.

SONG, G. H., VENKATRAMAN, V., HO, K. Y., CHEE, M. W. L., YEOH, K. G. & WILDER-SMITH, C. H. 2006. Cortical effects of anticipation and endogenous modulation of visceral pain assessed by functional brain MRI in irritable bowel syndrome patients and healthy controls. *Pain,* 126**,** 79-90.

SORENSEN, G. 2013. Mechanistic Neuroimaging: The New New Thing. Massachusetts General Hospital: Imaging Group. Available: http://www.massgeneralimaging.org/alumni/hampton2011/03_sorensen_mech_neuroimaging.pdf [Accessed 17 December 2013].

SPALLONE, V., MORGANTI, R., D'AMATO, C., GRECO, C., CACCIOTTI, L. & MARFIA, G. A. 2012. Validation of DN4 as a screening tool for neuropathic pain in painful diabetic polyneuropathy. *Diabet Med,* 29**,** 578-85.

SPECTOR, T. D. & MACGREGOR, A. J. 2004. Risk factors for osteoarthritis: genetics. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society,* 12 Suppl A**,** S39-44.

SPIELBERGER, C. D., GORSUCH, R. L., LUSHENE, R. E. & VAGG, P. R. 1983. State-Trait Anxiety Inventory (STAI). *BiB 2010***,** 180.

SPITZER, R. L., MD, K. K. & WILLIAMS, J. B. Diagnostic and statistical manual of mental disorders. American Psychiatric Association, 1980. Citeseer.

SPREEN, E. S. 1998. *A Compendium of Neuropsychological Tests: Administration, Norms, and Commentary*, Oxford Univ. Press, New York.

SRIKANTH, V. K., FRYER, J. L., ZHAI, G., WINZENBERG, T. M., HOSMER, D. & JONES, G. 2005. A meta-analysis of sex differences prevalence, incidence and severity of osteoarthritis. *Osteoarthritis and Cartilage,* 13**,** 769-781.

STANKEWITZ, A., VALET, M., SCHULZ, E., WOLLER, A., SPRENGER, T., VOGEL, D., ZIMMER, C., MUHLAU, M. & TOLLE, T. R. 2013. Pain sensitisers exhibit grey matter changes after repetitive pain exposure: a longitudinal voxel-based morphometry study. *Pain,* 154**,** 1732-7.

STANKIEWICZ, J. M., GLANZ, B. I., HEALY, B. C., ARORA, A., NEEMA, M., BENEDICT, R. H., GUSS, Z. D., TAUHID, S., BUCKLE, G. J., HOUTCHENS, M. K., KHOURY, S. J., WEINER, H. L., GUTTMANN, C. R. & BAKSHI, R. 2011. Brain MRI lesion load at 1.5T and 3T versus clinical status in multiple sclerosis. *J Neuroimaging,* 21**,** e50-6.

STAUD, R., VIERCK, C. J., CANNON, R. L., MAUDERLI, A. P. & PRICE, D. D. 2001. Abnormal sensitization and temporal summation of second pain (wind-up) in patients with fibromyalgia syndrome. *Pain,* 91**,** 165-75.

STEHLING, C., VIETH, V., BACHMANN, R., NASSENSTEIN, I., KUGEL, H., KOOIJMAN, H., HEINDEL, W. & FISCHBACH, R. 2007. High-resolution magnetic resonance imaging of

the temporomandibular joint: image quality at 1.5 and 3.0 Tesla in volunteers. *Invest Radiol,* 42**,** 428-34.

STEJSKAL, E. & TANNER, J. 1965. Spin diffusion measurements: spin echoes in the presence of a time- dependent field gradient. *The journal of chemical physics,* 42**,** 288-292.

STEVENS, F. L., HURLEY, R. A. & TABER, K. H. 2011. Anterior cingulate cortex: unique role in cognition and emotion. *J Neuropsychiatry Clin Neurosci,* 23**,** 121-5.

STUDENIC, P., RADNER, H., SMOLEN, J. S. & ALETAHA, D. 2012. Discrepancies between patients and physicians in their perceptions of rheumatoid arthritis disease activity. *Arthritis Rheum,* 64**,** 2814-23.

SULLIVAN, M. J., THORN, B., HAYTHORNTHWAITE, J. A., KEEFE, F., MARTIN, M., BRADLEY, L. A. & LEFEBVRE, J. C. 2001. Theoretical perspectives on the relation between catastrophizing and pain. *Clin J Pain,* 17**,** 52-64.

SULLIVAN, M. J. L., BISHOP, S. R. & PIVIK, J. 1995. The Pain Catastrophizing Scale: Development and validation. *Psychological Assessment,* 7**,** 524-532.

SUOKAS, A. K., WALSH, D. A., MCWILLIAMS, D. F., CONDON, L., MORETON, B., WYLDE, V., ARENDT-NIELSEN, L. & ZHANG, W. 2012. Quantitative sensory testing in painful osteoarthritis: a systematic review and meta-analysis. *Osteoarthritis Cartilage,* 20**,** 1075-85.

SYMONDS, L. L., GORDON, N. S., BIXBY, J. C. & MANDE, M. M. 2006. Right-lateralized pain processing in the human cortex: an FMRI study. *J Neurophysiol,* 95**,** 3823-30.

TAE, W. S., KIM, S. S., LEE, K. U., NAM, E.-C. & KIM, K. W. 2008. Validation of hippocampal volumes measured using a manual method and two automated methods (FreeSurfer and IBASPM) in chronic major depressive disorder. *Neuroradiology,* 50**,** 569-581.

TARDIF, C. L., COLLINS, D. L. & PIKE, G. B. 2010. Regional Impact of Field Strength on Voxel-Based Morphometry Results. *Human Brain Mapping,* 31**,** 943-957.

TASSAIN, V., ATTAL, N., FLETCHER, D., BRASSEUR, L., DEGIEUX, P., CHAUVIN, M. & BOUHASSIRA, D. 2003. Long term effects of oral sustained release morphine on neuropsychological performance in patients with chronic non-cancer pain. *Pain,* 104**,** 389-400.

TEUTSCH, S., HERKEN, W., BINGEL, U., SCHOELL, E. & MAY, A. 2008. Changes in brain gray matter due to repetitive painful stimulation. *Neuroimage,* 42**,** 845-9.

THOMPSON, D. K., WOOD, S. J., DOYLE, L. W., WARFIELD, S. K., EGAN, G. F. & INDER, T. E. 2009. MR-determined hippocampal asymmetry in full-term and preterm neonates. *Hippocampus,* 19**,** 118-23.

THOMPSON, P. M., HAYASHI, K. M., DE ZUBICARAY, G., JANKE, A. L., ROSE, S. E., SEMPLE, J., HERMAN, D., HONG, M. S., DITTMER, S. S., DODDRELL, D. M. & TOGA, A. W. 2003. Dynamics of gray matter loss in Alzheimer's disease. *Journal of Neuroscience,* 23**,** 994-1005.

THOMPSON, P. M., VIDAL, C., GIEDD, J. N., GOCHMAN, P., BLUMENTHAL, J., NICOLSON, R., TOGA, A. W. & RAPOPORT, J. L. 2001. Mapping adolescent brain change reveals dynamic wave of accelerated gray matter loss in very early-onset schizophrenia. *Proceedings of the National Academy of Sciences of the United States of America,* 98**,** 11650-11655.

TOLLE, T. R., KAUFMANN, T., SIESSMEIER, T., LAUTENBACHER, S., BERTHELE, A., MUNZ, F., ZIEGLGANSBERGER, W., WILLOCH, F., SCHWAIGER, M., CONRAD, B. & BARTENSTEIN, P. 1999. Region-specific encoding of sensory and affective components of pain in the human brain: a positron emission tomography correlation analysis. *Ann Neurol,* 45**,** 40-7.

TRACEY, I. & BUSHNELL, M. C. 2009a. How Neuroimaging Studies Have Challenged Us to Rethink: Is Chronic Pain a Disease? *The Journal of Pain,* 10**,** 1113-1120.

TRACEY, I. & BUSHNELL, M. C. 2009b. How neuroimaging studies have challenged us to rethink: is chronic pain a disease? *J Pain,* 10**,** 1113-20.

TRACEY, I. & MANTYH, P. W. 2007. The cerebral signature for pain perception and its modulation. *Neuron,* 55**,** 377-91.

TSAI, G. C. & COYLE, J. T. 1995. N-ACETYLASPARTATE IN NEUROPSYCHIATRIC DISORDERS. *Progress in Neurobiology,* 46**,** 531-540.

TSOU, K. & JANG, C. S. 1964. STUDIES ON THE SITE OF ANALGESIC ACTION OF MORPHINE BY INTRACEREBRAL MICRO-INJECTION. *Sci Sin,* 13**,** 1099-109.

TSUBOKAWA, T., KATAYAMA, Y., UENO, Y. & MORIYASU, N. 1981. Evidence for involvement of the frontal cortex in pain-related cerebral events in cats: increase in local cerebral blood flow by noxious stimuli. *Brain Res,* 217**,** 179-85.

TUZUN, E. H. 2007. Quality of life in chronic musculoskeletal pain. *Best Practice & Research in Clinical Rheumatology,* 21**,** 567-579.

VACHON-PRESSEAU, E., ROY, M., MARTEL, M. O., ALBOUY, G., CHEN, J., BUDELL, L., SULLIVAN, M. J., JACKSON, P. L. & RAINVILLE, P. 2012. Neural processing of sensory and emotional-communicative information associated with the perception of vicarious pain. *Neuroimage,* 63**,** 54-62.

VACHON-PRESSEAU, E., ROY, M., MARTEL, M. O., CARON, E., MARIN, M. F., CHEN, J., ALBOUY, G., PLANTE, I., SULLIVAN, M. J., LUPIEN, S. J. & RAINVILLE, P. 2013. The stress model of chronic pain: evidence from basal cortisol and hippocampal structure and function in humans. *Brain,* 136**,** 815-27.

VALDES, A. M., LOUGHLIN, J., VAN OENE, M., CHAPMAN, K., SURDULESCU, G. L., DOHERTY, M. & SPECTOR, T. D. 2007. Sex and ethnic differences in the association of ASPN, CALM1, COL2A1, COMP, and FRZB with genetic susceptibility to osteoarthritis of the knee. *Arthritis and Rheumatism,* 56**,** 137-146.

VAN ESSEN, D. C. 1997. A tension-based theory of morphogenesis and compact wiring in the central nervous system. *Nature,* 385**,** 313-8.

VEALE, J. F. 2014. Edinburgh Handedness Inventory - Short Form: a revised version based on confirmatory factor analysis. *Laterality,* 19**,** 164-77.

VELDHUIJZEN, D. S., NEMENOV, M. I., KEASER, M., ZHUO, J. C., GULLAPALLI, R. P. & GREENSPAN, J. D. 2009. Differential brain activation associated with laser-evoked burning and pricking pain: An event-related fMRI study. *Pain,* 141**,** 104-113.

VIDEBECH, P. & RAVNKILDE, B. 2004. Hippocampal volume and depression: A meta-analysis of MRI studies. *American Journal of Psychiatry,* 161**,** 1957-1966.

VILLANUEVA, L. & BERNARD, J.-F. 1999. *The Multiplicity of Ascending Pain Pathways. In: Handbook of behavioral state control : cellular and molecular mechanisms,* Boca Raton, Fla. ; London, CRC Press. pp. 564-575.

VOGT, B. A. 2005. Pain and emotion interactions in subregions of the cingulate gyrus. *Nat Rev Neurosci,* 6**,** 533-44.

VOGT, B. A., BERGER, G. R. & DERBYSHIRE, S. W. 2003. Structural and functional dichotomy of human midcingulate cortex. *Eur J Neurosci,* 18**,** 3134-44.

VOGT, B. A., DERBYSHIRE, S. & JONES, A. K. 1996. Pain processing in four regions of human cingulate cortex localized with co-registered PET and MR imaging. *Eur J Neurosci,* 8**,** 1461-73.

VOGT, B. A., NIMCHINSKY, E. A., VOGT, L. J. & HOF, P. R. 1995. Human cingulate cortex: surface features, flat maps, and cytoarchitecture. *J Comp Neurol,* 359**,** 490-506.

VOGT, B. A., SIKES, R. W. & VOGT, L. J. 1993. *Anterior cingulate cortex and the medial pain system. In: Neurobiology ofcingulate cortex andlimbic thalamus.,* Boston, Basel, Berlin, Birkhauser pp. 313-344.

VRENKEN, H., POUWELS, P. J., GEURTS, J. J., KNOL, D. L., POLMAN, C. H., BARKHOF, F. & CASTELIJNS, J. A. 2006. Altered diffusion tensor in multiple sclerosis normal-appearing brain tissue: cortical diffusion changes seem related to clinical deterioration. *J Magn Reson Imaging,* 23**,** 628-36.

WALLACE, G. L., ROBUSTELLI, B., DANKNER, N., KENWORTHY, L., GIEDD, J. N. & MARTIN, A. 2013. Increased gyrification, but comparable surface area in adolescents with autism spectrum disorders. *Brain,* 136**,** 1956-67.

WANG, C., CHENG, Y., LIU, T., LI, Q., FILLINGIM, R. B., WALLACE, M. R., STAUD, R., KAPLAN, L. & WU, R. 2008. A computational model for sex-specific genetic architecture of complex traits in humans: Implications for mapping pain sensitivity. *Molecular Pain,* 4.

WARTOLOWSKA, K., HOUGH, M. G., JENKINSON, M., ANDERSSON, J., WORDSWORTH, B. P. & TRACEY, I. 2012. Structural changes of the brain in rheumatoid arthritis. *Arthritis Rheum,* 64**,** 371-9.

WATSON, C., ANDERMANN, F., GLOOR, P., JONESGOTMAN, M., PETERS, T., EVANS, A., OLIVIER, A., MELANSON, D. & LEROUX, G. 1992. ANATOMIC BASIS OF AMYGDALOID AND HIPPOCAMPAL VOLUME MEASUREMENT BY MAGNETIC-RESONANCE-IMAGING. *Neurology,* 42**,** 1743-1750.

WHALEN, P. J., BUSH, G., MCNALLY, R. J., WILHELM, S., MCINERNEY, S. C., JENIKE, M. A. & RAUCH, S. L. 1998. The emotional counting Stroop paradigm: a functional magnetic resonance imaging probe of the anterior cingulate affective division. *Biol Psychiatry,* 44**,** 1219-28.

WHEELER-KINGSHOTT, C., BARKER G.J., S., GARETH, STEFAN, S. & VAN BUCHEM, M. 2003. the Diffusion of water. *In:* PAUL, T. (ed.) *Quantitative MRI of the Brain. Measuring Changes Caused by Disease. 1st ed.* Chichester: John Wiley and Sons.

WHITE, T. & HILGETAG, C. C. 2011. Gyrification and neural connectivity in schizophrenia. *Dev Psychopathol,* 23**,** 339-52.

WHITWELL, J. L. 2009. Voxel-based morphometry: an automated technique for assessing structural changes in the brain. *J Neurosci,* 29**,** 9661-4.

WIGNALL, E. L., DICKSON, J. M., VAUGHAN, P., FARROW, T. F. D., WILKINSON, I. D., HUNTER, M. D. & WOODRUFF, P. W. R. 2004. Smaller hippocampal volume in patients with recent-onset posttraumatic stress disorder. *Biological Psychiatry,* 56**,** 832-836.

WILLIS, W. D. & WESTLUND, K. N. 1997. Neuroanatomy of the pain system and of the pathways that modulate pain. *J Clin Neurophysiol,* 14**,** 2-31.

WOLF, H., GRUNWALD, M., KRUGGEL, F., RIEDEL-HELLER, S. G., ANGERHOFER, S., HOJJATOLESLAMI, A., HENSEL, A., ARENDT, T. & GERTZ, H. J. 2001. Hippocampal volume discriminates between normal cognition; questionable and mild dementia in the elderly. *Neurobiology of Aging,* 22**,** 177-186.

WOOLARD, A. A. & HECKERS, S. 2012a. Anatomical and functional correlates of human hippocampal volume asymmetry. *Psychiatry Res,* 201**,** 48-53.

WOOLARD, A. A. & HECKERS, S. 2012b. Anatomical and functional correlates of human hippocampal volume asymmetry. *Psychiatry Research-Neuroimaging,* 201**,** 48-53.

WOOLF, C. J. 1983. Evidence for a central component of post-injury pain hypersensitivity. *Nature,* 306**,** 686-8.

WOOLF, C. J. 2011. Central sensitization: implications for the diagnosis and treatment of pain. *Pain,* 152**,** S2-15.

WRIGHT, I. C., MCGUIRE, P. K., POLINE, J. B., TRAVERE, J. M., MURRAY, R. M., FRITH, C. D., FRACKOWIAK, R. S. J. & FRISTON, K. J. 1995. A Voxel-Based Method for the Statistical Analysis of Gray and White Matter Density Applied to Schizophrenia. *NeuroImage,* 2**,** 244-252.

WU, L. J., TOYODA, H., ZHAO, M. G., LEE, Y. S., TANG, J., KO, S. W., JIA, Y. H., SHUM, F. W., ZERBINATTI, C. V., BU, G., WEI, F., XU, T. L., MUGLIA, L. J., CHEN, Z. F., AUBERSON, Y. P., KAANG, B. K. & ZHUO, M. 2005a. Upregulation of forebrain NMDA NR2B receptors contributes to behavioral sensitization after inflammation. *J Neurosci,* 25**,** 11107-16.

WU, M. F., PANG, Z. P., ZHUO, M. & XU, Z. C. 2005b. Prolonged membrane potential depolarization in cingulate pyramidal cells after digit amputation in adult rats. *Mol Pain,* 1**,** 23.

WYLDE, V., DIEPPE, P., HEWLETT, S. & LEARMONTH, I. D. 2007. Total knee replacement: Is it really an effective procedure for all? *Knee,* 14**,** 417-423.

WYLDE, V., HEWLETT, S., LEARMONTH, I. D. & DIEPPE, P. 2011. Persistent pain after joint replacement: prevalence, sensory qualities, and postoperative determinants. *Pain,* 152**,** 566-72.

YESAVAGE, J. A., BRINK, T., ROSE, T. L., LUM, O., HUANG, V., ADEY, M. & LEIRER, V. O. 1983. Development and validation of a geriatric depression screening scale: a preliminary report. *Journal of psychiatric research,* 17**,** 37-49.

YOUNGER, J. W., CHU, L. F., D'ARCY, N. T., TROTT, K. E., JASTRZAB, L. E. & MACKEY, S. C. 2011. Prescription opioid analgesics rapidly change the human brain. *Pain,* 152**,** 1803-10.

YOUNGER, J. W., SHEN, Y. F., GODDARD, G. & MACKEY, S. C. 2010. Chronic myofascial temporomandibular pain is associated with neural abnormalities in the trigeminal and limbic systems. *Pain,* 149**,** 222-8.

YUCEL, K., TAYLOR, V. H., MCKINNON, M. C., MACDONALD, K., ALDA, M., YOUNG, L. T. & MACQUEEN, G. M. 2008. Bilateral hippocampal volume increase in patients with bipolar disorder and short-term lithium treatment. *Neuropsychopharmacology,* 33**,** 361-367.

YUSUF, E., KORTEKAAS, M. C., WATT, I., HUIZINGA, T. W. & KLOPPENBURG, M. 2011. Do knee abnormalities visualised on MRI explain knee pain in knee osteoarthritis? A systematic review. *Ann Rheum Dis,* 70**,** 60-7.

ZAMBREANU, L., WISE, R. G., BROOKS, J. C., IANNETTI, G. D. & TRACEY, I. 2005. A role for the brainstem in central sensitisation in humans. Evidence from functional magnetic resonance imaging. *Pain,* 114**,** 397-407.

ZHANG, Y., YU, C., ZHOU, Y., LI, K., LI, C. & JIANG, T. 2009. Decreased gyrification in major depressive disorder. *Neuroreport,* 20**,** 378-380.

ZHANG, Y., ZHANG, J., XU, J., WU, X., ZHANG, Y., FENG, H., WANG, J. & JIANG, T. 2014. Cortical gyrification reductions and subcortical atrophy in Parkinson's disease. *Mov Disord,* 29**,** 122-6.

ZHUO, M. 2014. Long-term potentiation in the anterior cingulate cortex and chronic pain. *Philos Trans R Soc Lond B Biol Sci,* 369**,** 20130146.

ZIGMOND, A. S. & SNAITH, R. P. 1983. The hospital anxiety and depression scale. *Acta psychiatrica scandinavica,* 67**,** 361-370.

ZILLES, K., ARMSTRONG, E., SCHLEICHER, A. & KRETSCHMANN, H.-J. 1988. The human pattern of gyrification in the cerebral cortex. *Anatomy and embryology,* 179**,** 173-179.

ZIMMERMAN, M. E., PAN, J. W., HETHERINGTON, H. P., LIPTON, M. L., BAIGI, K. & LIPTON, R. B. 2009. Hippocampal correlates of pain in healthy elderly adults: a pilot study. *Neurology,* 73**,** 1567-70.

4.2 Appendices:

4.2.1 APPENDIX 1. ETHICAL COMITTEE APPROVAL

Approval for the study has been granted by the following regulatory bodies:

- **Nottingham Local Research Ethics Committee 2**

(Local NHS research ethics committee regulating medical research involving patients and healthy volunteers)

REC reference number: 10/H0408/115

- **Queen's Medical Centre Research and Development Department**

(Regulating research performed within QMC)

R&D reference: 11RH001 (CSP 57467)

- The ethical approval was processed for the whole neuroimaging studies in our division including along with my structural MRI studies; functional MRI, 7 Tesla, pre-post knee replacement and longitudinal studies. My involvement was limited to structural imaging related issues during the application process, which was administered mainly by Laura Condon (post-doc), Sharon Forman (divisional secretary), under supervision by the head of the division and principal investigator Professor Dorothee Auer.

4.2.2 APPENDIX 2. FORMS USED IN THE STUDY

Latest approved forms will be presented in the following order:

- Participant information sheet for knee OA patients
- Participant information sheet for healthy controls
- Consent form for knee OA patients
- Consent form for healthy controls

Nottingham University Hospitals NHS
NHS Trust

The University of Nottingham

PATIENT INFORMATION SHEET

"Imaging to Understand Pain in Osteoarthritis"

Name of Investigators*: Prof Dorothee Auer, Prof David Walsh, Prof Brigitte Scammell, Prof Michael Doherty, Prof Nadina Lincoln, Dr Laura Condon, Dr Jennifer Dixon, Mr Hamza Alshuft, Ms Anita French, Dr Naranjargal Dashdorj, Ms Maggie Wheeler*

You have been invited to take part in a research study. Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives if you wish to. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part or not. If you decide to take part you may keep this leaflet. Thank you for reading this.

Background

People with Osteoarthritis (OA) feel pain in their joints, particularly in the knee. This means that they may experience a persisting chronic pain which is processed in a specific way by the brain. By comparing the brains of patients with OA, patients with other musculo-skeletal pain, and healthy volunteers with no pain, we hope to better understand the nature of knee pain in OA.

Why have you been chosen?

You have been asked to take part because you have either:

- Been diagnosed with OA,
- Been diagnosed with musculo-skeletal lower back pain.
- You have previously taken part in research in Academic Rheumatology at The University of Nottingham.
- You have previously taken part in this program of research via questionnaire study (REC reference: 10/H0403/70),

What will happen to me if I take part?

If you volunteer you will be asked to complete some health questionnaires by post and attend the Division of Academic Radiology at the Queen's Medical Centre, or the Sir Peter Mansfield Magnetic Resonance Centre at the University of Nottingham on up to three occasions. During your visits you may be asked to do some or all of the following:

- Undergo an MRI scan of your knee.
- Undergo an MRI scan of your brain
- Undergo a quantitative sensory test (QST) to examine your threshold to different temperature and pressure sensations.
- Fill out some health questionnaires,
- Undergo an MRI scan of your brain as you feel manual pressure applied to your knee.
- You may also need to undergo an x-ray of your knee if there is no recent one available which was taken as part of your routine clinical care.

The MRI scans can take place in up to two or three separate visits. This will be discussed with you by the investigator upon your first visit. If you are taking any strong painkiller

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medications or NSAID's we would like you to stop taking them for 24 hours prior to your MRI scan and take paracetamol instead. Also, on the morning of your visit we may ask you to take a sample of your saliva in a container which we will provide for you and bring it along to your appointment.

Do you have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. Your level of ongoing care will not be affected should you choose to take part, or not to take part. If you do decide to take part, we would like to seek your permission to contact you again in the future regarding other research studies taking place at The University of Nottingham.

What will happen to any samples I give?

The samples will be analysed for levels of the hormone, cortisol, which is associated with stress. The samples will not become part of a human tissue bank and will be destroyed after the analysis is complete.

What are the benefits of taking part?

It is not thought that you will directly benefit from the research visits, as we are not trialling a new treatment or therapy. However, you will be helping us to better understand osteoarthritis pain which could in turn benefit you, and other osteoarthritis patients, in the future through the development of more effective treatment methods. The MRI scans are not, however, used for diagnostic purposes in individual cases, and are not reviewed for this purpose.

What are the possible disadvantages and risks of taking part?

In the event that a recent knee x-ray has not been carried out as part of routine clinical investigation we may need you to undergo a knee x-ray for which you will be exposed to a small amount of ionising radiation. This level of risk is deemed 'trivial' by The Health Protection Agency (HPA), and in radiation terms, is equivalent to approximately 34 days natural background radiation or a single flight to New York.

Part of the QST procedure uses a small device (Peltier Thermode) applied to the skin which will become warm, and cool in temperature, and part of the procedure uses another small device (Pressure Algometer) to press against your knee.

As described above, during one of the scans, you will feel a little pressure on your knee. This may be a little uncomfortable for you, and you can request that we stop at any time. Taking part will also mean taking time out of your normal activities, which may be for a few hours. This may inconvenience you, though we will be as flexible as we can with arranging a suitable time and day, and we will reimburse travel costs (30p per mile if you have driven or had a lift and full reimbursement of public transport travel upon production of the appropriate receipts).

It is possible that if the MRI scan is undertaken by a pregnant woman it will harm the unborn child. Pregnant women, or women who plan to become pregnant during the study period, must not therefore take part in this study. Women who are at risk of pregnancy may be asked to have a pregnancy test before taking part to exclude the possibility of pregnancy. Women who could become pregnant must use an effective contraceptive during the course of this study. Any woman who finds that she has

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become pregnant while taking part in the study should *immediately* tell the research doctor.

As the MRI scan uses a magnetic field you should not take part in this study if you have any metallic foreign bodies in your body (Eg. Pacemaker, aneurysm clips, shrapnel, other metal fragments). Body piercings are allowed providing they can be removed.

In the unlikely event that we should discover any significant unexpected findings on your MRI brain scans, MRI knee scans or knee x-ray, we will ask you to nominate whether you wish us to disclose this information to your GP, who will then discuss it with you. In some cases you will need to disclose a significantly abnormal finding to your employer, and it may affect your insurance status.

What if I don't want to carry on with the study?

Your participation is voluntary and you are free to withdraw at any time, without giving any reason, and without your legal rights being affected. If you withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis.

Involvement of the General Practitioner/Family doctor (GP)

We will seek permission to inform your GP should any abnormal findings be detected on your MRI brain scan, MRI knee scan, or knee x-ray, who will then inform you of the findings, rather than the research clinician.

What if something goes wrong?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. Please contact Professor Dorothee Auer in the first instance, telephone 0115 823 1178. If you remain unhappy and wish to complain formally, you can do this through NHS complaints. Details can be obtained from the Patient Advice and Liaison Service (PALS) at QMC by calling 0800 1830204

Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept on a password protected database and is strictly confidential. Any information about you which leaves the research unit will have your name and address removed so that you cannot be recognised from it.

What will happen to the results of the research study?

The results will be published in medical journals and will be submitted as part of a PhD thesis. You will not be identified in any way in any report or publication. If you ask, we can send you a summary of our findings when the project finishes.

Who is organising and funding the research?

This work is supported by the University of Nottingham and Arthritis Research UK.

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Who has reviewed the study?

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by Nottingham Research Ethics Committee.

Expenses & Payments

Your travel expenses will be reimbursed upon production of a receipt/ticket. Alternatively, if you have travelled to your appointment using your own vehicle, your travel expenses will be reimbursed at 30p per mile.

Contact for Further Information

If you have any questions please feel free to contact:

Maggie Wheeler
Division of Academic Rheumatology, University of Nottingham, CSB Building, City Hospital Campus, Nottingham, NG5 1PB
Tel: 0115 823 1676 Email: maggie.wheeler@nottingham.ac.uk

Or the Chief Investigator:

Professor. Dorothee Auer, Division of Academic Radiology, University of Nottingham, QMC Campus, Derby Road, Nottingham, NG7 2UH
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Faculty of Medicine & Health Sciences
Version 1.6 27/07/12 Patients

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HEALTHY VOLUNTEER'S INFORMATION SHEET

"Imaging to Understand Pain in Osteoarthritis"

Name of Investigators*: Prof. Dorothee Auer, Prof. David Walsh, Prof. Brigitte Scammell, Prof. Michael Doherty, Prof. Nadina Lincoln, Dr. Laura Condon, Dr. Jennifer Dixon, Mr. Hamza Alshuft, Ms Maggie Wheeler*

You have been invited to take part in a research study. Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives if you wish to. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. If you decide to take part you may keep this leaflet. Thank you for reading this.

Background

Patients with Osteoarthritis (OA) feel pain in their joints, particularly in the knee. This means that they may experience a persisting chronic pain which is processed in a specific way by the brain. By comparing the brains of patients with OA and the brains of healthy volunteers we hope to better understand the nature of knee pain in OA.

What does the study involve?

If you volunteer you will be asked to complete some health questionnaires by post and attend the Division of Academic Radiology at the Queen's Medical Centre, or the Sir Peter Mansfield Magnetic Resonance Centre at the University of Nottingham on up to three occasions. During these visits you may:

- Have an x-ray of your knee
- Undergo an MRI scan of your knee.
- Undergo an MRI scan of your brain at rest
- Undergo a quantitative sensory test (QST) to examine your threshold to different temperature and pressure sensations.
- Fill out some health questionnaires,
- Undergo an MRI scan of your brain as you feel manual pressure applied to your knee.
- Undergo an MRI scan of your brain after you have walked up and down for a little while.

The MRI scans can take place in up to three separate visits. This will be discussed with you by the investigator upon your first visit. Also, we may ask you to take a sample of your saliva in a container (which we will provide for you) and bring it along to your appointment.

Why have you been chosen?

You have been asked to take part because we need to compare patients with OA to healthy people of a similar age.

Do you have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. If you decide to take part in the study it may be that you are not called upon because we need to age-match the healthy control group to the OA patient group, however, we would like to seek your permission to contact you again in the future regarding other research studies taking place at The University of Nottingham.

What will happen to any samples I give?

The samples will be transferred to a commercial laboratory where they will be analysed for levels of the hormone, cortisol, which is associated with stress. They will not become part of a human tissue bank and will be destroyed after the analysis is complete.

What are the possible benefits of taking part?

It is not thought that you will directly benefit from the research visits. However, you will be helping us to better understand osteoarthritis pain which could in turn benefit osteoarthritis patients in the future through the development of more effective treatment methods.

The MRI scans are not used for diagnostic purposes in individual cases, and are not reviewed for this purpose.

What are the possible disadvantages and risks of taking part?

We will need you to undergo an x-ray of your knee to confirm the health of your joint, which will mean that you will be exposed to a small amount of ionising radiation. This level of risk is deemed 'trivial' by The Health Protection Agency (HPA), and in radiation terms, is equivalent to approximately 34 days natural background radiation or a single flight to New York.

Part of the QST procedure uses a small device (Peltier Thermode) applied to the skin which will become warm, and cool in temperature, and part of the procedure uses another small device (Pressure Algometer) to press against your knee.

As described above, during one of the scans, you will feel a little pressure on your knee. This may be a little uncomfortable for you, and you can request that we stop at any time. Taking part will also mean taking time out of your normal activities, which may be for a few hours. This may inconvenience you, though we will be as flexible as we can with arranging a suitable time and day, and we will reimburse travel costs (30p per mile if you have driven or had a lift and full reimbursement of public transport travel upon production of the appropriate receipts).

It is possible that if the MRI scan is undertaken by a pregnant women it will harm the unborn child. Pregnant women must not therefore take part in this study, neither should women who plan to become pregnant during the study. Women who are at risk of pregnancy may be asked to have a pregnancy test before taking part to exclude the possibility of pregnancy. Women who could become pregnant must use an effective

contraceptive during the course of this study. Any woman who finds that she has become pregnant while taking part in the study should *immediately* tell her research doctor.

As the MRI scan uses a magnetic field you should not take part in this study if you have any metallic foreign bodies in your body (Eg. Pacemaker, aneurysm clips, shrapnel, other metal fragments). Body piercings are allowed providing they can be removed.

In the unlikely event that we should discover any significant unexpected findings on your MRI brain scans, MRI knee scans or knee x-ray, we will ask you to nominate whether you wish us to disclose this information to your GP, who will then discuss it with you. In some cases you may need to disclose a significantly abnormal finding to your employer, and it may affect your insurance status.

What if I don't want to carry on with the study?

Your participation is voluntary and you are free to withdraw from the study at any time, without giving a reason, and without your legal rights being affected. If you withdraw from the study then the information collected so far will not be erased and this information will be carried forward to be used in the project analysis.

Involvement of the General Practitioner/Family Doctor (GP)

We will seek permission to inform your GP should any significant unexpected findings be detected on your MRI brain scan, MRI knee scan, or knee x-ray, who will then inform you of these findings, rather than the research clinician.

What if something goes wrong?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. Please contact Professor Dorothee Auer in the first instance, telephone 0115 823 1178. If you remain unhappy and wish to complain formally, you can do this through NHS complaints. Details can be obtained from the Patient Advice and Liaison Service (PALS) at QMC by calling 0800 1830204

In the unlikely event that you suffer injury to yourself or damage to your property as a result in taking part in this research, the University does have an insurance policy to cover harm arising as a result of the defect in the design of the study. In addition, all medical practitioners taking part in the research have personal medical negligence cover.

Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept on a password protected database and is strictly confidential. Any information about you which leaves the research unit will have your name and address removed so that you cannot be recognised from it.

What will happen to the results of the research study?

The results will be published in medical journals and will be submitted as part of a PhD thesis. You will not be identified in any way in any report or publication. If you ask, we can send you a summary of our findings when the project finishes.

Who is organising and funding the research?

This work is supported by the University of Nottingham and Arthritis Research UK.

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by Nottingham Research Ethics Committee.

Expenses & Payments

Your travel expenses will be reimbursed upon production of a receipt/ticket. Alternatively, if you have travelled to your appointment using your own vehicle, your travel expenses will be reimbursed at 30p per mile.

Contact for Further Information

If you have any questions please feel free to contact:

Maggie Wheeler
Division of Academic Rheumatology, University of Nottingham, CSB Building, City Hospital Campus, Nottingham, NG5 1PB
Tel: 0115 823 1676, Email: maggie.wheeler@nottingham.ac.uk

Or, the Chief Investigator:

Professor Dorothee Auer, Division of Academic Radiology, University of Nottingham, QMC Campus, Derby Road, Nottingham, NG7 2UH
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Nottingham University Hospitals NHS Trust

The University of Nottingham

CONSENT FORM
(Version 1.6 date 27/07/2012)

Title of Study: Imaging to Understand Pain in Osteoarthritis

REC ref: 10/H0408/115

Name of Researchers: Prof. Dorothee Auer, Prof. David Walsh, Prof. Brigitte Scammell, Prof. Michael Doherty, Prof. Nadina Lincoln, Dr. Laura Condon, Dr Jennifer Dixon, Mr. Hamza Alshuft, Ms Anita French, Dr Naranjargal Dashdorj, Ms Maggie Wheeler

Name of Participant: **Please initial box**

1. I confirm that I have read and understand the information sheet version number 1.6 dated 27/07/12 for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. I understand that should I withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis. ☐
3. I understand that relevant sections of my medical notes and data collected in the study may be looked at by authorised individuals from the University of Nottingham, the research group and regulatory authorities where it is relevant to my taking part in this study. I give permission for these individuals to have access to these records and to collect, store, analyse and publish information obtained from my participation in this study. I understand that my personal details will be kept confidential. ☐
4. I agree to my GP being informed of my participation in this study. ☐
5. Should there be any abnormal findings on either the MRI scans, or x-rays taken as part of this study, I agree to my GP being contacted.

GP name/address .....

6. I agree to having a saliva sample taken ☐
7. I agree to take part in the above study. ☐
8. **Only for those people who participated in the recent questionnaire study on knee pain in osteoarthritis (REC reference: 10/H0403/70):**
   - I give permission for access to my results from questionnaire study (REC reference: 10/H0403/70) (optional) ☐
9. I give permission for the data from this study to be accessed by researchers from the ARUK Pain Centre ☐
10. I agree to being contacted regarding future research studies (optional) ☐

Name of Participant	Date	Signature
Name of Person taking consent	Date	Signature

Version 1.6 27.07.12 Patient

Nottingham University Hospitals NHS Trust

The University of Nottingham

CONSENT FORM
(Version 1.5 date 27/07/12)

Title of Study: Imaging to Understand Pain in Osteoarthritis
REC ref: 10/H0408/115
Name of Researchers: Prof. Dorothee Auer, Prof. David Walsh, Prof. Brigitte Scammell, Prof. Michael Doherty, Prof. Nadina Lincoln, Dr. Laura Condon, Dr Jennifer Dixon, Mr. Hamza Alshuft, Ms Anita French, Dr Naranjargal Dashdorj, Ms Maggie Wheeler

Name of Participant: **Please initial box**

1. I confirm that I have read and understand the information sheet version number 1.5 dated 27/07/12 for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason and without my legal rights being affected. I understand that should I withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis. ☐
3. I understand that data collected in the study may be looked at by authorised individuals from the University of Nottingham, the research group and regulatory authorities where it is relevant to my taking part in this study. I give permission for these individuals to have access to these records and to collect, store, analyse and publish information obtained from my participation in this study. I understand that my personal details will be kept confidential. ☐

4a I agree to having a saliva sample taken. ☐

4b I agree to having an x-ray of my knee. ☐

5. Should there be any significant unexpected findings on either the MRI scans, or x-rays taken as part of this study, I agree to my GP being contacted ☐

Name/Address of GP .....
.....

6. I give permission for the data from this study to be accessed by researchers from the ARUK Pain Centre ☐
7. I agree to take part in the above study. ☐
8. I agree to being contacted regarding future research studies. (optional) ☐

Name of Participant	Date	Signature
Name of Person taking consent	Date	Signature

Version 1.5 27.07.2012 Healthy Volunteer

4.2.3 APPENDIX 3. Supplementary material for chapter 4 (systematic review)

Table 1. Search terms used in identifying relevant literature

Search engine: PubMed					
Search terms combination			**Records[4]**	**Potentially relevant[5]**	**Relevant[6]**
Osteoarthritis pain[1]	**AND**	Brain structure	45	6	4
		GM[3]	4	3	3
		GM density	1	1	1
		Brain volume/ GM volume	2	2	2
		Brain morphology	14	4	3
		Cort* (cortex, cortical)	107	3	1
		Cerebral	50	8	3
		Brain imaging	20	6	2
		Brain MRI	13	5	2
MSK pain[2]		GM	3	3	2
		GM density	1	1	0
		Brain volume	2	1	1
		Brain morphology	27	2	2
		GM volume	2	2	1
		Brain MRI	13	2	2
Chronic pain		GM	68	15	9
		GM density	12	3	3
		Brain volume	50	12	6
		Brain morphology	470	24	7
		GM volume	22	10	4
		Brain MRI	350	36	18

[1]In PubMed the search term "osteoarthritis pain" and "osteoarthritis AND pain" gives exactly same results. Searching was carried out 10-11June 2013. [2]MSK= musculoskeletal, [3]GM= grey matter. 4= records as they emerge from engine search without any filtering. 5= potentially relevant from information in title and abstract. 6= directly relevant as inferred from reading through the article.

Table 2. Quality assessment of studies included in the systematic review

Study*➔ CASP-Question§ ⬇	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Clearly focused issue	**5**	**5**	**5**	**4**	**5**	**4**	**4**	**5**	**4**	**5**	**4**	**5**	**5**	**4**	**3**	**5**	**5**	**4**
Appropriate method to answer the question	**5**	**5**	**5**	**4**	**5**	**4**	**4**	**5**	**4**	**5**	**4**	**5**	**5**	**4**	**3**	**4**	**5**	**4**
Cases recruited in an acceptable way	**4**	**5**	**5**	**3**	**5**	**3**	**4**	**5**	**4**	**5**	**4**	**5**	**5**	**4**	**4**	**4**	**5**	**4**
Controls selected in an acceptable way	**4**	**4**	**4**	**3**	**4**	**3**	**4**	**4**	**3**	**4**	**4**	**5**	**4**	**4**	**3**	**4**	**4**	**4**
The exposure was accurately measured to minimize bias	**5**	**5**	**5**	**4**	**5**	**4**	**4**	**5**	**4**	**3**	**4**	**5**	**5**	**4**	**4**	**4**	**5**	**4**
Controlled for confounding factors	**3**	**3**	**4**	**4**	**4**	**4**	**4**	**4**	**3**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
The results are precise and believable	**4**	**5**	**4**	**3**	**4**	**2**	**4**	**4**	**3**	**4**	**4**	**4**	**4**	**4**	**3**	**4**	**4**	**4**
The results can be applied to the local population	**4**	**4**	**4**	**1**	**2**	**1**	**4**	**4**	**3**	**4**	**3**	**4**	**4**	**3**	**3**	**4**	**4**	**4**
The results fit with other available evidence	**5**	**4**	**4**	**3**	**4**	**2**	**3**	**4**	**3**	**5**	**3**	**5**	**4**	**4**	**4**	**4**	**4**	**4**
Quality score (%)= (n/45)*100	**87**	**89**	**89**	**64**	**89**	**60**	**78**	**89**	**69**	**87**	**76**	**93**	**89**	**78**	**69**	**82**	**89**	**80**

§The answer to CASP questions was originally made possible under 3 replies: Yes, NO, or can't tell. I have made a slight modification to enable quantitative scoring so that the answer will carry a score from 0-5 where 0 is completely NOT met and 5 is strongly fulfilled. *1-18 refers to studies included in the review, see next page for each corresponding study.

Studies (1-18, in table 2) included in the review were as follow:

1) (Apkarian et al., 2004): Chronic back pain is associated with decreased prefrontal and thalamic grey matter density.

2) (Draganski et al., 2006): Decrease of thalamic grey matter following limb amputation.

3) (Schmidt-Wilcke et al., 2006): Affective components and intensity of pain correlate with structural differences in grey matter in chronic back pain patients.

4) (Kuchinad et al., 2007): Accelerated brain grey matter loss in fibromyalgia patients: premature aging of the brain?

5) (Schmidt-Wilcke et al., 2007): Striatal grey matter increase in patients suffering from fibromyalgia--a voxel-based morphometry study.

6) (Buckalew et al., 2008): Chronic pain is associated with brain volume loss in older adults: preliminary evidence.

7) (Hsu et al., 2009): No consistent difference in grey matter volume between individuals with fibromyalgia and age-matched healthy subjects when controlling for affective disorder.

8) (Rodriguez-Raecke et al., 2009): Brain grey matter decrease in chronic pain is the consequence and not the cause of pain.

9) (Buckalew et al., 2010a): Differences in brain structure and function in older adults with self-reported disabling and non-disabling chronic low back pain.

10) (Gwilym et al., 2010a): Thalamic atrophy associated with painful osteoarthritis of the hip is reversible after arthroplasty: a longitudinal voxel-based morphometric study.

11) (Younger et al., 2010): Chronic myofascial temporomandibular pain is associated with neural abnormalities in the trigeminal and limbic systems.

12) (Baliki et al., 2011): Brain morphological signatures for chronic pain.

13) (Gustin et al., 2011): Different pain, different brain: thalamic anatomy in neuropathic and non-neuropathic chronic pain syndromes.

14) (Moayedi et al., 2011): Contribution of chronic pain and neuroticism to abnormal forebrain grey matter in patients with temporomandibular disorder.

15) (Robinson et al., 2011): Grey matter volumes of pain-related brain areas are decreased in fibromyalgia syndrome.

16) (Seminowicz et al., 2011): Effective treatment of chronic low back pain in humans reverses abnormal brain anatomy and function.

17) (Wartolowska et al., 2012): Structural changes of the brain in rheumatoid arthritis.

18) (Rodriguez-Raecke et al., 2013): Structural brain changes in chronic pain reflect probably neither damage nor atrophy.

4.2.4 Appendix 4. Recruitment poster, scanning, and pain related questionnaires

1. Healthy volunteer recruitment poster

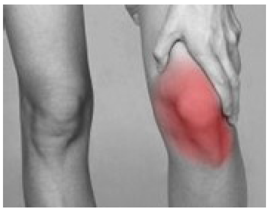
The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Radiological and Imaging Sciences
School of Clinical Sciences
Faculty of Medicine and Health

HEALTHY VOLUNTEERS REQUIRED, 45+

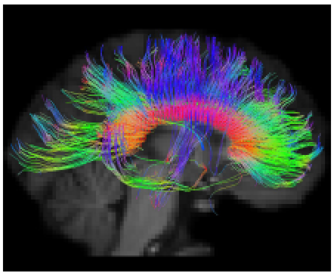
Imaging to understand pain in osteoarthritis

We are looking for healthy volunteers with no knee problems to take part in a brain imaging study aimed at better understanding the pain experienced by patients with Osteoarthritis.

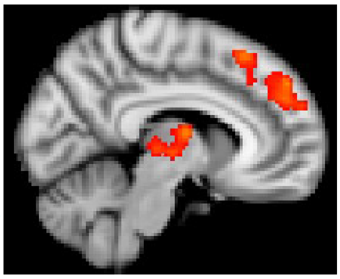


You will be required to attend the Queens Medical Centre, on up to three occasions during which you will be asked to complete a pain questionnaire and undergo an MRI brain scan, and an MRI scan of your knee.

YOU MUST BE:



- Over 45 years old;
- Not Pregnant;
- Have no significant medical conditions;
- Have no metal in your body (Eg. pacemaker, aneurism clips).



Final version 2.1 31.07.2012

FOR MORE INFORMATION, PLEASE CONTACT:

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2. MRI safety questionnaire

The University of Nottingham

HILF/Medical School MRI scanners

MR Volunteer Safety Screening Questionnaire:

NAME	Date of Scan	Date of Birth
ADDRESS	Volunteer Number	
	Ethics Code	
Phone number	Weight	Height if applicable

MR scanning uses strong magnetic fields. For your own safety and the safety of others it is **very important** that you do not go into the magnet halls with any metal in or on your body or clothing. Please answer the following questions carefully and ask if anything is not clear. All information is held in the strictest confidence.

1. Do you have any implants in your body? e.g. replacement joints, drug pumps Y/N
2. Do you have aneurysm clips (clips put around blood vessels during surgery)? Y/N
3. Do you have a pacemaker or artificial heart valve? *(These stop working near MR Scanners)* Y/N
4. Have you ever had any surgery? Please give brief details over. Y/N
   (We do not need to know about uncomplicated caesarean delivery, vasectomy or termination of pregnancy)
5. Do you have any foreign bodies in your body (e.g. shrapnel)? Y/N
6. Have you ever worked in a machine tool shop without eye protection? Y/N
7. Do you wear a hearing aid or cochlear implant? Y/N
8. Could you be pregnant? (Pregnancy tests are available in the female toilets) Y/N
9. Have you ever suffered from tinnitus? Y/N
10. Do you wear dentures, a dental plate or a brace? Y/N
11. Are you susceptible to claustrophobia? Y/N
12. Do you suffer from blackouts, epilepsy or fits? Y/N
13. Do you have any tattoos? (If yes, you may be asked to read and sign another form) Y/N
14. Do you have any body piercing jewellery that cannot be removed? Y/N
15. Do you have any skin patches (trans-dermal patches)? Y/N
16. Do you have a coil in place (IUD) for contraception? Do you know what type? Y/N
17. Do you have any condition that may affect your ability to control your temperature ?
    (e.g. Do you have a fever, cardiovascular disease, hypertension, diabetes or cerebrovascular disease?) Y/N
18. Will you remove all metal including coins, body-piercing jewellery, false-teeth, hearing aids etc. before entering the magnet hall? *(lockers available by the changing rooms)* Y/N
19. Is there anything else you think we should know? Y/N

I have read and understood all the questions **Signature:**	**Date:**
Verified by: **Scanner Operator Only:**	**Date:**

HILF/MIU

3. Edinburgh handedness inventory

Please indicate your preferences in the use of hands in the following activities **by putting a tick in the appropriate column.** Where the preference is so strong that you would never try to use the other hand, unless absolutely forced to, **put 2 ticks**. If in any case you are really indifferent, **put a tick in both columns.**

Some of the activities listed below require the use of both hands. In these cases, the part of the task, or object, for which hand preference is wanted is indicated in parentheses.

Please try and answer all of the questions, and only leave a blank if you have no experience at all with the object or task.

	Left	Right
1. Writing	☐ ☐	☐ ☐
2. Drawing	☐ ☐	☐ ☐
3. Throwing	☐ ☐	☐ ☐
4. Scissors	☐ ☐	☐ ☐
5. Toothbrush	☐ ☐	☐ ☐
6. Knife (without fork)	☐ ☐	☐ ☐
7. Spoon	☐ ☐	☐ ☐
8. Broom (upper hand)	☐ ☐	☐ ☐
9. Striking Match (match)	☐ ☐	☐ ☐
10. Opening box (lid)	☐ ☐	☐ ☐

4. Educational level

Educational level **Date: / /**

Study ID: .....

Please tick the appropriate box that best describes your educational attainment

(Information will be kept private and confidential)

	Level 1	Higher degree and professional qualifications, Postgraduate degree/qualifications
	Level 2	Undergraduate degree
	Level 3	Diploma, certificate (tertiary qualification below degree level)
	Level 4	Other sub-degree (technical and business qualifications above A-level but below degree level)
	Level 5	A and AS level GCE
	Level 6	Below AS level (O-level GCE/ GCSE, CSE, vocational qualifications below sub-degree level)
	Level 7	Apprenticeship
	Level 8	None

Reference: modified from Muriel Egerton and Killian Mullan (2006)

Pain duration=.....

Body weight=... **Kg**

Body height=..... **cm**

BMI=

Notes:.....

5. Pain catastrophizing scale (PCS)

PCS

Client No.: _____ Age: _____ Sex: M(__) F(__) Date: _____

Everyone experiences painful situations at some point in their lives. Such experiences may include headaches, tooth pain, joint or muscle pain. People are often exposed to situations that may cause pain such as illness, injury, dental procedures or surgery.

We are interested in the types of thoughts and feelings that you have when you are in pain. Listed below are thirteen statements describing different thoughts and feelings that may be associated with pain. Using the following scale, please indicate the degree to which you have these thoughts and feelings when you are experiencing pain.

0 – not at all **1** – to a slight degree **2** – to a moderate degree **3** – to a great degree **4** – all the time

When I'm in pain …

1. ☐ I worry all the time about whether the pain will end.
2. ☐ I feel I can't go on.
3. ☐ It's terrible and I think it's never going to get any better.
4. ☐ It's awful and I feel that it overwhelms me.
5. ☐ I feel I can't stand it anymore.
6. ☐ I become afraid that the pain will get worse.
7. ☐ I keep thinking of other painful events.
8. ☐ I anxiously want the pain to go away.
9. ☐ I can't seem to keep it out of my mind.
10. ☐ I keep thinking about how much it hurts.
11. ☐ I keep thinking about how badly I want the pain to stop.
12. ☐ There's nothing I can do to reduce the intensity of the pain.
13. ☐ I wonder whether something serious may happen.

… Total

6. McGill pain questionnaire

McGILL PAIN QUESTIONNAIRE

Client's name: . . . . . **Age:** . . . . .

File no.: . . . . . **Date:** . . . . .

Clinical category (e.g. cardiac, neurological, etc.): . . . . .

Diagnosis: . . . . .

. . . . .

. . . . .

Analgesic (if already administered):

1. Type . . . . .
2. Dosage . . . . .
3. Time given in relation to this test . . . . .

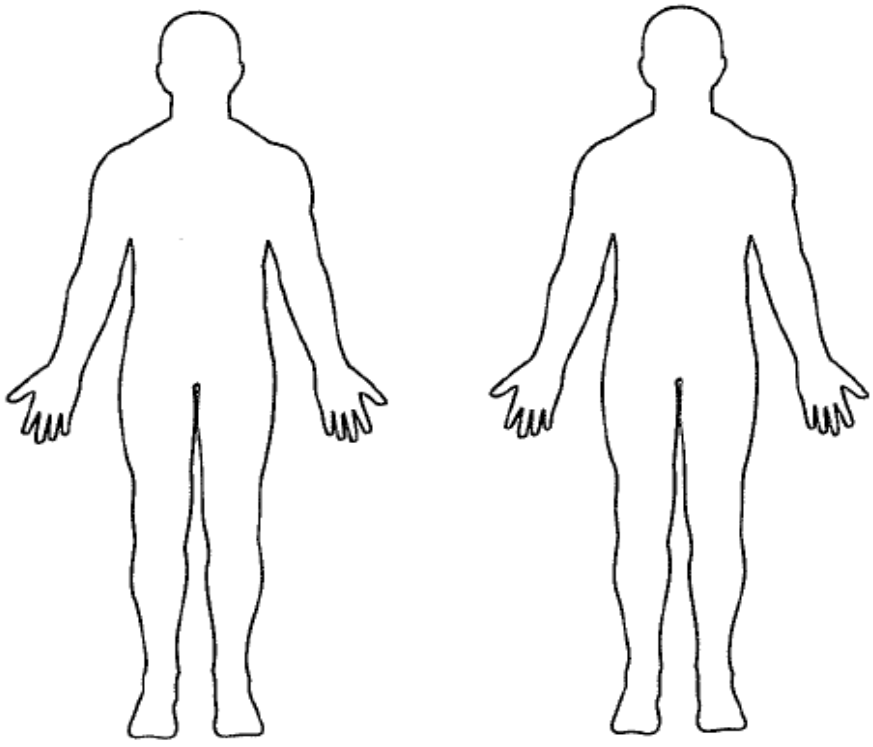
This questionnaire has been designed to tell us more about your pain. Four major questions we ask are:

1. Where is your pain?
2. What does it feel like?
3. How does it change with time?
4. How strong is it?

It is important that you tell us how your pain feels now. Please follow the instructions at the beginning of each part.

Part 1. **Where is your pain?**

Please mark, on the drawing below, the areas where you feel pain. Put E if external, or I if internal, near the areas which you mark. Put EI if both external and internal.



FRONT BACK

Part 2. What does your pain feel like?

Some of the words below describe your *present* pain. Circle *ONLY* those words that best describe it. Leave out any category that is not suitable. Use only a single word in each appropriate category – the one that applies best.

1	2	3	4
Flickering	Jumping	Pricking	Sharp
Quivering	Flashing	Boring	Cutting
Pulsing	Shooting	Drilling	Lacerating
Throbbing		Stabbing	
Beating		Lancinating	
Pounding			
5	6	7	8
Pinching	Tugging	Hot	Tingling
Pressing	Pulling	Burning	Itchy
Gnawing	Wrenching	Scalding	Smarting
Cramping		Searing	Stinging
Crushing			
9	10	11	12
Dull	Tender	Tiring	Sickening
Sore	Taut	Exhausting	Suffocating
Hurting	Rasping		
Aching	Splitting		
Heavy			
13	14	15	16
Fearful	Punishing	Wretched	Annoying
Frightful	Gruelling	Blinding	Troublesome
Terrifying	Cruel		Miserable
	Vicious		Intense
	Killing		Unbearable
17	18	19	20
Spreading	Tight	Cool	Nagging
Radiating	Numb	Cold	Nauseating
Penetrating	Drawing	Freezing	Agonizing
Piercing	Squeezing		Dreadful
	Tearing		Torturing

Part 3. How does your pain change with time?

1. Which word or words would you use to describe the *pattern* of your pain?

1	2	3
Continuous	Rhythmic	Brief
Steady	Periodic	Momentary
Constant	Intermittent	Transient

2. What kind of things *relieve* your pain?

3. What kind of things *increase* your pain?

Part 4. How strong is your pain?

People agree that the following 5 words represent pain of increasing intensity. They are:

1	2	3	4	5
Mild	Discomforting	Distressing	Horrible	Excruciating

To answer each question below, write the number of the most appropriate word in the space beside the question.

1. Which word describes your pain right now? .....
2. Which word describes it at its worst? .....
3. Which word describes it when it is at its least? .....
4. Which word describes the worst toothache you ever had? .....
5. Which word describes the worst headache you ever had? .....
6. Which word describes the worst stomach-ache you ever had? .....

7. PainDETECT

painDETECT

SCHMERZ-FRAGEBOGEN

Datum: Patient: Name: Vorname:

Wie würden Sie Ihren Schmerz **jetzt** im Augenblick einschätzen?

0 1 2 3 4 5 6 7 8 9 10

kein max

Wie stark war der **stärkste** Schmerz in den letzten 4 Wochen?

0 1 2 3 4 5 6 7 8 9 10

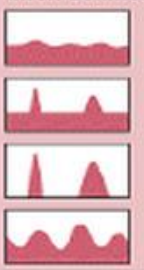
kein max

Wie stark war der Schmerz in den letzten 4 Wochen im **Durchschnitt?**

0 1 2 3 4 5 6 7 8 9 10

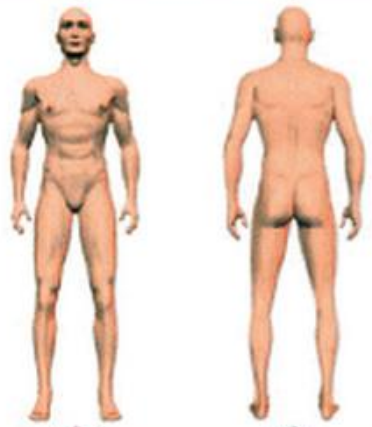
kein max

Kreuzen Sie das Bild an, welches Ihren Schmerzverlauf am besten beschreibt:



- **Dauerschmerzen mit leichten Schwankungen** ☐
- **Dauerschmerzen mit Schmerzattacken** ☐
- **Schmerzattacken dazwischen schmerzfrei** ☐
- **Schmerzattacken dazwischen Schmerzen** ☐

Bitte kennzeichnen Sie Ihren **Hauptschmerzbereich**



Strahlt Ihr Schmerz in weitere Körperregionen aus? ja ☐ nein ☐

wenn ja, dann zeichnen Sie bitte die Richtung ein, wohin der Schmerz ausstrahlt.

Leiden Sie in den eingezeichneten Bereichen an einem Brenngefühl (z.B. Brennnesseln)?

nie ☐ kaum ☐ gering ☐ mittel ☐ stark ☐ sehr stark ☐

Haben Sie im Bereich Ihrer Schmerzen ein Kribbel- oder Prickelgefühl (wie Ameisenlaufen, Stromkribbeln)?

nie ☐ kaum ☐ gering ☐ mittel ☐ stark ☐ sehr stark ☐

Ist leichte Berührung (Kleidung, Bettdecke) in diesem Bereich schmerzhaft?

nie ☐ kaum ☐ gering ☐ mittel ☐ stark ☐ sehr stark ☐

Haben Sie im Bereich Ihrer Schmerzen blitzartige, elektrisierende Schmerzattacken?

nie ☐ kaum ☐ gering ☐ mittel ☐ stark ☐ sehr stark ☐

Ist Kälte oder Wärme (Badewannenwasser) in diesem Bereich gelegentlich schmerzhaft?

nie ☐ kaum ☐ gering ☐ mittel ☐ stark ☐ sehr stark ☐

Leiden Sie in den von Ihnen eingezeichneten Bereichen unter Taubheitsgefühl?

nie ☐ kaum ☐ gering ☐ mittel ☐ stark ☐ sehr stark ☐

Löst ein leichter Druck z.B. mit dem Finger in diesem Bereich Schmerzen aus?

nie ☐ kaum ☐ gering ☐ mittel ☐ stark ☐ sehr stark ☐

(vom Arzt auszufüllen)

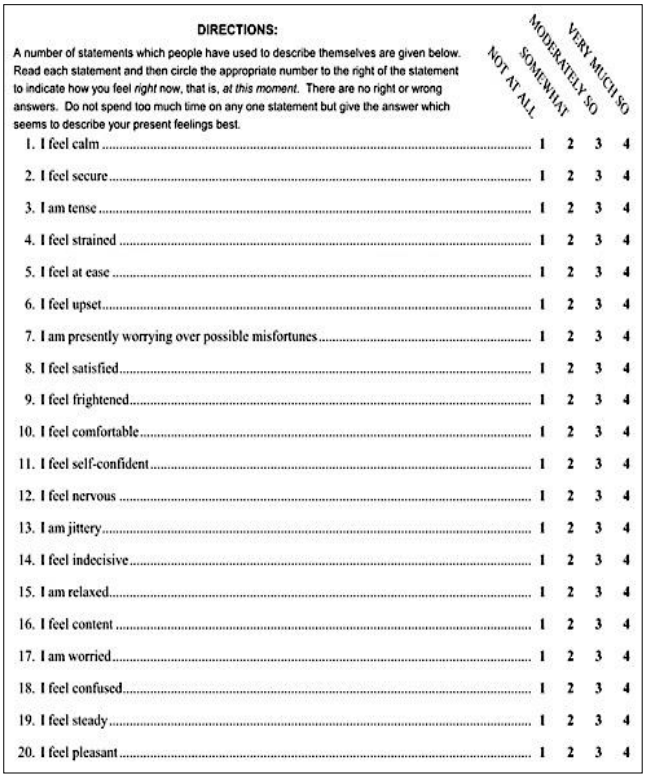
nie	kaum	gering	mittel	stark	sehr stark
x 0 = 0	x 1 =	x 2 =	x 3 =	x 4 =	x 5 =

Score-Gesamtsumme ☐☐ **von 35**

8. Beck's depression inventory (BDI)

- Due to copyright restrictions BDI cannot be shown here

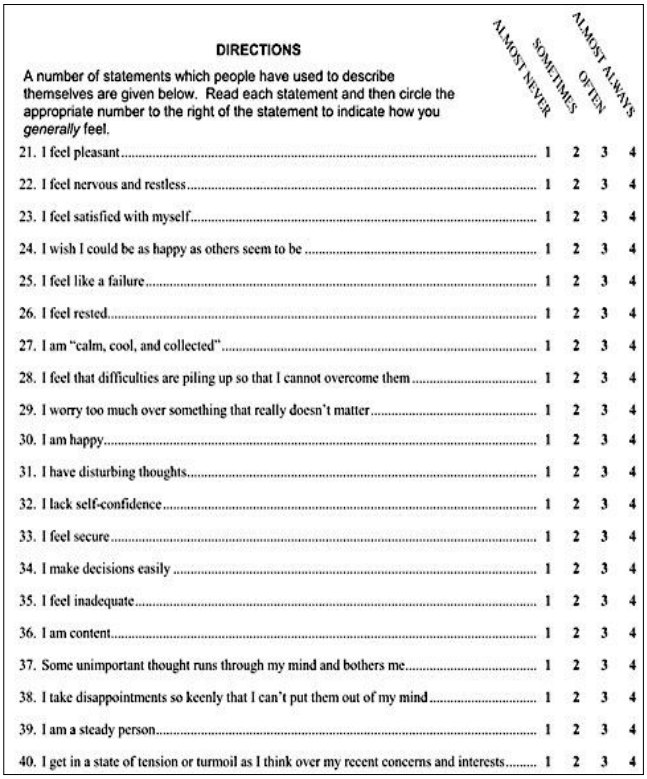
9. Spielberger, State-Trait Anxiety Inventory



DIRECTIONS:

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you feel *right* now, that is, *at this moment*. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

	NOT AT ALL	SOMEWHAT	MODERATELY SO	VERY MUCH SO
1. I feel calm	1	2	3	4
2. I feel secure	1	2	3	4
3. I am tense	1	2	3	4
4. I feel strained	1	2	3	4
5. I feel at ease	1	2	3	4
6. I feel upset	1	2	3	4
7. I am presently worrying over possible misfortunes	1	2	3	4
8. I feel satisfied	1	2	3	4
9. I feel frightened	1	2	3	4
10. I feel comfortable	1	2	3	4
11. I feel self-confident	1	2	3	4
12. I feel nervous	1	2	3	4
13. I am jittery	1	2	3	4
14. I feel indecisive	1	2	3	4
15. I am relaxed	1	2	3	4
16. I feel content	1	2	3	4
17. I am worried	1	2	3	4
18. I feel confused	1	2	3	4
19. I feel steady	1	2	3	4
20. I feel pleasant	1	2	3	4

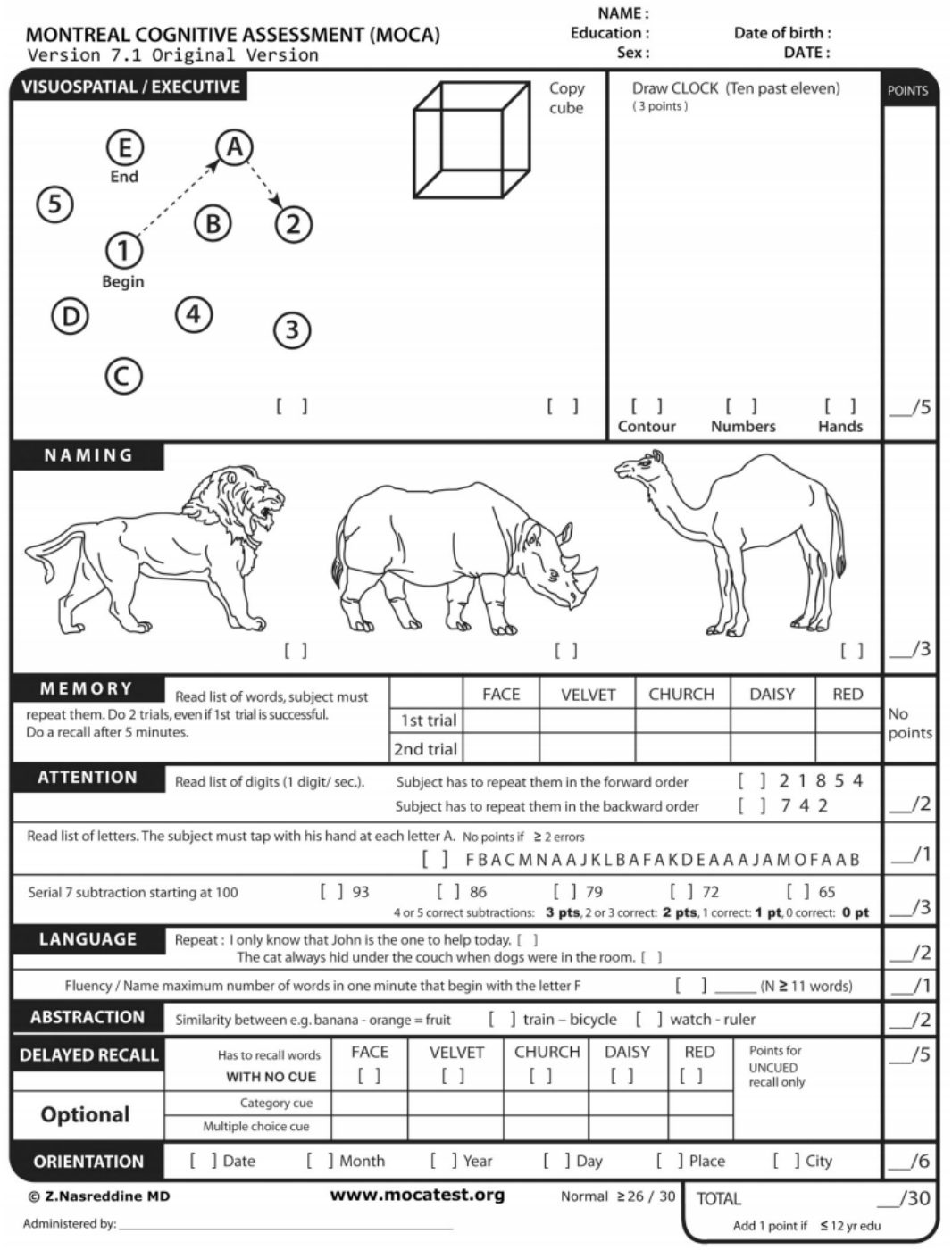


DIRECTIONS

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you *generally* feel.

	ALMOST NEVER	SOMETIMES	OFTEN	ALMOST ALWAYS
21. I feel pleasant	1	2	3	4
22. I feel nervous and restless	1	2	3	4
23. I feel satisfied with myself	1	2	3	4
24. I wish I could be as happy as others seem to be	1	2	3	4
25. I feel like a failure	1	2	3	4
26. I feel rested	1	2	3	4
27. I am "calm, cool, and collected"	1	2	3	4
28. I feel that difficulties are piling up so that I cannot overcome them	1	2	3	4
29. I worry too much over something that really doesn't matter	1	2	3	4
30. I am happy	1	2	3	4
31. I have disturbing thoughts	1	2	3	4
32. I lack self-confidence	1	2	3	4
33. I feel secure	1	2	3	4
34. I make decisions easily	1	2	3	4
35. I feel inadequate	1	2	3	4
36. I am content	1	2	3	4
37. Some unimportant thought runs through my mind and bothers me	1	2	3	4
38. I take disappointments so keenly that I can't put them out of my mind	1	2	3	4
39. I am a steady person	1	2	3	4
40. I get in a state of tension or turmoil as I think over my recent concerns and interests	1	2	3	4

10. Montreal cognitive assessment (MoCA)



MONTREAL COGNITIVE ASSESSMENT (MOCA)
Version 7.1 Original Version

NAME :
Education : Date of birth :
Sex : DATE :

VISUOSPATIAL / EXECUTIVE

Copy cube [ ] [ ]

Draw CLOCK (Ten past eleven) (3 points) [ ] Contour [ ] Numbers [ ] Hands

POINTS ___/5

NAMING [ ] [ ] [ ] ___/3

MEMORY Read list of words, subject must repeat them. Do 2 trials, even if 1st trial is successful. Do a recall after 5 minutes.

	FACE	VELVET	CHURCH	DAISY	RED
1st trial					
2nd trial					

No points

ATTENTION Read list of digits (1 digit/ sec.).
Subject has to repeat them in the forward order [ ] 2 1 8 5 4
Subject has to repeat them in the backward order [ ] 7 4 2 ___/2

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors
[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B ___/1

Serial 7 subtraction starting at 100 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65
4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt** ___/3

LANGUAGE Repeat : I only know that John is the one to help today. [ ]
The cat always hid under the couch when dogs were in the room. [ ] ___/2

Fluency / Name maximum number of words in one minute that begin with the letter F [ ] _____ (N ≥ 11 words) ___/1

ABSTRACTION Similarity between e.g. banana - orange = fruit [ ] train – bicycle [ ] watch - ruler ___/2

DELAYED RECALL

	Has to recall words WITH NO CUE	FACE [ ]	VELVET [ ]	CHURCH [ ]	DAISY [ ]	RED [ ]	Points for UNCUED recall only
Optional	Category cue						
	Multiple choice cue						

___/5

ORIENTATION [ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City ___/6

© Z.Nasreddine MD www.mocatest.org Normal ≥ 26 / 30

TOTAL ___/30
Add 1 point if ≤ 12 yr edu

Administered by: _____

Supplementary Table 1: Characteristics of Knee OA patients

ID	Sex	Age (Y)	Handedness	Educ. level	Pain duration (Y)	Pain severity	PRI	PPI	Pain-DETECT	BDI	MoCA	Dominant knee	PCS	S/T Anxiety	Medications
1	M	68	L	4	10	6	48	21	25	7	28	L	11	12/12	Paracetamol
2	F	72	R	3	20	5	26	19	16	1	27	R	10	15/17	Atenolol, Simvastatin
3	F	54	R	8	38	3	8	16	15	5	26	L	5	15/25	Tranexamic acid
4	M	77	R	6	15	8	29	25	9	9	27	L	13	12/12	Thyroxin, Omeprazole, Silenium
5	F	45	L	4	4	7	21	16	15	7	26	R	23	18/21	None
6	M	66	R	4	15	2	8	13	5	14	29	R	10	10/13	None
7	F	54	R	4	2	10	10	14	0	2	25	R	19	15/37	None
8	F	59	NA	NA	25	6	NA	NA	19	7	20	L	NA	NA	None
9	F	59	R	4	3	4	15	19	12	1	28	L	24	16/25	None
10	M	80	R	3	8	2	6	16	8	7	29	L	7	10/14	None
11	F	71	R	6	14	3	15	15	6	1	26	L	1	10/11	Paracetamol
12	M	65	R	8	5	5	12	16	6	1	27	L	0	10/12	None
13	F	70	R	6	10	5	30	20	14	19	29	L	17	22/28	Losartan, Bendroflumethiazide, Prochlorperazine
14	M	72	R	2	12	1	0	16	16	4	24	L	1	10/12	Atropine eye drops
15	F	63	R	3	20	4	22	19	12	13	29	R	24	18/31	Paracetamol
16	M	56	R	3	10	3	13	15	12	5	30	R	2	13/13	None
17	M	67	R	4	10	2	10	12	7	7	28	R	7	15/15	Glucosamine, Bendroflumethiazide, Simvastatin
18	M	80	R	2	10	2	16	12	17	8	27	L	8	10/22	Amlodipine
19	M	57	R	8	10	4	10	16	10	8	29	R	1	21/17	None
20	M	63	R	6	9	5	0	21	1	0	29	R	10	17/11	Paracetamol, Aspirin, Co-codamol, Lansoprazole, Glucosamine
21	F	64	R	6	3	6	24	19	23	0	29	R	7	10/19	Enalapril, Levothyroxine, Bendroflumethiazide, Amitriptyline
22	M	61	R	3	1	2	8	14	6	2	29	R	7	10/15	None
23	M	63	R	2	5	2	5	14	6	1	30	L	2	10/14	None
24	F	65	R	1	6	7	6	24	10	10	22	L	14	15/19	None
25	M	65	R	6	3	5	26	12	15	4	.	R	6	10/18	None
26	F	67	R	1	5	7	10	17	4	5	30	R	2	24/21	None
27	M	48	R	3	7	6	7	16	12	13	27	R	17	16/25	None
28	F	65	R	3	7.5	7	25	17	18	13	27	R	41	14/20	Amlodipine
29	F	72	R	2	5	7	21	20	18	14	28	R	34	26/25	Ramipril, Atorvastatin
30	M	65	R	3	2.5	10	56	20	9	8	26	R	15	14/18	Amlodipine
31	F	56	R	7	1	2	21	21	10	0	30	L	16	11/13	None

Handedness= based on Edinburgh Handedness Inventory, Educational level= 1-8 (1 is post-graduate levels and 8 is none, PRI and PPI= Pain Rating and Present Pain Indices from McGill pain questionnaire, PainDETECT, BDI= Beck's Depression Inventory. MoCA= Montreal Cognitive Assessment, PCS= Pain Catastrophizing Scale, S/T= state and Trait anxiety.

4.2.5 APPENDIX 5. Supplementary material for study 1, chapter 8

Supplementary Table 1: Detailed description of regions included within each cluster that exhibited significant negative correlation with pain duration in knee OA patients

Cluster	Size (mm2)	Sub-clusters
R 1	12382.5	**Posterior ramus of lateral sulcus, temporal plane, superior temporal gyrus, supramarginal gyrus, post-central sulcus, intraparietal sulcus, angular gyrus, subcentral gyrus, superior parietal lobule, parieto-occipital sulcus, cuneus, calcarine sulcus, precuneus, superior occipital gyrus, superior parietal lobule**
2	9714.8	**Fronto-marginal gyrus and sulcus, transverse fronto-polar gyri and sulci, orbital gyri and sulci, inferior and middle frontal sulci, inferior part of the precentral sulcus, precentral gyrus, middle frontal gyrus, superior frontal gyrus and sulcus, anterior part of the cingulate gyrus and sulcus, middle-anterior and middle posterior parts of the cingulate gyrus and sulcus**
3	556.9	**Inferior frontal sulcus, inferior part of the precentral, sulcus, opercular part of the inferior frontal gyrus**
4	568.5	**Temporal pole**
5	364.7	**Marginal branch of the cingulate sulcus, precuneus**
6	121.1	**Anterior transverse temporal gyrus (of Heschl), inferior segment of the circular sulcus of the insula**
7	97.6	**Superior temporal sulcus (parallel sulcus)**
8	23.4	**Middle temporal gyrus**
9	27.3	**Opercular part of the inferior frontal gyrus**
L 10	11949.8	**Lateral aspect of the superior temporal gyrus, superior temporal sulcus, temporal plane, posterior ramus of the lateral sulcus, supramarginal gyrus, post-central sulcus, superior parietal lobule, intraparietal sulcus, angular gyrus, sulcus intermedius primus, parieto-occipital sulcus, precuneus, subparietal sulcus, posterior-dorsal and middle-posterior parts of the cingulate gyrus and sulcus, marginal branch of the cingulate sulcus, posterior-ventral part of the cingulate gyrus and para-central lobule and sulcus**
11	254.6	**Posterior medial part of superior frontal gyrus**
12	88	**Superior part of inferior frontal sulcus**
13	49.8	**Superior anterior part of frontal gyrus**

Supplementary Figure1.

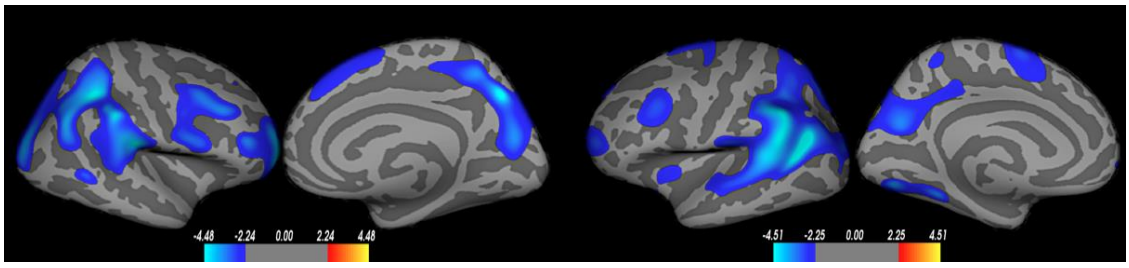


Figure 1: Surface map of progressive cortical thinning over pain duration in female Knee OA participants (p<0.025 FDR, controlled for age)

Supplementary Table 2: Detailed description of regions included within each cluster that exhibited significant negative correlation with pain duration in FEMALES only patients

Cluster	Size (mm2)	Sub-clusters
R 1	12401.5	**Posterior ramus of lateral sulcus, temporal plane, superior temporal gyrus and sulcus, sulcus intermedius primus, supramarginal gyrus, post-central sulcus, intraparietal sulcus, angular gyrus, superior parietal lobule, transverse occipital sulcus, middle occipital gyrus, middle occipital and lunatus sulcus, parieto-occipital sulcus, cuneus, calcarine sulcus, precuneus, subparietal sulcus, superior occipital gyrus, marginal branch of the cingulate sulcus, superior parietal lobule**
2	3315.9	**Fronto-marginal gyrus and sulcus, transverse fronto-polar gyri and sulci, orbital gyri and sulci, inferior and middle frontal sulci, middle frontal gyrus, superior frontal gyrus and sulcus,**
3	2761.6	**Opercular part of the inferior frontal gyrus, Inferior frontal sulcus, inferior part of the precentral sulcus, precentral gyrus, posterior part of middle frontal gyrus.**
4	242.3	**Posterior part of middle temporal gyrus**
5	1084.8	**Superior frontal gyrus**
6	274.1	**Temporal pole**
7	12	**Superior frontal sulcus**
8	8.8	**Anterior part of inferior temporal gyrus**
L 9	13201.0	**Lateral aspect of the superior temporal gyrus, superior temporal sulcus, anterior transverse temporal gyrus (of Heschl), temporal plane, posterior ramus of the lateral sulcus, supramarginal gyrus, subcentral gyrus and sulcus, post-central sulcus, superior parietal lobule, intraparietal sulcus, angular gyrus, sulcus intermedius primus, middle occipital gyrus, transverse occipital sulcus, cuneus, parieto-occipital sulcus, precuneus, subparietal sulcus, marginal branch of the cingulate sulcus**
10	1016	**Posterior transverse collateral sulcus, collateral and lingual sulcus and gyrus, medial occipito-temporal gyrus**
11	1504	**Superior frontal gyrus, middle-anterior part of the cingulate gyrus and sulcus, superior part of pre-central sulcus**
12	856.9	**Inferior frontal sulcus, middle frontal gyrus**
13	814.1	**Middle frontal gyrus and sulcus, fronto-marginal gyrus and sulcus, transverse fronto-polar gyrus and sulcus**
14	299.4	**Short insular gyrus, superior segment of the circular sulcus of the insula, long insular gyrus and central sulcus of the insula**
15	206	**Superior frontal gyrus**
16	125	**Precuneus, marginal branch of the cingulate sulcus**

4.2.6 APPENDIX 6. Quality assurance of freesurfer analysis output

Table 1. Evaluation of freesurfer analysis output data by a second researcher with freesurfer expertise using criteria shown in the table

Subject	Holes	Bumps	Dura/skull	Missing part of Gyrys/ Sulcus
002	1	0	0	0
007	0	0	0	2
013	0	0	0	0
017	0	0	0	2
022	0	0	0	0
027	0	0	0	2
031	0	0	0	1
037	0	0	0	0
048	0	0	0	1
053	0	0	0	0
057	0	0	0	1
062	0	0	0	0
066	0	0	0	0