Investigation of cortical and sub-cortical processing of pressure pain transduction in healthy controls and chronic lumbar radicular pain pre and post oxycodone treatment- An event related fMRI Study.

TRINITY COLLEGE DUBLIN

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A thesis submitted in partial fulfilment of the requirements for the degree of Doctor in Medicine at Trinity College Dublin.

Declaration

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Summary

Thesis motivation

The objective of this thesis was to explore the response of healthy controls versus chronic pain patients to pressure pain stimuli using fMRI in order to better understand the central pain processing in health and disease. Furthermore we investigated the effect of oxycodone therapy in patients with chronic pain and its effect on brain responses to pressure pain stimuli applied to the non-dominant thumb and it was always left thumb nail bed.

Introduction

Pain is universally accepted as both subjective and complex and the methods used to assess pain seem primitive and crude. The interaction between daily functioning, emotion, pain perception and wellbeing is individual and complicated. There are numerous pain assessment tools for acute pain, chronic pain, and neuropathic pain. The most commonly clinically utilized tools in Ireland are VAS, 0–10 Numeric Pain Rating Scale, Wong-Baker FACES Pain Rating Scale pain quality assessment scale for acute pain assessment, the McGill pain questionnaire and Brief Pain Inventory for chronic pain, the LEEDS and DN4 for neuropathic pain. Personality traits and affective states are also known to impact pain perception. These can be measured using tools such as the Minnesota Multiphasic Personality Inventory, The Beck Depression Inventory to assess depressive symptoms and the Hospital Anxiety and Depression Scales (HADS). This list is far from exhaustive however none of these tools provide consistently accurate, reproducible and clinically useful information that is applicable to the patient population attending pain clinics. While these tools can help to diagnose and chart changes in pain they are far from definitive and have a supportive role only. It is well recognised that each person will experience and rate their pain differently, so one person's subjective rating of five, on a scale of one to ten, could be worse than another person's seven, whilst a nine may or may not be bad enough to keep someone else from working. As the brain is the final terminal for sensory and emotional processing leading to perception it is reasonable to conclude that the objective answer of pain processing should lie in the brain[1].

Overview of chronic pain.

Experimental acute pain stimulus in healthy controls can predict with more than 90% accuracy whether a thermal stimulus is just warm or painfully hot[2]. Unlike acute pain, chronic pain patients show a shift in brain pain signatures. Activation of the insula is commonly associated with acute pain while in chronic pain brain activations are observed in the medial prefrontal cortex, which is associated with higher cognitive abilities such as memory and decision-making, and the amygdala, which plays a central role in emotional processing[3].

In neuroimaging studies, mechanical (pressure) and electrical stimuli have not been commonly used in either chronic pain patients or healthy volunteer groups because the use of hand-held pressure algometer to induce pressure pain has several limitations. The application of consistent pressure is very difficult and in fMRI pain studies is limited by failure to synchronise algometer activation with MR pulse sequence[4]. However, the experimental pressure pain stimulus modality is considered a relevant experimental model for clinical pain experienced by chronic pain patients[5]. In the current study, a novel, custom-built electronic pressure algometer was used to deliver pressure stimuli of different magnitudes.

Although they are widely prescribed for chronic and acute pain management, opioids are not curative for pain in general or for neuropathic pain specifically. Compelling evidence exists however for the efficacy of opioids as a core part of multimodal and multi-drug therapy for chronic neuropathic pain[6].

We hypothesized that healthy subjects would show less brain activation in response to experimental pain stimuli compared to chronic pain patients before treatment with oral oxycodone, and, that the greater magnitude of brain activation in chronic pain patients would be blunted by oral oxycodone administration. However, contrary to our prediction, we saw less cortical and sub-cortical activation in brain areas including cerebellar activation in chronic pain patients compared to healthy controls and also found more activation in brain areas including cerebellum in patients with chronic pain after treatment with oxycodone. These findings are consistent with multiple researchers reporting that neuronal regeneration is possible with effective treatment of chronic pain.

Acknowledgements

First and foremost, I thank my academic advisor, Professor Connail Mc Crory, Dean, Faculty Of Pain Medicine of Ireland for accepting me into his group. During my tenure, he contributed to a rewarding graduate school experience by giving me intellectual freedom in my work, supporting my attendance at various conferences, and engaging me in new ideas and demanding a high quality of work in all my endeavours. The door to his office was always open whenever I ran into trouble or had a question about my research or writing. He consistently allowed this research to be my own work, but steered me in the right direction whenever he thought I needed it.

I am very thankful to Prof. Arun Bokde, Professor of Bio-Statistical Imaging, Trinity College Dublin and co-supervisor for collaborating in this research with us and guiding me through every step. Every result described in this thesis was accomplished with the help and support of our collaborators.

I am very grateful to Prof. Bokde and Mr Fran Hegarty, Principal Physicist, St. James's Hospital, Dublin for innovating a novel pressure pain device for this research. Without their passionate participation and input, this research could not have been successfully conducted.

My special thanks to Dr Liz Kehoe, Post-doc, Trinity College Dublin for her constant support and without her efforts my job would have undoubtedly seemed impossible. I greatly benefited from her keen scientific insight, her knack for solving seemingly intractable practical difficulties, and her ability to put complex ideas into simple terms.

I would also like to acknowledge Mr Sojo Joseph, MRI technical group at Trinity College for his support, ideas and for excellent imaging skills.

I am indebted to Mrs Patricia Duff, Mrs Carmel Daly, Mrs Deidra O'Dowd and Mrs Lynn Rob, Chronic Pain Clinical Nurse Managers and Surgical day ward

staff, St James's Hospital Dublin, for their constant encouragement and support.

Finally, I must express my very profound gratitude to my wife Jayashri and children Shashank, Kushal and Vishal for providing me with unfailing support and continuous encouragement throughout my years of research. This accomplishment would not have been possible without them.

Thank you.

Author

[Hari Gopal K.S.]

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1. Introduction

1.1 Overview of pain and chronic pain.

Nociception and pain are an integral part of life; however, nociception and pain are not identical. Nociception represents the neural circuitry that underlies the perception of pain and is the sensory process that provides the signal that triggers pain.[7] Pain on the other hand is a subjective negative evaluation of sensory inputs. It is a perceptual rather than a purely sensory experience similar to the experience of hearing or seeing as a consequence of the activation of the auditory or visual systems. As seeing comprises more than vision and hearing comprises more than audition, pain comprises more than just nociception, but also relies upon psychological factors such as pain appraisal, mood and individual differences in pain threshold and experience[8].

Pain is difficult to assess clinically as there is large inter-individual variation in pain reporting in response to the same pain stimulus. Pain is the most frequent reason for patients seeking medical care and effective therapy is a pressing and growing clinical challenge. Chronic pain is defined as any pain lasting more than 12 weeks. Whereas acute pain is a normal sensation that alerts us to possible injury, chronic pain is very different. Chronic pain persists often for months or even longer[9] and internationally recognized as a major health care problem[10]. Although acute pain plays an important role in protecting a person against injury and the consequences of injury, chronic pain is not protective and is now recognised as a disease in its own right with no apparent physiological benefit. The origin of pain and its dual role as both a key physiological function but also as a debilitating disease when chronicity is established, has fascinated philosophers and scientists for centuries[11].

Pain is a complex, multi-dimensional experience. Major progress has been made in understanding the molecular and cellular mechanisms involved in the generation and conduction of neural signals resulting in pain perception. Pain remains primarily subjective with huge inter-individual interpretations of identical noxious stimuli [12-14].

1.2 Pain Receptors

Specialized peripheral sensory neurons known as nociceptors alert us to potentially damaging stimuli at the skin by detecting extremes in temperature and pressure and injury-related chemicals, and transducing these stimuli into long-ranging electrical signals that are relayed to higher brain centres. The activation of functionally distinct cutaneous nociceptor populations and the processing of information they convey and provide a rich diversity of pain qualities. Current work in this field is providing researchers with a more thorough understanding of nociceptor cell biology at molecular and systemic levels, and it is hoped that this information will promote the targeted design of novel pain therapies[15].

Sensory neurons which respond to noxious stimuli and give rise to pain sensations are classified as A-Beta (A-β), A-Alpha (A-δ) or unmyelinated C-fibres based on the degree of myelination and speed of neuronal transmission. They are further classified according to sensory modality such as thermoreceptors, nociceptors and mechanoreceptors[16]. The traditional view amongst pain researchers and clinicians was that only small diameter unmyelinated C fibres and myelinated A-δ fibres conduct nociceptive information. However that view is being challenged by recent evidence from primates and non-primates suggesting that a substantial fraction of the A-fibre nociceptors may conduct in the A δ -β conduction velocity and that the signal from nociceptors is carried by unmyelinated C- fibres[17, 18].

Each receptor is specially adapted to detect mechanical, chemical, or thermal stimuli. Activation of any of these receptors is converted into neural impulses and this sensory input is conveyed by specialized myelinated (A β and A δ fibres) or unmyelinated polymodal (c- fibres) to their respective nuclei in the central nervous system (CNS). The sensory information is then further processed, as it progresses, via the ascending sensory systems to the cerebral cortex or to the cerebellum. Sensory information is also relayed to other parts of the CNS where it may result in reflex responses.

The skin has homeostatic and immunological barrier functions, but also acts as a complex sensory organ. The neuroimmunocutaneous system (NICS) is responsible for the cutaneous sensation of touch, pressure, temperature and pain[19]. The Meissner corpuscles are widely regarded as low-threshold mechanoreceptors. These corpuscles are multi-afferented receptor organs with Aα-β-fibres that are closely intertwined with endings of peptidergic C-fibres Substance P (SP) and Calcitonin gene related peptide (CGRP) that may have nociceptive capabilities[20].

1.3 Sensory pathways

The ascending sensory pathways are classified according to the functional components they carry as well as by their anatomical location. They comprise the general somatic afferent (GSA) system and the general visceral afferent (GVA) system. The GSA system transmits sensory information such as touch, pressure, vibration, pain, temperature, stretch and position sense from somatic structures. The GVA transmits sensory information such as pressure, pain and other visceral sensation from visceral structures. Anatomically, the ascending sensory systems consist of three distinct pathways: the anterolateral system (ALS), the dorsal column–medial lemniscal (DCML) pathway, and the somatosensory pathways to the cerebellum.

The ALS is the main ascending sensory pain pathway and includes the spinothalamic (STT), spinoreticular (SRT), spinomesencephalic (SMT), spinotectal, and spinohypothalamic tracts (SHT). These tracts relay predominantly pain and temperature sensations to the brain as well as non-discriminative (crude or poorly localized) touch, pressure, and some proprioceptive sensation. Recent evidence suggest approximately 15% of nociceptive fibres project directly to the thalamus via the STT and the remaining 85% of fibres are relayed to the thalamus via extra thalamic pathways[21]. The SMT is involved in nociception[22] but, it is unclear if it contributes to the sensory-discriminative aspect or the motivational, affective aspects of pain [23](Figure 1).

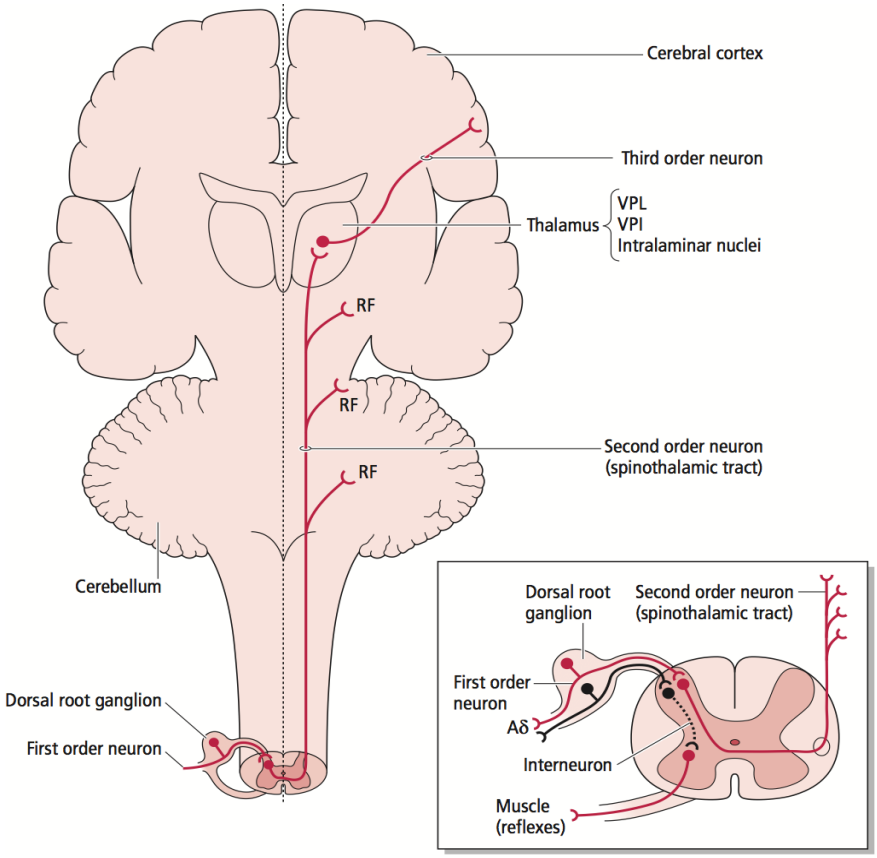

Figure 1. The direct pathway of the anterolateral system. Note the first order neuron in the dorsal root ganglion, the second order neuron in the dorsal horn of the spinal cord, and the third order neuron in the thalamus. The second order neuron sends collaterals to the reticular formation (RF). VPI, ventral posterior inferior; VPL, ventral posterior lateral thalamus (Text book of neuroanatomy by Maria A. Patestas and Leslie P. Gartner; Chapter 10)

The dorsal column medial lemniscal pathway (DCMP) relays fine tactile information, whilst the somatosensory pathways to the cerebellum, which include the anterior, posterior, and rostral spinocerebellar, as well as the cuneocerebellar tracts, relay primarily proprioceptive and also some pain and pressure information[21]. The cerebellum is regarded as a part of the CNS that is implicated mainly in motor behaviour and its coordination. However, numerous studies have shown it to have a broad diversity of functions and may be involved in a wide range of functions including attention, associative learning, practice-related learning, procedural learning, motor skill acquisition, declarative memory, working memory, semantic association, conditioned anxiety, mental exploration, complex reasoning and problem solving as well as motor control

and sensory operations [24, 25]. Data indicating that the cerebellum is also involved in nociception has been explored in recent years[26]. A recent elegant study investigating cerebellar clustering and functional connectivity during pain processing inferred that the cerebellum is involved in various aspects of nociception reflecting the multidimensional aspect of pain[27] (Figure 2).

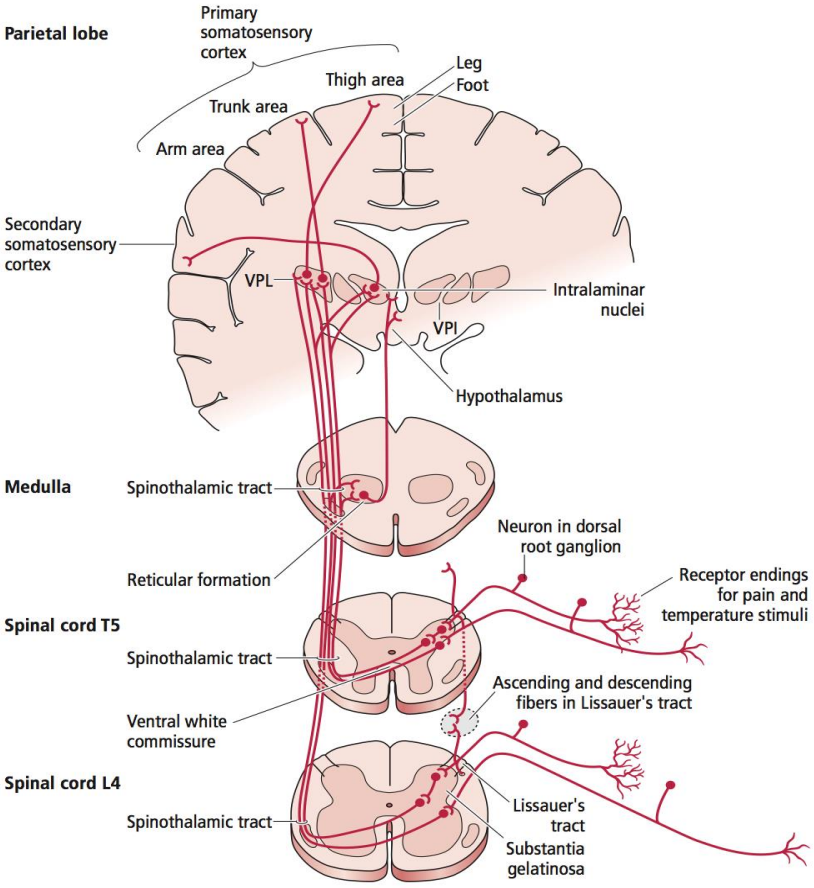

Figure 2. The ascending sensory pathway that transmits non-discriminative (crude) touch, pain and temperature sensations from the body (Textbook of neuroanatomy by Maria A. Patestas and Leslie P. Gartner; Chapter 10)

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1.4 Role of non-invasive brain imaging in pain

A large number of experimental paradigms and imaging techniques such as positron emission tomography (PET), functional magnetic resonance imaging (fMRI) and magneto encephalography (MEG) employing different modalities of acute experimental pain induction in healthy subjects have shown a consistent and reproducible activation of a set brain regions labelled as “acute nociceptive pain-related brain activity”. Considerable research has been undertaken to demystify the functional properties and the distinction between the activated areas for stimulus intensity, lateralisation relative to stimulus and the brain areas reflecting sensory in contrast to affective properties of pain and those areas that are better modulated by attention, mood or presence of other sensory inputs[9]. The currently available data shows that acute painful stimulus in healthy controls commonly activates somatosensory, insular and cingulate cortical regions[28-34]. Chronic pain is often associated with spontaneous pain (which occurs in the absence of a stimulus) and changes in sensitivity to various somatosensory stimuli[35]. Since chronic pain is associated with constant pain in the background these patients show double dissociation between brain regions signalling the perception of on going chronic pain from the perception of acute experimental pain. The neuroimaging studies in this group consistently has shown activation of prefrontal cortex, limbic and paralimbic regions [9].

Non- Invasive brain imaging techniques such as functional magnetic resonance imaging (fMRI) have opened exciting new avenues for studying how the human brain gives rise to complex behaviours, cognitive processes and emotions. Recent advances in fMRI has revolutionized our concept of central pain processing in health and disease[36]. fMRI has revealed that chronic pain preferentially activates prefrontal, limbic and paralimbic brain areas more than acute pain, which explains why treatments for acute pain have little effect on chronic pain[9]. This technology has opened the door to biofeedback pain control as well as improved testing of new drugs and other therapies[5, 36].

Chronic pain is a common condition affecting 35.5 % of the Irish population and chronic low back pain has emerged as the most common syndrome. The study

also revealed that 12% are unable to work or had reduced work hour and 15% met the criteria for clinically relevant depression. The mean cost per chronic pain patient was estimated at 5665 euros per year. The total cost for adults with chronic pain was estimated at 4.76 billion euros per year, representing 2.55% of Irish GDP in 2008;however its pathophysiology is poorly understood[37, 38]. Functional neuroimaging offers a method of exploring the central processing of pain in chronic pain patients who may differ in their experience of pain compared to healthy controls. A better understanding of how the brain responds to pain in patients with chronic pain is important as it may help to elucidate how chronicity is maintained and may be used to guide new therapeutic interventions.

1.5 Experimental pain induction methods

Experimental pain can be induced by several techniques. In addition to diversity in stimulus intensity and pain measurements, different modalities of pain stimuli have also been used across studies, such as heat and cold stimuli and electro cutaneous stimuli [39, 40]. Pressure (i.e. algometry) has most frequently been used for comparing pain perception between chronic pain patients and healthy controls since this modality is considered a relevant experimental model for clinical pain experienced by chronic pain patients[41-45], and pressure pain is thought to be mediated by A-δ and C fibers[46, 47]. In neuroimaging studies, mechanical (pressure) and electrical stimuli have not been commonly used either in chronic pain patients or healthy volunteer groups because the use of hand-held pressure algometers to induce pressure pain has several limitations[36, 48]. They do not enable accurate reproducibility of pressure profiles, they are operator-dependent, and they cannot provide a fast and precisely controlled stimulus required for conducting controlled experiments such as neuroimaging studies[49-51]. An improved method of inducing pressure pain was therefore required for use in experimental studies and in this current study an automated method of pressure pain induction was developed and employed. The pressure pain stimulus was applied to left thumb nail bed.

The complexity of the pain experience is poorly understood. For example sensory stimulation activates the insula and ACC. This is likely to reflect the negative emotional aspects of pain, especially the insula, which is involved with other unpleasant sensory experiences such as disgust. Activation of the amygdala suggests negative emotionality and also salience, and the pre-frontal cortices (PFC), which may be suggestive of increased attention to painful experiences, or possible top-down control.

1.6 Functional Magnetic Resonance Imaging

Few scientific developments have been more striking than the ability to measure the activity of the functioning human brain. As the name implies magnetic resonance imaging (MRI) uses strong magnetic fields to create images of biological tissue. To create images the MR scanners uses a series of changing magnetic gradients and oscillating electromagnetic fields known as a pulse sequence. Depending on the frequency of the electromagnetic fields, energy may be absorbed by atomic nuclei. For the MRI, scanners are tuned to the frequency of hydrogen nuclei, which are the most abundant in the human body due their prevalence in water molecules. After it is absorbed, the electromagnetic energy is later emitted by the nuclei and the amount of emitted energy depends on the number and types of nuclei present[52, 53].

Depending on the pulse sequence used, the MRI scanner can detect different tissues properties and distinguish between tissue types. MRI can be used to study the physical structure of the tissue such as the volume of grey and white matter, however these measurements are limited in that they cannot identify short-term physiological changes associated with the active functioning of the brain. Functional MRI (fMRI) studies can help overcome this limitation both by identifying the different parts of the brain where particular cerebral processes occur and by characterising the patterns of brain activation associated with this process.

Neuronal activity has metabolic consequences. Specifically, energy is required for the maintenance and restoration of neuronal membrane potentials. That

energy is not stored within the brain, but must continuously be supplied by the vascular system through the delivery of glucose and oxygen[54, 55].

The haemoglobin molecule has magnetic properties that differ depending on whether or not it is bound to oxygen. The oxygenated haemoglobin (Hb) is diamagnetic; that is it has no unpaired electrons and zero magnetic moment. In contrast, the deoxy haemoglobin (dHb) is paramagnetic; it has both unpaired electrons and a significant magnetic moment. Because paramagnetic substances can distort the surrounding magnetic field, nearby protons will experience different field strengths and thus process at different frequencies, resulting in the more rapid decay of transverse magnetisation (therefore a shorter T 2 *). Thus, MR pulse sequences sensitive to T2 * should show more MR signal where blood is highly oxygenated and less MR signal where blood is highly deoxygenated.

For the MRI to be useful in measuring physiology, it would need to be sensitive to some indirect measure of metabolism. One possibility is blood flow, since metabolic processes require oxygen that is supplied through haemoglobin within red blood cells (RBC). The MR signal increases during neuronal activity even though deoxygenated blood decreases the MR signal. This disparity between oxygen utilisation and oxygen delivery means more oxygen is supplied to a brain region than is consumed. As the excess oxygenated blood flows through active regions, it flushes the deoxygenated haemoglobin from the capillaries supporting the active neural tissue and from the downstream venules. So the Blood Oxygenation Level Dependant (BOLD) contrast following neuronal activity occurs not because the oxygenated haemoglobin increases the MR signal but because it displaces the deoxygenated haemoglobin that had been suppressing the MR signal intensity[56]

This research was designed to investigate and characterise cortical and sub-cortical activation in response to innocuous and noxious pressure stimulus induced by an automated pressure pain induction system using an event-related functional magnetic resonance imaging (e fMRI) technique in a healthy

volunteer population and in patients with chronic pain. To the best of our knowledge, our experimental paradigm is unique.

2. Opioid peptides and their receptors

2.1 Role of opioids in chronic pain

The efficacy of opioids in the management of acute and chronic cancer pain is well recognised, and there are guidelines recommending their use in patients with persistent moderate to severe pain, in particular when other treatments have failed[57].

Opioids are a large family of biologically active peptides that bind to and activate receptors in humans and can reduce pain and induce euphoria[58]. Historically, concerns about addiction have contributed to the under-treatment of disorders widely considered to be appropriate for opioid therapy, including cancer pain, pain at the end-of-life, and acute pain. The use of opioids for chronic non-malignant pain (CNMP) remains controversial. Following publication of reports on the safety and efficacy of opioids prescribed to a small numbers of patients with CNMP and the publication of an article entitled “The Tragedy of Needless Pain”[59], the use of opioids to treat CNMP began to be more widely practiced and incorporated into clinical guidelines. Nevertheless despite the advances in pain medicine and increase in employment of interventional analgesic techniques there has been an increasing use of opioids for patients with chronic non-malignant pain conditions. However there is still considerable controversy surrounding patient selection, clinical goals and the safety and effectiveness of opioids in patients with CNCP[60].

The endogenous opioid systems play a critical role in modulating a large number of sensory, motivational, emotional, and cognitive functions. As inhibitory neuropeptide transmitters, they fine-tune neurotransmission across a wide range of neuronal circuits. In addition, they have served as prototypes for understanding many structural and functional features of peptidergic systems. Scientific studies of these systems over the past three decades have uncovered a complex and subtle system that exhibits diversity in terms of the number of endogenous ligands and convergence to only three major receptors.

2.2 Genes and proteins

Three genes encode the opioid precursor proteins: pre-proopiomelanocortin, pre-proenkephalin, and pre-pro-dynorphin. The major opioid peptide encoded by pre-proopiomelanocortin is β-Pre-proenkephalin which encodes multiple copies of Met-enkephalin. Pre-pro-dynorphin encodes three opioid peptides of various lengths that all begin with the Leu-enkephalin sequence: dynorphin A, dynorphin B, and neoendorphin.

The three opioid receptors; μ-opioid receptors (MORs), δ-opioid receptors (DORs), and κ-opioid receptors (KORs) have been identified and cloned. All three receptors belong to the superfamily of seven transmembrane-spanning G protein-coupled receptors. A high degree of structural similarity exists between the three opioid receptors. These are identified in transmembrane domains 2, 3, and 7 and the first and second intracellular loops. The extracellular loops differ among the three-receptor classes, and this divergence may explain differences in ligand selectivity among the opioid receptors. The relationship between the opioid peptides and their receptors is complex[61].

The KOR displays the greatest selectivity across the endogenous ligands, Further research into the isolation of novel opioid receptors resulted in the identification of high structural homology between the opioid clones with very little or no binding affinity for the opioid ligands. The structural similarity between this opioid receptor-like (ORL-1) and the opioid receptors is highest in the transmembrane regions and cytoplasmic domain and lowest in the extracellular domains critical for ligand selection [62, 63].

2.3 Signal transduction mechanisms and their adaptation after chronic stimulation.

The opioid receptors couple to G proteins inhibiting adenylyl cyclase, activating inwardly rectifying K^+ channels and decreasing the conductance of voltage-gated Ca^{2+} channels[64]. The opioid receptors may bind to an array of other second messenger systems, which include activation of the mitogen-activated protein (MAP) kinases and the phospholipase C- mediated cascade leading to

the formation of IP3 (inositol- 1,4,5-triphosphate) and diacyl glycerol. Prolonged exposure to opiates results in adaptations at multiple levels within these signalling cascades. The significance of these adaptations can lead to conditions such as tolerance, sensitization, and withdrawal[65-67].

2.4 Pharmacology of Oxycodone:-

Oxycodone (14-hydroxyl-7, 8-dihydrocodeine) is a semi synthetic derivative of naturally occurring thebaine and is commonly used as an alternative to morphine. Both oxycodone and morphine can be administered orally, rectally, intrathecally and parenterally. Even though oxycodone and morphine share many similarities the pharmacology of oxycodone is different from that of morphine in many clinically relevant ways. The main difference between the two opioids is high oral bioavailability of oxycodone, more than 60% with mean ranging from 42-87% compared to morphine which ranges between 22%-48%[68-70].

Pharmacokinetics of oxycodone

In humans, the main oxidative metabolic route of oxycodone is N-demethylation at the 17 –position by CYP 3A4/5 to nor-oxycodone and to a lesser extent by a 3-O- demethylation to oxymorphone. Nor-oxycodone is metabolized by O-demethylation to Noroxymorphone by CYP 2D6. The oxymorphone is formed after an oxidative reaction mainly catalysed by CYP 2D6 in humans[71]. Oxymorphone is extensively conjugated and excreted in urine. Also, a minor route, with 20-30 fold lower rate compared with noroxycodone O-demethylation is by N-demethylation of oxymorphone to noroxymorphone via CYP 3A4 and 2D6 [71].

Oxycodone is extensively metabolized in the liver and only 8-14% is excreted unchanged or in conjugated forms in urine[72 73]. A study by Kaiko et al. found that the clearance of oxycodone in female healthy controls was 25% slower compared to men[74].

In patients with liver and renal failure the clearance of oxycodone is significantly reduced compared to healthy controls and in an elderly population a twofold higher plasma oxycodone concentration was noted[75]. The plasma protein binding and physicochemical properties of oxycodone are reported to be similar to those of morphine[76]. Clinical studies have reported that oxycodone has predictable pharmacokinetics with no apparent dose ceiling and that it has an adverse profile in line with other opioids[70, 74]. In experimental animal studies the unbound concentrations of oxycodone are 2.5 – 6 times higher in the brain than in the blood, suggesting an active transport of oxycodone across blood brain barrier (BBB) to the central nervous system (CNS) after intravenous administration[77].

Pharmacodynamics

Oxycodone is selective for the μ opioid receptor compared to δ and κ receptors and has 5- 40 times lower affinity compared to morphine[76]. Oxycodone activates the intra-cellular G- protein via μ opioid receptors[71].The anti-

nociceptive effect of oxycodone can be antagonised with selective μ opioid receptor antagonist's β- funaltrexamine and clocinnamox [78]. The main metabolite nor-oxycodone has four times lower affinity to μ opioid receptor compared to oxycodone[71].

After oral administration of oxycodone, noroxymorphone, a secondary oxidative metabolite of oxycodone, has been found in relative high plasma concentrations in humans[71]. Because of the high hydrophilicity of noroxymorphone its anti-nociceptive properties are poor after subcutaneous administration but it induces potent anti-nociceptive effect after intra thecal (i.t) administration in rats[79].

Role of Oxycodone in neuropathic pain

Opioids are not curative for pain in general nor for neuropathic pain specifically. However compelling evidence exists for the efficacy of opioids as a core part of multimodal and multi-drug therapy for chronic neuropathic pain. The natural course of neuropathic pain and its manifestations in terms of symptoms and signs are influenced strongly not only by etiologic, pathophysiological and pain mechanisms, but also by the presence of other types of coexisting pain, such as musculoskeletal pain, as well as the frequent coexistence of psychological and psychiatric comorbidities. Opioids are not considered the primary option in the treatment of neuropathic pain; instead anti-depressant and anti-epileptic medications are recommended[80]. However compelling evidence exists for the efficacy of opioids as a core part of multimodal and multi-drug therapy for chronic neuropathic pain[6].

3.0 Cortical and sub-cortical activations- Insights from other experimental pain studies.

Pre-clinical neuroimaging studies have implicated a number of cortical and subcortical brain regions in pain processing. The most commonly reported areas include the insula, thalamus, anterior cingulate cortex (ACC), the cerebellum, the prefrontal cortex (PFC), the motor cortex and the temporal lobe.

The insula is one of the most frequently activated brain regions in imaging studies[33]. Research has shown that touch evokes physiological and psychological effects and can activate a number of brain areas including insular cortex [79,81, 82]. The human insula has the largest concentration of cortical connections ranging from the limbic cortex to primary sensory and multi modal association cortices [83, 84].

The exact role played by insular cortex in processing tactile information is largely unknown[85]. The Insula is involved in processing many aspects of the conscious experience of pain such as affect[86, 87], autonomic activity[88], interoception[89] and temperature[90]. A recent fMRI study in humans has shown functional differentiation in the insular-cortical network.[91].The anterior insula receives input from the peripheral autonomic receptors and may become activated during affective tasks or perception of pain due to changes in the haemodynamic parameters[88, 92].

The Insula is a major site for emotional processing and also processes sensory-discriminative aspects of pain perception. The Posterior insular cortex is involved in processing both painful and innocuous somaesthetic inputs[93]. Imaging studies consistently report signal increases in the posterior and anterior insula during noxious stimuli[33]. A recent study demonstrated that the insula is somatopically organised with respect to muscle and cutaneous pain and that this organization is further separated according to the tissue in which the pain originates[94]. Further evidence demonstrates a noticeable activation of the insula for neutral touch and activation of anterior insula for painful stimuli [95]. Mouraux et al reported activation of both anterior and posterior insula for both nociceptive and non-nociceptive somatosensory stimuli[96]. In a PET imaging study Craig et al observed significant activation with innocuous cooling stimuli in contralateral

forebrain and middle/posterior insula and concluded that this region represents discriminative thermal sensation[90]. A recent meta-analysis of pain related neuroimaging data concluded that bilateral anterior insula and the left dorsal posterior insula had a significant likelihood of being activated by noxious stimuli[97].

The thalamus has been described as the “gate keeper” for the cerebral cortex. It monitors and controls the amount of information passing to and being received from the cerebral cortex” depending on the behavioural state of the animal [98]. The thalamus contains structurally and functionally well-defined nuclei [99-101]. Following peripheral tissue damage nociceptive information is conveyed through many ascending sensory pathways to the brain and the information signalling injury cross over to or is shared between these pathways.[102]. The thalamus forms a major relay system for conveying afferent input to the cortex through spinothalamic (STT) and thalamocortical tracts. The cells of origin for STT are located mainly in lamina I and IV-VI and few STT neurons are also located in laminae VII, VIII and X [103-106] of the spinal cord dorsal horn. While traditionally it was believed that most of the STT project to contra lateral thalamus, available experimental animal and human clinical studies confirm that a significant proportion of STT project to ipsilateral thalamus also[107]. In primates and non-primates the evidence suggest that STT projects to both the lateral and medial thalamus and terminate in ventral posterior lateral nucleus (VPL) and ventral posterior inferior nucleus(VPI) [108]. In primates STT cells project to the central lateral nucleus (CL) in the medial thalamus and may send collateral branches to the lateral thalamus and have a very large receptive field[109]. Craig et al has shown lamina I in primates project to ventral medial posterior (VMPo), ventral posterior inferior (VPi) nuclei which also receive convergent input from lamina V and the dorsal column nuclei and to medial thalamus. VMPo topographically project to insular cortex and area 3a. The medial thalamus project to anterior cingulate[110].

Several other pathways to extra thalamic structure accompany the STT. The spinomesencephalic (SMT) and spinoreticular (SRT) relay in the reticular formation in areas that project heavily to medial thalamic nuclei, especially the

intralaminar nuclei. The SMT project bilaterally and is involved in nociception[22, 111].In an experimental animal study it was observed that para brachial nucleus projects mainly to amygdala and the hypothalamus and the spino-parabrachio amygdala nocispecific neuronal chain is involved with intensity of pain [102, 112].

The postsynaptic dorsal column pathway projects to the dorsal column nuclei, which in turn project to the VPL and POm nuclei. In an elegant electron microscopic study Raltson et al has shown convergence of medial lemniscal and spinothalamic tract input onto the proximal dendritic trees of thalamocortical neurons [113], [114]. The currently available primate and non-primate evidence show that substantial fraction of A-fibre nociceptors may conduct in A-Beta conduction velocity range and has questioned the widely held view that only small diameter myelinated fibre A- delta fibre and unmyelinated 'c' fibre transmit nociceptive signal [17, 18].

Thalamic activation is observed in large number of imaging studies of cognition[115-117], pain[30, 34, 118], memory[119], and sexual arousal [120]. Interestingly, most pain imaging studies show bilateral thalamic activation, which may be partly due to attention processing along with sensory, processing[121, 122]. The Medio dorsal thalamus (MDT) is the main part of the limbic thalamus[123] and connects the basal ganglia and cortex[124]. The MDT provides strong cortical and sub cortical connections, and plays a major role in cognition, emotion, memory and motivation in monkeys[125] and sexual arousal in human males[126]. Bilateral ventrolateral thalamus (VL) activation was reported for a simple attentional task as a function of arousal and attention [127]. In another study, bilateral VL thalamus activation was observed during thermal pain application, which the authors attributed to the "attentional component of pain processing.[128]. The VL thalamus due to its connectivity with cerebellum is long considered to be involved with motor functions but a recent imaging study in a patient with stroke has shown that VL thalamus plays a prominent role in sensory processing[129].

Thalamic nuclei project too many parts of the cingulate cortex rather than a single nociceptive nuclei and there are multiple thalamic nuclei that process nociceptive input to cingulate cortex. Anterior cingulate cortex (ACC) is involved in other pain unrelated functions [130]. No region or area appears to be

nociceptive specific[131]. Many limbic structures activation's are noted during noxious stimulation of the body.

Vogt et al based on a neurobiological model divided cingulate cortex into four regions and further sub regions to predict the outcomes of information processed in the cingulate gyrus. The sub regions in ACC to sub genual ACC (s ACC) and peri genual (p ACC); middle cingulate cortex (MCC) into anterior (a MCC) and posterior (p MCC); posterior cingulate cortex (PCC) into ventral (V PCC) and dorsal (d PCC) and retrosplenial cortex (RSC)[130].

ACC is involved in coding for the reward properties of a particular behaviour[95, 132]. A study looking at displaying sad event activated s ACC and happy moments activated p ACC[133]. Thus different areas of ACC may be implicated in processing different emotions. In terms of pain processing, Ploner et al observed activation in p ACC for a thermal stimuli and concluded that this sub region of ACC may be associated with 'c" fibre activation[134].

In an animal experiment the neurons in ACC responded to noxious stimuli including pressure and thermal stimuli over 46 ^{0} c and this was the same as projections from thalamic nuclei with large and bilateral receptive fields that are mainly nociceptive[131]. The caudal part of a MCC was activated by both innocuous touch and noxious stimulus and the authors concluded that it might be related to cognitive processing other than nociception and intensity rating [135]. It is postulated that p MCC, and d PCC may be involved in orienting the body towards innocuous touch and noxious somatosensory stimuli.

Historically, the cerebellum is regarded as the little brain involved in motor behaviour and co-ordination[24] Recent structural and functional studies have demonstrated that the cerebellum is involved in multiple other non-motor activities such as attention, executive control, language, working memory, learning, pain, emotion and addiction[26]. The cerebellum has extensive interconnections with the cerebral cortex[26]. The cerebellar vermis is subdivided into 10 lobules, Lobules III, IV and V and receives major afferent input through the ventral spinocerebellar tract[136].

Neuroanatomical tracer and experimental neurophysiological studies have provided direct evidence that the cerebellum receives cutaneous nociceptive fibres and c fibres nociceptive signals via the postsynaptic dorsal column [137-139]. The dorsal spinocerebellar tract transmits pressure and pain sensation, and is known to represent a wide spectrum of integrated sensorimotor signals and recent animal studies show the existence of bilaterally projecting spinocerebellar neurons in the cerebellum[21, 140, 141].

The dentate nucleus is a complex three dimensional structure. The region of the dentate nucleus that contains neurons that project to the primary motor area constitutes only 30% of the total and the remaining substantial portion project to non-motor areas[26]. The available evidence shows that the dentate is the largest of all cerebellar nuclei and mainly involved in the non-motor functions in humans and great apes[142]. A study in non- human primates using transneuronal transport of neurotrophic viruses to examine topographic organization of circuits linking the cerebellar cortex with the arm area of primary motor cortex (M1) and with a non-motor Area 46 in dorsolateral prefrontal cortex demonstrated that the M1 receives input from purkinje cells located mainly in lobules IV- VI of the cerebellar cortex and BA 46 receives input from purkinje cells located mainly in crus II of the ansiform lobule without overlap of the system. This confirms the separation of motor and non-motor functions seen in the dentate nucleus extending to the level of cerebellar cortex[143].

A number of imaging studies has demonstrated bilateral cerebellar activations to pain-related visual stimuli[33, 144-146], noxious heat[147],[148], unpleasant images[148], physical and social pain[149], and cognition[150].

Few studies have suggested that the cerebellar anterior lobe is principally engaged in sensorimotor control. The cerebellar vermis is involved in affective processing, and the posterior cerebellum contributes to complex cognitive operations[151, 152]. A differential pattern of activation was noted to noxious thermal stimuli in the deep cerebellar nuclei, anterior vermis and bilaterally in the hemispheric lobule VI[147]

The prefrontal cortex (PFC) is known to play an important role in cognitive control and its ability to combine thought and action in accordance with internal goals. The PFC is critical in situations when the mappings between sensory inputs, thoughts, and actions either are weakly established relative to other existing functions or are rapidly changing. [153]. Human neuroimaging studies have provided insight into the role of left and right Inferior frontal cortex (IFC). The IFC is one of the most heavily connected regions of the PFC, receiving polymodal input from posterior cortical areas, and communicating heavily with other PFC regions [153]

In an event related fMRI study, it was shown that left inferior frontal cortex (LIFC) plays a crucial role in processing the selection and retrieval of semantic or linguistic knowledge and cognitive control of memory[154, 155]. The left IFC plays a fundamental role in integration; in particular the integration of information in the temporal domain[156, 157] and also serves as a critical neural substrate for unification operations during language comprehension[154, 155, 158].

The right inferior pre frontal cortex (RIPFC) is more important for monitoring the sensory signals[159]. Middle frontal cortex (MFC) is involved in broader range of cognitive processing such as simple action rules and spatial maps associated with planning and working memory[160]. There are positron emission tomography (PET) studies which found bilateral prefrontal cortex activation of the orbitofrontal, perigenual cingulate and dorsolateral prefrontal cortices after application of a heat stimuli on capsaicin treated skin[161, 162].

The superior frontal cortex (SFC) is also implicated in self-awareness and in co-ordination with sensory system[163]. A recent study has demonstrated functional connectivity between lateral SFC and the Broca's area and suggest a close relationship between this pathway and language organization[164].

The temporal gyrus has three major convolutions of the external surface of the temporal lobe and is divided into superior, middle and inferior temporal gyri. A recent fMRI investigation of BOLD response in brain pain processing areas to acupuncture before and after injection of local anaesthesia solution lignocaine 0.2mls at BL60 acupoint (centre of the depression between the prominence of

the lateral malleolus of fibula and the calcaneal tendon behind the ankle joint) concluded that temporal gyrus activation was observed even after anaesthetizing the stimulation point and concluded temporal gyrus activation was unrelated to pain activity[165]. Acoustic analysis of words is carried out in the superior temporal cortex and visual analysis of words in the posterior inferior temporal cortex and temporo-occipital cortex[166].

The posterior middle temporal gyrus (MTG) and inferior frontal gyrus (IFG) are two critical areas of the language network in brain and MTG is involved in the retrieval of lexical syntactic information and the IFG in unification operations[158]. A recent fMRI study confirmed that left inferior frontal gyrus (LIFG) is involved in the integration process and MTG in the retrieval of lexical-syntactic information. The lexical information has to be available for longer time intervals than during the processing of random word sequences and they concluded that the syntactic unification process requires the dynamic interplay between LIFG and MTG[167]. The superior temporal gyrus (STG) is involved in auditory processing, including language, but also has been implicated as a critical structure in social cognition[168 169].

The Inferior parietal lobe is subdivided into supra marginal gyrus (SMG/ Rostral IPL) and angular gyrus (ANG/ Caudal IPL). SMG is believed to be involved in motor planning and action related function and is a part of human mirror neurons but, also in conscious phonological decision making[170, 166].

A probabilistic fibre tract analysis in humans has shown SMG areas are mainly connected to (pre-) motor, inferior frontal, somatosensory, superior parietal and posterior temporal areas.[171]. A recent human post mortem study using quantitative in vitro auto radiography of multi receptor mapping in IPL has confirmed that SMG is most likely connected with ventral pre motor cortex and ANG with temporal areas.[170].

The somatosensory system reflects an organizational principle common to all sensory systems. Mechanoreceptors in the skin send their axons, which terminate in gracile or cuneate nuclei in caudal medulla. These second order neurons project directly to the contralateral thalamus, terminating in ventral

posterior lateral nucleus. The third order neurons in the thalamus send axons to the primary somatosensory cortex (S-I), located in the post central gyrus of the parietal lobe. Most thalamic fibres terminate in areas 3a and 3b, and the axons from 3a and 3b project to areas BA 1 and 2. Thalamic neurons also send a small projection directly to BA 1 and 2. Areas 3b and 1 receive information from receptors in the skin, where as 3b and 2 receive information from receptors in muscles and joints. However, all these regions are extensively interconnected, so that both serial and parallel processing are involved in higher-order elaboration of sensory information[98].

In the human somatosensory system, the contralateral primary somatosensory cortex (SI) is presumed to process and encode type and intensity of the sensory inputs, whereas the bilateral secondary somatosensory cortex (SII) is believed to perform higher order functions including sensorimotor integration, integration of information from the two body halves, attention, learning and memory[172].

The role of SI in the perception of pain has been disputed[97]. An electrophysiological study in primates has demonstrated the presence of intermingled low-threshold mechanoreceptive (LTM) neurons with nociceptive neurons, and wide dynamic range (WDR) and nocispecific neurons (NS) were identified in close proximity[173]. Recent brain imaging studies reported mixed SI activations[33, 97]. Surprisingly, a recent meta-analysis has shown likelihood of activation in SI to noxious stimulus was significant[97].

Examination of the activation pattern only in SI and SII has shown that stimulation of the thumb resulted in a larger activated area in SI than stimulating the middle finger which coincided with the larger representation of the thumb at SI. These studies also concluded that directing attention to the stimuli enlarged activated areas at SI and SII compared to activations due to same stimulation while the subjects were performing arithmetic tasks. Robust activations were observed due to strong stimulation and keeping the finger immobilized by experimenter's pressure around the finger [174-176]. Experimental observation of finger representation in the somatosensory cortex concluded that the first digit (D1), middle digit (D3) and little finger (D5) were all represented in the SI in all participants, but only 75% of the participants digits were represented in SII[176].

SII is the target of independent pathways for the processing and integration of non-painful and painful somatosensory stimuli salient for further high-order elaborations[172].This region is the pre motor cortex and is mainly involved in complex, co-ordinated movements. Changes in motor cortex excitability in chronic pain patients are in consistent. Some studies have reported increased activity and other studies reported reduced activity as this group of patients have other sensorimotor defecits[177].

Role of fMRI in understanding brain pain processing in health and disease.

The advent of non-invasive human brain imaging has made it possible to understand the role of the human brain in processing pain in both acute and chronic pain patients[178]. Current evidence suggests that the chronic pain patients process acute pain differently than healthy controls. For example, chronic pain patients have shown higher pain ratings and enhanced neural responses when acute experimental pain stimuli are presented to them[179-181]. There is some evidence to suggest that there is altered supraspinal pain processing between chronic pain patients and healthy controls[9]. In chronic pain patients the anterior insular cortex is shifted to more rostral regions compared to healthy controls[182]. There are many differences in activation patterns across studies but the activation pattern in healthy controls are mostly noticed in sensory, limbic, associative and motor areas[179]. Functional brain activity has now repeatedly demonstrated that acute painful stimuli in healthy subjects give rise to a consistent and reproducible activation of anatomical brain regions[34]. Chronic pain patient populations are complex and inhomogeneous. They employ diverse analgesics regimens and may have multiple co-morbidities[9]. Recent research has demonstrated gray and white matter abnormalities in chronic pain patients[183]. These changes are partially reversible when the underlying pain is treated effectively[9]. A recent study has demonstrated that when chronic pain is treated effectively, specific regional gray matter decreases are reversed, and this reversal is related to the extent of pain relief[184].

Lumbar radicular pain remains one of the most common reasons for patients to attend pain clinics in Ireland. With chronic lumbar radicular pain there is a

widespread amplification of pressure stimuli extending beyond the somatotopic distribution of chronic pain and therefore the chronicity of pain in this study being generated by lumbar radiculopathy results in widespread central amplification. Therefore pressure applied at the thumb is potentially amplified in brain. There is good evidence that chronic pain such as fibromyalgia is associated with central amplification. Recent research looking at the structural changes in the brain a day prior to lumbar discectomy and a follow-up scan four weeks after the operation has demonstrated that chronic lumbar radicular pain due to lumbar disc herniation can induce structural alterations of the brain that are potentially reversible after successful lumbar discectomy[185, 186].

4. The current study

The aim of this research was to explore and characterize, using fMRI, the cortical and subcortical activation in response to pressure pain stimuli in patients with chronic pain compared to healthy controls, and to examine how this neural activation is modulated by treatment with oxycodone in patients with chronic pain. The pressure pain stimulus was applied to the left thumb nail bed. The results of the fMRI pain task in healthy controls will be described first in order to explore the neural bases of pain processing in a healthy population. We then compared the activation to that of chronic pain patients in order to establish and then characterize differences in the central processing of pain between healthy controls and a chronic pain population. This study will provide information regarding the central neural mechanisms involved in chronic pain processing *in vivo*. This will allow characterization of the different neural responses between healthy controls and chronic pain patients in response to pressure pain stimulation, and it will also identify different responses to varying pressure pain stimuli. We hypothesized that there would be more brain activation in chronic pain patients compared to controls, due to pre-existing background chronic pain in this group.

The final part of this work will examine the effect of oxycodone, which is a commonly used analgesic agent with anti-neuropathic properties, in modulating response to different pressure pain stimuli in patients with chronic pain. This will provide insights into the neural mechanisms underlying the experience of pain in chronic pain patients and how it may differ to the experience of pain in a healthy population. We hope to gain further information regarding the central neural effects of oxycodone treatment for chronic pain, as the central basis of its analgesic properties have not been well characterised. We predict that post oxycodone treatment the chronic pain patients will show less activation, as their neural response to pain should be blunted by the central effects of oxycodone.

5. Materials and methods

Any ethical framework for human studies requires that subjects take part without coercion and have the opportunity to give informed consent. They must be fully educated with respect to potential risks and the adequacy of measures taken to minimise risk in the study group. In addition, the investigators must take responsibility for the identification and exclusion of any volunteer on the basis of clinical assessment of individual risk. To facilitate this, recruitment and participation were specifically separated.

5.1 Healthy controls

Twenty-four right-handed healthy controls (thirteen female, eleven male; mean age = 44.2 ± 8.58 years, age range = 29 -63 years) were recruited. Local advertising invited volunteers to make contact and an initial screening visit was arranged. Initial visits provided an opportunity for medical assessment of the volunteer's suitability for participation and for volunteer's education in all operational aspects of the proposed experiment. Subjects gave informed consent in accordance with full ethical approval by the St. James's Hospital and AMNCH Research Ethics Committee, Dublin, Ireland. Subjects with a history of current medical, neurological, or psychiatric illness, claustrophobia, head trauma, use of psychotropic drugs including haloperidol, Tricyclic anti-depressants(TCA's), Selective serotonin noradrenaline uptake inhibitors (SNRI), Selective serotonin re-uptake inhibitors (SSRI's) Mono amino oxidase inhibitors (MAO's), prescribed opioids or NSAIDs or "over the counter" analgesic drugs and anxiolytic drugs or current smokers, as assessed by the Physician Investigators, and women who were pregnant or breast feeding were excluded from the study. After data collection, data from four subjects (two female, two males) was excluded from the analysis. Two subjects did not complete the pressure pain task, and two were excluded for excessive head motion in the scanner. None of the participants received any financial or other reward for participating in the study.

5.2 Chronic pain patients

Ethical approval from the St. James's Hospital and AMNCH Research Ethics Committee, Dublin, Ireland was sought and obtained. Seventeen right-handed patients (nine female, eight male; mean age =46.29±10.90 years, age range 28 -70 years) with a diagnosis of chronic lumbar radicular pain as defined by history, physical exam and MRI scan, were recruited from the Chronic Pain Clinic in St. James's Hospital, Dublin. After a grace period all subjects gave written informed consent in accordance with Ethical Committee guidelines. Subjects with a history of medical, neurological, or psychiatric illness, claustrophobia, head trauma, or use of psychotropic drugs including haloperidol, Tricyclic anti-depressants(TCA's), Selective serotonin noradrenaline uptake inhibitors (SNRI), Selective serotonin re-uptake inhibitors (SSRI's) Mono amino oxidase inhibitors (MAO's), and opioids, NSAIDs or "over the counter" analgesics and anxiolytic drugs, gabapentinoids or current smokers, as assessed by the Physician Investigators were excluded from the study. Chronic pain subjects included in the study received 10 milligrams oral Oxycodone twice daily after first scan for seven days (7 days) prior to the second scan.

5.3 Equipment

A custom-built electronic pressure algometer was used to deliver pressure stimuli of different magnitudes. The applicator of this device is based on a pressure algometer design originally described by Richard H. Gracely [44, 181]. The non-dominant hand left thumbnail bed of the participant was placed in a plastic cylinder. A 20 cc syringe with 1-cm^2 round rubber application surface was mounted at right angles to this cylinder such that the syringe plunger was pointing toward the centre of the cylinder. When a pneumatic pressure was applied to the syringe, the plunger with 1-cm^2 round rubber application surface was pushed toward the participant's left thumbnail bed and a blunt pressure was applied which was proportional to pressure being applied to the syringe. All of the materials used to fabricate the pressure applicator apparatus were made of plastic and verified as MRI compatible. (See Figure 3 for the algometer).

Figure 3. The custom-built pressure algometer and air compressor.

The novel aspect of the system was the custom designed pneumatic controller. This was microprocessor controlled and could deliver different pre-set pressures to the syringe as described above. Pressures of one, two and three bar were applied resulting in three different stimuli intensities. We defined one bar pressure as touch (i.e. baseline condition), two bar pressure as mild pain and three bar pressure as moderate pain after testing these pressures on a pilot sample of 20 volunteers before running the fMRI experiment. In the fMRI analysis we compared the brain activation to mild pain versus touch (2 bar versus 1 bar), moderate pain to mild pain (3 bar versus 2 bar), and moderate pain to touch (3 bar versus 1 bar).

The pneumatic controller delivered four different, predetermined randomized pressure stimulus sequences. These will be referred to as the four experimental sequences and labelled A, B, C, and D. Each experimental sequence consisted of 60 stimuli, 20 at each of the three pre-set pressures, and each with a stimulus duration of five seconds. The four sequences differed in terms of the order of stimuli presentation, which was randomized and then programmed as a predetermined sequence. The inter-stimulus duration within each sequence varied from 5 to 14 seconds and the duration of each stimulus was also randomized. Each participant in the study experienced one of the four pressure sequences. This prevented the subject from predicting the next stimulus intensity.

The pneumatic controller was interfaced to the fMRI scanner. In this way the timing of the delivery of the randomized stimuli and the scanner acquisition times was synchronized. The pneumatic controller had a number of safety features. In the event of a failure of electrical power the device would automatically release any pressure. The front panel had a mechanical pressure gauge that displayed the applied pressure at any given time and this was independent of any electronics. The front panel also had an emergency manual over ride switch which would disconnect the electronic pressure algometer from the pressure generator and vent any pressure generated by the device. This controller was kept outside the MRI scanning room and was connected to the

pressure algometer through plastic tubing. The device was tested on 20 healthy controls for the reliability and reproducibility of the pressure pain stimuli before commencing the experimental pressure pain fMRI study. The results from device testing were reliable and were consistently reproducible.

5.4 Functional MRI

The participants were scanned in a 3T Philips Achieva MRI scanner (Best, The Netherlands) at the Institute of Neuroscience, Trinity College Dublin, Ireland, using an eight-channel SENSE radiofrequency (RF) head coil. An angled mirror was mounted on the head coil, enabling the participants to view the projection screen behind the MRI scanner. Throughout the pressure pain experiment the time remaining was displayed on the screen.

Before the scanning session all participants completed the Beck Depression Inventory (BDI) [187] in order to examine for presence of depressive symptoms, and the State Trait Anxiety Inventory (STAI) [188] to ascertain a measure of both trait anxiety and situational anxiety on the day of the scan. A Becks depression score of more than 10 was an exclusion criterion. Everyone can have depressive symptoms from time to time but not to the extent that it is diagnosed as a depressive disorder. In order for the diagnosis of depressive disorder to be made the symptoms need to be persistently moderate to severe with associated significant functional impairment. It is imperative that other medical causes of similar symptoms are excluded. These medical conditions include thyroid dysfunction, porphyria, metabolic disease, medication side effects and substance abuse.

The symptoms include: low mood, diminished interest, loss of socialization skills, poor work performance, weight change, sleep change, agitation, fatigue, poor concentration, and feelings of worthlessness and hopelessness which may lead to suicidal ideation. This symptom complex leads to significant functional impairment. The timeframe needs to be a minimum of two weeks but often longer. While many people experience some of these symptoms, to varying degrees from time to time, this does not constitute a depressive disorder. Becks Depression Inventory is not a diagnostic questionnaire but provides a

depression symptom rating. A disorder can only be diagnosed from a clinical interview by an appropriately trained doctor.

Prior to the start of the experiment the participants tested the three different pre-set pressure levels so that they were familiar with the stimuli before the commencement of the scan by switching automatic to manual mode on the device. No in scanner VAS scores were collected because the resource was simply not available. The possibility exists that a given participant may not have reported the same VAS scores for each given stimulus in and out of the scanner. However each participant complied with study methodology. Following the scan the participants completed a version of the visual analogue scale (VAS) to measure their subjective pain experiences of the three stimulus levels. The VAS ranged from 0 to 10.

During the MRI scan the participants wore ear-plugs and MR-compatible electrostatic headphones to reduce the noise of the scanner and facilitate communication with MR technician or researcher. Foam padding was placed around the participant's head to minimize movement, and they were instructed not to nod their head when communicated with by the MR technician or researcher. There was a single experimental session during which 60 blunt pressure stimuli were presented and 450 volumes of MR data were acquired.

Echo-planar imaging continued throughout the blunt pressure paradigm and used the following parameters: parallel imaging –SENSE 2, FOV-240x 240mm, matrix size 80x80, slice thickness of 3.2mm, order of acquisition axial, ascending sequential of whole brain. The blood oxygenation dependent (BOLD) signal changes were measured using a T2*-weighted echo-planar imaging sequence with TR = 2000ms, TE = 27ms and flip angle = 90^0. A 3D T1-weighted (axial) high resolution anatomical scan was acquired for each participant with an isotropic voxel size of 0.9mm. This image was used for structural localization of the functional results.

5.5 Experimental design

Each participant was scanned while being stimulated by the electronic algometer running one of the four experimental sequences. The four different experimental sequences were used in order to avoid order effects in the presentation of the stimuli, and each participant was randomly assigned to one of the four groups. Equal numbers of sequence A, B, C and D were put in a sealed envelope and the participants were asked to pick an envelope. The experimental design was optimized for efficiency by a fixed blunt pressure stimulus of 5 seconds and varying inter-stimulus duration between 4 and 14 seconds.

A small emergency button was also given to subjects, and participants were instructed to press at any time to attract the attention of the MRI technician and researcher. During scanning subjects were monitored from the control room via the window and a video camera. The total duration of scanning was 45 minutes and included clinical scans; high resolution anatomical scans, resting state fMRI (results not shown in the current study) and the experimental pressure pain fMRI task.

6.0 MRI Data Analysis

6.1 Pre-processing

The MRI data were analysed using AFNI [189] (http://afni.nimh.nih.gov/afni/) and FSL (FMRIB Software Library- http://www.fmrib.ox.ac.uk/fsl/). Pre-processing was conducted in AFNI and consisted of slice timing correction to the first slice, motion correction by realignment to the first volume of the first run, global mean-adjustment by proportional scaling and smoothing with a 6 mm full-width-at-half-maximum Gaussian-kernel.

6.2 First level analysis

A general linear model (GLM) analysis was conducted in AFNI. For the first level analysis (run for the control and patient groups), the AFNI program 3dttest++ with the option '-set A' was used to test the mean of the group against 0. The datasets, which were input, were contrast images for each subject. For example, bar 2 vs. bar 1, which is mild pain versus touch. These contrasts were generated from the first level (single subject) analysis, using the '–glt' option in AFNI's 3dDeconvolve programme which runs a series of general linear tests at the first level of analysis. Several regressors of no interest were included to model the following sources of variance: six motion parameters to model head motion and eight regressors to model low frequency noise. In addition, in order to control further for the possible confounding effect of subject motion on the fMRI results, an option was used in AFNI (-censor) to exclude any data points from the analysis in which the head motion in any direction was greater than 1mm. One subject was excluded from the analysis because they moved excessively at 57 time points (the total run lengths was 450 TRs), leaving a final sample of 20 participants for the analysis.

The mean BOLD signal in response to the three different pressure pain levels was modelled with three regressors of interest. These modelled the mean response to bar 1 (touch), bar 2 (mild pain) and bar 3 (moderate pain) pressure levels. A series of general linear tests (GLTs) were run at the first level to model

the difference in response between bar 1 and 2, bar 2 and 3 and bar 1 and 3. These beta maps were the contrasts generated from the general linear tests in the first level analysis, i.e. bar 2 vs. bar 1, bar 3 vs. bar 1, bar 3 vs. 2.

The experience of touch gives rise to sensations concerning both sensory (e.g., tactile sensitivity and discrimination) and emotional (e.g., pleasant, painful) aspects. It is well known that tactile sensitivity varies across the skin, where the fingertips and facial skin are particularly receptive to touch. Differences in tactile sensitivity can be related to the underlying neurophysiology of the skin. Recent research has shown that the perception of touch varies across the skin according to tactile pleasantness, sensitivity and direction discrimination. Previous studies testing the pleasantness perception of stroking on glabrous skin have found variable results. Top-down, cognitive processes may integrate these signals to interpret how pleasant a tactile stimulus is, especially as pleasantness can be interpreted through changes in friction during exploratory touch using the glabrous skin. Other affective tactile input (e.g., irritation, nociception) must also be included in the interpretation of how pleasant touch is[190].

6.3 Transformation of Results into Standard Space

All statistical analyses were calculated in the participant's native space, and the results were then warped to the MNI brain template (Montreal Neurological Institute/International Consortium for Brain Mapping 152 standard atlas as provided in the FSL software package) using FSL's linear registration tool, FLIRT. The transformation matrix (12 parameter affine) from native space to MNI space was calculated using the high-resolution structural images from each participant.

6.4 Second Level Analysis

The second-level (group) statistical analyses were based on a random-effects model, and the average activation maps across the group resulting from the various GLM analyses were computed with a series of ANOVAs and t-tests. The results of the second GLM, i.e. the model which examined the BOLD response to the three different pressure pain levels, were analysed with a one-way repeated-measures ANOVA. There was a single factor, pressure pain level, with three levels (bar 1, bar 2 and bar 3). The beta co-efficient maps for each subject for these three responses were entered into the ANOVA to test for a main effect of pressure level on the BOLD signal. The result of this F-test was used to create a mask to limit the post-hoc t-tests to regions of the brain which showed a statistically significant main effect in the ANOVA. In order to create the mask the F statistical map was thresholded at the voxel level at $p < 0.005$ ($F\ 2.54 \geq 5.85$) and then binarised to create a mask consisting of 1s (areas showing main effect of pressure level) and 0s (rest of brain). The F-test result of the main effect was thresholded but not corrected for family-wise errors at this point, as it was simply used to create binary mask to constrain the t-test results to those areas of the brain only showing a significant main effect in the ANOVA. It was not corrected because the t-tests to which the mask was applied were then corrected for family-wise error, using an appropriate voxel-level *p*-value < 0.001 and a minimum cluster size of 391µl generated for the F-test mask region. The voxel-level *p*-value was chosen to be quite stringent for all of the

cluster-extent based thresholding, as per recommendations by Woo et al. (2014) and others.

This mask was then applied to the general linear tests conducted at the first level, which served as post-hoc t-tests to examine the difference in BOLD response to the different pressure pain levels.

6.5 Correction for Multiple Comparisons

The three post-hoc t-tests were corrected for multiple comparisons across the regions included in the F-test mask. Significant voxels passed a voxel wise statistical threshold of $p \leq 0.005$, and a minimum cluster size of 391 mm^3 of contiguous statistically significant voxels was applied. This cluster size was calculated using a Monte Carlo simulation (with the AFNI program 3dClustSim) to obtain a family-wise error (FWE) corrected statistical significance level of $p< 0.05$ in the t-tests. The t-tests were corrected for multiple comparisons using a cluster-based correction as described above, however they were not further corrected (e.g. using Bonferroni adjusted *p* value) for the number of t-tests. Applying a method such as Bonferroni correction would change the voxel *p*-value threshold from 0.001 to 0.00033 (e.g. to correct for the three t-tests for control group). This would increase the *t*-value threshold from 3.88 to 4.235. This would not have a major impact on the results, as the majority of cluster peak values exceed this. Rather than subtracting the touch condition (using as a baseline), the BOLD activation between conditions was compared. This indicated regions that differed between touch and pain, thereby isolating those regions of the brain that specifically activated to pain.

The AFNI version used was: AFNI_2011_12_21_1014

It is possible that the version of 3dClustSim included in this release may have been susceptible to the inflation of type I errors as outlined by Eklund et al. (2016). However, a relatively stringent voxel-level *p*-value was employed ($p < 0.001$), which would minimize this possibility [191].The VAS correlation results were corrected for multiple comparison (family-wise error) across the whole brain using $p <0.001$ **thresholds** at the voxel level and minimum cluster size.

The STAI and BDI (Beck Depression Inventory) were administered to participants.

The SPM anatomy toolbox V1.7b, [192] was used to localize activation clusters; however where there were no probabilistic cytoarchitectonic labels available a Brodmann area is given in the results table instead.

7. FMRI Results

7.1 Healthy controls

Behavioural results

All subjects completed the pressure pain task. The pressure pain intensity was assessed from VAS scores obtained immediately after the subjects completed the experimental task and the average rating of intensity of stimulus was reported as 0.8 ± 0.69; mean ±SD [range 0-2] at 1 bar (touch condition), 2.65 ± 1.66; mean ± SD (range1-6) at 2 bar (mild pain) and 5.55 ± 2.21; mean ± SD (range1-9) at 3 bar (moderate pain) pressure stimulus. Because we used an event related design, we do not expect that there would be habituation effects, as commonly seen in block- stimulation design.

As can be seen from the t-test analyses, the chronic pain patients had statistically significantly higher levels of depression scores on the BDI, and higher state and trait anxiety scores as measured by the STAI compared to controls. These findings are in agreement with previous studies, which have found higher levels of mood and anxiety symptoms in chronic pain populations[193]. Following treatment with oxycodone, the pain patients reported significantly lower levels of state and trait anxiety, but their depression scores did not change significantly.

Table 1: Demographic details of the participants. Scores are mean (standard deviation). The healthy controls underwent one fMRI and testing session however the patients were re-assessed after treatment with oxycodone. These scores are indicated as “Post-drug”. All scores were compared between groups with independent t-tests, except for gender which was compared using a Chi-squared test. Baseline versus post-drug scores for the patients were compared with paired t-tests. All statistical tests are two-tailed and statistically significant results are indicated in bold.

Variables	Healthy Controls (HC)	Chronic Pain Patients (P)	HC vs. P difference *p*-value	Patients only: Baseline vs. Post-drug *p*-value
Gender	11 M, 9 F	8 M, 9 F	0.75	
Age	44.20 (8.58)	46.29 (10.90)	0.35	
Baseline BDI-II	2.45 (4.03)	12.24 (9.42)	< 0.001	
Post-drug BDI-II	-	9.41 (9.58)	-	0.089
Baseline STAI-S	25.00 (5.33)	38.53 (11.1)	< 0.001	
Baseline STAI-T	32.15 (8.81)	41.24 (6.61)	0.001	
Post-drug STAI-S	-	32.88 (9.14)		0.011
Post-drug STAI-T	-	35.59 (9.1)		0.006

Baseline VAS				
Bar 1 (touch)	0.80 (0.70)	0.65 (1.11)	0.14	
Bar 2 (mild pain)	2.65 (1.66)	3.12 (1.80)	0.88	
Bar 3 (mod pain)	5.55 (2.21)	6.06 (2.33)	0.56	
Post-drug VAS				
Bar 1 (touch)	-	0.47 (0.51)		0.53
Bar 2 (mild pain)	-	2.59 (1.46)		0.17
Bar 3 (mod pain)	-	6.24 (2.05)		0.68

Key: BDI-II = Beck's Depression Inventory, revised edition; STAI-S: State-Trait Anxiety Inventory, state anxiety score, STAI-T: State-Trait Anxiety Inventory, trait anxiety score; VAS = visual analogue scale to assess pain, ranging from 0 – 10.

Main findings:

Clinical questionnaires measuring depression and anxiety

At baseline, chronic pain patients had statistically significantly higher levels of depression scores on the BDI, and higher state and trait anxiety scores as measured by the STAI compared to controls. These findings are in agreement with previous studies which have found higher levels of mood and anxiety symptoms in chronic pain populations[193, 194]. Following treatment with oxycodone, the pain patients reported significantly lower levels of state and trait anxiety, but their depression scores did not change significantly.

Pain ratings:

Subjective pain ratings of the pressure stimulus used in the fMRI paradigm did not differ significantly between the patients and controls at baseline, and did not change in the patient group following treatment with oxycodone.

7.2 Pressure Pain Level Analysis

One-way ANOVA

The average BOLD response across the brain to the three different pressure pain levels was compared with a one-way ANOVA. The results of the F-test indicated a main effect of pressure level in the following brain regions: insula, thalamus, cerebellum, temporal lobe, cingulate cortex, frontal, occipital and parietal lobes. The results of this F-test are shown in Figure 4.

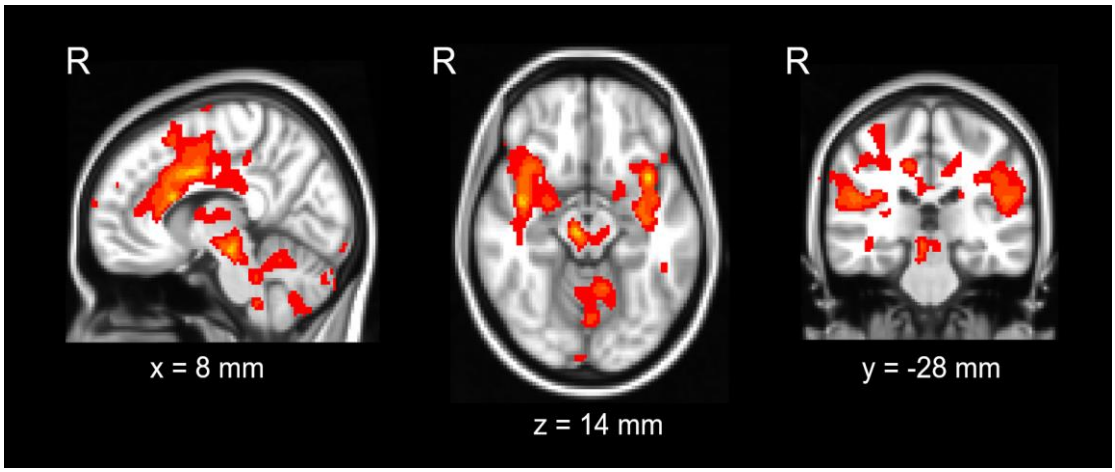


Figure 4: F-test result showing the main effect of the pain paradigm in the healthy controls. Activation in these regions in response to the different pressure levels was further explored with a series of t-tests, described below.

The results of this F-test were further explored with a series of t-tests, in order to compare activation differences between levels 1 and 2, 2 and 3, and 3 and 1. For the t-tests the results were confined to a mask of the regions of the brain where there was a significant main effect in the ANOVA ($F \geq 5.85$).

7.3 Post-hoc t-tests controls

7.4 Brain activation of mild pain versus touch

In the controls the statistical comparison of the contrast mild pain versus touch found greater BOLD response to mild pain in numerous cortical and subcortical regions, including bilateral ventral lateral thalamus, bilateral cerebellar lobules, bilateral anterior, middle and posterior cingulate, bilateral middle temporal gyrus, bilateral middle frontal gyrus and left supramarginal gyrus. These results are shown in (Figure 5 and Table 2).

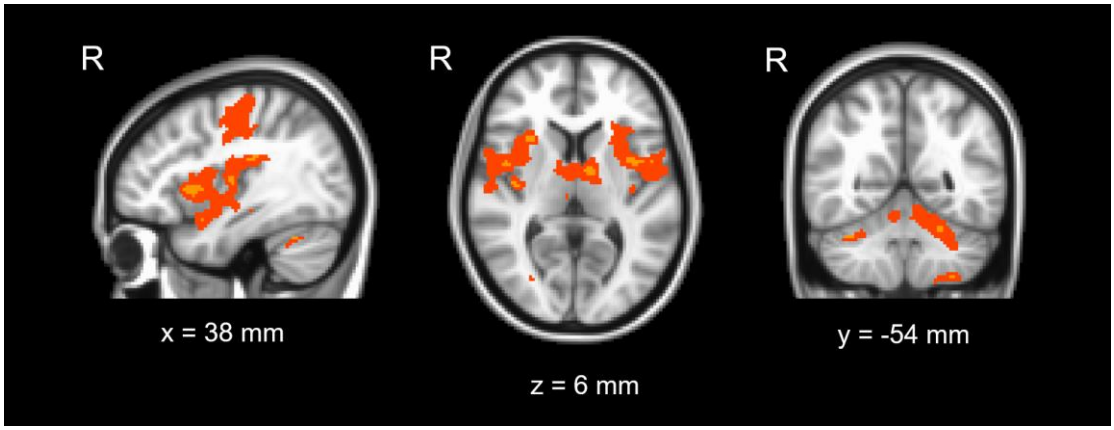


Figure 5: - Healthy controls – average brain activation in response to mild pain versus touch (Results are in MNI space, where negative co-ordinates indicate left, posterior and inferior directions (-LPI)).

Table 2. T- tests activation in healthy controls comparing mild pain to touch stimulus.

Cluster No.	No. Of Voxels	Brain Area	T-Stat	MNI Co-ords	Area: prob or BA
1	10507	Left Insula lobe	8.15	-42, -4, -6	BA 13
		Left Thalamus	8.04	-16, -8, 12	Left Ventral Lateral thalamus
		Right Insula lobe	7.82	44, 10, -8	BA 13
		Right Insula lobe	7.5	32, -26, 22	BA 13
		Left Thalamus	7.46	-16, - 14, 14	Left Ventral Lateral thalamus
		Right Thalamus	4.54	18, -8, 16	Right Ventral Lateral thalamus
2	1629	Left Cerebellum	6.44	-20, -62, -20	Lobule V1 (hem)
		Left Cerebellum	6.23	-30, -52, -56	Lobule VIII a (hem)
		Left Cerebellum	5.96	-14, -58, -14	Lobule VI (hem)
		Left Cerebellum	5.94	-24, -56, -26	Lobule VI (hem)
3	213	Right Cerebellum	6.50	38, -54, -30	Lobule VII a Crus I (hem)
		Cerebellar vermis	4.85	6, -56, -16	Lobule V
		Right Cerebellum	4.80	42, -66, -30	Lobule VII a Crus I (hem)
		Right Cerebellum	4.72	18, -58, -18	Lobule VI (hem)
4	140	Right dorsal posterior cingulate cortex	6.75	18, -32, 44	BA 31
		Right dorsal posterior cingulate cortex	6.24	20, -32, 36	BA 31
		Right dorsal posterior cingulate cortex	4.10	24, -40, 44	BA 31
5	130	Left dorsal posterior cingulate	5.67	-28, -68, 12	BA 31
		Left middle temporal gyrus	4.41	-26, -60, 20	BA 39
6	95	Left Insula	5.70	-26, -32, 12	BA 13
		Left Insula	5.47	-28, -44, 24	BA 13
		Left Supra marginal Gyrus	5.43	-30, -48, 22	BA 40
		Left Insula	5.47	-28, -36, 20	BA 13
7	88	Right dorsal posterior cingulate cortex	5.59	24, -42, 24	BA 31
		Right Caudate	5.15	16, -40, 18	
8	73	Cerebellar vermis	5.55	-2, -70, -12	Lobule VI (Vermis)
9	59	Right Cerebellum	6.06	18, -42, -48	Lobule VIII b
10	48	Right Middle Frontal Gyrus	4.95	30, 36, 22	BA 46

11	28	Left Middle Frontal Gyrus	5.02	-32, 36, 20	BA 46
		Left Middle Frontal Gyrus	4.07	-26, 38, 18	BA 46
12	28	Right Middle Temporal Gyrus	5.58	46, -40, -8	BA 21
13	22	Right Middle Occipital Gyrus	4.54	30, -76, 6	BA 18
		Right Middle Occipital Gyrus	4.32	34, -74, 10	BA 18
14	21	Cerebellar Vermis	5.08	6, -62, -42	Lobule VIII b (Vermis)

The average brain activation in response to mild pain in healthy controls was more compared to touch.

7.5 Brain activation of moderate pain versus mild pain

In the controls the statistical comparison of the contrast moderate pain versus touch found greater BOLD response to moderate pain in numerous cortical and subcortical regions including bilateral ventral lateral thalamus, supramarginal gyrus, anterior, middle and posterior cingulate and bilateral cerebellar lobules, right superior temporal and left middle temporal gyrus; right superior, middle and inferior frontal gyrus left middle frontal gyrus and also left primary somatosensory area. These results are shown in (Figure 6 and Table 3.)

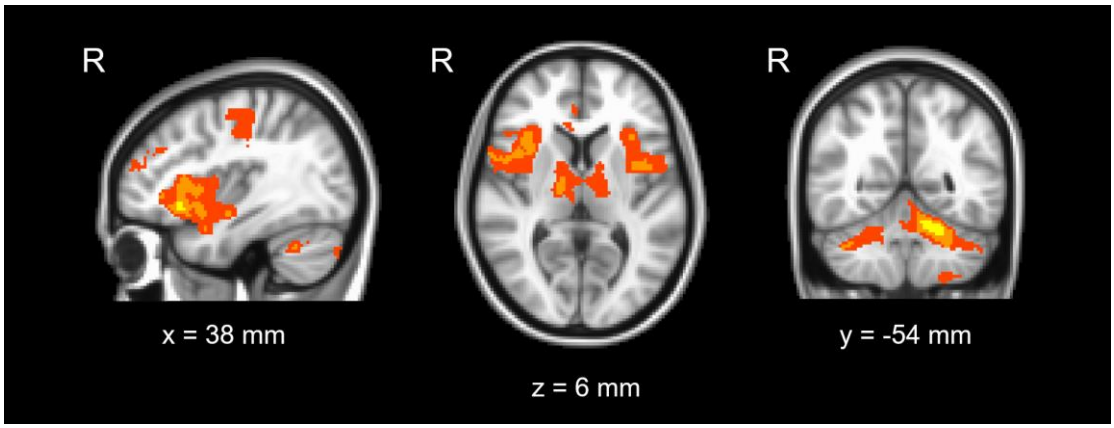


Figure 6: - Healthy controls – average brain activation in response to moderate pain versus mild pain (Results are in MNI space, where negative co-ordinates indicate left, posterior and inferior directions (-LPI).

Table 3. T- tests activation in healthy controls comparing moderate pain to mild pain stimulus.

Cluster No.	No.of Voxel	Brain Area	T-stat	MNI co-ords	Area:prob or BA
1	3869	Right insula	10.27	46, 10, 2	BA13
		Right superior temporal gyrus	9.41	58, 14, -2	BA 22
		Right superior temporal gyrus	8.72	60, 8, 0	BA 22
		Right inferior frontal gyrus	8.37	40, 22, -10	BA 47
		Left thalamus	7.32	-14, -12, -2	Left Ventral Lateral Nucleus
		Right thalamus	7.10	14, -12, 2	Right Ventral Lateral Nucleus
2	3578	Right ventral anterior cingulate gyrus	7.08	6, 12, 34	BA 24
		Right supra marginal gyrus	6.95	60, -38, 36	BA 40
		Right ventral anterior cingulate gyrus	6.89	8, 4, 36	BA 24
		Right ventral anterior cingulate gyrus	6.86	8, 40, 10	BA 24
3	2350	Left cerebellum	9.79	-22, -58, -24	Lobule VI (hem)
		Left cerebellum	7.94	-22, -48, -28	Lobule VI (hem)
		Left cerebellum	6.29	-30, -74, -30	Lobule VII a Crus I (hem)
		Left cerebellum	6.24	-4, -58, -12	Lobule V
4	1757	Left insula	7.77	-32, 18, 0	BA 13
		Left insula	7.55	-34, 10, 2	BA 13
		Left Insula	7.15	-38, 0, 10	BA 13
		Left insula	7.02	-42, 0, 12	BA 13
		Right insula	6.39	34,16, 4	BA 13
		Right insula	5.42	42, 16, 4	BA 13
5	679	Left supra marginal gyrus	7.52	-58, -30, 28	BA 40
		Left supra marginal gyrus	5.91	-52, -24, 26	BA 40
		Left supra marginal gyrus	5.74	-52, -38, 28	BA 40
		Left post central gyrus	5.74	-46, -24, 36	BA 2
6	518	Right cerebellum	6.80	20, -72, -26	Lobule VII a Crus I (hem)
		Right cerebellum	6.21	40, -54, -36	Lobule VII a Crus I (hem)
		Right cerebellum	5.23	26, -60, -30	Lobule VI (hem)
		Right dentate and nodule	5.08	16, -56, -34	Lobule VI (hem)

7	303	Left cerebellum	5.58	-28, -48, -54	Lobule VIII a
		Left cerebellum	5.37	-28, -42, -54	Lobule VIII b
		Left cerebellum	5.19	-14, -72, -52	Lobule VIII a
		Left cerebellum	4.97	-22, -60, -60	Lobule VIII a
8	265	Right middle frontal gyrus	6.37	30, 50, 22	BA 10
		Right superior frontal gyrus	6.10	26, 50, 18	BA 10
		Right middle frontal gyrus	5.66	36, 54, 20	BA 10
		Right middle frontal gyrus	4.34	38, 34, 30	BA 10
9	169	Right cerebellum	5.73	30, -82, -42	Lobule VII a Crus III (hem)
		Right cerebellum	5.26	16, -86, -38	Lobule VII a Crus III (hem)
		Right cerebellum	5.13	30, -86, -38	Lobule VII a Crus III (hem)
		Right cerebellum	4.84	12, -84, -36	Lobule VII a Crus III (hem)
10	101	Right superior frontal gyrus	5.28	4, 18, 58	BA 6
		Right superior frontal gyrus	4.32	6, 18, 68	BA 6
11	82	Right dorsal posterior cingulate gyrus	5.27	28, -48, 24	BA 31
		Right dorsal posterior cingulate gyrus	4.93	22, -44, 26	BA 31
12	65	Right cerebellum	5.92	4, -38, -46	Peri aqueductal gray
13	36	Left middle frontal gyrus	5.26	-30, 50, 14	BA 10
14	35	Right cerebellum	4.68	10, -70, -52	Lobule VIII a (hem)
		Right cerebellum	4.58	10, -66, -48	Lobule VIII b
15	27	Left middle temporal gyrus	4.59	-28, -60, 16	BA 21
16	21	Left dorsal posterior cingulate gyrus	4.76	-24, -48, 22	BA 31
		Left insula	4.21	-30, -42, 18	BA 13

The average brain activation in response to moderate pain in healthy controls was more compared to mild pain.

7.6 Brain activation to moderate pain versus touch

In the controls the statistical comparison of the contrast moderate pain versus touch found greater BOLD response to moderate pain in numerous cortical and subcortical regions, such as bilateral insula, cerebellar lobules, anterior, middle and posterior cingulate. These results are shown in Figure 7 and Table 4.

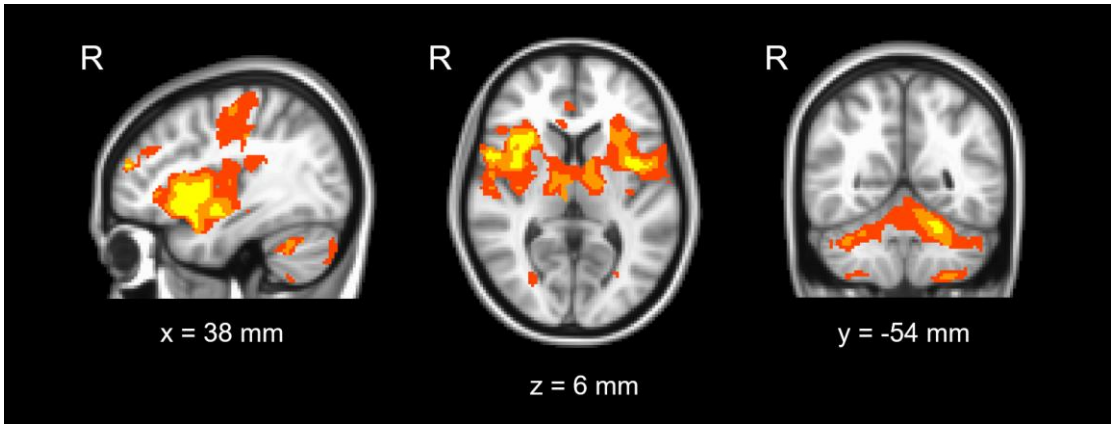


Figure 7: - Healthy controls – average brain activation in response to moderate pain versus touch (Results are in MNI space, where negative co-ordinates indicate left, posterior and inferior directions (-LPI).

Table 4. T-tests activation in healthy controls comparing moderate pain to touch stimulus.

Cluster No.	No. Of Voxels	Brain Area	T-stat	MNI co-ords	Area:prob or BA
1	21574	Right insula lobe	10.90	42,12, -8	BA 13
		Left insula lobe	10.02	-38, 2, 6	BA 13
		Right insula lobe	9.28	48,10, -2	BA 13
		Right superior temporal gyrus	9.25	52, 8, 2	BA 22
		Left thalamus	7.18	-14, -12, 8	Left Ventral Lateral Thalamus
		Right thalamus	6.54	4, -18, 10	Right Medial Dorsal Thalamus
2	5146	Left cerebellum	8.66	-22, -58, -26	Lobule VI (hem)
		Left cerebellum	7.53	-22, -50,-28	Lobule VI (hem)
		Left cerebellum	7.06	-30,-50,-54	Lobule VIIIa (hem)
		Left cerebellum	7.00	-28, -52,-56	Lobule VIIIa (hem)
3	378	Right middle frontal gyrus	9.17	36, 54, 18	BA 10
		Right middle frontal gyrus	7.56	-8,-40,-46	BA 10
		Right middle frontal gyrus	6.03	26,-42,-52	BA 10
4	313	Right cerebellar tonsil	7.12	6, -40, -48	Brain stem
		Right cerebellar tonsil	6.63	-8, -40, -46	Brain stem
		Right cerebellum	5.42	26, -42, -52	Lobule VIII b
		Right cerebellum	5.34	22, -38, -50	Lobule X (hem)
5	180	Left dorsal posterior cingulate	5.45	-28, -68, 14	BA 31
		Left dorsal posterior cingulate	5.16	-26, -58, 18	BA 31
6	142	Right dorsal posterior cingulate	7.10	24, -42, 24	BA 31
		Right dorsal posterior cingulate	6.91	24, -44, 30	BA 31
		Right dorsal posterior Cingulate	5.05	14, -38, 20	BA 31

7	115	Left Insula	6.84	-28, -44, 22	BA 13
		Left Insula	5.61	-28, -34, 16	BA 13
		Left Insula	5.57	-26, -32, 14	BA 13
8	103	Left pre central gyrus	6.05	-32, -8, 34	BA 6
		Left cingulate gyrus	5.26	-26, -10, 36	BA 31
9	67	Right middle temporal gyrus	5.26	34, -64, 16	BA 21
		Right agranular retrolimbic	5.26	30, -72, 8	BA 30
10	52	Right culmen	5.61	6, -36, -30	-
11	44	Right post central gyrus	5.01	24, -46, 66	BA 5
12	28	Right middle temporal gyrus	5.96	54, -42, -10	BA21
		Right middle temporal gyrus	5.35	46, -40, -8	BA21

The average brain activation in response to moderate pain in healthy controls was more compared to touch.

7.7 Correlations between subjective pain scores and the brain activation to pain

The participants' pain scores (VAS scores) for the three pressure levels were correlated against the mean BOLD response to each level in order to explore whether there was a relationship between subjective ratings of pain and the neural response to pain. There was no significant linear relationship between the scores and activation in response to either touch or mild pain. However, at the moderate pain stimulation level (activation to bar 3) there was a positive correlation (there were no negative correlations) between the BOLD response and VAS scores in a number of brain regions, including bilateral cerebellum, inferior temporal cortex and parietal lobe, the right superior orbital gyrus, the left hippocampus, left pallidum and right brain stem. This suggests that healthy controls who rated the bar 3 pressure level as more painful were more likely to show increased brain activation in these regions in response to the stimulus. These results are presented in Figure 8 (a) and (b) and Table 5.

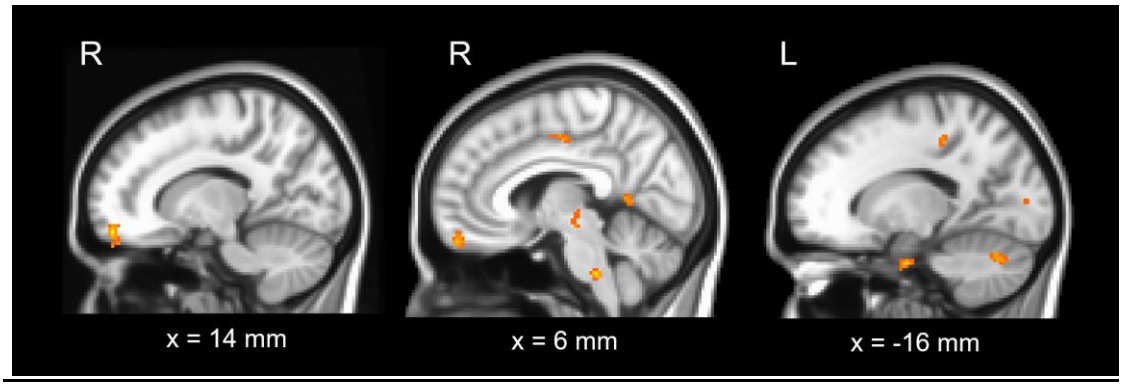
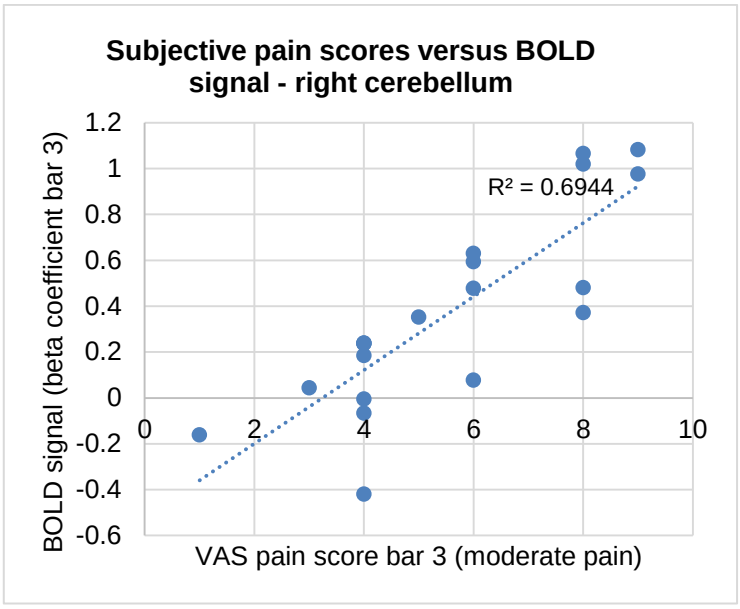


Figure 8 (a): Correlation between brain activation to moderate pain stimulus and pain ratings in healthy controls.

(a)



(b)

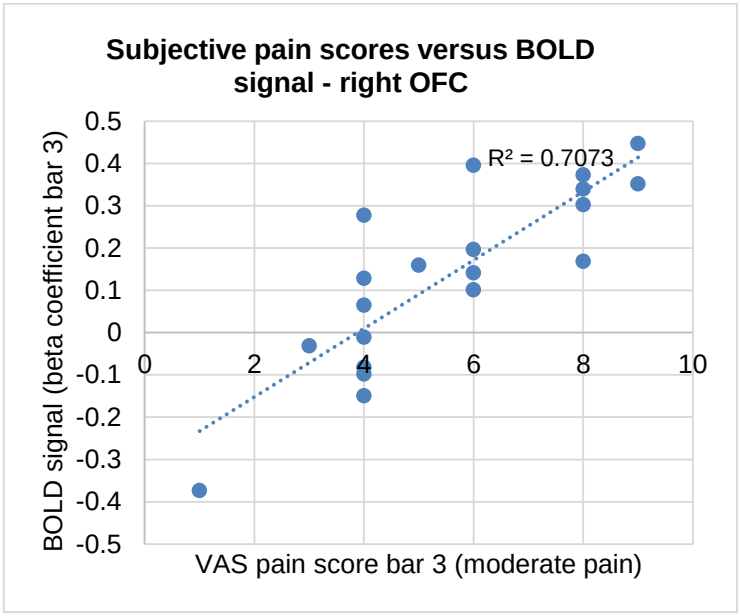


Figure 8 (b) A correlation analysis indicated that higher subjective pain sores were associated with stronger BOLD activation in a number of brain regions. Scatterplot of the VAS scores and the mean BOLD response of the healthy controls in the (b) right cerebellum and (c) right superior orbital gyrus. (OFC). (Results are in MNI space, where negative co-ordinates indicate left, posterior and inferior directions (-LPI).

Table 5. T- tests activation in healthy controls VAS correlations.

Cluster No.	No. Of Voxels	Brain Area	T-Stat	MNI Co-ords	Area: prob or BA
1	320	Right cerebellum	6.39	-34, 40, -38	Lobule VI (hem)
		Right cerebellum	6.14	-30, 42, -50	Lobule VIII b (hem)
		Right cerebellum	5.88	-52, 60, -38	Lobule VII a Crus I (hem)
		Right cerebellum	5.64	-42, 48, -54	Lobule VIII b (hem)
2	215	Left supramarginal gyrus	5.89	62, 28, 24	BA 40
		Left Pre central gyrus	5.60	48, 14, 12	BA 4
		Left superior temporal gyrus	5.03	64, 12, 10	BA 41
		Left post-central gyrus	5.03	64, 20, 24	BA 40
3	167	Left inferior temporal gyrus	5.82	68, 12, -24	BA 20
		Left inferior temporal gyrus	5.09	56, 10, -26	BA 20
		Left inferior temporal gyrus	4.93	50, 20, -24	BA 20
		Left inferior temporal gyrus	4.76	54, 22, -26	BA 20
4	166	Left cerebellum	5.56	18, 68, -30	Lobule VI (hem)
		Left cerebellum	5.08	30, 70, -30	Lobule VII a Crus I (hem)
		Left cerebellum	4.55	26, 64, -28	Lobule VI (hem)
5	125	Right superior orbito temporal gyrus	6.6	-14, -56, -16	BA 11
		Right superior orbito temporal gyrus	5.41	-12, -54, -12	BA 11
		Right superior orbito temporal gyrus	4.96	-14, -56, -26	BA 11
6	113	Left post-central gyrus	6.22	56, 24, 50	BA 40
		Left post-central gyrus	4.74	48, 26, 56	BA 40
		Left Inferior Parietal Lobe	4.25	52, 30, 56	BA 40
7	106	Right superior temporal gyrus	7.18	-58, -10, -6	BA 41
8	91	Right insula	5.47	-46, 14, 20	BA 13
		Right post central gyrus	4.71	-60, 16, 16	BA 40
		Right post central gyrus	4.51	-60 22, 16	BA 40
9	67	Right middle cingulate cortex	4.59	-6, 14, 44	BA 24
		Left middle cingulate cortex	4.32	8, 10, 46	BA 24
		Right middle cingulate cortex	4.15	-8, 4, 46	BA 24

		Left middle cingulate cortex	4.09	0, 8, 48	BA 24
10	55	Left cerebellum	4.92	32, 68, -46	Lobule VII a Crus II (hem)
		Left cerebellum	4.20	26, 76, -50	Lobule VII a Crus II (hem)
11	54	Brain stem	5.78	-4, 32, -44	
12	52	Left cerebellum	4.69	12, 44, -20	Lobule V
13	51	Left middle cingulate cortex	4.75	14, 32, 48	BA 24
14	47	Right middle temporal gyrus	4.81	-46, 64, 12	BA 19
15	44	Left amygadala	5.28	22, 4, -2	Left amygadala
16	42	Right middle temporal gyrus	4.71	-48, 22, -20	BA 19
		Right middle temporal gyrus	4.14	-48, 32, -20	BA 19
		Right Inferior Temporal Gyrus	3.98	-46, 18, -26	BA 20
17	37	Left pre-central gyrus	4.74	56, -4, 8	BA 4
		Left Pre central gyrus	4.64	54, -2, 16	BA 4
18	37	Left superior temporal gyrus	5.21	34, -18, -48	BA 41
		Left superior temporal gyrus	4.50	32, -18, -42	BA 41
19	33	Left Middle Temporal Gyrus	4.57	50, 62, 8	BA 19

Healthy controls those rated higher pain scores to moderate pressure stimuli showed more average brain activations.

8. Post hoc t-tests results pre oxycodone patients.

8.1 Brain activation to mild pain versus touch.

The statistical comparison of the contrast mild pain versus touch in pre-oxycodone patients indicated a greater BOLD response to mild pain in contralateral pre-central gyrus, medial frontal gyrus, insula and lentiform nucleus; bilateral middle cingulate with contra lateral dominant activation and ipsilateral cerebellum lobule V and VI (hem) (Figure 9 and Table 6).

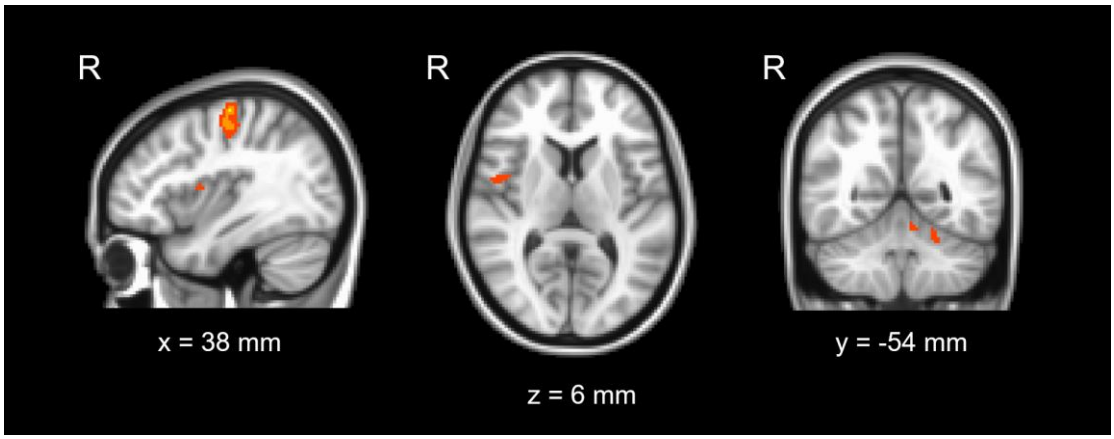


Figure 9: - Chronic pain patient's pre-drug treatment – average brain activation in response to mild pain versus touch (Results are in MNI space, where negative co-ordinates indicate left, posterior and inferior directions (-LPI).

Table 6. T- tests activations in chronic pain patient's pre-oxycodone comparing mild pain to touch stimulus.

Cluster No.	No. Of Voxels	Brain Area	T-Stat	MNI Co-ords	Area: prob or BA
1	402	Right pre central gyrus	8.73	-34, 20, 62	BA 4
		Right pre central gyrus	6.54	-38, 16, 52	BA 4
		Right pre central gyrus	6.36	-36, 20, 50	BA 4
		Right pre central gyrus	6.25	-40, 16, 56	BA 4
2	330	Right medial frontal gyrus	5.91	-8, 2, 64	
		Right middle cingulate gyrus	4.88	-4, 2, 46	BA 24
		Right middle cingulate gyrus	4.78	-10, 6, 50	BA 24
		Left middle cingulate cortex	4.70	4, -4, 40	BA 24
3	92	Right lentiform nucleus	5.34	-28, -6, -6	
		Right lentiform nucleus	4.80	-22, -8, -6	
		Right lentiform nucleus	4.17	-24, 0, -10	
4	67	Right insula	5.12	-34, -4, 10	
		Right pre central gyrus	5.03	48, 0, 8	
5	43	Right insula	5.52	-48, 20, 14	
6	32	Left cerebellum	4.52	20, 58, -20	Lobule VI (hem)
7	22	Left cerebellum	5.47	16, 54, -16	Lobule V

The average brain activations in chronic pain patient's response to mild pain before treatment with oral oxycodone was more compared to touch.

8.2 Brain activation to moderate pain versus touch.

The statistical comparison of the contrast moderate pain versus touch in pre-oxycodone patients indicated a greater BOLD response to moderate pain in contralateral superior temporal gyrus, pre-central gyrus, parahippocampal gyrus, middle cingulate, medial frontal gyrus and bilateral anterior cingulate, insula with ipsilateral dominance, amygdala, post-central gyrus, thalamus, inferior parietal lobe, lentiform nucleus, inferior and superior frontal gyrus and cerebellum lobules VI and VIII a (hem) (Figure 10 and Table 7).

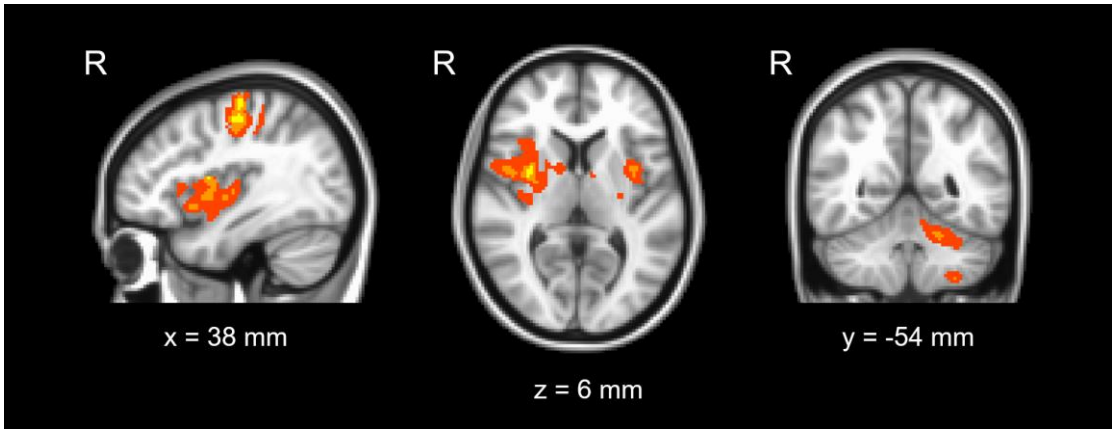


Figure 10: - Chronic pain patient's pre-drug treatment – average brain activation in response to moderate pain versus touch (Results are in MNI space, where negative co-ordinates indicate left, posterior and inferior directions (-LPI).

Table 7. T- tests activation in chronic pain patient's pre- oxycodone comparing moderate pain to touch stimulus.

Cluster No.	No. Of Voxels	Brain Area	T-Stat	MNI Co-ords	Area: prob or BA
1	2640	Right superior temporal gyrus	9.93	-52, 20, 12	BA 22
		Right insula	8.65	-34, -2, 10	BA 13
		Right para hippocampal gyrus	8.37	-22, -6, -20	BA 34
		Right insula	7.45	-46, -2, 8	BA 13
2	1067	Right middle cingulate	6.42	-4, -4, 38	BA 24
		Left anterior cingulate	5.68	0, -10, 36	BA 24
		Right middle cingulate	5.67	-6, 8, 52	BA 24
		Right medial frontal gyrus	5.28	-4, -6, 52	BA 10
3	1026	Right precentral gyrus	9.13	-36, 20, 62	BA 4
		Right precentral gyrus	7.57	-36, 18, 50	BA 4
		Right precentral gyrus	6.63	-48, 12, 50	BA 4
		Right precentral gyrus	6.42	-32, 22, 70	BA 4
4	619	Left insula	6.66	38, 4, -10	BA 13
		Left insula	6.09	36, -2, 8	BA 13
		Left insula	5.80	36, 16, -2	BA 13
		Left inferior frontal gyrus	5.04	28, -14, -18	BA 47
5	407	Left cerebellum	6.36	20, 58, -26	Lobule VI (hem)
6	239	Left post central gyrus	8.53	56, 24, 24	BA 5
		Left inferior parietal lobe	7.20	54, 38, 26	BA 3
		Left inferior parietal lobe	7.05	56, 36, 24	BA 3
7	106	Right anterior cingulate	5.38	-4, -28, 22	BA 24
8	96	Left lentiform nucleus	4.64	10, -2, -6	-
		Left lentiform nucleus	4.36	8, 0, 2	-
		Left thalamus	4.36	10, 4, 2	-
9	79	Left cerebellum	6.01	30, 54, -54	Lobule VIII a (hem)
10	31	Left superior frontal gyrus	6.73	16, 6, 68	BA 10
11	29	Right middle cingulate	5.05	-10, 20, 34	BA 24
12	29	Left amygdala	4.62	22, 6, -18	-
		Left amygdala	4.41	24, 8, -12	-
13	16	Left lentiform nucleus	4.54	28, 16, 10	-

The average brain activations in chronic pain patient's response to moderate pain before treatment with oral oxycodone was more compared to touch.

8.3 Brain activation to moderate pain versus mild pain

The statistical comparison of the contrast moderate pain versus mild pain in pre-oxycodone patients indicated a greater BOLD response to moderate pain in bilateral insula, post central gyrus with ipsilateral dominant activation in contra lateral pre central gyrus, lentiform nucleus, anterior and middle cingulate, thalamus and medial frontal gyrus and in ipsilateral cerebellum lobules VI, VII a, VIII a and b (hem) (Figure11 and Table 8).

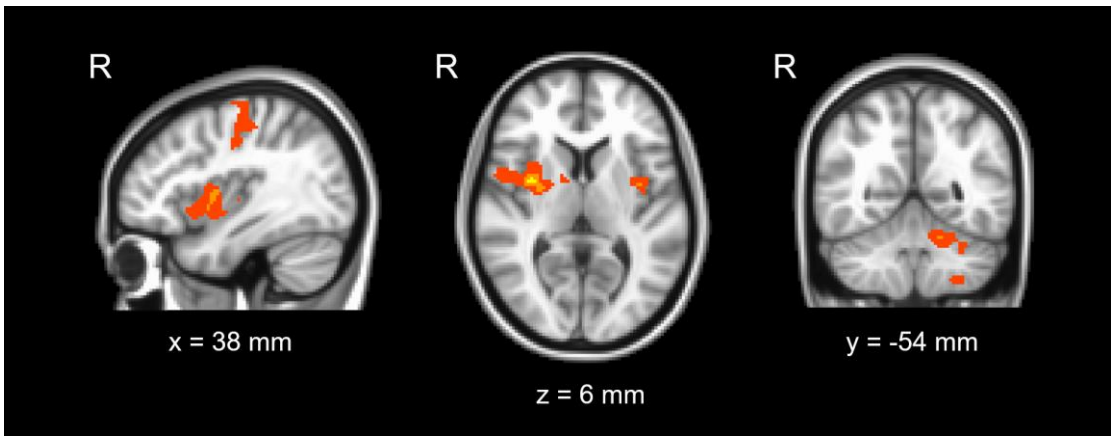


Figure 11: - Chronic pain patient's pre-drug treatment – average brain activation in response to moderate pain versus mild pain (Results are in MNI space, where negative co-ordinates indicate left, posterior and inferior directions (-LPI).

Table 8. T-tests activation in chronic pain patient's pre-oxycodone comparing moderate pain to mild pain stimulus.

Cluster No.	No. Of Voxels	Brain Area	T-Stat	MNI Co-ords	Area: prob or BA
1	1654	Right lentiform nucleus	10.71	-28, 0, 2	Right lentiform nucleus
		Right Insula lobe	9.65	-34, 0, 6	BA 13
		Right lentiform nucleus	8.06	-26, -4, -2	Right lentiform nucleus
		Right insula lobe	6.97	-38, -4, -2	BA 13
2	910	Right pre-central gyrus	7.76	-48, 10, 50	BA 4
		Right pre-central gyrus	7.30	-44, 12, 60	BA 4
		Right pre-central gyrus	6.91	-42, 14, 40	BA 4
		Right pre-central gyrus	6.88	-44, 14, 46	BA 4
3	613	Right middle cingulate	7.18	-8, -2, 40	BA 23
		Right middle cingulate	7.07	-8, 20, 36	BA 23
		Right middle frontal gyrus	5.96	-6, 8, 52	BA 46
		Right medial frontal gyrus	5.32	-4, 2, 54	BA 46
4	514	Left insula	7.93	36, 2, 4	BA 13
		Left insula	7.72	40, 18, -8	BA 13
		Left insula	6.82	40, 18, -8	BA 13
		Left insula	5.34	38, 6, -1	BA 13
5	332	Left cerebellum	7.11	16, 52, -24	Lobule VI (hem)
		Left cerebellum	5.62	30, 50, -26	Lobule VI (hem)
		Left cerebellum	5.17	32, 54, 34	Lobule VII a (hem)
6	196	Left post central gyrus	6.48	58, 36, 24	BA 40
		Left post central gyrus	6.31	56, 22, 22	BA 40
		Left post central gyrus	6.04	62, 26, 18	BA 40
		Left post central gyrus	5.39	50, 24, 18	BA 40
7	175	Right insula	8.15	-52, 22, 12	BA 13
		Right post central gyrus	5.09	-50, 22, 20	BA 40
		Right post central gyrus	4.65	-58, 24, 18	BA 40
8	67	Right thalamus	6.71	-10, 22, -4	Right ventral thalamus
9	66	Left cerebellum	6.25	30, 54, -52	Lobule VIII a (hem)
		Left cerebellum	4.92	20, 60, -52	Lobule VIII b (hem)
10	31	Left thalamus	5.02	14, 14, -2	Left ventral thalamus
11	25	Right anterior cingulate	4.24	-6, -22, 26	BA 24

The average brain activations in chronic pain patient's response to moderate pain before treatment with oral oxycodone was more compared to mild pain.

8.4. T-tests patients pre-drug VAS correlations.

The participants' pain scores (VAS scores) for the three pressure levels were correlated against the mean BOLD response to each level in order to explore whether there was a relationship between subjective ratings of pain and the neural response to pain. There was no significant linear relationship between the scores and activation in response to either touch or mild pain. However at the moderate pain stimulation level (activation to bar 3) there was a positive correlation between the BOLD response and VAS scores in the following brain regions: right anterior cingulate, left middle cingulate, right cerebellum, left inferior parietal lobe, left pre-cuneus and cuneus (Figure 12 and Table 9).

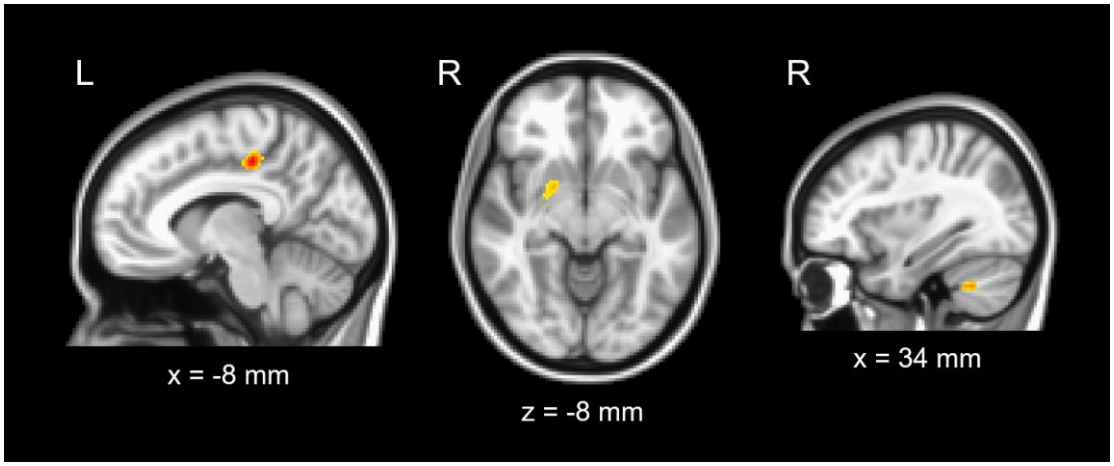


Figure 12:- Linear correlation between brain activation to moderate pain stimulus and pain ratings in patient's pre-drug treatment (Results are in MNI space, where negative co-ordinates indicate left, posterior and inferior directions (-LPI).

Table 9. T- tests activation chronic pain patient's pre-oxycodone VAS correlations.

Cluster No.	No. Of Voxels	Brain Area	T-Stat	MNI Co-ords	Area:prob or BA
1	206	Left middle cingulate	7.57	8, 28, 44	BA 24
2	42	Right lentiform nucleus	5.01	-20, -2, -8	-
3	36	Right cerebellum	5.86	-34, 52, -40	Lobule VII a (hem)
4	31	Right anterior cingulate	4.46	-6, -10, 32	BA 24
		Right anterior cingulate	4.18	-6, -20, 28	BA 24
		Right anterior cingulate	4.00	-4, -18, 30	BA 24
5	25	Left precuneus	5.40	22, 74, 22	-
		Left cuneus	4.09	22, 78, 30	-
6	23	Right cerebellum	5.31	-12, 66, -58	Lobule VIII b (hem)
		Right cerebellum	4.61	-18, 64, -60	Lobule VIII b (hem)
7	22	Left inferior parietal lobe	5.49	52, 40, 26	BA 3

Chronic pain patients before treatment with oral oxycodone those who rated higher pain scores to moderate pressure stimuli showed more average brain activations

9.0 Comparisons in activation between patients and controls

Behavioural Results – Patients

All subjects completed the pressure pain task. The pressure pain intensity was assessed from VAS scores obtained immediately after the subjects completed the experimental task and the average rating of intensity of stimulus before treatment with oxycodone was reported as 0.64 ± 1.11; mean ±SD [range 0-2] at 1 bar (touch condition) , 3.11 ± 1.79; mean ± SD (range 0-6) at 2 bar (mild pain) and 6.05 ± 2.33; mean ± SD (range2-9) at 3 bar (moderate pain) pressure stimulus and after treatment with oxycodone for seven days was reported as 0.47 ± 0.51; mean ±SD [range 0-1] at 1 bar (touch condition) , 2.58 ± 1.46; mean ± SD (range 0-4) at 2 bar (mild pain) and 6.23 ± 2.04; mean ± SD (range4-9) at 3 bar (moderate pain) pressure stimulus.

9.1 Brain activation to mild pain versus touch

The statistical comparison between controls and patients of the contrast (mild pain versus touch) found greater signal in the controls compared to patients. The activation regions were confined to the right fusiform gyrus and right inferior temporal gyrus, and the left cerebellar hemisphere in lobule VIIIa and VIIIb (Figure 13 and Table 10). There were no increased activation in patients compared to controls.

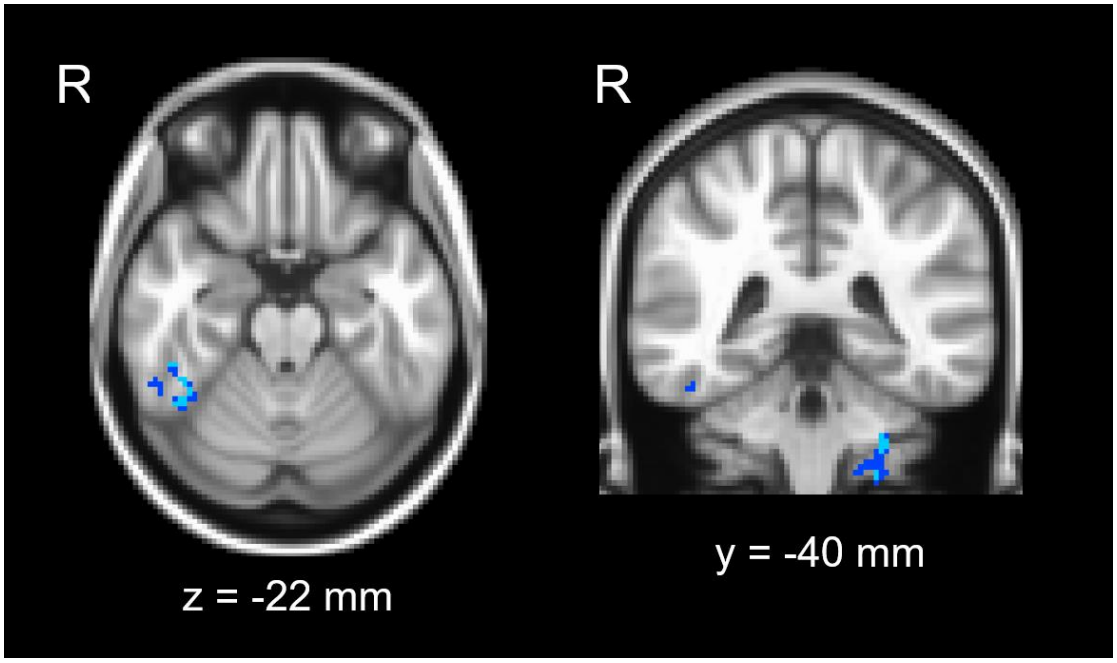


Figure 13:- Healthy controls versus patients pre-drug: Greater brain activation in response to mild pain versus touch in controls (Results are in MNI space, where negative co-ordinates indicate left, posterior and inferior directions (-LPI).

Table 10. T-test activation in chronic pain patient's pre-oxycodone versus controls comparing mild pain to touch stimulus.

Cluster No.	No. Of Voxels	Brain Area	T-Stat	MNI Co-ords	Area:prob or BA
1	143	Right fusiform gyrus	-4.63	-42, 54, -20	BA 37
		Right fusiform gyrus	-4.57	-40, 48, -22	BA 37
		Right fusiform gyrus	-4.23	-44, 44, -24	BA 37
		Right inferior temporal gyrus	-4.10	-46, 38, -22	BA 36
		Right inferior temporal gyrus	-3.91	-58, 48, -20	BA 37
2	85	Left cerebellum	-4.13	26, 40, -44	Lobule VIII a (hem)
		Left cerebellum	-4.09	24, 40, -56	Lobule VIII b (hem)
		Left cerebellum	-3.74	18, 38, -54	Lobule VIII b (hem)
		Left cerebellum	-3.62	18, 42, -53	Lobule VIII b (hem)
		Left cerebellum	-3.42	22, 46, -50	Lobule VIII b (hem)

The average brain activation in response to mild pain comparing to touch in healthy controls was more compared to chronic pain patients.

9.2 Brain activation of moderate pain versus touch

The statistical comparison between controls and patients of the contrast (moderate pain versus touch) found greater signal in the controls compared to patients. The activations were noted in the right supramarginal gyrus, inferior parietal lobe, superior temporal gyrus and precuneus. There were bilateral cerebellar hemisphere activations in lobules VI, VIIIa, VIIIb, IX, and X. (Figure 14 and Table 11). There was no increased activation in patients compared to controls.

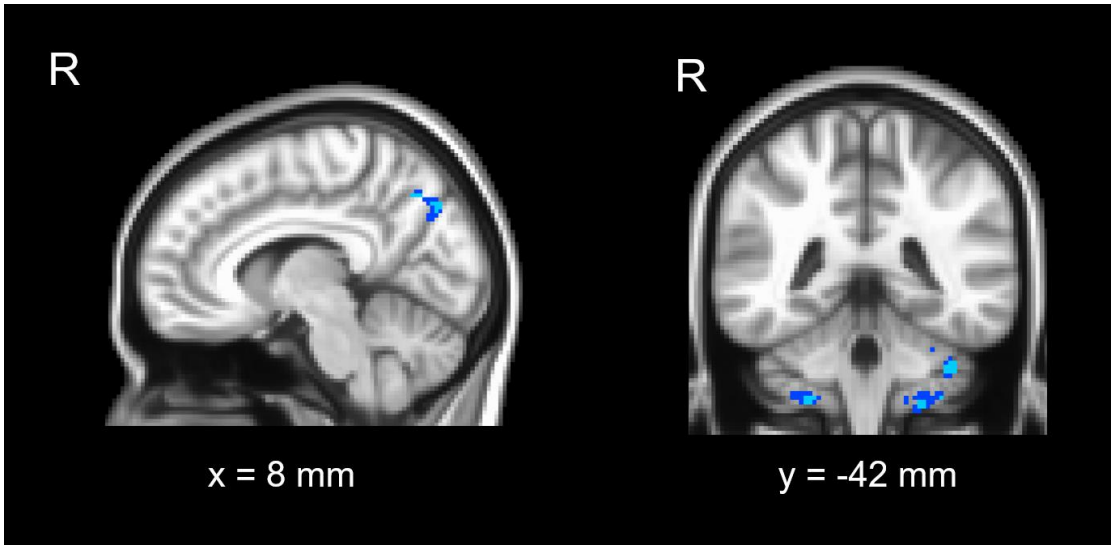


Figure 14: - Healthy controls versus patients pre-drug: Greater brain activation in response to moderate pain versus touch in controls (Results are in MNI space, where negative co-ordinates indicate left, posterior and inferior directions (-LPI).

Table 11. T-tests activation in chronic pain patients pre-oxycodone versus controls comparing moderate pain to touch stimulus.

Cluster No.	No. Of Voxels	Brain Area	T-Stat	MNI Co-ords	Area:prob or BA
1	265	Right supra marginal gyrus	-6.44	-46, 48, 34	BA 40
		Right supra marginal gyrus	-4.73	-58, 54, 34	BA 40
		Right inferior parietal lobe	-4.68	-54, 60, 40	BA 39
		Right inferior parietal lobe	-4.30	-52, 58, 44	BA 40
		Right superior temporal gyrus	-3.23	-50, 46, 22	BA 13
2	234	Left cerebellum	-4.59	38, 40, -40	Lobule VI (hem)
		Left cerebellum	-4.12	20, 36, -52	Lobule VIII b (hem)
		Left cerebellum	-4.00	24, 40, -54	Lobule VIII b (hem)
		Left cerebellum	-3.74	28, 40, -46	Lobule VIII a (hem)
		Left cerebellum	-3.71	34, 42, -52	Lobule VIII a (hem)
3	98	Right cerebellum	-4.46	-26, 42, -52	Lobule VIII b (hem)
		Right cerebellum	-3.71	-28, 38, -48	Lobule X (hem)
		Right cerebellum	-3.65	-22, 48, -48	Lobule VIII b (hem)
		Right cerebellum	-3.58	-34, 48, -54	Lobule VIII a (hem)
		Right cerebellum	-3.53	-20, 48, -52	Lobule VIII b (hem)
4	87	Right precuneus	-4.19	-8, 66, 44	--
		Right precuneus	-4.02	-8, 76, 40	-
		Right precuneus	-3.53	-6, 72, 34	-
5	86	Left cerebellum	-4.31	4, 56, -48	Lobule X (hem)
		Left cerebellum	-4.14	-2, 64, -52	Lobule IX (hem)
		Left cerebellum	-3.59	-4, 60, -48	Lobule IX (hem)

The average brain activation in response to moderate pain comparing to touch in healthy controls was more compared to chronic pain patients.

9.3 Brain activation to moderate pain versus mild pain.

There was no statistically significant difference detected between patients and controls for the contrast moderate pain versus mild pain.

10. Comparison of chronic pain patients pre- and post-oxycodone

Behavioural Results – Patients pre-oxycodone and post oxycodone

All subjects completed the pressure pain task. The pressure pain intensity was assessed from VAS scores obtained immediately after the subjects completed the experimental task and the average rating of intensity of stimulus before treatment with oxycodone was reported as 0.64 ± 1.11; mean ±SD [range 0-2] at 1 bar (touch condition) , 3.11 ± 1.79; mean ± SD (range 0-6) at 2 bar (mild pain) and 6.05 ± 2.33; mean ± SD (range2-9) at 3 bar (moderate pain) pressure stimulus and after treatment with oxycodone for seven days was reported as 0.47 ± 0.51; mean ±SD [range 0-1] at 1 bar (touch condition) , 2.58 ± 1.46; mean ± SD (range 0-4) at 2 bar (mild pain) and 6.23 ± 2.04; mean ± SD (range4-9) at 3 bar (moderate pain) pressure stimulus.

10.1 Brain activation to mild pain versus touch

The statistical comparison between pre and post-oxycodone of the contrast mild pain versus touch found greater signal in post-oxycodone patients in the contra lateral cerebellum in lobule V and VI (Figure 15 and Table 12).

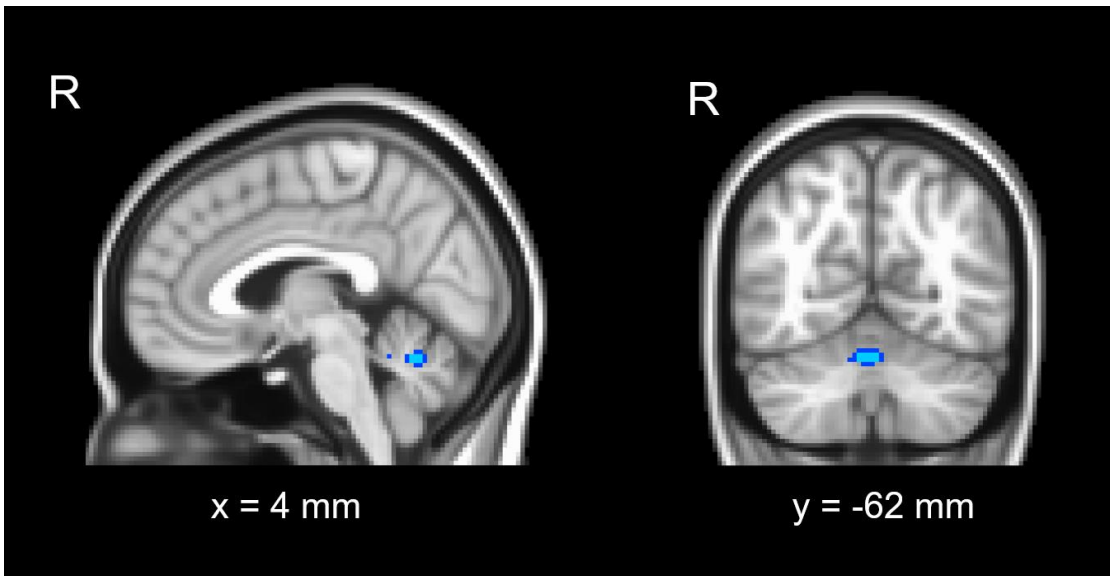


Figure 15: - Chronic pain patients pre- versus post-drug treatment: average brain activation in response to mild pain versus touch. Post drug patients

showed more activation to mild pain. (Results are in MNI space, where negative co-ordinates indicate left, posterior and inferior directions (-LPI).

Table 12. T-tests activation in chronic pain patients comparing pre-oxycodone versus post oxycodone to mild pain versus touch stimulus.

Cluster No.	No. Of Voxels	Brain Area	T-Stat	MNI Co-ords	Area: prob or BA
1	123	Right cerebellum	-4.42	-2, 60, -24	Lobule V
		Right cerebellum	-4.06	-12, 54, -24	Lobule VI (hem)
		Right cerebellum	-3.76	-10, 50, -22	Lobule V

The average brain activations were more in chronic pain patients treated with oxycodone compared to pre-treatment patients for mild pain.

10.2 Brain activation to moderate pain versus touch

The statistical comparison between pre- and post-oxycodone of the contrast moderate pain versus touch found greater signal in post-oxycodone patients in ipsilateral cerebellum lobules VIII a (hem) and VIII a crus III (Figure 16 and Table 13).

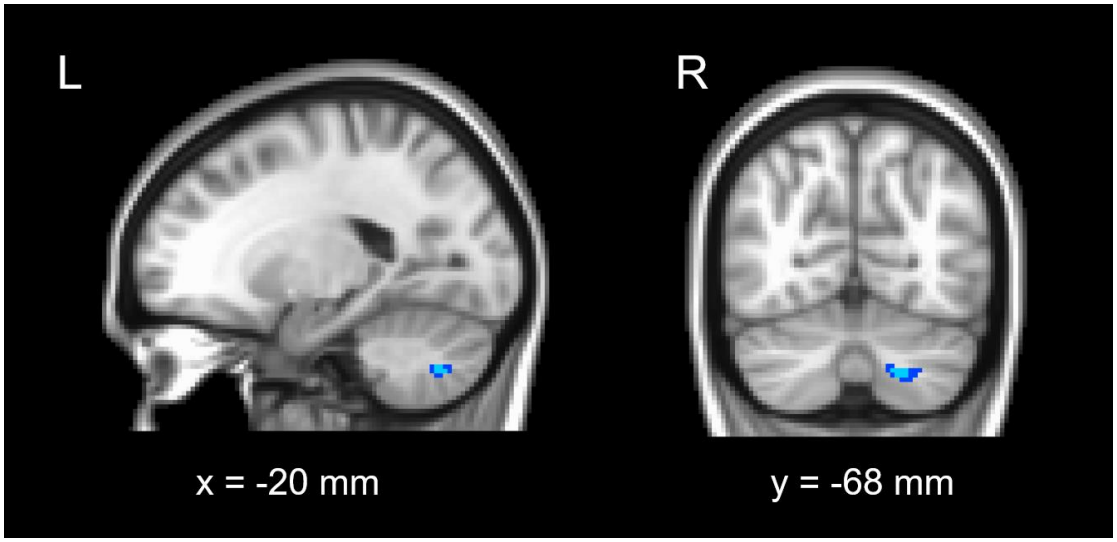


Figure 16: - Chronic pain patients pre- versus post-drug treatment: average brain activation in response to moderate pain versus touch. Post drug patients showed more activation to moderate pain (Results are in MNI space, where negative co-ordinates indicate left, posterior and inferior directions (-LPI).

Table 13. T-tests activation comparing pre- oxycodone versus post oxycodone to moderate pain to touch stimulus.

Cluster No.	No. Of Voxels	Brain Area	T-Stat	MNI Co-ords	Area: prob or BA
1	135	Left cerebellum	-4.92	18, 68, -42	Lobule VIII a (hem)
		Left cerebellum	-4.23	28, 76, -44	Lobule VIII a crus III (hem)
		Left cerebellum	-3.53	16, 76, -42	Lobule VIII a crus III (hem)
		Left cerebellum	-3.43	12, 70, -38	Lobule VIII a (hem)

The average brain activations were more in chronic pain patients treated with oxycodone compared to pre-treatment patients for moderate pain.

10.3 Brain activation to moderate pain versus mild pain

There was no statistically significant difference between pre- and post-oxycodone patients in the contrast moderate pain versus mild pain.

11. T-tests results comparing patients post-drug versus controls.

11.1 Brain activation to mild pain versus touch.

The statistical comparison between post-oxycodone versus controls of the contrast mild pain versus touch found greater signal in controls to mild pain in ipsilateral insula, caudate, claustrum, middle and posterior cingulate, precuneus and contralateral cuneus, anterior, middle cingulate cortex, middle occipital and temporal gyrus, claustrum, pre and post central gyrus and inferior frontal gyrus (Figure 17 and Table 14).

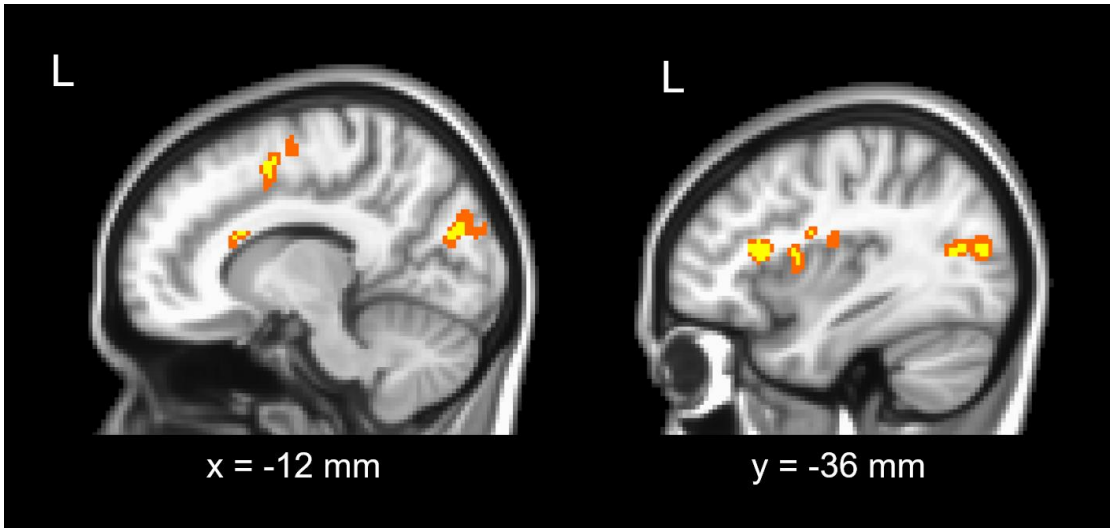


Figure 17. Chronic pain patient's post-drug versus controls, mild pain versus touch; average brain activation in response to mild pain versus touch. Healthy controls showed more activation. (Results are in MNI space, where negative co-ordinates indicate left, posterior and inferior directions (-LPI).

Table 14. T- tests activation comparing post oxycodone versus healthy controls to mild pain versus touch stimulus.

Cluster No.	No. Of Voxels	Brain Area	T-Stat	MNI Co-ords	Area: prob or BA
1	611	Right cuneus	4.82	-16, 78, 20	BA 18
		Right cuneus	4.07	-6, 82, 26	BA 18
		Right middle occipital gyrus	3.98	-38, 78, 10	BA 19
		Right middle temporal gyrus	3.97	-40, 78, 22	BA 21
2	398	Left insula	4.54	44, 8, 20	BA 13
		Left insula	4.33	32, -8, 14	BA 13
		Left insula	4.23	30, -10, 16	BA 13
		Left insula	4.06	34, 12, 18	BA 13
3	384	Right claustrum	4.44	-24, -20, 14	-
		Right anterior cingulate	3.92	-10, -16, 22	BA 24
		Right anterior cingulate	3.88	-8, -10, 22	BA 24
		Right anterior cingulate	3.80	-12, -20, 20	BA 24
4	372	Left insula	5.63	38, -22, 14	BA 19
		Left insula	4.09	28, -14, 18	BA 19
		Left caudate	4.05	16, -14, 18	-
		Left claustrum	3.65	26, -24, 16	-
5	342	Left middle occipital gyrus	4.57	42, 88, 16	BA 19
		Left middle occipital gyrus	4.14	30, 68, 12	BA 19
		Left middle temporal gyrus	3.98	32, 12, 16	BA 21
		Left middle occipital gyrus	3.80	38, 82, 14	BA 19
6	301	Right middle cingulate	4.04	-14, -6, 50	BA 24
		Right middle cingulate	3.90	-16, -8, 44	BA 24
		Right middle cingulate	3.88	-18, -10, 42	BA 24
		Right middle frontal	3.84	-14, 6, 64	BA 46
7	218	Right precentral gyrus	4.41	-52, 2, 26	BA 4
		Right postcentral gyrus	3.72	-54, 12, 18	BA 3
		Right inferior frontal gyrus	3.50	-50, 2, 18	BA 44
		Right precentral gyrus	3.17	-48, 0, 10	BA 4

8	149	Left caudate	3.93	20, 12, 26	-
		Left middle cingulate	3.64	16, 0, 26	BA 24
		Left thalamus	3.61	22, 18, 16	-
		Left middle cingulate	3.41	18, 20, 28	BA 24
9	147	Left precuneus	3.73	24, 56, 26	=
		Left posterior cingulate	3.63	24, 58, 20	BA 24
		Left inferior parietal lobe	3.61	28, 48, 22	BA 40

The average brain activations were more in chronic pain patients treated with oxycodone compared to pre-treatment patients for mild pain.

11.2 Brain activation to moderate pain versus touch.

The statistical comparison between post-oxycodone versus controls of the contrast moderate pain versus touch found greater in controls to moderate pain in bilateral cerebellum lobule VII a Crus I (hem), insula and claustrum; ipsilateral cerebellum lobule VIII b (hem), middle cingulate and occipital gyrus, and contralateral anterior, middle and posterior cingulate, para hippocampal gyrus, inferior temporal gyrus, fusiform gyrus and middle frontal gyrus (Figure 18 and Table 15).

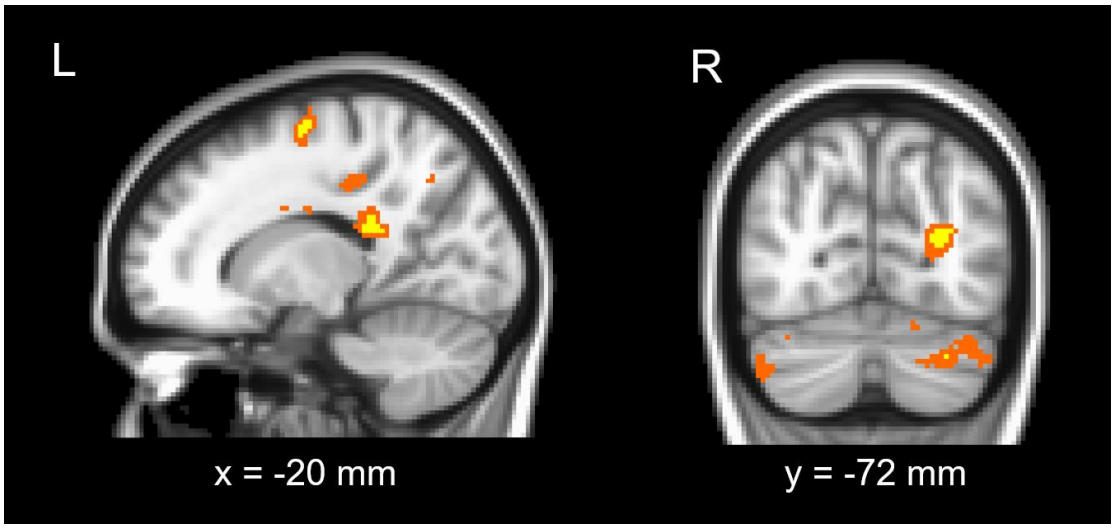


Figure 18. Chronic pain patients post-drug versus controls, average brain activation in response to moderate pain versus touch. Healthy controls showed more activation to moderate pain. (Results are in MNI space, where negative co-ordinates indicate left, posterior and inferior directions (-LPI).

Table 15. T-tests activation comparing post-oxycodone versus controls to moderate pain versus touch stimulus.

Cluster No.	No. Of Voxels	Brain Area	T-Stat	MNI Co-ords	Area: prob or BA
1	2866	Left insula	5.76	26, 44, 24	BA 13
		Left middle occipital gyrus	5.47	28, 70, 16	BA 19
		Left middle cingulate	5.29	20, 16, 28	BA 24
		Left claustrum	4.95	26, -22, 12	-
2	1809	Right caudate	6.55	-24, 40, 22	
		Right posterior cingulate	4.92	-24, 36, 30	BA 24
		Right middle cingulate	4.80	-22, 16, 42	BA 24
		Right insula	4.75	28, 34, 26	BA13
3	577	Left cerebellum	4.35	50, 64, -38	Lobule VII a crus I (hem)
		Left cerebellum	4.28	30, 72, -34	Lobule VII a crus I (hem)
		Left cerebellum	3.89	50, 56, -34	Lobule VII a crus I (hem)
		Left cerebellum	3.86	46, 68, -32	Lobule VII a crus I (hem)
4	336	Right claustrum	6.27	-24, -24, 18	
		Right anterior cingulate	4.27	-12, -18, 20	BA 24
5	212	Left cerebellum lobule VIIIb (hem)	5.34	18, 40, -56	Left cerebellum
		Left cerebellum lobule VIIIa (hem)	4.46	34, 44, -54	Left cerebellum
		Left cerebellum lobule VIIIb (hem)	4.23	28, 42, -56	Left cerebellum
		Left cerebellum lobule VI (hem)	4.05	34, 40, -40	Left cerebellum
6	136	Right cerebellum)	4.06	-46, 74, -42	Lobule VII a crus I (hem
		Right cerebellum	3.85	-48, 72, -36	Lobule VII a crus I (hem)
		Right cerebellum	3.68	-44, 80, -36	Lobule VII a crus I (hem)
		Right cerebellum	3.62	-42, 80, -44	Lobule VII a crus I (hem)
7	130	Right cerebellum	3.88	-38, 86, -36	Lobule VII a crus I (hem)
		Right cerebellum	3.61	-32, 78, -28	Lobule VII a crus I (hem)
		Right cerebellum	3.32	-20, 80, -22	Lobule VII a crus I (hem)
		Right cerebellum	3.28	-30, 80, -38	Lobule VII a crus I (hem)
8	96	Right parahippocampal gyrus	3.59	-42, 30, -14	-
		Right inferior temporal gyrus	3.51	-60, 32, -24	BA 20

		Right fusiform gyrus	3.50	-48, 26, -22	BA 37
		Right inferior temporal gyrus	3.45	48, 20, -26	BA 20

The average brain activations were more in chronic pain patients treated with oxycodone compared to pre-treatment patients for moderate pain.

11.3 Brain activation to moderate pain versus mild pain.

The statistical comparison between post-oxycodone versus controls of the contrast moderate pain versus mild pain found greater signal in controls to moderate pain in insula, middle and posterior cingulate cortex, caudate, cerebellum, middle and inferior temporal gyrus (Figure19 and Table 16).

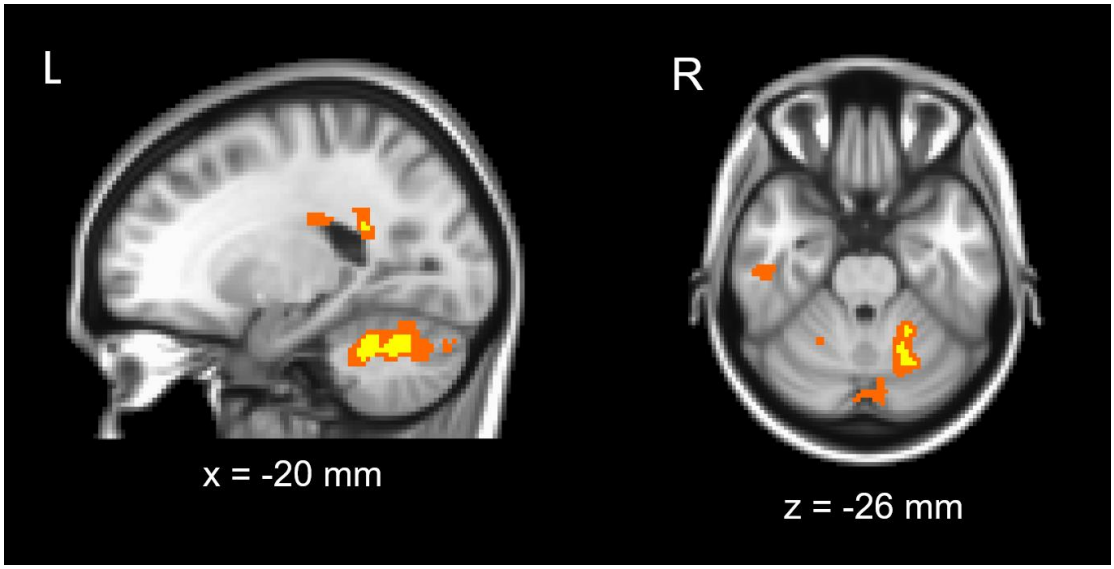


Figure 19. Chronic pain patients post-drug versus controls, average brain activation in response to moderate pain versus mild pain. Healthy controls showed more activation to moderate pain. (Results are in MNI space, where negative co-ordinates indicate left, posterior and inferior directions (-LPI).

Table 16. T-tests activation comparing post-oxycodone versus controls to moderate pain versus mild pain stimulus.

Cluster No.	No. Of Voxels	Brain Area	T-Stat	MNI Co-ords	Area: prob or BA
1	1268	Left cerebellum	5.26	28,72, -34	Lobule VIII a crus III (hem)
		Left cerebellum	4.88	20, 48, -30	Lobule VII a crus I (hem)
		Left cerebellum	4.78	20, 62, -30	Lobule VI (hem)
		Left cerebellum	4.63	38, 68, -34	Lobule VII a crus I (hem)
2	576	Right posterior cingulate	4.86	-14, 36, 26	BA 24
		Right posterior cingulate	4.49	-28, 48, 24	BA 24
		Right caudate	4.46	-24, 28, 24	-
		Right insula	4.23	-30, 34, 26	BA13
3	320	Right cerebellum	4.03	50, 64, -38	Lobule VII a crus II (hem)
		Left cerebellum	3.88	30, 72, -34	Lobule VIII a crus II (hem)
		Right cerebellum	3.81	50, 56, -34	Lobule VII a crus I (hem)
		Left cerebellum	3.72	46, 68, -32	Lobule VII a crus II (hem)
4	132	Left posterior cingulate	4.31	22, 48, 22	BA 24
		Left insula	3.91	-12, -18, 20	BA 13
5	125	Right middle temporal gyrus	3.82	-48, 30, -20	BA 37
		Right inferior temporal gyrus	3.73	-48, 18, -26	BA 37
		Right inferior temporal gyrus	3.73	-52, 28, -22	BA 37
		Right inferior temporal gyrus	3.65	-50, 22, -24	BA 37
6	115	Left caudate	4.51	22, 26, 26	-
		Left middle cingulate	3.60	-48, 72, -36	BA 24
7	115	Right cerebellum	3.60	-26, 50, -32	Lobule VII a crus II (hem)
		Right cerebellum	3.61	-24, 58, -32	Lobule VI (hem)
		Right cerebellum	3.32	-20, 80, -22	Lobule VII a crus I (hem)
		Right cerebellum	3.28	-30, 80, -38	Lobule VII a crus I (hem)
8	90	Right cerebellum	3.60	-30, 82, -40	Lobule VII a crus III (hem)
		Right cerebellum	3.55	-38, 80, -40	Lobule VII a crus III (hem)
		Right cerebellum	3.52	-36, 84, -36	Lobule VII a crus I (hem)

		Right cerebellum	3.49	-32, 86, -38	Lobule VII a crus III (hem)

The average brain activations were more in chronic pain patients treated with oxycodone compared to pre-treatment patients for moderate pain.

12. Discussion

There are various commercially available and custom-built experimental pain induction methods. Some of the commonly utilized experimental pain stimulus methods include mechanical, chemical, thermal and electrical techniques. Mechanical stimulation of skin can provide exact and reproducible pressure and the response can be assessed quantitatively. The pressure pain stimulation activates both Aδ and c fibers. The commercially available and custom-built handheld pressure algometers have number of limitations. This equipment has to be used in a consistent way by one examiner to induce pressure pain to minimize the variations in rate of pressure increase. To overcome these issues it is recommended that experiments using handheld pressure algometers should use fixed rate and one experimenter to apply pressure stimulus to enhance the reliability. But unfortunately, this has not shown to be a reliable experimental pain induction method in fMRI pain studies[46, 195, 196].

The experimental heat pain is applied by a heating thermode. This method also activates Aδ and c fibers. Slow heat is shown to preferentially activate c fibers. This technique has few limitations; though the skin surface temperature is controlled, stimuli applied with different methods are not necessarily comparable, because the intracutaneous temperature profiles may be different depending on wavelength or type of contact. This makes comparisons difficult between, for example, different drug studies. The speed of conventional cutaneous heating is usually slow and does not allow for an appropriate study of neural phenomena[197, 198].

The novel aspect of this study was the custom designed electronic pressure algometer. This was microprocessor controlled and could deliver different pre-set pressures to the syringe as described previously. Pressures of one, two and three bar were applied resulting in three different stimuli intensities. The pneumatic controller was interfaced to the fMRI scanner. In this way the timing of the delivery of the randomized stimuli and the scanner acquisition times was synchronized. The electronic pressure algometer had a number of safety features. In the event of a failure of electrical power the device would

automatically release any pressure. The front panel had a mechanical pressure gauge that displayed the applied pressure at any given time and this was independent of any electronics. The front panel also had an emergency manual over ride switch which would disconnect the electronic pressure algometer from the pressure generator and vent any pressure generated by the device. This controller was kept outside the MRI scanning room and was connected to the pressure algometer through plastic tubing. The device was tested on 20 healthy controls for the reliability and reproducibility of the pressure pain stimuli before commencing the experimental pressure pain fMRI study. The results from device testing were reliable and were consistently reproducible. This electronic pressure algometers has more potential for further studies by including more pain levels or comparing the effects of longer pain stimulus versus shorter pain stimulus and by dynamically varying levels.

We investigated the cortical and subcortical neural activation in response to pressure pain stimuli in a healthy volunteer population. After this we investigated the cortical and subcortical neural activation in response to pressure pain stimuli in a cohort of patients with a diagnosis of chronic lumbar radicular pain. Further to this the effect of oxycodone administration was examined in this chronic lumbar radicular pain population. We explored the brain activation patterns in these groups in response to mild pain versus touch, moderate pain versus mild pain and moderate pain versus touch. In order to do this we used a novel event-related paradigm and specially designed and commissioned an electronic pressure algometer. We compared our data with other published pain studies as well as with a recently published meta-analysis of neuroimaging data[97]. Although there were marked similarities in the cortical and sub cortical activation pattern to all levels of pressure stimulation and between patients and controls, the analyses also revealed a number of differences, which are discussed below.

12.1 Healthy controls

The cortical and subcortical bases of pressure pain processing

Early neuroimaging studies looking at haemodynamic correlates of pain involving healthy subjects indicated that multiple cortical and sub-cortical region activations during painful cutaneous heat stimulation[34]. The most commonly activated regions included primary S1 and S2. These areas are perceived to process perception of sensory features of pain. ACC and IC cortices have been implicated in the affective processing of pain, PFC and parietal association areas activations may be related to cognitive variables, such as memory or stimulus evaluation. Motor and pre-motor cortical areas activations may be related to suppression of movement or actual pain-evoked movements themselves. Subcortical activations are also observed in thalamus (Th), basal ganglia, and cerebellum. The differences and similarities of these activation patterns may be to some extent due to different technical procedures and differences in statistical analyses and power[199, 200].

The present study demonstrated greater activations in bilateral anterior insula cortex, thalamus, p ACC, a MCC and p MCC and d PCC, cerebellum, superior, middle (also known as dorso lateral PFC (DLPFC) and inferior frontal gyrus, middle temporal gyrus, SMG, S I, SII and motor cortex.

The anterior insula receives input from the peripheral autonomic receptors and may become activated during affective tasks or perception of pain due to changes in the haemodynamic parameters[88, 92]. A recent human fMRI study has shown functional differentiation in the insular-cortical network.[91]. There has been some uncertainty regarding the involvement of the anterior or posterior insula. This study finding indicates that the anterior insula plays an important role in pressure pain processing. It is yet unclear whether the Broadmann's Area 13 (BA 13) which is anterior insular cortices or posterior insular cortices (BA 14-16) are predominantly involved in pain perception[84, 201]. Previous studies have reported that Insula is involved in processing many aspects of the conscious experience of pain such as affect[86, 87], autonomic activity[88], interoception[89] and temperature[90].

The present study demonstrated greater activations in thalamus for pressure pain stimulus. The thalamus is one of the structures that receive projections from multiple ascending pain pathways. The structure is not merely a relay centre but is involved in processing nociceptive information before transmitting the information to various parts of the cortex. Interestingly, most pain imaging studies show bilateral thalamic activation which may be partly due to attention processing along with sensory processing[121, 122].Our findings indicate that with higher levels of discomfort MDT is activated. It may be the case that MDT activation in our study is associated with increasing levels of discomfort. Previous studies have reported that thalamic activation is observed in imaging studies of cognition[115-117], pain[30, 34, 118], memory[119] and sexual arousal [120].

This study demonstrated greater activation in p ACC, a MCC and p MCC and d PCC, for pressure pain stimulus. Our findings indicate that cingulate cortex plays an important role in pressure pain transduction and also confirm the role of d PCC in processing touch and nociceptive information. Previous studies have reported that ACC is involved in coding for the reward properties of a particular behaviour[95, 132], noxious stimuli including pressure and thermal stimuli over 46 ^{0} c[131]. The p MCC, and d PCC may be involved in orienting the body towards innocuous touch and noxious somatosensory stimuli. The caudal part of, a MCC was activated by both innocuous touch and noxious stimulus and the authors concluded that it might be related to cognitive processing other than nociception and intensity rating [135]. It has been suggested that p MCC may share some functions with d PCC[130]..

To date animal data has indicated PCC receives a direct projection from the main pain and temperature transmitting STT and also contains nociceptive neurons. [202] Most imaging pain studies report activation of ACC and MCC because these regions are thought to be involved in processing information related to affective component and rarely PCC activation is observed.[130] Two studies looking at painful and non-painful electrical stimulation of muscle and simple finger movement have reported activation of d PCC [203, 204]. A recent article concluded that irrespective of side of stimulation the cingulate response could be contralateral or bilateral[205]. Sub region cingulate activation may vary

depending on the mode of stimulation, rostral cingulate is commonly activated in tonic experimental pain, while caudal cingulate is activated in response to phasic pain experiments[206].

This study demonstrated bilateral cerebellar activation with ipsilateral hemisphere dominance to all stimulation levels. At mild pain versus touch comparison, we observed bilateral activation of lobule VI hemisphere (hem), VIII $_b$; ipsilateral lobule VIII $_a$ and contralateral lobule V and cerebellar vermis. At moderate pain versus mild pain comparison, we observed bilateral activation in lobules VI (hem) with ipsilateral lobule V and VIII $_a$ and contralateral VII a crus I and crus III (hem). At moderate pain versus touch comparison, we observed activations in ipsilateral VI (hem), VIII $_a$, and contralateral brainstem, lobules VIII $_b$, X and dentate.

This study has clearly demonstrated strong ipsilateral activations for all stimulus levels in lobule V and VI with some contralateral activation. This confirms that these vermal lobules are associated with sensorimotor processing. It may also infer that dorsal spino cerebellar tract may terminate in lobule VI-VIII. Further studies are needed to confirm the termination of dorsal spino cerebellar tract. A recent meta-analysis of neuroimaging data of cerebellar activation for various stimuli concluded that lobule V and VI processes sensorimotor tasks, language tasks activate lobule VI crus I, executive function tasks activated lobule VII B and emotional processing in crus I and medial VII[207]. Another study comparing healthy subjects and trigeminal neuropathic patients stated that in healthy subjects, noxious heat increased activation in cognitive processing lobules crus II and VIIB, and in sensory-motor integration lobule VI and brush stimuli in patient group resulted in activation in areas lobules IV, V, and VI which is considered as sensory motor integration area, and lobule VIII$_b$ which is considered as secondary sensory processing area, lobules VII$_b$ crus I, crus II, and dentate nucleus is considered to process cognition and only noxious stimuli activated cerebellar areas and not innocuous stimuli[208]. In another study comparing neutral stimulus, pleasant touch and painful touch showed bilateral activation of brainstem to neutral stimulus, activation of brainstem to pleasant

touch and parts of brainstem including peri-aqueductal grey and in primary motor cortex to painful touch[95].

This study demonstrated greater activation in DLPFC and VLPFC for pressure pain stimulus. Other investigators using positron emission tomography (PET) found bilateral prefrontal cortex activation of the orbitofrontal, perigenual cingulate and dorsolateral prefrontal cortices using heat stimuli on capsaicin-treated skin[161, 162]. Previous studies have reported that right inferior pre frontal cortex (RIPFC) is more important for monitoring the sensory signals[159]. Middle frontal cortex (MFC) is involved in a broader range of cognitive processing such as simple action rules and spatial maps associated with planning and working memory[160]. Our study indicates that DLPFC and VLPFC plays a major role in pressure pain processing.

This study demonstrated greater activations in contralateral activation of superior temporal gyrus at moderate pain versus mild pain, which might be due to the noise generated by MRI. Currently available neuroimaging studies have suggested that the temporal gyrus is not involved in processing noxious stimulus[166]. In our study, we noticed activation of the middle temporal gyrus. We suggest that activation may be associated with reading a written message displayed on the projection screen, “time remaining for the completion of scan is …. minutes”, which counted backwards from 15 minutes until the completion of the task. The present study of activations in both left inferior frontal gyrus (LIFC) and middle temporal gyrus (MTG) do accord with findings from other studies of pain processing[173]. The posterior middle temporal gyrus (MTG) and inferior frontal gyrus (IFG) are two critical areas of the language network in the brain and MTG is involved in the retrieval of lexical syntactic information and the IFG in unification operations[158]. A recent fMRI study confirmed that left inferior frontal gyrus (LIFG) is involved in the integration process and MTG in the retrieval of lexical-syntactic information. The lexical information has to be available for longer time intervals than during the processing of random word sequences and this concluded that the syntactic unification process requires the dynamic interplay between LIFG and MTG[167]. The superior temporal gyrus (STG) is involved in auditory processing, including language, but also has been

implicated as a critical structure in social cognition.[168] We also observed contralateral activation of superior temporal gyrus at moderate pain versus mild pain which might be due to the noise generated by MRI. Acoustic analysis of words is carried out in the superior temporal cortex, and visual analysis of words in the posterior inferior temporal cortex and temporo-occipital cortex[166].

This study demonstrated contralateral activation of the primary somato sensory area (SI) in response to mild pain versus touch, bilateral SI activation in response to moderate pain versus mild pain, and in response to moderate pain versus touch a stronger ipsilateral SI and contralateral SII activation was observed. Our study findings from the healthy volunteers showed ipsilateral activation only in the motor cortex (M1) to moderate pain versus touch stimulus. We attribute this to the fact that subjects were required to grasp the applicator in their hand and insert their thumb into the apparatus.

Analysis of the relationship between VAS pain scores and BOLD response demonstrated no relationship between VAS and BOLD response to either touch or mild pain (activation to bar 1 and 2). However at moderate pressure pain stimulus (bar 3) there was a positive correlation between VAS scores and BOLD response in a number of anatomical regions including: bilateral cerebellum, inferior temporal cortex and parietal lobe, the right superior orbital gyrus, the left hippocampus, left pallidum and right brain stem. There were no negative correlations observed. This suggests that healthy controls who rated the bar 3 pressure level as most painful were more likely to show increased brain activation in aforementioned regions in response to moderate pressure pain stimulus (bar 3). To our knowledge these findings are not reported in earlier studies.

12.2 Chronic Pain Patients

12.3 Chronic pain patients and controls.

There is a general perception that chronic pain patients have enhanced pain processing and in addition this cohort of patient's also process acute pain differently compared to healthy controls. It is believed that chronic pain patients with diagnosis such as fibromyalgia and irritable bowel syndrome (IBS) show higher pain ratings and enhanced pain-evoked neural responses to experimental pain stimuli[178].

Imaging chronic pain remains challenging due to the presence of background pain that is difficult to experimentally manipulate. Also chronic pain patients are inhomogeneous, and medicated with various analgesic regimes and assessment is further complicated by the presence of other co-morbid conditions[9]. Opioid therapy for chronic non-cancer pain is indicated when all other therapies have failed. Morphine and aspirin, introduced for the treatment of pain more than a century ago, continue to dominate biomedical publications despite their limited effectiveness in many areas (e.g., neuropathic pain) and multiple serious adverse effects[209].

We had hypothesized that the chronic pain patients would demonstrate greater brain activation to the pressure pain stimuli than the controls, due to the presence of constant or spontaneous background pain. However, contrary to our prediction, the present study found less cortical and sub-cortical activation in brain areas including cerebellar activation in patients with chronic pain compared to healthy controls. This study demonstrated activations in superior, middle and inferior frontal gyrus, anterior and middle cingulate, lentiform nucleus, insula, cerebellum, superior temporal gyrus, para-hippocampal gyrus, primary somatosensory area, inferior parietal lobe, thalamus and amygdala.

Although this was an unexpected finding, one possible explanation is that this reduced activation in the chronic pain group may be due in part to neurodegenerative processes in both gray and white matter, the cerebellum and neural pathways[9]. In recent times multiple investigators have demonstrated that

these changes can be partly reversed when the underlying pain is treated appropriately[210, 211]. It has also been demonstrated that acute painful stimuli activates somatosensory, insular and cingulate cortical regions while spontaneous pain and allodynia activate PFC and the limbic system.[9]

Studies investigating the effect of acute pain on attentional networks and the default mode network give inconsistent and conflicting results. In an elegant fMRI study involving healthy controls, using painful electric shocks as stimuli, found that the default mode network activity in response to pain was biphasic. This study concluded that increased pain perception results in greater activity in default mode network and the anti-correlation between the default mode network and dorsal attentional network disappear in chronic pain patients. The brain regions included in the default mode network include pre-cuneus, posterior cingulate cortex, bilateral supramarginal gyrus and ventro-medial pre-frontal gyrus[212]. Another study comparing executive function in chronic pain patients and healthy controls concluded that patients with higher anxiety or fatigue levels showed increased activity in the IC and IFG and patients with low anxiety or fatigue levels showed increased activation in right inferior temporal gyrus and fusiform gyrus that connects to the pre-SMA during inhibition[213]. In an elegant review by Moultan et al examining the cerebellar activations in patients with trigeminal neuralgia compared to healthy controls inferred that in controls noxious heat showed increased activation in areas thought to be in involved in cognitive processing (lobules Crus II and VIIB), as well as sensory-motor integration (lobule VI) whereas brush stimuli applied to the affected V2 region produced allodynia in these subjects and resulted in activation in areas involved in sensory-motor integration (lobules IV, V, and VI), secondary sensory processing (lobule VIIIB), cognition (lobules VIIB, Crus I, Crus II), and the dentate nucleus. By contrast, painful heat and painful brushing in chronic neuropathic pain patients both activated cerebellar areas related to cognitive processing. This suggests that cognitive processing areas in the cerebellum may be related to the encoding of pain, possibly as a cognitive modulator[214]. A recent brain structural covariance study in fibromyalgia (FM) patients suggest that in FM, the medial prefrontal/orbitofrontal cortex loses connections with

other neighbouring frontal regions, and instead gains connections with the cerebellum.

This study demonstrated more activation in insula in controls compared to chronic pain patients. The present study findings are consistent with the findings from other studies[3]. The insula plays a major role in identification of the emotional significance of environmental stimuli, production of affective states and regulation of autonomic responses to emotive stimuli. It has been suggested that the insula participates in the evaluation of distress and that it is involved in the evaluative, experiential, or expressive aspects of self-induced or internally generated emotions[215-217]. Insular activity may be modulated by opioid analgesics[215]. Determining changes within the motor cortex in chronic pain patients is difficult as this group of patients have other associated sensorimotor deficits, which may have an impact on motor-cortex excitability. Other researchers have reported activation in motor cortex in chronic pain patients which may be attributed to decreased motor threshold or reduced intra-cortical inhibition[177] .

12.4 Chronic pain patient's pre-oxycodone and post–oxycodone.

The brain of chronic pain patients responds differently to stimuli than the normal brain[218]. The anterior cingulate cortex, prefrontal cortex, insula, and amygdala are parts of the opioid circuitry that are involved in the affective experience of pain and other sensations. Chronic pain patients show higher pain ratings and enhanced pain-evoked neural responses when experimental pain stimuli are presented to them[178]. Currently, there is no data to indicate whether commonly used analgesics for chronic pain modulate or reverse this cortical reorganization[218].

We had hypothesized that the chronic pain patients after oral oxycodone treatment would demonstrate lesser brain activation to the pressure pain stimuli than pre-oxycodone treatment, as the opioid system is also involved in the modulation of emotions, anxiety, reward, stress, learning, and memory acquisition[215]. However, contrary to our prediction, the present study demonstrated more cortical and sub-cortical activation in brain areas including cerebellar activation in patients with chronic pain after treatment with oxycodone.

There was more activation in patients with chronic pain after treatment with oral Oxycodone and this was particularly noted in brain regions including cerebellum, superior, middle and inferior temporal gyrus, supra marginal gyrus, insula, culmen, inferior parietal lobe, parahippocampal gyrus, post-central gyrus, inferior frontal gyrus, lentiform nucleus, thalamus, amygdala, and uncus.

In an elegant review article by Apkarian states "*we can now resolutely refute the simplistic notion that brain activity for chronic pain is enhanced activity of the "neuro matrix" as identified for acute pain"* [219]. Recent research literature has shown that the human brain in chronic pain is abnormal. Chronic pain is associated with reduced gray matter changes and impaired cognitive ability[184, 220]. Multiple recent studies have demonstrated reversibility in the chronic pain disease process associated with improvement in brain gray matter changes with effective treatment[184, 221].This reversal is related to the extent of pain relief and also renormalization of cognitive abilities[184]. A study comparing intravenous morphine with saline reported increased activations in reward circuitry regions

of brain that included nucleus accumbens, sub-lenticular extended amygdala, orbitofrontal cortex, and hippocampus. They also reported decreased activation with Morphine in cortical areas in a similar manner to sedative-hypnotic drugs[222]. After morphine infusion, they also observed decreased BOLD signal in the brainstem, medial thalamic nuclei and in cortical regions involved in sedation including prefrontal and parietal regions[222]. The SI and SII are commonly activated in both healthy controls and chronic pain patients but, SII appears to be more consistently activated than SI [34].

The existing imaging data suggest that significant changes are present in PFC both in health and disease. In chronic pain patients the activity in medial PFC is increased and is shown to be attenuated by analgesics[223]. The PFC is known to be important for cognitive control, enabling behaviour to be at once flexible yet task focused[224]

In this study when chronic pain participants' pain scores (VAS scores) for the three pressure levels before treatment with oral oxycodone were analysed there was no relationship between VAS and BOLD response to either touch or mild pain (activation to bar 1 and 2). However at moderate pressure pain stimulus (bar 3) there was a positive correlation between VAS scores and BOLD response in a number of anatomical regions including: right anterior cingulate, left middle cingulate, right cerebellum, left inferior parietal lobe, left pre-cuneus and cuneus. This suggests that chronic pain patients who rated the moderate pressure pain stimulus (bar 3) pressure level as most painful were more likely to show increased brain activation in these regions in response to the stimulus. There were no negative correlations observed. To our knowledge these findings have not been reported in earlier studies.

13. Conclusions

The objective of this thesis was to explore the neural response of healthy controls and chronic pain patients to pressure pain stimuli using fMRI in order to better understand the central pain processing in health and disease. Furthermore we investigated the effect of oxycodone therapy in patients with chronic pain and its effect on brain responses to pressure pain stimuli.

Neuroimaging has provided evidence of structural and functional brain changes in the majority of chronic pain syndromes[225]. We had hypothesized that patients with chronic pain would have greater brain activation compared to healthy controls due to the presence of constant or spontaneous pain. However, our study demonstrated that healthy controls responded to pressure pain stimuli with more brain activation than chronic pain patients and the pressure pain stimuli preferentially activated somatosensory, insular and cingulate cortical regions. Interestingly more activation was noticed in bilateral cerebellar lobules.

While in chronic pain patients many researchers have demonstrated activations in PFC, limbic system and basal ganglia our study demonstrated brain activations in response to pressure pain stimulation in superior, middle and inferior frontal gyrus, anterior and middle cingulate, lentiform nucleus, Insula, cerebellum, para-hippocampal gyrus, primary somatosensory area, inferior parietal lobe, thalamus and amygdala. These findings have not been previously reported.[9].

Though the role of cerebellum in pain processing is unclear, the available neuroimaging data suggests that DLPFC is heavily connected to cerebellar lobules IV-VI[136]. There is evidence that the dorsal spinocerebellar transmits pressure and pain processing[226] . This study demonstrated significant cerebellar activation in both controls and chronic pain patients and that oxycodone therapy resulted in increased cerebellar activation in patients with chronic pain.

Finally, we expected that oral oxycodone therapy would blunt brain activation in response to pressure pain stimuli in patients with chronic pain however this was not the case. Oxycodone therapy resulted in increased brain activation in the following areas: cerebellum, superior, middle and inferior temporal gyrus, supra marginal gyrus, insula, culmen, inferior parietal lobe, parahippocampal gyrus, post-central gyrus, inferior frontal gyrus, lentiform nucleus, thalamus, amygdala, and uncus.

Healthy volunteers demonstrated more brain activation compared to both pre and post oxycodone treated chronic pain patients. The Oxycodone therapy resulted in more brain activation in patients with chronic pain and this may be explained by gray matter regeneration. This is consistent with multiple researchers reporting partial reversibility in neuronal degeneration with effective treatment of chronic pain[9].

14. Study strengths; limitations and Future directions

The novel aspect of this study was the custom designed electronic pressure algometer. This was microprocessor controlled and could deliver different pre-set pressures. The results from device testing were reliable and were consistently reproducible. This electronic pressure algometer has more potential for further studies by including more pain levels or comparing the effects of longer pain stimulus versus shorter pain stimulus and by dynamically varying pressure pain levels.

In our investigation, we compared brain activations to pressure pain stimulus of varying intensities in healthy controls and chronic pain patients before and after treatment with oxycodone. Our results indicate that with effective treatment of chronic pain using oxycodone we can partially reverse or improve the progression of neural changes associated with chronic pain. This study has also shown that the experimental pressure pain paradigm may give us an insight into pain processing in chronic pain patients and healthy controls. We have shown extensive cerebellar activations in both health and disease which may suggest that dorsal spino-cerebellar tract, which is implicated in processing pressure and pain, may be important in both health and disease.

Our study limitation is primarily limited to a small sample size. Due to this, with rigorous statistical testing, and if examined in a larger population of subjects, our data may be unconvincing. But multiple research centres with almost similar sample size have shown partial reversibility in gray matter changes if underlying pain is treated effectively.

Diffusion Tensor Imaging (DTI) and invitro viral tract studies in particular looking at the relationship between pressure pain stimulus and cerebellar activations may give meaningful evidence regarding the role of cerebellum in processing pain in both health and disease. And also a simple task independent resting state network scans of 6- 10 minutes before tasks are given will allow

characterizing the difference between resting state networks and healthy volunteers and those in chronic pain before and after treatment with opioids.

Appendix A:- Becks Depression Inventory

1.	0	I do not feel sad.
	1	I feel sad
	2	I am sad all the time and I can't snap out of it.
	3	I am so sad and unhappy that I can't stand it.
2.	0	I am not particularly discouraged about the future.
	1	I feel discouraged about the future.
	2	I feel I have nothing to look forward to.
	3	I feel the future is hopeless and that things cannot improve.
3.	0	I do not feel like a failure.
	1	I feel I have failed more than the average person.
	2	As I look back on my life, all I can see is a lot of failures.
	3	I feel I am a complete failure as a person.
4.	0	I get as much satisfaction out of things as I used to.
	1	I don't enjoy things the way I used to.
	2	I don't get real satisfaction out of anything anymore.
	3	I am dissatisfied or bored with everything.
5.	0	I don't feel particularly guilty
	1	I feel guilty a good part of the time.
	2	I feel quite guilty most of the time.
	3	I feel guilty all of the time.

6. 0 I don't feel I am being punished.
1 I feel I may be punished.
2 I expect to be punished.
3 I feel I am being punished.

7. 0 I don't feel disappointed in myself.
1 I am disappointed in myself.
2 I am disgusted with myself.
3 I hate myself.

8. 0 I don't feel I am any worse than anybody else.
1 I am critical of myself for my weaknesses or mistakes.
2 I blame myself all the time for my faults.
3 I blame myself for everything bad that happens.

9. 0 I don't have any thoughts of killing myself.
1 I have thoughts of killing myself, but I would not carry them out.
2 I would like to kill myself.
3 I would kill myself if I had the chance.

10. 0 I don't cry any more than usual.
1 I cry more now than I used to.
2 I cry all the time now.
3 I used to be able to cry, but now I can't cry even though I want to.

11. 0 I am no more irritated by things than I ever was.
1 I am slightly more irritated now than usual.
2 I am quite annoyed or irritated a good deal of the time.
3 I feel irritated all the time.

12. 0 I have not lost interest in other people.
1 I am less interested in other people than I used to be.
2 I have lost most of my interest in other people.
3 I have lost all of my interest in other people.

13. 0 I make decisions about as well as I ever could.
1 I put off making decisions more than I used to.
2 I have greater difficulty in making decisions more than I used to.
3 I can't make decisions at all anymore.

14. 0 I don't feel that I look any worse than I used to.
1 I am worried that I am looking old or unattractive.
2 I feel there are permanent changes in my appearance that make me look unattractive.
3 I believe that I look ugly.

15. 0 I can work about as well as before.
1 It takes an extra effort to get started at doing something.
2 I have to push myself very hard to do anything.
3 I can't do any work at all.

16. 0 I can sleep as well as usual.
1 I don't sleep as well as I used to.
2 I wake up 1-2 hours earlier than usual and find it hard to get back to sleep.
3 I wake up several hours earlier than I used to and cannot get back to sleep.

17. 0 I don't get more tired than usual.
1 I get tired more easily than I used to.
2 I get tired from doing almost anything.
3 I am too tired to do anything.

18. 0 My appetite is no worse than usual.
1 My appetite is not as good as it used to be.
2 My appetite is much worse now.
3 I have no appetite at all anymore,

19. 0 I haven't lost much weight, if any, lately.
1 I have lost more than five pounds.
2 I have lost more than ten pounds.
3 I have lost more than fifteen pounds.

20. 0 I am no more worried about my health than usual.
1 I am worried about physical problems like aches, pains, upset stomach or constipation.
2 I am very worried about physical problems and it's hard to think of much else.
3 I am so worried about my physical problems that I cannot think of anything else.

21. 0 I have not noticed any recent change in my interest in sex.
1 I am less interested in sex than I used to be.
2 I have almost no interest in sex.
3 I have lost interest in sex completely.

Interpreting the beck depression inventory.

Now that you have completed the questionnaire, add up the score for each of the twenty-one questions by counting the number to the right of each question you marked. The highest possible total for the whole test would be sixty-three. This would mean you circled number three on all twenty-one questions. Since the lowest possible score for each question is zero, the lowest possible score for the test would be zero. This would mean you circles zero on each question. You can evaluate your depression according to the table below.

Total Score	Levels of Depression
1-10	These ups and downs are considered normal
11-16	Mild mood disturbance
17-20	Borderline clinical depression
21-30	Moderate depression
31-40	Severe depression
Over 40	Extreme depression

A persistent score of 17 or above indicates that you may need Medical treatment.

Thank you for filling this form.

Name of the person: _____.

Date of Birth: _____.

Contact address: _____

_____

Appendix B:- Healthy controls registration form

Department Of Pain Medicine, St. James's Hospital And Trinity College Institute Of Neurosciences, Trinity College, Dublin.

Healthy Volunteer Registration Form

Dear Healthy volunteer thank you for your general interest in volunteering for a Brain Imaging Study at the Trinity College Institute of Neurosciences.

We are undertaking an experimental pressure pain study that requires healthy controls to be scanned by MRI (Magnetic Resonance Imaging), and we have advertised for such volunteers.

This study is undertaken through the Department of Pain Medicine, St James's Hospital and Trinity College Institute of Neuroscience, Trinity College Dublin. This research has been approved by an AMNCH Research Ethics Committee to ensure that both the scientific aims and methods of the study are sound and well reviewed. All the information that volunteers receive is properly vetted and all scans and personal information are kept securely to ensure strict confidentiality. The time you need to be in the scanner and what the scan actually entails will vary from 45 minutes up to 1 hour and all details are included in the Patient/ Healthy Volunteer information sheets, which will be provided to you in good time, to allow you to consider your involvement.

For this study, you need to attend one scanning session, which can take up to one hour. For this reason, participants need to consider the time and commitment required in taking part in this research.

Volunteers are not normally paid for their involvement with the study, but reasonable travel expenses are usually reimbursed.

Should you like to register your interest in being a healthy volunteer for this imaging study please complete the form below and contact Dr Hari Gopal on 087-2181697 or e-mail: -doc_harigopal@yahoo.com to discuss what is

involved; please do not take this form as confirmation of your involvement for this study until you are clinically assessed by Dr Hari Gopal.

Many thanks for your interest.

1) **Name of the healthy volunteer: -**

   Address: -

   Contact details:

   Phone: -

   E-mail: -

2) **General Practitioner's Details: -**

   Name: -

   Address: -

   Phone: -

   E-mail:-

Appendix C:- Patient Information leaflet

SJH/AMNCH RESEARCH ETHICS COMMITTEE

Patient / Healthy Volunteer Information leaflet and consent form.

1. Investigation of cortical and sub-cortical processing of pressure pain transduction in healthy controls and chronic lumbar radicular pain pre and post oxycodone treatment- An event related fMRI Study.

2. Introduction: - Chronic pain syndrome affects approximately 2-4% of the population. The cause for this condition remains largely unknown, although there is support of notion that altered central pain processing is a factor in the presentation of this condition.

The purpose of this study is to investigate brain pain processing in chronic pain subjects utilizing functional brain scans (MRI) and compare the data obtained from healthy controls to see if the pain processing in the brain is different between healthy controls and chronic nerve pain subjects or not.

In chronic pain subjects, also to investigate the brain site of action of painkiller (Oxycodone) and also the effectiveness of medication in reducing chronic pain and changes those occur in the brain with improved pain relief.

This is done by application of three different levels of pressure pain on non-dominant thumb nail bed before and after commencing the pain killer medication in chronic pain subjects; without administering medication in healthy volunteer subjects (Healthy volunteer will not receive any medication.)

Also to investigate if there are any differences when the brain is at rest i.e. when the brain is not actively involved in any physical or mental process.
You are invited to participate in this study as a Healthy Volunteer
Or
You are suffering from chronic pain disorder such as nerve pain (Neuropathic pain).

Scan location: - The scans are done at Trinity College Institute of Neuroscience, Lloyd's Building (near science gallery, Pearse street) Trinity College Dublin.
The duration of brain scan is approximately 45 minutes to 1 hour. Few forms need to be completed before the scan and this may take approximately 30 minutes.

Three different brain scans are done in a single session (ie.no break in between scans).

First scan is a clinical scan (this will be sent for a review by a Consultant Radiologist at Tallaght Hospital), if any incidental findings are noticed; this will be informed to participant's GP (healthy controls and chronic pain subjects). This scan takes approximately 20 minutes. After the first scan you will asked to look at a red cross for 7 minutes (To check how a brain at rest works). Finally, 15 minutes of pressure pain testing during which three different levels of pressure pain stimulus is applied starting with 1bar, 2bar and 3bar. The duration of each pressure pain stimulus is 5 seconds. The three levels of pressure pain stimulus are applied at different times (ranging from 4 seconds to 14 seconds randomly).

Measurement of how pressure stimulus activates pain perception areas in brain will be analysed by functional magnetic resonance imaging (fMRI). This is a non-invasive method and does not cause any radiation exposure like x-ray's/CT scans and no contrast is needed to visualize brain.

MRI's are noisy and you will be provided with earplugs and headphone. A small buzzer will be given to you; this can be pressed at any time to attract attention of the scanner operator and researcher. During MRI scanning regular breaks will occur when scanner operator /researcher will communicate with you. During scanning you will be monitored from the control room via the window and a video camera. The duration scanning of scan is 45 minutes to a maximum of 1 hour or until you express a desire to terminate the session. You will be then be

moved from the scanner, following which you are free to leave Trinity College Institute of Neuroscience.

It is very important not to move your head. Please do not nod your head while answering any questions when communicated with you during the scan.
Healthy controls will be scanned once only.

Note for chronic pain subjects: -
You will undergo scans twice: - once before the commencement of pain killer medication and another after seven days after the commencement of medication. You will be prescribed oral Oxycodone as a part your treatment after clinical evaluation. This medication needs to be taken twice a day for seven days for this study (will be continued for longer period of time until your pain is manageable or you no longer need this medication).

4.Participants
Healthy controls of either sex or chronic pain patients aged between 25 years – 60 years and, if female participants cannot be pregnant or breastfeeding and chronic nerve pain patients who remain unresponsive to regularly prescribed painkillers (anti-inflammatory agents or first line weak opioid).

After a clinical decision chronic nerve pain patients will be prescribed oxycodone and a prescription for the same will be given. You need to take this medication as advised by your Pain Physician for at least seven days before second scan. If deemed clinically necessary before commencing this medication we may ask you to get blood tests and radiological investigations done at St. James's Hospital Dublin-8 for free of charge.

5.Benefits
You will receive no direct benefit from taking part in this study. Your participation may, however provide information about the mechanism of pain and help develop new medications/procedures to treat chronic nerve pain patients, but this benefit is not assured.

6. Risks

There are no known adverse effects of the magnetism or radio waves used in MRI. There is, however, the potential risk related to the machine itself attracting metal. Therefore, people who have metal within them (e.g., aneurysm clips or pacemakers) will be excluded from the study (Subjects with dental fillings can be studied without risks). Entering the scanner with metal implants can have very serious consequences. There are known dangers from MRI to people with heart pacemakers, with the possibility that the pacemaker may malfunction, as well as to people with implanted metal devices such as aneurysm clips that might be moved in a dangerous manner. Should there be any uncertainty regarding the presence of metal you will be asked to consent to a further procedure, an x-ray study, to rule out the presence of metallic fragments.

Another risk is if you have tattooed eyeliner. Certain metallic pigments used in the tattooing process can be affected by the electromagnetic fields used for MRI procedures and some people with tattooed eyeliner have experienced short-term skin irritations or swelling associated with MRI procedures. People who have tattooed eyeliner will be excluded from the study.

Some people become claustrophobic, a feeling of anxiety or nervousness associated with being in a small, confined space, while in the machine. Should this occur, the examination will be terminated immediately and you will be rapidly removed from the scanner. This is a common adverse event (occurs in 10-25% of people (10-25 out of 100 people) that is usually mild in severity and quickly resolved upon leaving the scanner.

Risks with oxycodone (For chronic nerve pain patients only)

1. Constipation – although oxycodone is a good painkiller many people who takes it get constipated. Diet, liquids or laxatives as required can easily overcome this.

2. Vomiting **–** about a third of people starting oxycodone can feel sickly even vomit in the first week of treatment. Fortunately, the sickly feelings usually

disappear. If this troubles you side effect an anti- sickness tablet can be prescribed for you to help you through this time. Bear in mind that there are other causes of vomiting and nausea it may not be the oxycodone – if you are concerned speak to your doctor or nurse.

3. Drowsiness – sometimes when starting oxycodone or after increasing the dose, people feel more sleepy or drowsy than usual for a few days. For most people this quickly wears off. If it affects you in this way, you MUST not drive or operate dangerous machinery.

Less common side effects while taking oxycodone are;
Unsteadiness, confusion, sweating, blurring of vision and a dry mouth.

7. Exclusion from participation:

Your doctor has told you that you cannot be in this study if any of the following are true:

Known allergy to opioids or previous treatment with oxycodone
Participant refusal.
Clinical depression
Substance abuse
Pregnant women
Impaired kidney function.
Patients in whom MRI is contraindicated

Scanning using fMRI has certain conditions that exclude patients from participating, these include

-Cardiac pacer
-Aneurysm clip
-Cochlear implant
-Shrapnel
-Tattooed eyeliner
-Neurostimulators or any other metal device.

8. Alternative treatment

You do not have to be a part of this study to be treated.

Your treatment will not be altered in anyway. You will be a patient who agreed to enter the study or would prefer not to enter the study will be treated in exactly the same way. There are other medications available that can be used to treat your complaint and your doctor has discussed this with you.

9.Confidentiality

Any information obtained from you regarding this research will be absolutely confidential. All records relating to your involvement in this research study will be stored in a password-protected computer. Numbers will identify you not by name. The information linking your number to your identity will be kept separate from the research records in a separate password protected computer. Only researchers listed in the first page of this form will have access to your research records. Regarding publication of study results you will not be identified.

10.Compensation

Your doctors are covered by standard medical malpractice insurance. Nothing in this document restricts or curtails your rights. The medical practitioners involved in this study have current medical malpractice insurance cover by St. James Hospital, Dublin. The Investigators will comply with the ABPI guidelines and Irish Law (statutory and otherwise) in the unlikely event of your becoming ill or injured as a result of participation in this clinical study."

11. Voluntary Participation

You have volunteered to participate in this study. You may quit at any time. If you decide not to participate, or if you quit, you will not be penalised and will not give up any benefits, which you had before entering the study.

12. Stopping the study

You understand that your doctor or the sponsoring company may stop your participation in the study at any time without your consent.

13. Permission

All the research in the St. James's Hospital is looked at by Hospital Research Ethics Committee approval to protect your safety, rights, well-being and dignity.

14. Further information

You can get more information or answers to your questions about the study, your participation in the study, and your rights, from Dr Hari Gopal who can be telephoned at 087-2181697.If your doctor learns of important new information that might affect your desire to remain in the study, he will tell you.

In the event of an irregularity being found both in healthy controls and Chronic nerve pain patients, the Consultant Radiologist, [Dr William Torreggiani, of The Adelaide and Meath Hospital Incorporating the National Children's Hospital] will inform you as the participants GP that a proper clinical scan may be required to determine whether or not an irregularity is of clinical significance

SJH / AMNCH RESEARCH ETHICS COMMITTEE.

CONSENT FORM

Title of Research Study

Investigation of cortical and sub-cortical processing of pressure pain transduction in healthy controls and chronic lumbar radicular pain pre and post oxycodone treatment- An event related fMRI Study.

This study and this consent form have been explained to me. My doctor has answered all my questions to my satisfaction. I believe I understand what will happen if I agree to be part of this study.

I have read, or had read to me, this consent form. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. I freely and voluntarily agree to be part of this research study, though without prejudice to my legal and ethical rights. I have received a copy of this agreement and I understand that, if there is a sponsoring company, a signed copy will be sent to that sponsor.

Name of sponsor:

PARTICIPANT'S NAME:

PARTICIPANT'S SIGNATURE:

Date:

Date on which the participant was first furnished with this form:

Where the participant is incapable of comprehending the nature, significance and scope of the consent required, the form must be signed by a person competent to give consent to his or her participation in the research study (other than a person who applied to undertake or conduct the study). If the subject is a minor (under 18 years old) the signature of parent or guardian must be obtained: -

NAME OF CONSENTOR, PARENT or GUARDIAN: ----------
SIGNATURE: ----------
RELATION TO PARTICIPANT: ----------

Where the participant is capable of comprehending the nature, significance and scope of the consent required, but is physically unable to sign written consent, signatures of two witnesses present when consent was given by the participant to a registered medical practitioner treating him or her for the illness.

NAME OF FIRST WITNESS:
SIGNATURE:

NAME OF SECOND WITNESS:
SIGNATURE:

Statement of investigator's responsibility: I have explained the nature, purpose, procedures, benefits, risks of, or alternatives to, this research study. I have offered to answer any questions and fully answered such questions. I believe that the participant understands my explanation and has freely given informed consent.

Physician's signature:
Date:

Appendix D: - State Trait Anxiety Inventory form Y-1 and Y-2

mind garden

SELF-EVALUATION QUESTIONNAIRE
STAI Form Y-1

Please provide the following information:

Name_____ Date_____ S_____

Age_____ Gender (*Circle*) **M** **F** T_____

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

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SELF-EVALUATION QUESTIONNAIRE
STAI Form Y-2

Name_____ Date_____

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

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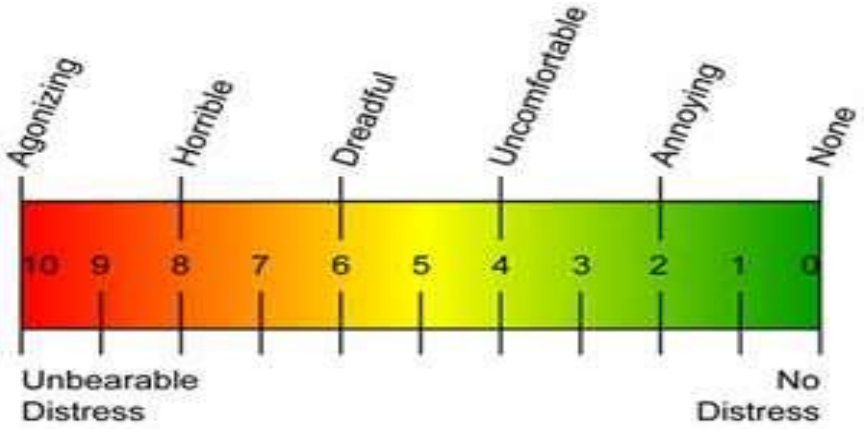
Appendix E:- Visual analogue scale

VAS scores at varying intensity of pressure pain stimulus



Task _____

Date _____ *Start* _____ *End* _____

a) 1 bar pressure: - VAS score _____

b) 2 bar pressure: - VAS score _____

c) 3 bar pressure: - VAS score _____

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