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A Converging Approach to Sex differences and Pain; an Examination of Social and Biological Mechanisms that May Account for Differences Between Male and Female Pain Reports

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A Converging Approach to Sex differences and Pain; an Examination of Social and Biological Mechanisms that May Account for Differences Between Male and Female Pain Reports.

Alexander G. J. Currie

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Contents

Abstract

The Neuromatrix of Pain is a comprehensive theory that has been designed to account for a majority of mediatory processes that influence pain perception, yet one aspect that does not appear to have been explicitly considered as a predominant factor in the research is that of biological sex, despite several articles and reviews that have highlighted the importance of it. Originating as an examination of anticipatory processes, this thesis evolved to examine how males and females experience pain differently in social contexts, and possible neurometabolic differences that may account for these disparities. From the social approach, we examined the experimenter gender effect, which demonstrates that the experimenter's sex alters pain perception. Not only were the results concurrent with previous literature, but it was demonstrated that the presence and gender of an additional observer also influences pressure-pain threshold (PPT), predominantly in males; the observer effect could operate as either an extension of the experimenter effect, or a facilitating factor to it. It was also found that, in females, the personality trait Openness correlated significantly with PPT, which may reflect previous findings of females' coping mechanisms. From the biological approach, proton magnetic resonance spectroscopy (^{1}H-MRS) was used to examine the neurometabolic concentrations in the insula of healthy males and females based on findings in clinical populations. While the results were not replicated, it was found that there was a significant difference between glutamate concentrations between males and females in the anterior insula (A.I.), and also that glutamate in the A.I. also correlated significantly with PPT in males. These findings demonstrate and support evidence for how males and females adopt differential anticipatory mechanisms to predict and limit potential tissue damage. Overall, this thesis provides evidence for gender differences in pain perception that holds implications for both the experimental and clinical fields of study.

Chapter 1: Introduction

"Pain is just a state of mind. You can think your way out of everything, even pain"

–Rodman Philbrick (author)

Freak the Mighty (1993)

"The pain of the mind is worse than the pain of the body."

- *Publilius Syrus (Roman writer and poet)*

Pain is a universal phenomenon of sensation and in attempt to examine and understand it humanity has not limited itself to scientific examination. The examination of pain has extended into philosophical and theological disciplines, often raising it beyond the realms of empirical apprehension to more intangible, abstract planes. Arguably, this philosophical perspective has attributed pain with characteristics that extend beyond its evolutionary developed purpose. Likely due to its common presence in our everyday lives and due to the strong connections between pain and emotion. Moving past philosophy's attempts to impose and convey a much deeper, more significant purpose on pain, the question that scientists have been examining for decades is; "what is pain, and how does it work"? According to the International Association for the Study of Pain (IASP, 1994), pain is defined as "a conscious awareness of an unpleasant sensory and emotional experience associated with actual or potential tissue damage", and typically any stimuli that is harmful or causes a painful feeling is referred to as noxious. The unpleasant sensation of pain is believed to have evolved as an evolutionary mechanism that is designed to evoke an individual's withdrawal from noxious stimuli to prevent damage to tissue (Lynn, 1984), and remains to be an important aspect of this multi-dimensional phenomenon.

Evolution of pain theories.

Some of the earliest attempts to understand and define pain can be seen in the workings of ancient philosophers such as Hippocrates (c. 460 BC – c. 370 BC) and Aristotle (384 BC – 322 BC). Hippocrates proposed that pain was the result of an intrinsic imbalance of the four humours, which were believed to be important mediators in factors of emotion and behaviour. Aristotle, however, postulated that it was evil spirits entering the body through injury that caused pain. In both instances, neither of these theories considered that the brain had any involvement in the perception of pain (Linton, 2005). Indeed, prior to the scientific renaissance, most theories related to defining and understanding what pain is, as well as its cause, were largely external to the individual. For example, within Christianity there were two opposing views; one was that pain was delivered to man as a punishment from God for their sins, whilst the other view was that pain was sent from God as a test of man's faith, similar to some of the trials that Jesus may have faced (Linton, 2005). In fact, two of the root words for the word 'pain' come from the Latin *poena*, meaning 'punishment' or 'penalty', and the Greek word *poine,* meaning 'retribution' or 'penalty'. It was not until Descartes' 'Treatise of Man' was published in 1664 that a more internal, biological approach to somatosensation became more widely accepted (cited in Melzack, 1993; Linton 2005).

Forming the basis for virtually all contemporary theories and research investigating pain perception, Descartes' work in the 17th Century took a new approach to anatomical and physiological studies. In his work, 'Treatise of Man', Descartes stipulated that although man is in possession of a soul (or 'mind'), the body is designed to function as a machine, much like that of an animal's (as cited in Melzack, 1993). In his proposal, Descartes put forward that noxious stimulation excites specific receptor cells within the body that are attached to hollow tubes, through which spirits flowed in a mechanical fashion. These tubes were the nerves, and it was put forward that they connected and relayed the information relating to the noxious stimulation to the body's sensory centre, the brain (Linton, 2005). This led to a dualistic view of body and mind (wherein the body is mechanical in nature, though governed by a rational soul/mind) that, though not entirely accurate, revolutionised both the theories of physiological processes, and the research investigating the functionality and processes of the human body. According to Melzack (1993), the implications of Descartes theory paved the way for much of the research conducted over the past century as researchers have conducted the experiments to investigate the idea of dedicated receptors, fibres and neural areas that have evolved to experience and process noxious stimulation. This became one of the earliest theories of perception, known as the Specificity theory of pain.

Taken from Descartes' workings, the specificity theory Von Frey (1895, cited in Rey, 1995; Moayedi & Davis, 2013) stated that there were specific receptors, fibres and cortical areas that have evolved dedicated to processing noxious stimulation, in a similar fashion to the other senses, such as auditory and ocular systems. This theory is a ridged model that regarded pain as a straight through sensory projection system that, for a time, determined how patients with chronic pain were treated (usually with neurosurgically-induced lesions) (Melzack, 1993). The specificity theory, though initially widely accepted amongst the scientific community, was strongly opposed by another concept of pain perception; the Pattern theory by Goldscheider (1894, as cited in Melzack and Wall, 1965). The pattern theory postulates that, rather then the presence of a peripheral system specifically designed for the detection of painful sensation there is a collection of fibres in the dorsal horn of the spinal cord that results in the experience of unpleasant sensation once a certain threshold of stimulation has been breeched. Goldscheider maintained that in the body there are no specific nerve fibres or endings and that the sensation of pain is caused by the intensity and pattern of stimulation at the site of excitation. Other pattern theories have been proposed based on Goldscheider's work (Livingstone, 1943; Noordenbos, 1959) that discuss the non-specific fibres accumulating in the dorsal horn, but one of the main criticisms of these theories is that

they do not take into account or specify the involvement or function of the brain, other then designating it the receiver of the pain impulses (Melzack, 1993, 1996). These theories formed the basis for one of the most widely accepted theories; Melzack and Wall's (1965) Gate Control theory of pain perception.

In their theory, Melzack and Wall postulated that afferent nerve fibres carry information to transmission (T) cells in the dorsal horn of the spinal cord, which is regulated by a 'gating' mechanism that, in turn, is affected by input from two forms of fibres. These fibres differ in terms of what information they convey; the large (L) diameter fibres conduct information related to normal pressure and tactile stimulation, whereas the small (S) diameter fibres conduct information that is related to noxious stimulation. When the input from the S-diameter fibres exceeds a certain level, this causes output from T cells in the dorsal horn of the spinal cord to surpass the threshold of normal tactile stimulation and allow for the transmission of stimulation as noxious, enabling the information to continue to cerebral areas associated with painful stimulation for further processing. To put this system into its simplest terminology, input from the S-diameter fibres result into an excitatory effect, 'opening the gate' and allowing the noxious stimulation to proceed for further processing and perception, while input from the L-diameter fibres result in an inhibitory effect upon this system, essentially 'closing the gate'. This system also receives input from a separate set of L-diameter fibres that descend from the brain and allow for a cognitive-based regulatory modulation of the gating mechanism. Once the output of the T cells surpasses the tactile threshold, the information is relayed to an action system in the brain, which is responsible for characterisation of stimuli and the behavioural patterns given in response, usually facilitating and producing aversive behaviour in order to withdraw from the stimuli and prevent further tissue damage.

With the understanding of the involvement of the peripheral nervous system the recognition and transmission of noxious stimulation brought about by the gate control theory, researchers then began to examine what role the central nervous system may play. Melzack and Casey (1968) proposed that the sensory and emotional aspects of noxious stimulation are, in fact, modulated and processed not only by separate structures in the brain, but also in parallel of one another. It has been postulated that pain is represented in three different dimensions: The sensory-discriminative, the affective-motivational and the cognitive-evaluative, each developed to process a specific aspect of pain perception. The sensory-discriminative could be considered the most simplistic of the three dimensions, associated with the basic perception of noxious stimulation, specifically related to location, intensity and

duration. In parallel to this, the affective-motivational aspect relates to the emotional experience of pain perception, specifically the afferent experiences of unpleasantness associated with noxious stimulation and the innate desire to withdraw. The third and final dimension is possibly the most complex of the three, the cognitive-evaluative. This is associated with higher cognitive functions, including the ability to assign reasoning and value to the experience to the extent that it may induce a conscious effort to override the innate withdrawal reflex, or attempt to reassign attention away from the stimulation ('blocking it out') if escape is not an available option. To examine this in the sense of an evolutionarily developed system, the sensory-discriminative is paramount in the recognition and assessment of stimulation, the affective-motivational imperative to preservation of tissue and minimalizing the risk of damage, and the cognitive-evaluative can act as a moderator to the other two systems, asserting control over them and overriding their initial reactions in order to influence behaviour.

The neuromatrix of pain.

The current model used to explain the perception on pain stems from Melzack's work on the gate control theory and the dimensional aspects of pain, as well as supporting research and examinations into phantom limb pain. All of these observations have been incorporated into a singular theory; that of the existence of a Neuromatrix of Pain processing within the brain (Melzack, 1993; 1996; 1999; 2001). The neuromatrix (also known as the body-self matrix, Melzack, 2001) is postulated to incorporate a widespread neuronal network that consists of loops between the Thalamus and cortex, as well as between the cortex and limbic system (Melzack, 2001). The basis for this matrix stems from genetically determined synaptic links of functionality that are later built up based on the sensory inputs and experiences that elicit these inputs. The matrix forms a converging loop of cyclically processed nerve impulses through these cortical and sub-cortical structures that form what Melzack refers to as a characteristic neurosignature. As previously discussed, separate structures within the brain appear to process varying aspects of impulses related to noxious stimulation in parallel (referred to as 'neuromodules'), and it is suggested that the outputs from these structures are incorporated into the overall neurosignature, which in turn is projected out from the neuromatrix to structures in the brain so that the stream of nerve impulses are converted into a continually fluctuating stream of awareness. This stream of awareness is then attached to the afore-mentioned 'action systems', which interpret the activity within this process and then form appropriate responses to any potentially harmful inputs (i.e. aversive behaviour, withdrawal etc.). In its most simplistic form, the neuromatrix

is constantly evaluating inputs into the continual nerve stream in order to produce awareness of noxious stimulation, whereupon the action system is engaged to elicit a pattern of behaviours appropriate to the situation.

Overall, the neuromatrix is a multi-dimensional model that is much more complicated than the original theories stipulated. Melzack has built the model to consist of the body-self matrix as the central facet, consisting of sensory, affective and cognitive neuromodules. The main inputs comprise of characteristics that are grouped into the three dimensions of pain outlined by Melzack and Casey (1968), with sensory inputs (not just limited to noxious stimulation) comprising the sensory-discriminative dimension, cortical structures and biological mechanisms making up the affective-motivational and psychological aspects (such as personality, past experiences, attention and anxiety) grouped under the cognitive-evaluative. Outputs of the body-self matrix include the overall experience of pain perception as defined by previous models, the aforementioned 'action' systems and associated behaviours, and the stress-regulatory system put in place by the homeostatic regulatory system. Overall, what creates the experience that is known as pain stems from a combination and integration of several factors, which include sensory inputs (visual, tactile and audio), which influences how the stimulation may be interpreted cognitively; cognitive and emotional inputs from varying cortical structures; cognitive awareness and interpretation of the context of the stimulation and the activity of the bodies' stress-regulatory system (Melzack, 1999). This model for pain perception can account for how both internal and external factors can influence how an individual's sensory and affective experiences of noxious stimulation can be altered.

Another characteristic to consider within this matrix is related to the bodies' predilection to maintain homeostasis. From a purely sensory perspective, when the body is injured or receives input relating to noxious stimulation, the homeostatic regulatory system is disrupted, producing biological stress (Melzack, 2001). Upon disruption, the homeostatic regulatory system then attempts to restore balance by instigating a series of designated protocols specifically designed to combat it (Melzack, 2001), usually in the form of hormonal release and regulation of neurometabolites. The outputs from the homeostatic regulatory system are, like the involvement of the action system, a compensatory strategy in order to prevent further tissue damage and are related to alterations in pain perception.

While the neuromatrix of pain is widely accepted as an approach that accounts for a variety of influences, or inputs, there are those who may refer to it as the "Pain Matrix", as opposed to the neuromatrix of pain. This view would indicate that there are a set number of

cortices, regions, or structures that are dedicated to the processing of pain, much in the same way that the auditory cortex is dedicated to processing auditory stimuli or the occipital cortex is dedicated to sight (Iannetti & Mouraux, 2010). This, however, would not be the case. One factor that adds to the complexity of examining the processing of nociceptive perception is that there are no observed structures that are strictly dedicated to the processing of pain. Although some research can reliably predict neuronal responses or social behaviour related to pain, there are a number of avenues of investigation report inconsistent results between studies (Racine, Tousignant-Laflamme, Kloda, Dion, Dupuis & Choinere, 2012a; 2012b). As previously discussed, pain is believed to have evolved as a form of early warning system, and, as there is no dedicated pain structure present in the mammalian brain, it must logically have developed around, or been incorporated into pre-existing structures (which is one argument that has been put forth to explain gender differences in pain; Bodnir, Commons & Pfaff, 2002; Mogil, 2012). As such, one theory of the neuromatrix is that it has essentially developed as a saliency network, designed around the need to detect and modulate inputs that may potentially, or actually, impact upon homeostasis (Iannatti & Mouraux, 2010). This theory, based around saliency, may also account for the role that stress and anxiety may play within pain perception, though research in the field is largely inconclusive on that subject (Racine et al. 2012b). Another argument put forward by Iannatti and Mouraux (2010) is that regions in some cortical structures associated with pain perception show activity both during and not during nociceptive processing, indicating that regions within these structures are not specified for pain. As well, it is possible for the brain to interpret non-noxious stimulation as painful, and vice-versa. Iannatti and Mouraux put forward a stipulation that rather than these areas being dedicated to specific aspects of both nociceptive and non-nociceptive stimuli, they are actually demonstrating equivalent activity in a variety of salient-sensory inputs. This theory would account for a variety of consistent and inconsistent findings associated with biological, and behavioural, reactions to pain.

While these two perspectives of a neuromatrix and a saliency network can appear at odds to one another, they can actually compliment each other quite effectively. The generalizability of the neuromatrix can be viewed as both a blessing and a curse; an umbrella theory that can account for a lot of factors (ranging from biological to psychological to social and more) unspecifically, with a more broad understanding of the neurological processes. In contrast, the saliency network approach can make up for the generalizable nature of the neuromatrix, accounting for how each integrated structure can work together like biological cogs in the machine, each structure and function feeding into the network, integrating multi-

modal sensory elements to produce perceptual feelings from pre-existing corticies that are not dedicated to nociception. However, it can be difficult for the saliency network to account for externalized influences, while the broader nature of the neuromatrix model allows it to account for how certain individual, behavioural, or social contexts can modulate nociceptive processing. Some advice that has stayed with me over the years is that in any given situation or altercation, there will be at least three stories; one of each side involved and an unbiased 'truth', which will usually be somewhere in the middle. During my research, it appears that most theories of cognitive function, especially in regards to pain, tend to approach previous theories as a rival; that is, trying to address the weaknesses in other theories and, at times, reinterpreting other factors that may better suit their model. While there is always merit in these theories, if they are examined in a manner to compliment previous theories rather than oppose, we may develop a better understanding of the underlying processes and functions, which could be somewhere in the middle of the two perspectives. I believe one of the most important perspectives to maintain when addressing the development of pain theories, is that of its origin. Pain has evolved into a complex phenomenon, but at its most basic level it began as a survival instinct, a simple stimulus-and-response mechanism to bring attention to potential tissue damage that could prove life threatening, and implement aversive action; to withdraw, limiting damage to the organism and avoid an untimely end. Those who lived longer through successful mechanisms will have passed these genetic advantages through the generations, and as higher cognitive function has evolved, so has methods of detection and avoidance, fitting around and integrating into pre-existing, specialized cortical structures. At some point humans began adapting their environment to suit there needs, rather than adapting to the environment, and evolve socially. Taking this perspective into account, pain has grown to become incredibly complex, more so than could have been anticipated by the earliest organisms (that is, if they could anticipate). The scale of evolutions can be in the form of millions of years, far beyond our individual grasps, and sometimes it can be difficult to remember where our species have come from, and where they will go.

As well as biological stress, psychological stress has been shown to alter the processing of noxious stimulation, both subjectively and neurologically. Psychological stress appears to stem from an individuals internalised anxiety, both conscious and unconscious. In studies examining correlations between pain and psychological anxiety, it has been demonstrated that a combination of high-trait and high-state anxiety in populations that are not defined as clinically anxious result in similar behavioural reactions to stimuli as the clinically anxious populations, as this combination may increase the likelihood of interpretive

biases towards threat-related stimuli (Mogg & Bradley, 1998; Bishop, 2007). It has been found that there is a relatively stable trait construct that has been attributed to anxiety-based disorders, which results in the tendency to interpret somatic stimuli as noxious in anxiety-based contexts. This anxiety sensitivity (A.S) is considered to be an important predictor of pain response, much like the anticipatory anxiety associated with procedural pain (Siddall, Taylor, McClelland, Rutkowski & Cousins, 1999; Tsao, Myers, Craske, Bursch, Kim and Zeltzer, 2004).

Tsao et al. (2004) examined the influence of A.S. trait anxiety and state-specific anticipatory anxiety across three methods of noxious stimulation (cold pressor, thermal and pressure) in a population of healthy children and adolescents. It was found that anticipatory anxiety significantly predicted incremental variance in pain intensity for all three tasks, though not pain response, which supports findings from experiments in clinical populations, indicating that anticipatory anxiety of noxious stimulation results in an altered perception of the stimuli. This indicates that state-specific anxiety in anticipation of pain is an important predictor of pain report, and that one clinical implication of these findings could be that interventions designed to target anticipatory anxiety may be able to reduce the perception of pain, in children and adolescents.

Stress and anxiety (as well depression and other psychiatric disorders, McWilliams, Cox and Enns, 2003) are highly prevalent in populations of chronic pain sufferers, and as such the link between anxiety, stress and pain has come under much scrutiny (Racine et al., 2012b), aimed at examining an almost chicken-and-egg paradigm in the clinical population; has chronic pain impacted on individuals lives to the extent that it has caused severe depression and anxiety, or has the stress and anxiety contributed to the neurosignature within the body-self matrix to the extent that, in some cases, they have developed an increased sensitivity to pain? Of course the latter of these two questions would not be true for all forms of chronic pain, some have definitive biological sources, but it can be difficult to examine extensively as researchers typically only have access after the fact, that is to say once the individual has developed a condition. In an attempt to better understand the relationships between pain and anxiety, some researchers have set their sights on examining anxiety within healthy populations in order to determine the impact that one facet may have upon another, i.e. do more anxious participants have a lower pain threshold or tolerance that those who are less anxious, or by experimentally manipulating anxiety levels of participants and then assessing changes within their reports of noxious stimuli. Whichever way this may be viewed, the link between chronic pain and stress is a tentative subject, and those suffering

from both conditions may find that it severely impacts upon their quality of life (Weich & Tracey, 2009). The current understanding of the relationship between stress, anxiety and pain is limited, some researchers have found a strong link between the two in clinical populations, whereas those examining the two in healthy populations have reported mixed results, and reviews of the literature have lead to inconsistent findings across studies (Racine at al., 2012b). Understanding how these two interact, like with most inputs into the neuromatrix, may bring us a step closer to gaining effective insight into the phenomena of pain, in turn aiding in the treatment of those whose lives have been detrimentally impacted upon by chronic pain, potentially improving their quality of life and responses to treatment.

Where does sex and gender fit in?

While it has not been explicitly incorporated into working models of the theory of the neuromatrix, sex appears to be an important factor to consider. This is due to multiple reports of disparities between males' and females' reports of pain, in both and experimental and clinical context or research (Fillingim, King, Ribeiro-Dasilva, Rahim-Williams & Riley, 2009; Mogil, 2012; Racine et al., 2012a; 2012b). In the early 1990's, two important reviews were published highlighting the importance of reporting and examining differences between sexes in the field of pain research (Berkley, 1992; Ruda, 1993), particularly when taking into consideration factors such as hormonal influences on both perceptions and behaviour. In the years following the publication of these articles, there was a marked increase of interest in examining gender differences in pain perception, though it is noted that not all publications adopted a common practice of reporting participant genders (Fillingim et al., 2009; Racine et al., 2012a). It was then established that the importance of examining these gender differences stemmed from obvious disparities between males and females suffering from chronic pain (or at least reports of chronic pain due to differential patterns of healthcare-seeking behaviour, Fillingim et al. 2009). Of course, research into gender differences is not limited to the clinical environment, though that is the predominant focus. By examining gender differences in an experimental setting, we may better understand the underlying mechanism behind these effects, potentially improving the efficacy of our approaches to treatments or examinations in clinical populations. For instance, if some chronic pain syndromes are the result of an extreme reaction to a nominal continuum of pain perception (with low sensitization on one end and high sensitization on the other), rather than a binary 'pain/no-pain' paradigm, we may find more effective methodologies to inhibit or influence chronic pain, improving quality of life in those afflicted. While this thesis is not focussed on examining chronic pain, and as there are a number of syndromes, each with a variety of potential causes, this thesis

will utilise Fibromyalgia (FM) as an exemplary chronic pain syndrome. Fibromyalgia is a musculoskeletal chronic pain disorder with a high (and potentially increasing) prevalence in society, with 2.0% of the population suffering from it. FM is also of interest due to the notable gender bias, with 3.4% of the general population suffering are female, and only 0.5% of the population afflicted are male (Wolfe, Ross, Anderson, Russell & Hebert, 1995; Neumann & Buskila, 2003). In part, this disparity may be attributable to the current understanding that psychological, as well as biological, factors contribute to gender differences of nociceptive processing and coping in the clinical observations (LeResche, 2011). While chronic pain disorders (particularly FM) are not the main focus of this thesis, they are among the primary motivations for examining gender differences in the healthy population, as well as attempting to replicate observations on determinants of pain sensitivity from clinical samples in healthy controls.

Reviews aimed at examining and assessing gender differences have not been limited to a single modality, taking into account and reporting results from studies utilizing a variety of different methodologies and forms of noxious stimulation. These methods include cold pain, either in the form of a cold pressor task (where a block of ice or sufficiently chilled cold pack is applied to an area of the body) or cold immersion task (where a body part, usually hand or foot, is submerged in ice water); heat, or thermal, task (where mechanically induced and controlled heat is applied to a site on the body); pressure pain (wherein, typically, force is gradually applied to a site on the body until the participant regards the stimuli as painful); electrical pain (where electrical stimulation is applied to an area on the participant); and ischemic pain (where a tourniquet is applied). Chemical (intradermal or topical application of capsaicin) and visceral (in this instance described as stimulation of the oesophagus) have also been examined in these reviews, but to a lesser extent (<5 studies altogether). These varying methodologies are examined differently due to inconsistencies both within studies of similar stimulations (such as method, task, stimulus application and aims) and between the different modalities i.e. how each stimuli affects perception. As such, is it only whilst reviewing a selection of studies does a clearer picture begins to become apparent. Fillingim et al. (2009) also noted that reports from clinical trials might inadvertently demonstrate healthcare bias, in which females are typically more likely to use healthcare systems more than males. Both Fillingim et al. (2009) and Racine et al. (2012a) aimed at examining evidence from research exploring gender differences using these various modalities over the course of the last 20 years (10-15 years at time of publication for Fillingim et al., 10 years at time of publication for Racine et al.). Each form of noxious stimulation reflects gender differences in a separate

manner. For cold-pain, Fillingim et al. (2009) found that the results supported the hypothesis of females having an increased sensitivity to cold pain, with 67% of reviewed papers reporting gender differences in cold-pain threshold. 93% of papers reported similar gender differences in cold pain tolerance elicited by cold-pressor task, as well as in 81% of the papers examining continuous subjective pain ratings during or following cold-water immersion. In contrast, Racine (2012a) observed no consistent pattern across papers, with the exception of pain tolerance. In this instance, Racine et al. reported 80% of the reviewed papers on the subject reported females as having a lower tolerance to cold pain than males. In regards to heat pain, Fillingim et al. (2009) observed a similar pattern of results to those of cold pain, where 81% of papers reported lower heat-pain thresholds in females, as wells as 94%n reporting a lower female heat-pain tolerance. As with cold-pain, Racine et al. (2012a) found that ≈80% of papers reported a lower heat-pain tolerance in females compared to males, but again no consistent patterns of results with observed. Fillingim et al. (2009) found that females were consistently reported as exhibiting a lower electrical-pain tolerance, though it was only as a result of reviewing 4 studies. Similarly, Racine et al. (2012a) found 4 of the 8 reviewed studies reported a significant difference between male and female electrical-stimulation thresholds, though they noted that the papers that failed to find a significant gender difference were underpowered according to the criterion established by Riley, Robinson, Wise, Myers and Fillingim (1998), which stipulated that in order for a study to have sufficient statistical power, there should be a minimum of 41 participants per group (i.e. 41 males and 41 females). Both Fillingim et al. and Racine et al. reported ischemic pain as being the most consistent both across studies and between genders, in that no significant difference was found between participant's threshold, tolerances and ratings of unpleasantness, though Racine et al. did note that 25% of the papers reported females as having higher ratings of pain intensity compared to males. With regards to pressure-pain, Fillingim et al. reported that studies examining pressure-pain produced the largest difference between male and female participants, with females exhibiting a significantly lower threshold and tolerance than males. Similarly, Racine et al. reported that 86% of their reviewed papers demonstrated females as having lower pressure-pain tolerances than males, and that (after controlling for adequate statistical power) all studies reported females as having a lower threshold than males. In summation, it is apparent that ischemic pain produces statistically similar experimental pain in males and females, pressure-pain reliably produces a disparity between genders, and that heat, cold, and electrical pain may result in a lower threshold in

females, though result can be inconsistent. Despite these well-reported differences in male and female pain perception, there has been a noted bias towards selecting male participants in experimental and pre-clinical pain research (Mogil & Chanda, 2005; Mogil, 2012).

Examinations of gender differences in pain have not just been limited to the perception of pain, but also to examine potential modulatory effects, such as diffuse noxious inhibitory control (DNIC). DNIC refers to the modulation of pain during the application of two concurrent noxious stimuli; one stimulus (usually the stronger or posing a more immediate danger) will typically modulate nociception and override the other stimuli (van Wijk & Veldhuijzen, 2010). While this is nominally investigated in terms of chronic pain patients, where acute stimuli may override the chronic pain symptoms and offer some respite, it has also been examined in terms of non-clinical populations to ascertain any differences between males' ad females' modulatory processes. Similar to the examination of sex differences between different methods of noxious stimulation, previous literature had not always provided the most coherent perspective on the phenomena. A systematic review by Popescu, LeResche, Truelove and Drangsholt (2010) identified methodology and results from a large selection of studies and concluded that, while experimental methodology and testing does appear to impact the results from examinations into DNIC or DNIC-like effects, it is apparent that males are more respondent to this form of pain modulation in a healthy sample. While diffuse noxious inhibitory control does not appear to be a factor in the context of this thesis, understanding how these modulatory effects can affect males and females differently is of particular interest in order for us to account for as many possible confounding factors that can influence the perception of pain.

Interpretations of sex differences.

There are a number of theories to interpret this disparity between genders and their experience of pain, from biological, psychological and social approaches. The first theory aimed at accounting for the apparent predisposition of females to chronic pain syndromes has already been mentioned, and this is of healthcare bias. Females have previously been shown to pursue professional healthcare advice and treatment in comparison to males (Briscoe, 1987). This means that reports of chronic pain conditions may not be truly representative of the actual prevalence, it is merely reflective of societal approaches and perspectives towards stereotypical male robustness, as well as considerations of needlessly concerning themselves with treatments of ailments or conditions, as seeking help or treatment may impact negatively upon their masculinity or reflect weakness. Conversely it is possible that females do have a higher sensitivity than males, in which case there may be observable, though not necessarily

comparable, differences in the functionality of their neuromatrix. The next question would be whether these differences are attributable to a biological difference in the ascending pain pathway differentially processing nociceptive inputs compared to males, or psychological differences in modulating inputs and responses (Mogil, 2012).

One possible theory as to why females perceive pain differently is related to hormone fluctuations associated with the menstrual cycle (Sherman & LeResch, 2006; Mogil 2012). In a review conducted by Sherman and LeResch (2006), papers focusing on the impact of the female menstrual cycle on pain perception was examined, and while some differences were noted, there were difficulties in establishing a consistent pattern due to differential approaches, methodologies, and even terminologies utilized by the researchers for phases in the cycle. This lead Sherman and LeResch to conclude that further research should establish a unified approach and standardized methodology for examining the relationship between hormone cycles and pain perception. Another biological theory to account for gender differences is derived from observations in rats. Put simply, the theory stipulates that neural circuitry relating to pain perception has developed around pre-existing reproductive circuitry, and as such there are gender differences in perception due to differences in reproductive neurology (Bodnar, Commons & Pfaff, 2002; Mogil, 2012). Considering evidence for the theory that the neuromatrix, rather than having evolved specifically for pain perception, is related to a much similar network based around homeostasis and saliency, this seemed like a viable option. Especially when taking into account that the neuromatrix is such a distributed network with a potentially large number of inputs influencing the output that we understand as pain (Iannatti & Mouraux, 2010).

From a psychosocial perspective, there are a number of potential influences modulating input into the neuromatrix that have come under examination with regards to gender differences, the first of which has already been discussed; stress and anxiety. As previously mentioned, ‘stress’ can refer to the by-product of the body being taken out of homeostatic equilibrium, anxiety has been highly correlated with chronic pain, and anxiety sensitivity (A.S.) has been established as an input into the neuromatrix. In the context of the current discussion, stress and anxiety refer to psychological constructs quantifiable in healthy participants with the use of scales and questionnaires such as the STAI, The Depression Anxiety Scale, and the Anxiety subscale of the Profile of Mood State, along with other visual analogue scales (Racine et al. 2012b). In the companion paper to their review of experimental gender differences, Racine et al. published a paper reviewing examinations into bio-psychosocial influences of gender differences in pain. They noted that some papers did not

report any significant correlations between anxiety and pain in both genders, while others reported significant correlations between stress/anxiety and pain in males but not females, and others reporting the opposite correlation (i.e. in females rather than males). Still more reported that males with anxiety scores above the median reported lower pain tolerance with higher intensity and ratings of unpleasantness, while no differences were observed between high- and low-anxiety females. Racine et al. (2012b) observed the tendency for anxiety to be a stronger predictor for pain in males rather than females, but found that the authors for the original papers were unable to replicate their findings. Examinations into experimentally manipulated anxiety found similar inconsistent results, ranging from no significant differences between males and females, to indications that anxiety had stronger effects on pain thresholds of males compared to females, to females in higher stress conditions reporting higher perceived pain intensity. As with most examinations into experimentally manipulated pain and gender effects, different methodologies between each experiment, as well as forms of measurement and manipulation, results are (almost consistently) inconsistent and difficult to reliably compare.

Non-biological influences.

Aside from anxiety and stress, there are other psychosocial factors that have been shown to modulate and influence inputs into the neuromatrix with regards to gender differences in pain reports. By manipulating expectations of performance, researchers have also proven to alter gender perceptions separately. Robinson, Gagnon, Riley III and Price (2003) manipulated gender role expectations in a task wherein participants were required to submerse their hand in an ice bath by informing the participant that "the average male/female can last X seconds in this task", as well as including a 'no expectation' condition wherein they were not given this information. They found that there was a significant gender effect in the no expectation condition and that female participants rated the stimulation as more intense, whereas there was no observable gender effect in the expectation conditions. This process may be attributable to the cognitive-evaluative inputs into the body-self matrix (as described by Melzack, 2001) wherein higher cognitive functions have put the stimulation into a context that manipulated their perceptions, in turn altering their perception.

In an experimental environment, one of the more interesting influences on differential gender perceptions of pain, external to the participant, is that of the experimenter's characteristics and, more specifically, their gender (Fillingim et al., 2009). In a study by Levine and DeSimone (1991), the effects of experimenter gender on pain reports were examined. Experimenters were selected for based upon their attractiveness in order to evoke

a gender-related response, and they were also required to dress in a way that accentuated their masculinity and femininity in order to enhance the effect. It was found that males reported significantly less pain in the presence of a female experimenter, and that females demonstrated a tendency towards a lower pain threshold in the presence of a male experimenter, but not to any level of significance. Similarly, Gijsbers and Nicholson (2005) found that males demonstrated a higher pressure-pain threshold when tested by a female experimenter, whereas female participants' pressure pain threshold was not influenced by experimenter gender. When examining autonomic responses (such as heart rate variability and skin conductance) as well as pain ratings, Aslaken, Myrbakk, Hoifoft and Flaten (2007) found a significant interaction between participant gender and experimenter gender, in a similar fashion to previous research, but found no physiological interactions. This is indicative that attractiveness may not have a part to play in this effect, and that it may be due to psychosocial processes that are distinctive from the stimulation itself. As well as gender, perceived professional status has been examined (Kallai, Barke & Voss, 2004). It was found that participants tolerated noxious stimulation for a longer period of time when tested, not only by an experimenter of the opposite sex, but also by one that they perceived to be of a higher professional status.

Sex/gender dissociation.

Although throughout this introduction the terms "sex" and "gender" are utilised interchangeably, research in recent years has started to differentiate between sex and gender, by which sex refers to the biological condition of being male or female, and gender refers to the social role that an individual relates as, essentially biological vs. psychological. As such, sex and gender may have alternate inputs into the neuromatrix broadening the perspective of gender research in the field of pain beyond just males versus females to a much more complex arena. As noted previously in regards to male vs. female healthcare-seeking behaviours, this interest in dissociating sex from gender may be reflective of a more acceptable form of understanding within societal viewpoints relating to sex and gender as two distinct, potentially parallel aspects of an individual. A recent review by Mathna (2015) examined this dissociation and what it may mean in relation to the neuromatrix. Mathna (2015) examined evidence and discussed that one potential theory as to why women may be more susceptible to chronic pain is due to sex hormones, specifically oestrogen, taking note that according to Melzack (2005), females predominantly suffer from chronic pain syndromes such as FM, as the oestrogen stimulates a reaction that ultimately results in increased cortisol release, which in turn leads to a build-up in bodily tissues resulting in

chronic pain. With regards to psychological gender, males and females have been noted as practising different coping strategies; females tend to utilize methods involving social support, whereas males tend to utilize behavioural distractions (Lynch, Kashikar-Zack, Goldschneider & Jones, 2007; Mathna, 2015). As such, sex and gender may have entirely separate, distinct influences on modulating inputs into the neuromatrix, and, as ever, a better understanding of how these aspects interact with other factors in the neuromatrix may further research in the field and lead to a more representative model of pain perception.

Evidence from neuroimaging.

With the advent of neuroimaging techniques over the last few decades, studies have been conducted to support the theory that aspects of the tri-dimensional theory of pain are processed separately and in parallel across multiple cortical structures. It has been found that while the sensory-discriminative process occurs in the primary somatosensory cortex, information regarding pain intensity and perceived unpleasantness does not (Coghill, Sang, Maisog & Iadarola, 1999). This would suggest that, rather then individual structures of the brain working in isolation to process aspects of noxious stimulation there is most likely a globally distributed system involving different sections of multiple structures. When examining the cognitive-evaluative dimension, Lorenz, Minoshima and Casey (2003) observed that frontal lobe activity is linked to attentional processing and, using positron emission tomography (PET) scanning techniques, found that the dorsolateral prefrontal cortex is activated bilaterally during noxious heat stimulation. This led them to conclude that the dorsolateral prefrontal cortex is able to modulate pain perception by actively controlling the cortical pathways between integrated structures. Lorenz, Minoshima and Casey noted that, although the dorsolateral prefrontal cortex is involved with the cognitive-evaluative aspect, other multiple structures (including the medial thalamus, anterior insula, anterior cingulate and orbitofrontal cortices) are involved with the perceived intensity and unpleasantness of pain, and it is the pathways between these structures that the dorsolateral prefrontal cortex exerts control over.

In order to examine the relationship between perceived intensity and unpleasantness of noxious stimulation, Hofbauer, Rainville, Duncan and Bushnell (2001) attempted to alter participant's perception of the sensation of pain and examined the cortical structures involved with intensity and unpleasantness processing. By using hypnotic suggestion to suppress the perception of the intensity of stimulation, Hofbauer et al. found that the anterior cingulate cortex is involved in the perception of unpleasantness and the characterisation of stimuli as noxious. This experiment was conducted as a follow-up to an earlier study by Rainville,

Duncan, Price, Carrier and Bushnell (1997), who used hypnotic suggestion to alter the affective component of pain, thereby suppressing the unpleasant component. Rainville et al. found that anterior cingulate cortex function was affected but activity in the somatosensory S1 and S2 areas remained intact, which is indicative of S1 and S2's involvement in perceived intensity. The findings of these experiments show that, although these structures are integrated into the processing of the experience of pain, they are responsible for the perception of different components that are relevant to the experience of pain as a whole.

Other evidence in support of a globally distributed mechanism has been observed by Coghill et al. (1999) using PET scanning techniques. Coghill et al. found that brain structures involved in motor, somatosensory, attentional and affective processing as well as autonomic function have all demonstrated activity with the application of noxious heat stimuli. This demonstrated that the processing of nociceptive information relative to the intensity of pain is reliant on an infrastructure, transmitting from the thalamus to areas such as S1, S2, the anterior cingulate cortex, premotor areas and the insular lobe, and the involvement of each structure is paramount in assessing the affective components of pain in parallel (Fulbright, Troche, Skudlarski, Gore & Wexler, 2001).

Though research on gender differences in pain has been deemed by some to be a topic of important scrutiny, not many appear to have examined neurological differences in great depth. In general, it has been reported that males have a higher percentage of white matter, females have a higher percentage of grey matter, regional differences are inconsistent, and females appear to have greater global blood flow in the brain (Cosgrove, Mazure & Staley, 2007). Some examinations have been conducted in relation to the BOLD response to pain and sex differences, where it was found that while spatially there was no difference in BOLD activation in response to thermal stimulation, females demonstrated a much lower signal amplitude in multiple regions (Moulton, Keaser, Gullapalli, Maitra & Greenspan, 2006). Regions in which females' signal amplitude was significantly lower than males included SI (primary somatosensory), mid-ACC and the dorsolateral prefrontal cortex. Further research has found that (whilst controlling for pain intensity) subjective pain unpleasantness was related to an increase in perigenial ACC activity in females, whereas in males it was related to decreased activity in the ventromedial prefrontal cortex (Girard-Trembley, Auclair, Daighe, Leonard, Whittingstall & Goffaux, 2014). As previous behavioural studies have demonstrated, these findings could reflect differential coping mechanisms in males and females. Studies aimed at examining neurochemistry in the field of pain research utilising Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) do not appear to have focussed on

gender differences, with exception to those investigating chronic pain conditions, such as FM. In these instances the participants were predominantly female.

Spectroscopy and pain.

As a complement to fMRI and PET scanning, ^{1}H-MRS is proving to be a useful insight into how the central nervous system operates in pain perception. As with fMRI, ^{1}H-MRS is a non-invasive imaging technique. However, unlike most imaging modalities that focus on acquiring a spatial representation of cortical structures and associated activity therein, ^{1}H-MRS is used to quantify metabolic concentrations within a specific voxel of interest (VOI) by utilizing specific resonance frequencies to excite protons, in turn generating a chemical-specific spectrum (Alger, 2010). Several metabolites are observable using ^{1}H-MRS, including (but not limited to) N-acetylaspartate (NAA), Creatine (Cr), Choline (Ch), Glutamate (Glu), Glutamine (Gln), and gamma-aminobutyric acid (GABA), the latter three are of particular interest in the field of pain research (Mountford, Stanwell, Lin, Ramadan & Ross, 2010; Mullins, Rowland, Jung & Sibbitt, 2005). Glu and GABA are the most abundant excitatory and inhibitory neurotransmitters in the brain, respectively. Gln is a by-product of Glu synthesis, and at 1.5T the Glu and Gln signals can overlap on a spectra, making them difficult to tease apart and effectively examine separately. As such the overlapping signals of Glu and Gln are commonly referred to as Glx (Mountford et al. 2010, Feraco, Bacci, Pedrabassi, Passamonti, Zonpagna, Malavolta & Leonardi, 2011). Among the first to demonstrate the link between neurometabolites and pain, Mullins et al. (2005) observed that Glu in the ACC, an area well established to be involved in nociception, increased dynamically upon the application of noxious cold stimuli, by as much as 9.3%. Gln was also shown to increase with the subjective experience of pain. Further research has found similar functional relationships in the anterior insula cortex (Gussew, Rzanny, Erdtel, Schollw, Kaiser, Mentzel & Reichenbach, 2009), wherein the concentration of Glu was shown to increase by as much as 18% upon the application of acute thermal stimuli. GABA has also been demonstrated to be involved in pain perception, the first evidence for which was reported by Kuper, Danielson, Kehlet, Christensen and Thomsen (2009). Kupers et al. (2009) found that GABA increased in the rostral ACC (rACC), and other areas believed to be related to the affective component of pain (Bornhovd, Quante. Glauche, Bromm, Weiller, & Buchel, 2002). The researcher believed that these results demonstrated a neurochemical underpinning for the increased blood-flow response in the rACC during pain. Similarly, studies examining GABA have reported findings that are indicative of reduced activity in the inhibitory systems. In this instance, Cleve, Gussew and Reichenbach (2015) examined the ACC and

occipital cortex. They found that upon noxious thermal stimulation, there was an increase in Glx and a decrease in GABA+. This may reflect an excitatory/inhibitory ratio in neurotransmission, indicating that nociceptive inputs are not purely dependent on excitation, but rather the relativity of excitation to inhibition. These results could be interpreted as supporting models within the neuromatrix theory, wherein inputs into the neurosigniture are dependent on an ascending excitatory influence or pathway, and a descending modulatory (inhibitory) pathway. Further research into this relationship may paint a more coherent picture, especially at higher MRI field strengths, at which point neurometabolites are easier to examine separately.

The use of ^{1}H-MRS in pain research has not been limited to the study of experimental pain. There have been a number of papers published examining the roles of regional concentrations of Glu, Gln and GABA in chronic pain patients, particularly in FM (Harris, Sundgren, Pang, Hsu, Petrou, McLean, Gracely, & Clauw, 2008; Harris, Sundgren, Craig, Kirshenbaum, Napadow, & Clauw, 2009; Valdes, Collado, Bargallo, Vazquez, Rami, Gomez, & Salamero, 2010; Fayed, Garcia-Campayo, Magallon, Andres-Bergareche, Luciano, Andres, & Beltran, 2010; Feraco et al., 2011; Harris & Clauw, 2012). Valdes et al (2010) examined 28 FM patients compared to 24 age-matched health controls (all females), and found a higher Glx concentration in the right amygdala of FM patients compared to healthy controls. Feraco et al. (2011) examined 12 FM patients and 12 age- and sex-matched controls (11 female, 1 male) and found higher Glx and Glu concentrations in the ventrolateral prefrontal cortex, as well as positive correlation between Glu in the left thalamus and scores of pain an a visual analogue scale. Fayed et al. (2010) found that FM patients had significantly higher concentrations of Glu and Glx in the posterior insula compared to age- and sex-matched controls (10 patients, 10 controls, gender does not appear to have been reported).

Among the first to report the link between altered neurochemistry and chromic pain, Harris et al. (2008) focussed on the anterior and posterior insula. They examined activity within these two regions using ^{1}H-MRS and fMRI before and after the application of a pain-reducing intervention. They found that both experimentally induced pressure-pain and clinical pain ratings observed from the short form of the McGill pain questionnaire were reduced after the intervention. Not only was there a reduction in pain ratings, but changes in Glu were negatively correlated with changes in experimental pain rating and negatively correlated in measure of clinical pain. These findings lead them to believe that changes in Glu in certain regional structures may serve as a useful biomarker in clinical patients. Further

examination into experimentally evoked pain and neurometabolic concentrations in the insula showed that FM patients had significantly higher concentrations of Glu and Glx in the right posterior insula compared to healthy controls (Harris et al., 2009). The researchers argued that this may potentially indicate that the insula is a region that exhibits increased neuronal sensitivity in FM. As one theory of the mechanism behind FM alludes to decreased inhibitory neurotransmission, insular GABA has also been examined (Foerster, Petrou, Edden, Sundgren, Schmidt-Wilcke, Lowe, Harte, Clauw & Harris, 2012). 16 FM and 17 healthy age- and sex-matched controls (all female) underwent pressure-pain testing during ^{1}H-MRS data acquisition. It was found that FM patients had lower concentrations of GABA in the right anterior insula compared to controls, potentially supporting the theory that aspects of FM may derive from a decreased inhibitory response, particularly in the anterior insula (Foerster et al, 2012; Harris & Clauw, 2012).

Spectroscopy is proving to be a valuable commodity in its contributions to understanding the neurological mechanisms underlying pain perception, particularly as it offers a unique approach to non-invasively examine neurometabolic activity and concentrations, especially in combination with other imaging techniques. However, there are still a number of issues that must be addressed, especially in multimodal imaging paradigms, before the results from these studies can be deemed sufficiently reliable or accurate in their observations and interpretations (Duncan, Wiebling & Northoff, 2014). At current, we are only getting a snapshot of a much more complex mechanism utilizing current methodologies. As a number of cortical structures are involved in nociceptive perception, this thesis will focus on two central structures that are of particular interest to this research.

Central Regions of Interest: The ACC

On the medial surface of the brain, surrounding the corpus callosum (one of the most central structures of the brain) is the cingulate cortex. The term “cingulate” has developed from the belt-like accessory that Roman men and women used to wear, and the cortex is thus named as it forms a ‘belt’ or ‘collar’ surrounding the corpus callosum (Allman, Hakeem, Erwin, Nimchinsky & Hof, 2001; Vogt, Hof & Vogt, 2004). Although the cingulate cortex appears to have structurally formed as a single and continuous entity, it has been shown that the cortex is in fact both functionally and structurally heterogeneous, insofar that different segments of the cortex have displayed a variety of connections and pathways to other structures within the nervous system (i.e. amygdala and parietal projections can distinguish between the rostral and caudal sections, connecting with the anterior cingulate, while showing minimal connections to the mid cingulate and posterior cingulate; Vogt, Finch

&Olson, 1992; Vogt, Nimchinsky & Hof, 1997; Vogt & Sikes, 2000). Traditionally, the cingulate cortex consists of sections identified as the Anterior Cingulate Cortex (ACC; Brodmann's areas 24, 24 and 32), the Posterior Cingulate Cortex (PCC; Brodmann's areas 23 and 31), and retrosplenial cortex (RSC; Brodmann's areas 29 and 30; Vogt et al., 1992). It is worth mentioning that it has been shown that Brodmann's areas do not necessarily identify individual structures that perform dedicated functions, but rather it has been demonstrated that these areas are highly interconnected, both physically and functionally (e.g. the PCC is area 23, but projections into area 31 have been observed (Braak, 1976). The ACC can further be divided into two subregions; the perigeniual anterior cingulate (pACC; located anteriorly), and the Mid Cingulate (MCC; located posteriorly), changing the traditional three-area model into a four-area model (Vogt, 1993; Vogt, Nimchinsky &Hof, 1997). All the connections entering and leaving the cingulate cortex do so via the Cingulum bundle, a white matter tract underlying the cingulate cortex. Neurosurgical procedures designed to alleviate the symptoms of chronic pain, anxiety, major depression and obsessive-compulsive disorder (OCD) have all targeted the cingulum bundle (Vogt, Hof & Vogt, 2004).

From a functional imaging perspective, the anterior cingulate cortex has been an area of much interest due to its high structural and functional interconnections with other structures, including dorsal connections to the prefrontal cortex and parietal lobe (Posner & DiGirolamo, 1998) and ventral connections to the amygdala, hypothalamus and anterior insula (Allman et al., 2001). These differential connections have lead researchers to consider the ACC as being divided in function; being defined as dorsal cognitive and ventral emotional connections, which correspond to the pACC and MCC (Bush, Luu & Posner, 2000; Vogt, Hof & Vogt, 2004). Functional roles of the ACC are quite varied, with one of the most commonly known being its involvement in error-detection, evident during tasks such as Stroop test; alternatively it is involved in conflict monitoring, during tests such as the Erikson Flanker task (Posner & DiGirolamo, 1998). It is plausible that the role of the ACC in error-detection is not just that it detects response errors during a task, but rather that it detects conditions in which errors are likely to occur, as it has been shown to not only be active during incorrect responses, but also during conditions of increased response competition (Carter, Braver, Barch, Botvinick, Noll & Cohen, 1998). In relation to error-detection, it has been found that the rostral ACC (rACC) and dorsal ACC (dACC) both activate during tasks, though they appear to be responsible for differential roles, with deactivation of the rACC being associated with accurate task performance, and it may also play a role in reward-based decision making (Polli, Barron, Cain, Thakkar & Rauch, 2005; Bush, Vogt, Holmes, Dale,

Greve, Jenike & Rosen, 2002), more specifically that it processes loss-related responses to errors (Taylor, Martis, Fitzgerald, Welsh, Abelson, Liberzon, Himle & Gehring, 2006) . The dACC has been shown to activate early during both erroneous and correct performance, though this early activation, or anticipatory response, has been associated with fewer errors during task performance (Polli et al., 2005). These studies provide support to the theory that the areas of the ACC (rACC and dACC in particular) make differential contributions to the evaluation and optimisation of cognitive performance over the course of a trial (Polli et al., 2005).

The ACC has been shown to be involved in anticipation (Murtha, Chertkow, Beauregard, Dixon & Evans, 1996) and it is also one of the structures in the brain to be widely observed in a role during the perception and experience of pain (Ingvar, 1999; Coghill, Sang, Maisog & Iadarola, 1999; Lorenz, Minoshima & Casey, 2003). Murtha et al. (1996) demonstrated increases in cerebral blood flow (CBF) using positron emission tomography (PET) scanning techniques in an anticipatory condition in comparison to control/baseline conditions during performance of a selection of cognitive-based tasks. The pattern of activity suggested that receiving instructions, preparing for and anticipating performance on a cognitive task rather than task-related processing itself is responsible for the increased CBF. This corresponds to the findings of Polli et al. (2005) and presents further evidence for the ACC's role in the evaluation and optimisation of performance over the course of a cognitive task, through anticipation of upcoming stimuli. It is plausible that the role of the ACC in pain processing and perception may be similar.

As areas of the cingulate cortex are defined by circuitry and function rather than structure, it is important to bear in mind that each region is not necessarily uniform in its function (Vogt, 2005). Typically the ACC is involved in the storage of emotional memories, though the subgenual ACC (sACC) has been shown to activate during sad events (George, Ketter, Parekh, Horwitz, Herscovitch & Post, 1995) and the pACC shown to activate during happy events (Vogt, Berger & Derbyshire, 2003). Interestingly enough, using magnetoencephalography (MEG), a pain response has been demonstrated in the pACC which may be associated with the affective component of pain (i.e. the feeling associated with suffering; Vogt, 2005; Ploner, Gross, Timmerman & Schnitzler, 2002).

There is further evidence to support the involvement of the ACC in the perception of the unpleasantness (synonymous with 'suffering') aspect associated with the experience of pain. For example, Rainville, Duncan, Price, Carrier and Bushnell (1997) used hypnotic suggestion to alter participants' experiences of the unpleasantness aspect associated with

noxious stimulation, while keeping the perception of intensity intact. Using PET scanning, they examined how modulating the affective experience of pain impacted upon activity within the ACC in comparison to the somatosensory areas of the brain. They found that there were significant changes in the ACC consistent with the encoding of perceived unpleasantness, whereas the primary somatosensory area remained unaffected. In a follow-up study, Hofbauer, Rainville, Duncan and Bushnell (2001) used hypnotic suggestion to modulate the sensory experience of the intensity of stimulation. They found that activity in S1 correlated with the modulation of the perceived intensity and the ACC remained unaffected. This double dissociation between the different dimensions of the experience of pain, particularly between the sensory and affective components, is indicative of a globally distributed matrix within the brain that processes the different aspects associated with feeling pain, and demonstrates the involvement of the ACC in the emotional encoding of the affective experience.

Central Regions of Interest: The Insula

Towards the lateral surface of the brain, within the Sylvian fissure, lies the Insular cortex, which is also known as the Island of Reil (after the German physician and anatomist Johann Christian Reil). Considered to be the fifth lobs of the brain, the insula is obscured (or insulated) by the fronto-orbital, fronto-parietal, and temporal operculum, and it is divided into two distinct portions by a central sulci. The larger, anterior portion (AI) consists of three short gyri and has been shown to have extensive connections to the frontal lobe, while the smaller, posterior section (PI) consists of two long gyri (anterior and posterior) and has numerous connections to the temporal and parietal regions (Murphy & Jones, et al, n.d.; Türe, Yasargil, Al-Mefty & Yasargil, 1999). Extensive investigations into the structural connectivity of the insula in primates and humans have found reciprocal connections (i.e. to- and from- both structures) between the insula and frontal regions, which include the frontal operculum, both lateral and posterior orbitofrontal cortex, and the prefrontal cortex. The insula also projects (but does not reciprocate) connections to frontal regions including the inferior frontal gyrus, the ventral granular frontal cortex, the medial portion of Broca's area, and Brodmann's area 6 (part of the frontal cortex adjoining the motor area). Similar connections can be observed between the insula and temporal cortex, with reciprocal connections with the temporal pole and superior temporal sulcus, while the insula also receives input fibres from the primary auditory and auditory association areas, post auditory cortex, and temporal operculum, but does not project to those areas. In relation to the parietal cortex, there are extensive fibres to and from the insula, which includes the anterior-inferior

parietal cortex, parietal operculum, primary and secondary somatosensory cortex, and the retroinsular area, which are all reciprocal. Insula connections can also be observed in subdivisions of the ACC, basal nuclei, amygdaloid body, and dorsal thalamus (Augustine 1996).

As well as outlining the insular cortex's structural connections, Augustine (1996) also provided a concise summation of some of the roles that can be attributed to its functions, the details of which have been the subject of extensive and expansive investigations since the review was published. The functions that were initially outlined included gustation and digestive processes, somatosensation (including pain, thermal, and tactile recognition and assessment), its involvement as a multifaceted sensory area (which includes processes associated with feeding and neglect), as a limbic integration area, as an autonomic area involved in cardiovascular function and homeostatic regulation, as well as its roles in motor association and as a language area. Due to the implication of the sheer number of processes attributed to the insular, more detailed examinations into both its structural and functional connectivity have been required over the years, aided by the development of functional imaging techniques that have subsequently provided a greater understanding of this cortex

As research techniques and technologies have evolved over the years the insula has been the subject of an array of different examinations, the range of which extending from the micro (single neuron responses), to the local (examination of regional differences within the stricter, encompassing functional imaging and electrode stimulation), to the global (functional connectivity between structures and within neural networks), in an effort to ascertain the 'how's', 'what's' and 'whys' of the insula's varying roles in cognition. When examining single neuron responses in rats, Hanamori, Kunitake, Kato and Kannan (1998) found that insular neurons received and responded to convergent inputs of nociceptive, gustatory, and visceral processes, which can account for the overlap in insular cortical areas in a variety of functions. Local regional examinations of the insula with the implantation of diagnostic electrodes during a pre-surgical evaluation in five epilepsy patients found that excitation of the PI elicited somatosensory reactions, with pain and warmth arising from stimulation of the dorsal portions of the PI. This has been evidence in a number of studies, with the PI being associated with the perceived intensity or unpleasantness of noxious and non-noxious stimulation (Sawamoto et al, 2000; Singer, Seymour, O'Doherty, Kaube, Dolan, & Frith, 2004). Stimulation with a more anteriorly located electrode demonstrated a viscerosensory reaction, with gustatory responses localized towards the central insula. However, no reactions were observed when the electrode in the AI was stimulated, though

the authors did admit that this might have been due to insufficient central coverage of the AI. It is also worth considering that due to the AI's connections to the frontal cortex and its role in high-level cognitive control, attention aspects and role in saliency detection may have caused a less noticeable reaction, or it may not have been sufficiently integrated with the patients interoceptive awareness (Menon and Uddin, 2010).

In contrast to the PI, the anterior portion appears to play a more covert role in cognition, especially in regards to pain. As the insular has been accepted as a structure that plays an important part in viscerosensory processing, researchers have attempted to examine and breakdown its mechinisms, particularly in regards to the integration physiological and somantic states can produce an emotional reaction, and what is commonly referred to as 'feelings', to contextual stimuli. It has been argued that the AI plays a central part in the production of feeling states by receiving minimally- or un-processed inputs relating to physiological, visceral states as well as affective states, integrates them together to map out an anatomical representation in a manner that is then available for the stream of consciousness, resulting in the conscious awareness of states (Singer, Critchley & Preuschoff, 2009). It has also been found that greater activity in the AI is recorded in cases of increased physiological arousal, as well as during declarative states of physiological states and responses (e.g. when the participant is aware of an increase in their heart rate), as well as a relationship between the AI and the overall experience of anxiety. These links also suggest that the AI is involved in the prediction of physiological states as a form of autonomic learning, which can be closely related to the neurobiological model of anxiety, as proposed by Paulus and Stein (2006). In this model, evidence from neuroimaging studies showed that sensory inputs into the insula allow the region to produce a signal that predicts the expected contextual physiological arousal. When there is then a discrepancy between the predictive signal and the actual level of arousal, an interoceptive prediction error is produced, resulting in subjective anxiety and avoidance behaviours. Singer, Critchley and Preuschoff (2009) also suggested that the integrated signal of the physiological, social, predictive and psychological states can also be modulated by uncertainty to produce the subjective feeling state, which could facilitate the regulation of homeostasis and in turn represent a physiological and emotional learning process that would accelerate the implementation of coping mechanisms or strategies. This model bears some similarity to other insula-based theories of prediction and integration of states during nociceptive processing.

In relation to nociceptive processing, the AI in particular has demonstrated to modulate the experience of noxious stimuli in a similar method to the anticipatory effect

shown in the ACC. Singer et al. (2004) found that while the PI was active during noxious stimulation, the anterior portion and the ACC were active both during and prior to the stimulus application, whether the participant was receiving stimulation or observing a loved one be stimulated. The predictive models that insula produces can also be affected by cognitive elements that relate to participants' perceptual decisions about the stimuli; i.e. the participants' expectation that the stimuli would be either safe (non-painful) or potentially noxious impacted upon their ratings of the stimuli, causing the potentially noxious expectation to result in a higher pain rating. It was also demonstrated that the anticipation and prediction of noxious stimulation increases pre-stimulus functional connectivity between the mid-cingulate cortex and the anterior insula (Wiech, Lin, Brodersen, Bingel, Ploner & Tracey, 2010). Further examinations into the role of the AI has discussed how these models of interoceptive perception, homeostatic regulation, and modulating cognitive inputs can fit together in the form of a saliency network, with the AI operating as a central processing hub.

Menon and Uddin (2010) proposed that the insula itself is sensitive to salient events and one of its primary roles is to appraise and catagorise salient stimuli for additional processing and initiate appropriate control signals. Once the AI has detected a salient event, it switches between functional networks to redirect or improve the attentional focus, whilst incorporating aspects of working memory. Connections between the AI and PI are then engaged to mediate autonomic reactivity to the stimuli, followed by increasing the functional connectivity between the AI and ACC in order to facilitate and rapidly instigate avoidance behaviours where necessary. This is particularly relevant in regards to noxious stimuli, which itself is highly salient. This is just one of several similarly-minded theories examining the role of the insula in not just pain, but also tactile and autonomic processes, which will be addressed in detail in the subsequent chapters. By examining the evidence put forward by these theories, it can be evident to see how these processes may have evolved throughout the development of the human brain, from a simpler avoidance mechanism to one of the most complex and subjective natural phenomena we are familiar with today.

Postulations and objectives.

Initially, this thesis began as an investigation into the neurometabolic mechanisms behind uncertain anticipation of noxious stimuli, but developed into something a little different. As such, the aim of this thesis has been to examine different aspects of gender influences on pain perception (or at least pain reports) from both a psychosocial and biological approach. What mechanisms, both physiologically and socially, can impact on our experiences and interpretations of noxious stimuli, and is it possible to experimentally

manipulate them without explicitly declaring so, such as in effects of experimenter gender? If so, is it possible to build upon the effect and utilize other social aspects to enhance it? This could indicate social considerations need to be implemented in both experimental and clinical contexts. Further then that, what biological contributions may need to be considered, and are observations made in clinical populations of FM sufferers replicable in healthy participants? If they are, could this indicate that sensitivity in FM patients reflect "normal" neurochemical patterns, but in the extreme? In instances of both biological and psychological approaches, is quantifiable stress or anxiety incorporated within or between subjects? If it is, does this account for any variations? This thesis will aim to answer a majority of these questions in the form of two experiments with different approaches; the first is aimed at examining social mechanisms in the form of the experimenter gender effect. While no previous evidence has been found to account for this effect, this thesis will examine a number of psychosocial aspects, including state and trait anxiety, personality via the BFI, and participant's ratings of the experimenter. This experiment will also investigate whether the presence of an observer during the collection of pain ratings also influences participant thresholds. If it does have an effect, it may demonstrate that the experimenter gender effect may either extend to the observer, or the observer might mediate the experimenter's influence. The second experiment examines the topic from a biological approach, and is aimed at replicating previous regional ^{1}H-MRS findings in the insular cortex of clinical populations in a healthy sample. Not only that, but as no males have been previously tested it is aimed at examining whether they exhibit the same response, as well as whether there are any regional differences between males and females, and whether this can account for any gender-based differences.

Though this thesis may not be able to answer all of these questions, it may shed some light onto the mystery that is the subjective and deeply personal experience of pain, as well as how sex and gender may fit into current models and theories of pain perception. As we uncover the answers piece-by-piece, we may one day develop a complete understanding. An understanding that may improve clinical practices, improve quality of life for so many of those who suffer, and find a method to reliably quantify measurements of pain consistently.

The road less traveled.

At its inception, this thesis began as an investigation into neurometabolic concentrations within the anterior cingulate during uncertain anticipation of pain, as discussed in Chapter 3. I was already interested in pain due to my mother who, despite being disabled and in pain every single day, yet still had a successful career as a specialist paediatrician, and being given the opportunity to begin a career in the field was something I couldn't pass up. During the early days, I had grandiose ambitions, intent on discovering as much as possible and developing a number of different potential experiments that either complimented the examination of the ACC or were completely unrelated. Most of these involved functional imaging; examining activity in the ACC during pain compared to an error awareness task; longitudinal correlations of pain and anxiety pre- and post-intervention for students experiencing difficulty with stress and anxiety; something to do with placebo manipulations and their impact on relevant neural structures; the list goes on. Most of these were beyond the scope of available resources and funding, and after developing the ACC examination and finding it did not function as intended, we took a step back while I got quotes for various other equipment and potential stimuli, all of which were far too expensive. During my Masters, I had already conducted a similar experiment to that of the observer study in Chapter 4; that one was a much smaller scale with about a dozen participants wherein an observer was present or not during measurement of pressure pain thresholds. While there did appear to be an effect, there were too few participants to adequately determine whether there was an effect, so we decided to expand on that project and incorporate experimenter and observer genders in order to examine what could have an impact on ratings; is it just when someone is in the room, or did it operate similarly to the experimenter gender effect? Even though that project was much more obtainable, it did prove to be a bit more of a challenge for a single person to conduct, having to arrange all the testing, recruitment, finding and ultimately hiring confederates and training them. It was difficult but mostly rewarding (expect when participants kept dropping out after getting the last few course credits they needed as they got 1 per session). Unfortunately between my studies, working part time off-campus, working as a teaching assistant, some smaller administrative roles and a handful of other duties, I stretched myself too thin and, after suffering for some back pain for some time, I work up one day in the worst pain I had ever suffered, which is saying something, considering during the previous couple of years I had punctured my lung and been thrown from a car (both totally unrelated). After six months of examinations and severely diminished motability, I was diagnosed with a genetic condition

wherein my immune system is attacking my spine, causing inflammation and ossification (i.e. the growth of bone shards around spinal tissue). Ironically, they genetics were from my father's side, who had no known spinal issues, rather than from my mother. Suffice it to say, this changed my outlook on a lot of things; originally I had little interest at this stage of my career at examining chronic pain in detail, but experiencing it first-hand helped me develop more of an interest. I realized that the implications from the observer experiment could have an impact on medical assessments, having had quite a few myself by then in which there was almost always a medical student, physiotherapist, pharmacologist, or nurse present. I also found myself reading more about [1]HMRS examinations in chronic pain. It was something my supervisor, Dr. Mullins, and I had discussed about the insular cortex in passing, and I started to read up more on the subject. One thing that struck me was how little information there seemed to be on males suffering from chronic pain, as it is predominantly something experienced by females. Whether this was due to with genetic representation or due to individual reports was unclear, but having already been interested in gender differences and pain perception, it was this that solidified my fascination on the subject. We decided to progress along this avenue of investigation, along with another aspect of pain perception relating to differences between healthy and clinical patients. Although there is evidence to support that elements of the brain change over the course of time in those suffering from chronic pain (such as increases in grey matter density), it seems to be a very binary acute vs. chronic pain field, with little discussion of the in-between stages. Again, having experienced it first hand I could attest that there is no sudden cut-off where the doctor or patient suddenly say "yep, that is no longer acute, it is now officially chronic pain"; you just find ways to adapt along the way and cope as well as you can, until you suddenly realize it has been (for me) over three years at the time of this writing. So, if that is the case, does that mean that there could be a spectrum, similar to a number of other conditions, when it comes to pain? This formed the basis of the investigation into insular neurometabolic concentrations in relation to individual pain tolerances; something that had already been investigated with fibromyalgia patients (who were predominantly female) but there was little to suggest whether there had been any similar investigations into healthy participants, both male and female. With that, the "theme" of my thesis became clear: examining gender differences in healthy participants' perceptions of pain, and what or how other factors can influence them separately. Needless to say this has been a long road and the end is not yet in sight; all I can do is keep going, give it my all and one day I'll be able to look back and appreciate just how far I will have gone, and know it was all worthwhile. In life, sometimes that is the best any of

us can hope for, and I am thankful every day for all the opportunities have I been granted and all the support I have had. I look forward to the rest of this journey and seeing just where it will take me.

Chapter 1 References

Alger, J. R. (2010) Quantitative Proton Magnetic Resonance Spectroscopy and Spectroscopic Imaging of the Brain: A Didactic Review. *Topics in Magnetic Resonance Imaging.* 21(2):115-128.

Allman, J. M., Kakeem, A., Erwin, J. M., Nimchinsky, E. and Hof, P. (2001). The anterior cingulate cortex: the evolution of an interface between emotion and cognition. *Annals of the New York Academy of Sciences.* 107-117.

Aslaken, P. M, Myrbakk, I., N., Hoifodt, R. S. and Flaten, M. A. (2007). The effect of experimenter gender on autonomic and subjective responses to pain stimuli. *Pain*. 129: 260-268.

Augustine, J. R. (1996) Circuitry and functional aspects of the insular lobe in primates including humans. *Brain Research Reviews*. 22: 229-244.

Berkley, K. J. (1992). Vive la difference. *Trends in Neurosciences.* 5: 331-332.

Bishop, S.J. (2007) Neurocognitive mechanisms of anxiety: an integrative account. *Trends in Cognitive Sciences.* 11: 307-16.

Bodnar, R. J., Commons, K. and Pfaff, D.W. (2002). Central neural states relating sex and pain. John Hopkins University Press.

Bornhövd, K., Quante, M., Glauche, V., Bromm, B., Weiller, C. and Büchel, C. (2002). Painful stimuli evoke different stimulus-response functions in the amygdala, prefrontal, insula and somatosensory cortex: a single-trial fMRI study. *Brain*. 125: 1326-1336.

Braak, H. (1976). A primitive gigantopyramidal field buried in the depth of the cingulate sulcus of the human brain. *Brain Research.* 109: 219-233.

Briscoe, M.E. (1987) Why do people go to the doctor? Sex differences in the correlates of GP consultation. *Social Science and Medicine*. 25(5): 507–13.

Bush, G., Luu, P. and Posner, M. I. (2000). Cognitive and emotional influences in anterior cingulate cortex. *Trends in Cognitive Sciences.* 4(6): 215-222.

Bush, G., Vogt, B. A., Holmes, J., Dale, A. M., Greve, D., Jenike, M. A. and Rosen, B. R. (2002). Dorsal anterior cingulate cortex: A role in reward-based decision making. *Proceedings of the National Academy of Sciences of the United States of America.* 99(1): 523-528.

Carter, C. S., Braver, T. S., Barch, D. M., Botvinick, M. M., Noll, D. and Cohen, J. D. (1998). Anterior cingulate cortex, error detection, and the online monitoring of responses. *Science.* 280(5364): 747-749.

Chang, L. J., Yarkoni, T., Khaw, M. W. and Sanfey, A. G. (2013). Decoding the Role of the Insula in Human Cognition: Functional Parcellation and Large-Scale Reverse Inference. *Cerebral Cortex*. 23: 739-749.

Cleve, M., Gussew, A. and Reichenback, J. R. (2015). In vivo detection o acute pain-induced changes of GABA+ and Glx in the human brain by using functional 1H MEGA-PRESS MR spectroscopy. *Neuroimage.* 105:67-75.

Coghill, R. C., Sang, C. N., Maisog, J. M. and Iadarola, M. J. (1999). Pain intensity processing within the human brain: A bilateral, distributed mechanism. *Journal of Neurophysiology.* 82: 1934-1943.

Cosgrove, K. P., Mazure, C. M. and Staley, J. K. (2007). Evolving Knowledge of Sex Differences in Brain Structure, Function and Chemistry. *Biological Psychiatry.*62(8): 847-855**.**

Duncan, N. W., Wiebking, C. and Northoff, G. (2014). Associations of regional GABA and glutamate with intrinsic and extrinsic neural activity in humans – A review of multimodal imaging studies. *Neuroscience and Biobehavioral Reviews.* 47, pp 36-52.

Fayed, N., Garcia-Campayo, J., Magallon, R., Andres-Bergareche, H., Luciano, J. V., Andres, E. and Beltran, J. (2010) Localized 1H-NMR spectroscopy in patients with fibromyalgia: a controlled study of changes in cerebral glutamate/glutamine, inositol, choline, and N-acetylaspartate. Arthritis Research and Therapy. 12. R134.

Feraco, P., Bacci, A., Pedrabissi, F., Passamonti, L., Zompogna, G., Malavolta, N. and Leonardi, M. (2011) Metabolic abnormalities in pain-processing regions of patients with fibromyalgia: a 3T MR spectroscopy study. *American Journal of Neuroradiology. Resonance in Medicine.* 55(6), pp 1219-1226.

Fillingim, R. B., King, C. D., Ribeiro-Sasilva, M. C., Rahim-Williams, B. and Riley III, J. L. (2009). Sex, gender and pain: A review of recent clinical and experimental findings. *The Journal of Pain*. 10 (5): 447-485.

Foerster, B. R., Petrou, M., Edden, R. A. E., Sundgren, P. C., Schmidt-Wilcke, T., Lowe, S. E., Harte, S. E., Clauw, D. J. and Harris, R. E. (2012). Reduced Insular γ-Aminobutyic Acid in Fibromyalgia. *Arthritis and Rheumatism*. 64(2), pp 579-583.

Fulbright, R. K., Troche, C. J., Skudlarski, P., Gore, J. C. and Wexler, B. E. (2001). Functional MR imaging of regional brain activation associated with the affective experience of pain. *American Journal of Roentgenology*. 177: 1205-1210.

Gasprovic, C., Song, T., Devier, D., Blockholt, H. J., Caprihan, A., Mullins, P. G.,

Posse, S., Jung, R.E., and Morrison, L. A. (2006) Use of tissue water as a concentration reference for proton spectroscopic imaging. *Magnetic Resonance in Medicine.* 55(6), pp 1219-1226.

George, M. S., Ketter, T. A., Parekh. P. I., Horwitz, B., Herscovitch, P. and Post, R. M. (1995). Brain activity during transient sadness and happiness in healthy women. *The American Journal of Psychiatry.* 152 (3): 341-351.

Gijsbers, K. and Nicholson, F. (2005). Experimental pain thresholds influenced by sex of the experimenter. *Perceptual Motor Skills*. 101: 803-807.

Girard-Tremblay, L., Auclair, V., Daigle, K., Leonard, G., Whittingstall, K. and Goffaux, P. (2014). Sex differences in the neural representations of pain unpleasantness. *Journal of Pain.* 15(8): 867-877.

Gussew, A., Rzanny, R., Erdtel, M., Scholle, H. C., Kaiser, W. A., Mentzel, H. J. and Reichenbach, J. R. (2010) Time-resolved functional 1H MR spectroscopic detection of glutamate concentration changes in the brain during acute heat pain stimulation. *NeuroImage*. 49(2). Pp. 1895-1902.

Hanamori, T., Kunitake, T., Kato, K. and Kannani, H. (1998) Responses of neurons in the insular cortex to gustatory, visceral, and nociceptive stimuli in rats. *Journal of Neurophysiology*. 79: 2535-2545.

Harris, R. E. and Clauw, D. J. (2012) Imaging central neurochemical alterations in chronic pain with proton magnetic resonance spectroscopy. *Neuroscience Letter*. 520: 192-192.

Harris, R. E., Sundgren, P. C., Craig, A. D., Kirshenbaum, E., Sen, A., Napadow, V. and Clauw, D. J. (2009) Elevated Insular Glutamate in Fibromyalgia Is Associated With Experimental Pain. Arthritis and Rheumatism. 60(10), pp 3146-3152.

Harris, R. E., Sundgren, P. C., Pang, Y., Hsu, M., Petrou, M., Kim, S. H., McLean, S. A., Gracely, R. H. and Clauw, D. J. (2008) Dynamic Levels of Glutamate Within the Insula Are Associated with Improvements in Multiple Pain Domains in Fibromyalgia. *Arthritis and Rheumatism*. 58(3), pp 903-907.

Hofbauer, R. K., Rainville, P., Duncan, G. H. and Bushnell, M. C. (2001). Cortical representation of the sensory dimension of pain. *Journal of Neurophysiology*. 86: 402-411.

Iannetti, G. D. and Mouraux, A. (2010). From the neuromatrix to the pain matrix (and back). *Experimental Brain Research.* 205:1-12.

Ingvar, M. (1996). Pain and functional imagining. *Philosophical Transcripts of the*

Royal Society. 354: 1347-1358.

International Association for the Study of Pain (1994). Classification of chronic pain: descriptions of chronic pain syndromes and definitions of pain terms. Task Force on Taxonomy. Vol. suppl. 3, p. S215. Seattle: IASP Press.

Kallai, I., Barke, A. and Voss, U. (2004). The effects of experimenter characteristics on pain reports in women and men. *Pain*. 112: 142-147.

Kupers, R., Danielsen, E. R., Kehlet, H., Christensen, R., & Thomsen, C. (2009). Painful tonic heat stimulation induces GABA accumulation in the prefrontal cortex in man. *Pain*. 142(1-2): 89–93.

LeResche L. (2011). Defining gender disparities in pain management. *Clinical Orthopaedics and Related Research.* 469: 1871-1877.

Levine, F. M. and DeSimone, L. L. (1991). The effects of experimenter gender on pain reports in male and female subjects. *Pain*. 44: 69-72.

Linton, S. J. (2005) Understanding Pain for Better Clinical Practice. Edinburgh, Scotland. Elsiver. P 9-18.

Livingston, W.K, (1943) Pain Mechanisms, NY. MacMillan,

Lorenz, J., Minoshima, S. and Casey, K. L. (2003). Keeping pain out of mind: The role of the dorsolateral prefrontal cortex in pain modulation. *Brain.* 126: 1079-1091.

Lynch, A.M., Kashikar-Zuck, S., Goldschneider, K.R., and Jones, BA. (2007). Sex and age differences in coping styles among children with chronic pain *Journal of Pain Symptom Management*. 33: 208-216.

Lynn, B. (1984) Cutaneous Nociceptors. In Holden, A., V. and Winlow, W. The Neurobiology of pain. Manchester, UK: Manchester University Press. P. 106.

Mantha, M. H. (2015). Application of the Neuromatrix Pain Theory to Understand Sex and Gender Differences in Chronic Pain. *AASCIT Journal of Medicine.* 1(5): 67-73.

McWilliams, L. A., Cox, B. J. and Enns, M.W. (2003). Mood and anxiety disorders associated with chronic pain: an examination in a nationally representative sample. *Pain*. 106(1-2): 127-133.

Melzack, R. (1993). Pain: Past, present and future. *Canadian Journal of Experimental Psychology.* 47: 615-629.

Melzack, R. (1996). Gate control theory: on the evolution of pain concepts. *Official Journal of the American Pain Society.* 5: 128-138.

Melzack, R. (1999). From the gate to the neuromatrix. *Pain.* 6: 121-126.

Melzack, R. (2001). Pain and the neuromatrix in the brain. *Journal of Dental Education.* 65(12):1378-1382.

Melzack, R. (2005). Evolution of the neuromatrix theory of pain. The Prithvi Raj Lecture: presented at the third World Congress of World Institute of Pain, Barcelona 2004. *Pain Practice.* 5(2): 85-94

Melzack, R. and Casey, K. L. (1968). Sensory, motivational and central control determinants of chronic pain: A new conceptual model. In Kenshalo, D. R. *The Skin Senses.* Springfield, Illinois: Thomas pp. 423-435.

Melzack, R. and Wall, P. D. (1965). Pain mechanisms: A new theory. *Science.* 150, 3699: 971-979.

Menon, V. and Uddin, L. Q. (2010). Saliency, switching attention and control: a network model of insula function. *Brain Structure and Function*. 214: 655-667.

Moayedi, M. and Davis, K. D. (2013). Theories of pain: from specificity to gate control. *Journal of Neurophysiology.* 109: pp 5-12.

Mogg, K. and Bradley, B. P. (1998). A cognitive-motivational analysis of anxiety. *Behavioral Research Therapy.* 36(9): 809-848.

Mogil, J. S. (2012). Sex differences in pain and pain inhibition: multiple explanations of a controversial phenomenon. *Nature Reviews Neuroscience.* 13: 859-866.

Mogil, J. S. and Chanda, M. L. (2005). The case for the inclusion of female subjects in basic science studies of pain. *Pain.* 117(1-2): 1-5.

Moulton, E. A., Keaser, M. L., Gullapalli, R. P., Maitra, R. and Greenspand, J. D. (2006). Sex differences in the cerebral BOLD signal response to painful heat stimuli. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology.* 291(2): 257-267.

Mountford, C. E., Stanwell, P., Lin, A., Ramadan, S. and Ross, B. (2010). Neurospectroscopy: the past, present and future. *Chemical Reviews.* 110(5).

Mullins, P. G., Rowland, L. M. Jung, R. E. and Sibbitt Jr., W. L. (2005). A novel technique to study the brain's response to pain: Proton Magnetic Resonance Spectroscopy. *NeuroImage.* 26. Pp 642-646.

Murphy, A. and Jones, J. et al (n.d.) Retrieved from: https://radiopaedia.org/articles/insular-cortex

Murtha, S., Chertknow, H., Beauregard, M., Dixon, R. and Evans, A. (1996). Anticipation causes increased blood flow in the anterior cingulate cortex. *Human Brain Mapping.* 4: 103-112.

Neumann, L. and Buskila, D. (2003) Epidemiology of Fibromyalgia. *Current Pain and Headache Reports.* 7. Pp 362-368.

Noordenbos, W. (1959). Pain. Amsterdam. Elsevier.

Paulus, M. P. and Stein, M. B. (2006) An Insular view of anxiety. *Biological Psychiatry*. 60: 383-340.

Ploner, M., Gross, J., Timmerman, L. and Schnitzler, A. (2002). Cortical representation of first and second pain sensation in humans. *Proceedings of the National Academy of Sciences of the United States of America.* 99: 12444-12448.

Ploner, M., Lee, M. C., Wiech, K., Bingel, U. and Tracey, I. (2010). Prestimulus functional connectivity determines pain perception in humans. *Proceedings of the National Academy of Science of the United States of America.* 107: 355-360.

Polli, F. E., Barron, J. J., Cain, M. S., Thakkar, K. N., Rauch, S. L. and Manoach, D. S. (2005). Rostral and dorsal anterior cingulate cortex make dissociable contributions during antisaccade error commission. *Proceedings of the National Academy of Sciences of the United States of America.* 102(43): 15700-15705.

Popescu, A., LeResche, L., Truelove, E. L. and Drangsholt, M. T. (2010) Gender differences in pain modulation by diffuse noxious inhibitory control: A systematic review. *Pain.* 150(2): 209-318.

Posner, M. I. and DiGirolamo, G. J. (1998). Executive attention: Confilct, target detection and cognitive control. *In* "The Attentive Brain" (R. Parasuraman ed), MIT Press, Cambridge, Mass.

Racine, M., Tousignant-Laflamme, Y., Kloda, L. A., Dion, D., Dupuis, G. and Choinere, M. (2012a). A systematic literature review of 10 years of research of sex/gender and experimental pain perception – Part 1: Are there really differences between women and men? *Pain.* 153: 602-618.

Racine, M., Tousignant-Laflamme, Y., Kloda, L. A., Dion, D., Dupuis, G. and Choinere, M. (2012b). A systematic literature review of 10 years of research of sex/gender and experimental pain perception – Part 2: Do biosocial factors alter pain sensitivity differently in women and men? *Pain.* 153: 619-635.

Rainville, P., Duncan, G. H., Price, D. D., Carrier, B. and Bushnell, M. C. (1997). Pain affect encoded in human anterior cingulate but not somatosensory cortex. *Science.* 277: 968-971.

Rey, R. (1995). The History of Pain. Cambridge, MA: Harvard University Press

Riley III, J. L., Robinson, M. E., Wise, E. A. Wyers, C. D and Fillingim, R. B. (1998). Sex differences in the perception of noxious experimental stimuli: a meta-analysis. *Pain.* 74(2-3): 181-187.

Robinson, M. E., Gagnon, C. M., Riley III, J. L and Price, D. D. (2003). Altering gender role expectations:effects on pain tolerance, pain threshold, and pain ratings. *The Journal of Pain*. 4 (5): 284-288.

Ruda, M. A. (1993). Gender and pain [editorial]. *Pain.* 53: 1-2.

Sherman, J. J. and LeResch, L. (2006). Does experimental pain response vary across the menstrual cycle? A methodological review. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology.* 29(2): 245-256.

Siddall, P. J., Taylor, D.A., McClelland, J. M., Rutkowski, S. B. and Cousins, M. J. (1999). Pain report and the relationship of pain to physical factors in the first 6 months following spinal cord injury. *Pain.* 81(1-2): 187-197.

Singer, T., Critchley, H. D. and Preuschoff, K. (2009) A common role of insula in feelings, empathy and uncertainty. *Trends in Cognitive Sciences*. 13(8): 334-340.

Singer, T., Seymour, B., O'Doherty, J., Kaube, H., Dolan, R. J. and Frith, C. D. (2004) Empathy for pain involves the affective but not sensory components of pain. *Science*. 303(5661): 1157-1162.

Stephani, C., Vaca, F-B., Maciunas, R., Koubeissi, M. and Lüders, H. O. (2011). Functional neuroanatomy of the Insular lobe. *Brain Structure and Function*. 216: 137-149.

Taylor, S. F., Martis, B., Fitzgerald, K. D., Welsh, R. C., Abelson, J. L., Liberzon, I., Himle, J. A. and Gehring, W. J. (2006). Medial frontal cortex activity and loss-related responses to errors. *Journal of Neuroscience.* 26(15): 4063-4070.

Tsao, J. C. I., Myers, C. D., Craske, M. G., Bursch, B., Kim, S. C. and Zeltzer, L. K. (2004). Role of anticipatory anxiety and anxiety sensitivity in children's and andolescent's laboratory pain responses. *Journal of Pediatric Psychology.* 29(5): 379-388.

Türe, U., Yasargil, D. C., Al-Mefty, O. and Yasargil, M. G. (1999). Topographic anatomy of the Insular region. *Journal of Neurosurgery.* 90: 720-733.

Valdes, M., Collado, A., Bargallo, N., Vazquez, M., Rami, L., Gomez, E. and Salamero, M. (2010) Increased glutamate/glutamine compounds in the brains of patients with fibromyalgia: a magnetic resonance spectroscopy study. Arthritis and Rheumatism. 62. Pp. 1829-1836.

van Wijk, G. and Veldhuijzen, D. S. (2010) Perspective on diffuse noxious inhibitory controls as a model of endogenous pain modulation in clinical pain syndromes. *The Journal of Pain.* 11(5): 408-419.

Vogt, B. A. (1993). Structural organisation of cingulate cortex: areas, neurons and somatodendritic transmitter receptors. *In* "Neurobiology of Cingulate Cortex and Limbic Thalamus" (B. A. Vogt and M. Gabriel, eds), pp. 19-70. Birkhauser, Boston.

Vogt, B. A., Berger, G. R. and Derbyshire, S. W. J. (2003). Structural and functional dichotomy of human midcingulate cortex. *European Journal of Neuroscience.* 18: 3134-3144.

Vogt, B. A., Finch, D. M. and Olson, C. R. (1992). Functional heterogeneity of cingulate cortex: The anterior executive and posterior evaluative regions. *Cerebral Cortex.* 2: 435-443.

Vogt, B. A., Hof, P. R. and Vogt, L. J. (2004) Cingulate gyrus. *In* "The Human Nervous System, 2nd ed" (G. Paxinox and J. K. Mai, eds), pp. 915-949. Elsevier Academic Press, San Diego.

Vogt, B. A., Nimchinsky, E.A and Hof, P. R. (1997). Human cingulate cortex: Surface features, flat maps and cytoarchitecture. *The Journal of Comparative Neurology.* 359: 490-506.

Vogt, B. A. and Sikes, R. W. (2000). The medial pain system, cingulate cortex, and parallel processing of nociceptive information. *In* "Progress in Brain Research" (E. A. Mayer and C. B. Sapier, eds), pp 915-949. Elevier, Amsterdam.

Wiech, K. Lin, C., Brodersen, K. H., Bingel, U., Ploner, M. and Tracey, I. (2010) Anterior Insula Integrates Information about salience into Perceptual Decisions about Pain. *The Journal of Neuroscience.* 30(48): 16324-16331.

Weich, K. and Tracey, I. (2009). The influence of negative emotions on pain: behavioral effects and neural mechanisms. *Neuroimage*. 47(3): 987-994.

Wolfe, F., Ross, K., Anderson, J., Russell, I. J. and Hebert, L. (1995). The prevalence and characteristics of fibromyalgia in the general population. *Arthritis and Rheumatism.* 38(1): 19-28.

Chapter 2: Methodology

Pressure-pain thresholds (PPT)

The examination of responses to noxious stimulation in the form of pressure-pain thresholds (PPTs) is widely practiced in both clinical and experimental environments (Fischer, 1987). This is most likely due to the relative operational ease and affordability that some of the instruments offer, such as algometers and Von Frey hairs. Particularly when taking into consideration the requirements for acquiring and operating other instruments of stimulation, such as those of a thermal or electrical nature. However, as some of the PPT methods are reliant on the judgement and execution of the researcher, such as hand-held algometry, both inter- and intra-researcher reliability has been brought into question. Chesterton, Sim, Wright and Foster (2007) examined interrater reliability with regards to algometry in order to establish the efficacy of conducting pain research with examinations of PPT. Algometry refers to the measurement of pressure applied to a specific pressure-point or site with the use of either a hand-held or hand-operated device, which is known as an algometer. The numerical units of measurement used to represent how much pressure is applied, in this instance, are recorded as either Newtons (N), or pound-force (lbf); it is worth noting that 1 lbf is equal to approximately 4.45 N. Pressure pain threshold is normally examined by applying pressure to a pre-defined area on the body, and gradually increasing the force with which the pressure is applied up to the point that the participant reports the sensation as becoming painful. The pressure point utilised as the site of stimulation, as well as the one examined by Chesterton et al. (2007), is that of the first dorsal interosseous muscle, which is located in the hand in the fleshy webbing between the thumb and forefinger. Chesterton et al. (2007) established interrater reliability by training five experimenters (all female) in algometry and then having each researcher (or 'observer' as they were referred to) measure the PPT of 13 (12 female) healthy participants using a fixed-angle pressure algometer, using protocol stipulations put forward by Goulet, Clark and Fleck (1993). Pressure was applied at a rate of five N/s for 10 seconds over five consecutive measurements, with each measurement 15 seconds apart. Chesterton et al. reported that training experimenters in algometry is a successful way to improve interrater reliability, and advised that the mean of at least three measurements is used to assess PPT in approaches employing more than one experimenter. This is due to their finding that the mean of several sessions had higher interrater reliability compared to consecutive single measurements. In the experiments reported upon in this thesis, experimenters were self-trained in the use of a Wagner FPX 50 algometer topped with a round rubber stub 1cm in diameter, and to apply pressure at a rate of 1 lbf/s at an angle between 0-45 degrees. Each measurement of PPT was taken a minimum of

10 minutes apart in order to reduce any effects of sensitisation, with two being taken for the imaging experiment, and three for the social (see Chapters 5 and 4, respectively).

^{1}H-MRS

The main method for intra-neuronal communication in the brain is by synaptic transmission. The electrical impulse travels along the axon of a neuron in the form of an action potential. When the action potential reaches the synapse, it results in the depolarisation of the axon terminal, causing calcium ions to be released. The influx, and subsequent increased concentration, of calcium ions at the synapse causes small vesicles containing chemicals called neurotransmitters to fuse with the synaptic membrane, resulting in the release of the neurotransmitters into the area between the pre-synaptic and post-synaptic membranes, known as the synaptic cleft. The neurotransmitters then diffuse across the synaptic cleft and fuse with specific receptor molecules, which initiate changes in the neuron via either depolarisation or hyperpolarisation (Gazzaniga, Ivry & Mangun, 2009). At the most basic level, there are two main classes of neurotransmitters; excitatory and inhibitory. Excitatory neurotransmitters cause depolarisation of the membrane, resulting in the generation of an action potential, whereas inhibitory neurotransmitters cause hyperpolerisation, which makes it less likely for the neuron to generate an action potential. Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) is one among a number of scanning techniques (e.g. ^{31}P-MRS, ^{13}C-MRS) that allows researchers to quantify and examine localised chemical concentrations of neurotransmitters *in vivo* (Ross & Bluml, 2001). It is developing into an important tool in diagnosis and treatment monitoring in pre-clinical fields, as well as improving knowledge relating to regional neurometabolic concentrations and dynamic fluctuations of metabolites in neuronal transmission (Duarte, Lei, Mlynarik & Gruether, 2012). ^{1}H-MRS has been utilised in clinical and experimental conditions to investigate both normal and abnormal neurometabolic behaviours in conditions such as hypoxic brain injury, brain lesions, epilepsy, ataxia, Alzheimer's, migraine, schizophrenia, and chronic pain (Duncan, 1996; Mountford, Sitwell, Lin, Ramadan & Ross, 2010; Duarte et al., 2012). The field of research associated with the examination of metabolic concentrations in the nervous system in relation to conditions such as these has become known as Neurospectroscopy (Ross & Bluml), The basic principles of practicing neurospectroscopy use the same concepts that should already be familiar to those acquainted with MRI methodology, insofar as ^{1}H-MRS involves investigating and examining the interactions of protons i.e. Hydrogen molecules, within an external magnetic field. The hydrogen nucleus is an ideal target for both MRI and MRS due to its abundance in living tissue, as well as its high

magnetic resonance sensitivity (Ende, 2015). The hardware required for ^{1}H-MRS is more or less the same as is required for MRI; radio frequency (RF) coils, which act as antennas to excite the protons and collect the data, or "signal"; gradients for localization; both an RF amplifier and an RF receiver; a computer to interpret and mathematically map the signal; a sample of the nuclei of interest; and, of course, a strong magnet or magnetic field; the stronger the magnetic field, the better the quality of the signal that is received (Ross & Bluml, 2001; Duarte et al. 2012; Ende, 2015). It is also worth noting that for some of the other forms of spectroscopy previously mentioned, different RF amplifiers are required as RF amplifiers are designed to operate and collect data from protons at specific resonance frequencies (Ross & Bluml, 2001).

The hydrogen nuclei are not static in nature; each proton exhibits gyroscopic 'spins', and as such they have both an orientation and a frequency, which can be altered when placed in a strong magnetic field. When an external magnetic field is applied there is a split in energy, resulting in a lower and a higher energy state. Each hydrogen nuclei can essentially be considered as an extremely small spinning magnet, and are sometimes referred to as "spins" as a result of this property. Once they are placed in a strong magnetic field they will rotate or 'precess' around the main magnetic field, and the protons will either align with the gyroscopic axis parallel to the magnetic field, or against it (anti-parallel; Ende, 2015) due to the gyromagnetic interaction between the spin and the external field. This results in a form of magnetic equilibrium, though more protons will align with the magnetic field as it occupies the lower energy state. However, the spins do not just align with the external magnetic field, they still precess around the principle axis of this field. The rate of this precession can be calculated using the Larmor equation, which establishes the characteristic frequency at which spins precess in a magnetic field, and which results from the physical properties of the spin and the strength of the magnetic field. The transferal of proton alignment from one energy state to another requires energy that is specific for each type of spin; as such the Larmor equation is used to calculate the exact frequency that is required to regulate transitions of the spins energy states (Huettel, Song & McCarthy, 2009).

Data acquisition follows the initial steps of MRI, i.e. the participant is placed within an alternating electromagnetic field generated by an MRI machine, wherein the RF pulses are applied to the proton resonance frequency, perpendicular to the external magnetic field, to induce a higher energy state and 'tip' the axis of the proton. Once the RF pulses are turned off, protons then precess around the magnetic field and return to the lower energy state, the process of which is known as relaxation. During relaxation, the protons emit the RF energy

that they had absorbed, (which is where the "resonance" in "Magnetic Resonance" comes from) via the precessing magnetisation of the proton, which then induces an alternating current in an adjacent RF coil, known as the free induction decay (FID) signal. This signal is acquired as time-domain data, which can then be mathematically transformed using a Fourier transform to frequency-domain data, producing a spectra of the different resonance frequencies detected by the signal (Ross & Bluml, 2001; Duarte et al., 2012; Ende, 2015). In ^{1}H-MRS, the frequency is essentially the measure of chemical structure, and with different neurometabolites, each with different chemical structures, comes different numbers of protons, resulting in the output of data as a spectrum (Ross & Bluml, 2001; Duarte et al. 2012). The hydrogen protons found in water all exhibit the same resonance frequency when they are subjected to the same magnetic field. However, the hydrogen nuclei that are found in other molecular compounds exhibit different resonance frequencies as they experience a slightly different electromagnetic environment. As well as the environment, the interactions between the precession or spin magnetic moments of other nuclei in the same molecule can result in time-dependent changes in signal deterioration, impacting upon relaxation times. This is known as spin-spin coupling or J-coupling, which can place limitations on the efficacy of detection and data collection of a number of metabolites. These limitations can be overcome by utilising a spin echo pulse, which refocuses the precessing spin magnetisation of the hydrogen nuclei to allow improved detection of the resonances of J-coupled metabolites. (Duncan, 1996; Mullins, Chen, Xu, Caprihan & Gasprovic, 2008; Ende, 2015).

As with most neuroimaging techniques, ^{1}H-MRS is not infallible. There are some neurometabolites, such as dopamine and serotonin, which are not present in sufficient quantities to be detectable by ^{1}H-MRS. Similarly, the process can be not sensitive enough to accurately quantify some metabolites separately, particularly in places on the spectra where signals overlap, or with J-coupled metabolites. This, however, can be less of an issue at MRI field strengths of 3T and above, as the sensitivity of metabolic detection increases proportionately to the strength of the magnetic field (Mountford et al., 2010; Duarte et al., 2012). Another limiting factor of ^{1}H-MRS is that the signal is also prone to "noise", which in this context refers to "random fluctuating signal present in the absence of artefacts associated with physiological characteristic, scanner performance or other nonideal factors…. and is often similar in amplitude to the metabolite signal levels" (Alger, 2010). Noise can be reduced when taking static readings of metabolic concentrations by taking several readings and averaging them together, as the measured signal increases in proportion to the number of

averaged while the noise signal grows in proportion to the square root of the number or averages (Alger, 2010).

There are a number of techniques, or sequences, that are used in ^{1}H-MRS of the brain, such as Image Selected, In vivo Spectroscopy (ISIS), Point RESolved Spectroscopy (PRESS), and STimulated Echo Acquisition Mode (STEAM; Ross & Bluml, 2001; Duarte et al. 2012). In the terms of this thesis, a sequence variant of PRESS, called MEscher-Garwood Point RESolved Spectroscopy, or MEGAPRESS (named for its developers; Mescher, Tannus, Johnson, Garwood & Gruetter, 1998), is of particular interest due to its application in quantifying the signal of gamma-aminobutyric acid (GABA). PRESS is a sequence that concentrates on a slice-selective excitation along the voxel of interest (VOI), followed by two frequency-selective refocusing RF pulses that result in a three-dimensional voxel (Duarte et al. 2012). In other words, the initial slice-selective excitatory pulse may be along the X-axis, the first refocusing pulse in the Y-axis, and the second refocusing pulse in the Z-axis, producing a single-cubed voxel that has received pulses from all three dimensions. Each pulse refocuses the spin-alignment of the protons and decreases the rate of signal deterioration within the voxel, allowing for clearer signal acquisition. This process renders the signal outside of the voxel neither excited or defocussed, reducing any noise from outside the selected VOI. MEGAPRESS builds upon this specifically for the detection of GABA, by collecting two simultaneous data sets, commonly referred to as the 'ON' spectra, and the 'OFF' spectra. The ON spectra reflects the signal that is acquired after the application of a pulse that selectively refocuses the proton spins in the GABA, exciting them and in turn increasing the signal for GABA, while the OFF spectra reflects the signal after a GABA-unspecified refocusing pulse is applied elsewhere. The GABA concentration is then calculated by subtracting the OFF edited spectrum from the ON edited spectrum, while the rest of the spectra is calculated by fitting the OFF edited spectra as a normal PRESS acquisition (Mullins, McGonigle, O'Gorman, Puts, Vidyasagar, Evans & Edden, 2014).

The applications of ^{1}H-MRS and neurospectroscopy have contributed significantly to the knowledge and understanding as to the roles and functions of neurometabolites, as well as their relationships with neurotransmission and processing, among other implications. Neurometabolites that can be examined using neurospectroscopy include N-Acetylaspartate (NAA), which is considered to be a neuronal marker, although others argue that it may be related to neuronal dysfunction rather than neuronal density; Choline (Cho), which may act as a membrane marker of cell density; Creatine (Cre), which plays a role in brain energy homeostasis; Glutamate (Glu) and Glutamine (Gln), the most abundant excitatory

neurotransmitter in the nervous system, though Glu and Gln can be difficult to separate on a spectra at lower field strengths, resulting in the combined signal being referred to as Glx; and, of course, GABA, the most abundant inhibitory neurotransmitter (Mountford et al., 2010; Rae, 2014). Glx and GABA are possibly the two metabolites that are of most interest in pain research due to experimental and clinical findings upon examination. As methods of *in vivo* measurement evolve and research progresses, we will be able to develop a more complete understanding of how these neuronal processes function, and eventually develop more comprehensive investigations and treatments in clinical instances of dysfunctioning neuronal chemistry.

Chapter 2 References

Alger, J. R. (2010). Quantitative proton magnetic resonance spectroscopy and spectroscopic imaging of the brain: A didactic review. *Topics in Magnetic Resonance Imaging.* 21(2): 115-128.

Chesterton, L. S., Sim, J., Wright, C. C. and Foster, N. E. (2007). Interrater reliability of Algometry in measuring pressure pain thresholds in healthy humans, using multiple raters. Clinical Journal of Pain. 23(9), pp 760-766.

Duarte, J. M. N., Lei, H., Mlynarik, V. and Gruether, R. (2012). The neurochemical profile quantified by *in vivo* ^{1}H-NMR. *NeuroImage*. 61: 342-362.

Duncan, J. S. (1996). Magnetic Resonance Spectroscopy. *Epilepsia.* 37(7): 598-605.

Ende, G. (2015). Proton magnetic resonance spectroscopy: relevance of Glutamate and GABA to neuropsychology. *Neuropsychological Reviews.* 25: 315-325

Fischer, A. A. (1987). Pressure threshold measurement for diagnosis of myofascial pain and evaluation of treatment results. *Clinical Journal of Pain.* 2: 207-215.

Gazzeiga, M. S., Ivry, R. B. and Mangun, G. R. (2009). Cognitive Neuroscience: The Biology of the Mind (3rd ed.) pp 47-56. New York: W. W. Norton & Company.

Goulet, J. P., Clark, G.T. and Flack, V. F. (1993). Reproducibility of experimenter performance for muscle and joint palpation in temporomandipular system follwoingtraining and calabration. *Community of Dental and Oral Epidemiology.* 21: 72-77.

Huettel, S. A., Song, A. W. and McCarthy, G. (2009). Functional Magnetic Resonance Imaging (2nd ed.). Sunderland, MA: Sinauer Associates, Inc.

Mescher, M., Tannus, A., Johnson, M. O., Garwood, M. and Gruetter, R. (1998). Simultaneous in vivo spectral editing and water suppression. *NMR Biomedicine.* 11(6), pp 266-272.

Mountford, C. E., Stanwell, P., Lin, A., Ramadan, S. and Ross, B. (2010). Neurospectroscopy: the past, present and future. *Chemical Reviews.* 110(5).

Mullins, P. G., Chen, H., Xu, J., Caprihan, A. and Gasparovic, C. (2008). Comparative reliability of proton spectroscopy techniques designed to improve detection of J-coupled metabolites. *Magnetic Resonance in Medicine.* 60: 964-969.

Mullins, P. G., McGonigle, D. J., O'Gorman, R. L., Puts, N. A. J., Vidyasagar, R., Evans, C. J., and Edden, R. A. E. (2014) Current practice in the use of MEGA-

PRESS spectroscopy for the detection of GABA. *NeuroImage*. 86, pp 43-52.

Rae, C. D. (2014). A Guide to the Metabolic Pathways and Function of Metabolites Observed in Human Brain ^{1}H Magnetic Resonance Spectra. *Neurochemical Research.* 39: 1-36.

Ross, B., and Bluml, S. (2001). Magnetic resonance spectroscopy of the human brain. *The Anotomical Record (New Anatomy)*. 265: 54-84.

Chapter 3: Development of a functional imaging paradigm to investigate anticipation to uncertain noxious stimulation – A pilot

"Of all the hardships a person had to face, none was more punishing than the simple act of waiting."

— *Khaled Hosseini (author)*

A Thousand Splendid Suns (2007)

"We need the sweet pain of anticipation to tell us we are really alive."

— *Albert Camus (philosopher and writer)*

"I see you shiver with antici…"

It has been shown that anticipation of noxious stimuli activates areas of the brain that are structurally close to, but different from, areas activated by noxious stimulation. Ploghaus et al. (1999) demonstrated that pain activated the caudal part of the anterior cingulate cortex, whereas anticipation of pain activated a more anterior region extending from the pACC to the frontal pole (anterior medial frontal cortex). They also found that, whereas the fMRI signal for pain remained constant through trials, the signal for anticipation actually increased over successive trials. They stated that this increase would be expected as the participants learn the stimulus indicators (in this study a coloured light preceded the stimulus and it was used to indicate whether the stimuli was to be noxious or not) and associate them with pain or not. It could therefore be inferred that this increase in fMRI signal is due to associative learning, as it can be argued that anticipation is a form of adaptive mechanism designed to establish behaviours that will prevent future harm by learning to recognise signals of impending pain (Ploghaus et al, 1999).

It has also been proposed that anticipatory responses such as these may operate as a form of predictive adaptation to noxious stimulation in order to prevent tissue damage and facilitate aversive behaviour. Utilizing the anterior insula as a hub for inputs from the thalamus and other cortical structures, a risk-weighting analysis is performed to ascertain a prediction of the stimuli utilising factors such as previous experiences, contextual information, and cues, in order to discern whether other cortical areas are required to process, or withdraw from the stimulus in order to limit the risk of damage. In this theory, the predictive model is then weighted against the incoming nociceptive signals, and where there is a large discrepancy between the prediction and stimuli (i.e. the stimuli > prediction), a higher error signal is produced, as well as higher uncertainty, resulting in an increased perception of pain (Morrison, Perini & Dunham, 2013). This fits in with previous research, which has highlighted how uncertainty and anticipation of noxious stimulation can result in hypersensitisation to pain (Ploghaus et al., 1999; Sawamoto et al., 2000). In fact, a recent fMRI study has demonstrated that increased sensitivity to pain in patients on the autistic spectrum correlates with increased activation in the rostral and dorsal ACC in anticipation to noxious stimulation, giving further evidence as to how activity prior to noxious stimulation can impact upon its perception (Gu, Zhou, Anagnostou, Soorya, Kolvezon, Hof & Fan, 2017)

When examining the neural activation associated with the anticipatory response to pain, a factor that has been shown to mediate the activation is the participant's expectation of the stimulus, i.e. whether they are aware that the impending stimulation is to be noxious or not. Sawamotto and colleagues conducted a study to directly examine whether expectation of

pain would enhance brain responses to stimulation in the ACC and the area including the parietal operculum/posterior insula using fMRI (Sawamoto, Honda, Okada, Hanakawa, Kanda, Fukuyama, Konishi and Shibasaki, 2000). In their study two experimental conditions were used: certain (wherein participants knew that the stimuli would not be painful) and uncertain (wherein the non-painful stimulus was randomly intermixed with painful). They found that there was a gradual signal increase prior to stimulation in all conditions, and that the signal associated with painful stimulation was higher in the non-painful uncertain condition then it was in the non-painful certain condition in the ACC. They also found that participant's rated the non-painful uncertain stimulus as more unpleasant then the non-painful certain. When combined with the increased BOLD signal observed in the ACC, this finding matches the association between ACC activity and the perception of unpleasantness (Vogt, 2005).

It has also previously been shown that noxious stimulation is rated as more intense when accompanied by a high-intensity visual cue, suggesting that expectation of pain intensity modulates perception of noxious stimulation throughout a distinct modulatory network (Keltner, Furst, Fan, Redfurn, Inglis & Fields, 2006). It has been theorised that certainty and uncertainty regarding noxious stimulation cause different adaptive behaviours and alter perceptions, with certain expectation eliciting a fear response, resulting in decreased sensitivity, and uncertain expectation eliciting an anxiety response, resulting in increased sensitivity (Ploghaus, Becerra, Borras & Borsook, 2003). It was also shown that uncertainty of outcome type resulted in activity in the ventro-medial prefrontal cortex, mid-cingulate and SI (Ploghaus et al., 2003). These results support the findings of Sawamoto et al. (2000), with uncertainty being linked to an anxiety response, resulting in increased sensitivity to noxious stimulation. This also links to Melzack's (1999) descriptions of input into the neuromatrix that modulate the perception of pain i.e. from the stress-regulatory system. These experiments all provide evidence as to how the individuals' expectation of pain can modulate activity in the ACC.

While the BOLD signal responses to anticipation of noxious stimuli would appear to be well understood, there are a few other measures of neural activity that it could be instructive to apply to this phenomenon. Of particular interest would be to understand how intra-neuronal communication might be modulated by anticipation. The main method for intra-neuronal communication in the brain is by synaptic transmission through the effects of neurotransmitters on the synapse. At the most basic level, there are two main classes of neurotransmitters; excitatory and inhibitory. Excitatory neurotransmitters cause

depolarisation of the membrane, resulting in the generation of an action potential, whereas inhibitory neurotransmitters cause hyperpolerisation, which makes it less likely for the neuron to generate an action potential.

The primary method used to examine the cerebral metabolism and changes in metabolite concentrations *in-vivo* is by using the non-invasive imaging technique of proton magnetic resonance spectroscopy (^{1}H-MRS), which essentially functions in the same capacity as other magnetic resonance imaging techniques. By analysing the resonance frequency of the atomic nuclei within tissue molecules, information can be obtained relating to chemical composition. This information can be quantified in the form of spectra by plotting signal intensity against frequency in order to examine the concentrations of the present metabolites (Duncan, 1996; Alger, 2010). Compounds that are observable using ^{1}H-MRS include metabolites such as Glutamate (Glu), Glutamine (Gln), gamma-aminobutyric acid (GABA), N-Acetylaspartate (NAA), Choline (Cho), Creatine (Cre), among others, though it is unable to obtain information relating to the concentrations of neurotransmitters such as dopamine and seratonin, as they are not present in sufficient concentrations to be detectable by ^{1}H-MRS (Duncan, 1996). Glu and GABA are the most abundant excitatory and inhibitory neurotransmitters in the nervous system respectively, both of which are amino acid based metabolites (Pinel, 2003). With regards to pain perception, Glu has been found to function predominantly in transmission, facilitating the conveyance of pain impulses (Mullins, Rowland, Jung & Sibbitt Jr., 2005). It has been demonstrated using ^{1}H-MRS that there was an increase in the levels of Glu in the anterior cingulate cortex in response to noxious stimulation, indicating that Glu is paramount in the transmission of pain (Mullins et al., 2005). They were also able to observe a correlation between another excitatory neurotransmitter, glutamine, and the participant's perception of the unpleasantness of pain. The results of this experiment were among the first to quantify the relationship between pain and Glu.

To add considerably to our current understanding of the interplay between anticipation and pain sensitivity, the use of event-related ^{1}H-MRS scanning techniques to investigate the possible role of Glu and other neurotransmitters both prior to and during certain and uncertain noxious stimulation would be of interest. However, measuring neurotransmitter flux through fMRS is still a relatively novel technique, and until more firmly established and accepted, it is best applied to well-established experimental paradigms. To this end, we wanted to ensure that previous experimental paradigms used in fMRI studies on the role of anticipation in noxious stimuli perception where replicable utilising the stimuli

and resources available. As such, we first designed an fMRI study following protocol from previous literature on the subject (Such as Sawamoto et al, 2000; Ploghaus et al. 1999) to act as a pilot for the paradigm to be employed in fMRS.

We hypothesised that our results would follow that of previous research; there would be an observable increase in BOLD activity in the ACC prior to painful or non-painful stimulation. In an uncertain condition, this activity would likely be high than in a certain condition, regardless as to weather the stimulus would be painful or not. We would then expect this increase in activity to correlate with an increased perception of pain, so that the participants would rate uncertain painful stimulation as higher than certain painful stimulation, possibly even to the extent that uncertain non-painful stimuli would be rated on par, or close to, the certain painful stimuli. If we were able to replicate these fMRI results, we then hoped to move forward with examining the effect using ^{1}H-MRS.

Methods

Participants

The participants were 16 Undergraduate and Postgraduate students at Bangor University (nine female), who were recruited via opportunistic sampling, via word of mouth, social media (email and university forums) and with the use of a poster. Originally 18 participants were recruited, but the fMRI data from participants one and two were unusable due to early issues with the scanning protocol, and as a result their rating files were also unusable. Participants received a monetary-based reward of £10, as well as a copy of their T1 structural image for taking part in the study. The participants were all healthy subjects, with no known peripheral nerve damage, no known medical condition that may influence scanning results (e.g. Raynaud's syndrome), no metallic or other form of medical implants, following standardised safety protocals for MRI, and did not suffer from any form of chronic pain syndrome or instances of longitudinal exposure to pain within three months prior to participation.

Materials

This experiment used a Phillips 3T Achieva MR scanner, equipped with spectroscopic capabilities and an 8-channel head coil, in the Bangor Imaging Unit. Part of the experimental paradigm was programed in Presentation®, from Neurobehavioural Systems (http://www.neurobs.com), and utilised an MRI-compatible joystick interface for pain perception monitoring. We utilized a modified cold pressor test in which freezer ice blocks are applied to the bottom of the participants foot to produce a noxious stimulus. Specifically, we used six freezerblocks (16.5cm x 11cm x 1.5cm), three frozen (-7 to -4 degrees Celsius),

and three chilled to just below room temperature (12-16 degrees Celsius). Freezer blocks were rotated between the scanning blocks in order to prevent them from warming too much, and they were kept in a chilled icebox during the experiment when not in use.

Design

This experiment is a within-subjects design, with each participant being subjected to each of the six (three visual 'anticipation', three tactile stimuli) conditions. Conditions were: presentation of a Green circle for 10 secs to signify the upcoming stimulus would be non-painful (Green), followed directly by application of a freezer block at slightly less then room temperature (Certain no-pain) for 60 secs; presentation of an Amber circle for 10 secs to signify the following stimulus type was unknown (Amber), followed directly by either the slightly less then room temperature freezer block (uncertain no-pain) for 60 secs, or the frozen freezer block (uncertain pain)for 60 secs; and finally presentation of a red circle for 60 secs to signify the following stimulus would be painful (red), followed by application of the frozen freezer block (certain pain) for 60 secs.

18 paired-blocks were used throughout the experiment, allowing six runs for each of the coloured conditions (Green/Amber/Red). Using the Presentation® software, each condition was delivered in a randomised order as to avoid eliciting any serial order effects. In order to prevent any sensitisation issues for the participants, the site of stimulation (base of the foot) was alternated between the left and right, block-to-block.

fMRI

High resolution T1-weighted images were collected for image registration and slice preparation as a 5-echo 3D MPRAGE sequence, the five echo images are then summed to produce a final image with improved signal-to-noise and contrast (TE1/TE2/TE3/TE4/TE5 = 3.5/5.2/6.8/8.5/10.2ms; TR = 12.05ms; T1 = 1150ms; Resolution = 0.7mm3, FOV = 240mm X 240mm X 130mm; NAS 1). fMRI data was collected using multi-slice EPI sequence (TE = 33ms; TR = 2000ms; 160 dynamics; Resolution = 3mm Isotropic; FOV = 240mm X 240mm, Number of slices = 36, slice thickness = 3mm.

fMRI Analysis

Analysis was conducted using FSL v. 5.0.6. Initially BET brain extraction was used at Robust, 0.4. First level FEAT analysis was conducted using the following settings; Misc: - Brain/Background threshold 10%; Noise level 0.66%; High pass filter- 60. Data: Temporal smoothness 0.34; Z-threshold=5.3. Number of volumes-1080, - 2.0. High pass filter - 60. Pre-stats: Motion correction-MCFLIRT, slice timing correction-regular, up; Spatial smoothing FWHM(mm) – 5; Temporal filtering – Highpass. Stats : General linear model, 7 EV's (all 3

column format) – Basic shape, using regressor/log files from fMRI. EVs were Green, Amber, Red (three visual prior to stimulus application), Green_n.p., Amber_n.p, Amber_p., Red_p.(four tactile conditions). Post-stats: Z-threshold – 2.3, Cluster corrected P threshold – 0.05. Registration: main structural image was the participant's T1, taken during scanning. Second level analysis was then conducted utilising the individual .gfeat output files from the first level analysis.

Procedure

The participant was informed as to the procedure of the experiment, though the full extent of what we were examining was not revealed during the consent (i.e. that we are investigating uncertain anticipation of noxious stimulation). The participant was exposed to the noxious stimuli prior to starting the experiment for familiarisation and in order to ascertain whether they wished to continue in their participation. They were then instructed on the use of a visual analogue scale using a joystick to register the level of pain experienced throughout the scans. Following consent and standardised screening of MR compatibility, participants were placed into the MRI scanner for acquisition of the structural and functional images. A member of the research team remained present within the scanning room in order to administer stimuli manually. The experiment was divided into 18 randomised, paired blocks (anticipation/tactile stimulation) of 100 seconds There were seven conditions in this experiment, three visual/anticipatory, where the participant was informed via a coloured circle as to which stimulation they will experience (green = certain no-pain, orange = uncertain stimuli, and red = certain pain) and four stimulation conditions (certain no-pain, uncertain-no pain, uncertain pain, and certain pain). The blocks began once the computer received a TTL signal from the scanner: a fixation cross was shown for 10s. Following that, the anticipatory visual stimulus was presented for 10s, which would also be a cue for the researcher as to which stimuli they were to use. Following the visual stimuli, one of the ice blocks was placed against the sole of the foot and held in place with a footrest to ensure that the stimuli was not held against the skin with any unequal or extra pressure. During this time, a rating scale of 0-9 was presented with an arrow, and the participants were asked to use the MRI-compatible joystick to rate the intensity of the stimuli, with 0 being no pain at all, and 9 being worst pain imaginable. The stimulus ratings were not collected autonomously, and as such the participants were asked to press a button on the joystick to record their ratings, with at least 3 ratings per block. The tactile stimulation would last for 60s, which was followed by a rest period of 20s the word 'REST' was shown, indicating a rest block. This process was then repeated until a total of 18 blocks were collected, with six blocks per participant, per

visual condition and certain tactile stimulus, and three blocks for each uncertain tactile stimulus per participant. Once the participant had completed their scanning sessions, they were debriefed and the aims of the experiment explained, before they were supplied with their participation fee and copy of their scan. Each testing session took approximately an hour, with 40 minutes per scanning session and around 20 minutes for consent, screening, setup, and debrief.

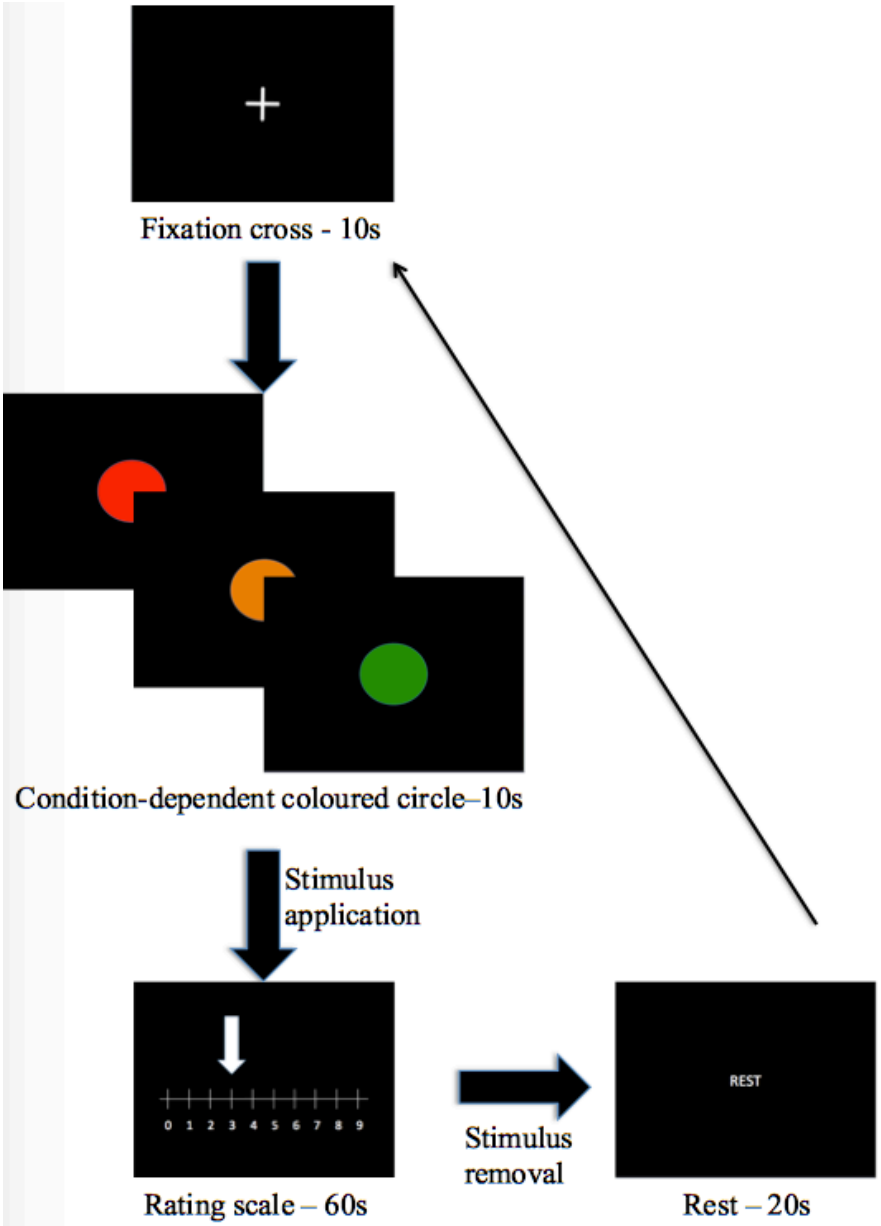


Figure 3.1: Example of fMRI visual stimulus presentation.

Results

From the 16 remaining participants (accounting for the unusable data from the first to participants mentioned above), nine were included in the final analyses. These were participants 3, 5, 6, 7, 12, 13, 15, 16 and 17. Participants 4, 8, 9, 10, 11 and 14 were removed

due to the identified movement artifacts, while participant 18's data was corrupted and unusable.

Statistical analysis – Pain ratings

A one-way repeated measure ANOVA was conducted in order to analyse average pain ratings in each condition. There was a statistically significant effect of condition, with both anticipated and uncertain pain resulting in a higher rating than anticipated and uncertain innocuous stimuli, Wilks Lambda =.135, $F(3, 6)=12.79$, $p = .005$, multivariate partial eta squared = .865 indicating a large effect size; means and standard deviations can be seen in Table 3.1.

Condition	N	Mean	Standard Deviation
Certain-No Pain	9	107.55	62.47
Uncertain-No Pain	9	124.07	47.92
Uncertain-Pain	9	437.94	173.69
Certain-Pain	9	451.37	153.08

Table 3.1. Descriptive statistics for pain ratings in each condition for participants included in the fMRI analysis

The same analysis was conducted including the pain ratings of the participants who were removed from the fMRI analysis (N=16), in order to determine whether there was sufficient power to report these findings. The output from the ANOVA showed very similar results; Wilks Lambda =.14, $F(3, 13)=23.33$, $p < .001$, multivariate partial eta squared = .859. See Table 3.2 for descriptive statistics.

Condition	N	Mean	Standard Deviation
Certain-No Pain	16	137.4	75.14
Uncertain-No Pain	16	188.83	120.77
Uncertain-Pain	16	507.26	204.27
Certain-Pain	16	520.06	191.71

Table 3.2. Descriptive statistics for pain ratings in each condition for all participants.

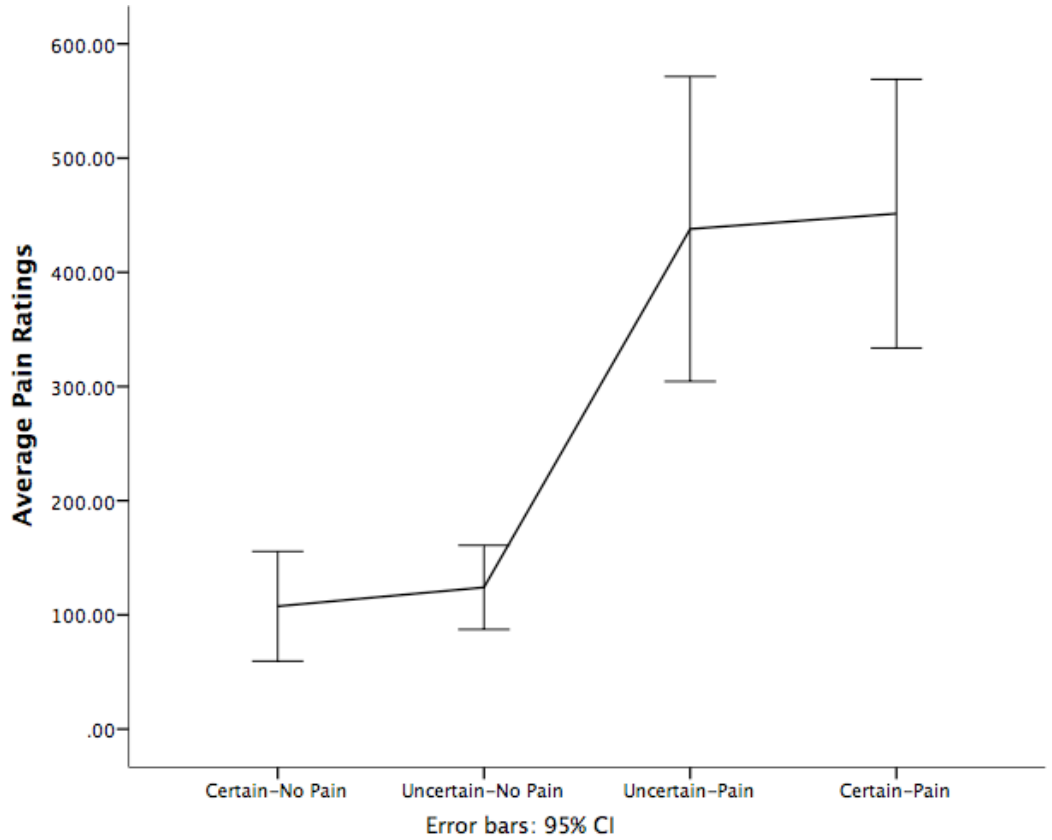


Figure 3.2. Output from one-way repeated measures ANOVA average pain ratings for each condition for participants included in fMRI analysis. Both certain and uncertain pain conditions were reported as significantly higher than both certain and uncertain no-pain conditions

fMRI analysis

During the FEAT analysis, 17 contrasts were set up; 1- 7 each condition versus baseline, and 10 further comparative analyses, which were as follows; 1. Certain no pain anticipation; 2. Uncertain pain anticipation; 3. Certain pain anticipation; 4. Certain no-pain; 5. Uncertain no-pain; 6. Uncertain pain; 7. Certain pain; 8. Certain pain anticipation(1) > Certain no-pain anticipation(-1); 9. Uncertain pain anticipation(1) > certain pain anticipation(-1); 10. Certain pain(1) > certain no-pain(-1); 11. Uncertain pain(1) > uncertain no-pain(-1); 12. Uncertain no-pain(1) > certain no-pain(-1); 13. Uncertain pain(1) > certain pain(-1); 14. Certain no-pain(1) > certain pain(-1); 15. Certain pain(1) > uncertain pain(-1); 16. Certain no-pain(1) > uncertain no-pain(-1); and 17. Uncertain no-pain(1) > uncertain pain(-1). Of these 17 contrasts, eight showed no activity (contrasts 1, 2, 7, 8, 9, 11, 12, 15), five showed small clusters of increases in areas such as the occipital cortex, somatosensory areas, and the intraparietal sulcus (see Figure 3.3; contrasts 3, 6, 10, 13, 16), and the remaining four showed widespread activation throughout a number of cortices, including occipital cortex, sensorimotor areas, ACC, insula, intraparietal sulcus, posterior parietal mid-

temporal lobe, and prefrontal cortex (see Figure 3.4; contrasts 4, 5, 14, 16). Table 3.3 provides a summary of the contrasts, clusters observed and in which areas activity was associated with.

Contrast	Number of Clusters	Range of Voxels	*P*	Structures active
1. Green (visual)	0	0	N/A	N/A
2. Amber (visual)	0	0	N/A	N/A
3. Red (visual)	2	216 - 863	<.001	Occipital cortex
4. Green (no pain)	14	224 - 3154	<.005 - <.001	Occipital cortex, Insula, Posterior Parietal/ intraparietal sulcus, Sensorimotor S1/M1, Ventromedial prefrontal cortex
5. Amber (no pain)	12	189 - 2907	<.01 - <.001	Ventromedial prefrontal cortex, intraparietal sulcus, Somatosensory cortex, Mid-Temporal cortex, Mid Anterior cingulate cortex
6. Amber (pain)	5	284 – 481	<.01 - <.001	Intraparietal sulcus, Somatosensory cortex
7. Red (pain)	0	0	N/A	N/A
8. Red(v) > Green(v)	0	0	N/A	N/A
9. Amber(v) > Red(v)	0	0	N/A	N/A
10. Red(p) > Green(n.p)	2	188 - 199	<.01	Occipital cortex
11. Amber(p) > Amber(n.p)	0	0	N/A	N/A
12. Amber(n.p) > Green(n.p)	0	0	N/A	N/A
13. Amber(p) > Red(p)	1	304	<.001	Occipital cortex
14. Green(n.p) > Red(p)	12	328 - 4363	<.001	Occipital cortex, Sensorimotor cortex(S1/M1), Posterior Parietal/ intraparietal sulcus, dorsolateral Prefrontal cortex, mid-Temporal cortex, Anterior Cingulate cortex, Insula
15. Red(p) > Amber(p)	0	0	N/A	N/A
16. Green(n.p) > Amber(n.p)	1	310	<.001	Somatosensory cortex
17. Amber(n.p) > Amber(p)	13	203 - 3135	<.005 - <.001	Occipital cortex, Somatosensory cortex, dorsolateral prefrontal cortex, Mid Anterior cingulate cortex, mid-Temporal cortex

Table 3.3. Summation of contrasts analysed, voxel clusters and cortical structures identified from the 2nd Level output. Green(n.p)=Certain no-pain, Amber(n.p)=Uncertain no-pain, Amber(p)=Uncertain

pain, Red(p)=Certain pain. Green/Amber/Red (visual) refers to the visual stimuli shown to participants preceding stimulation (i.e. anticipation condition)

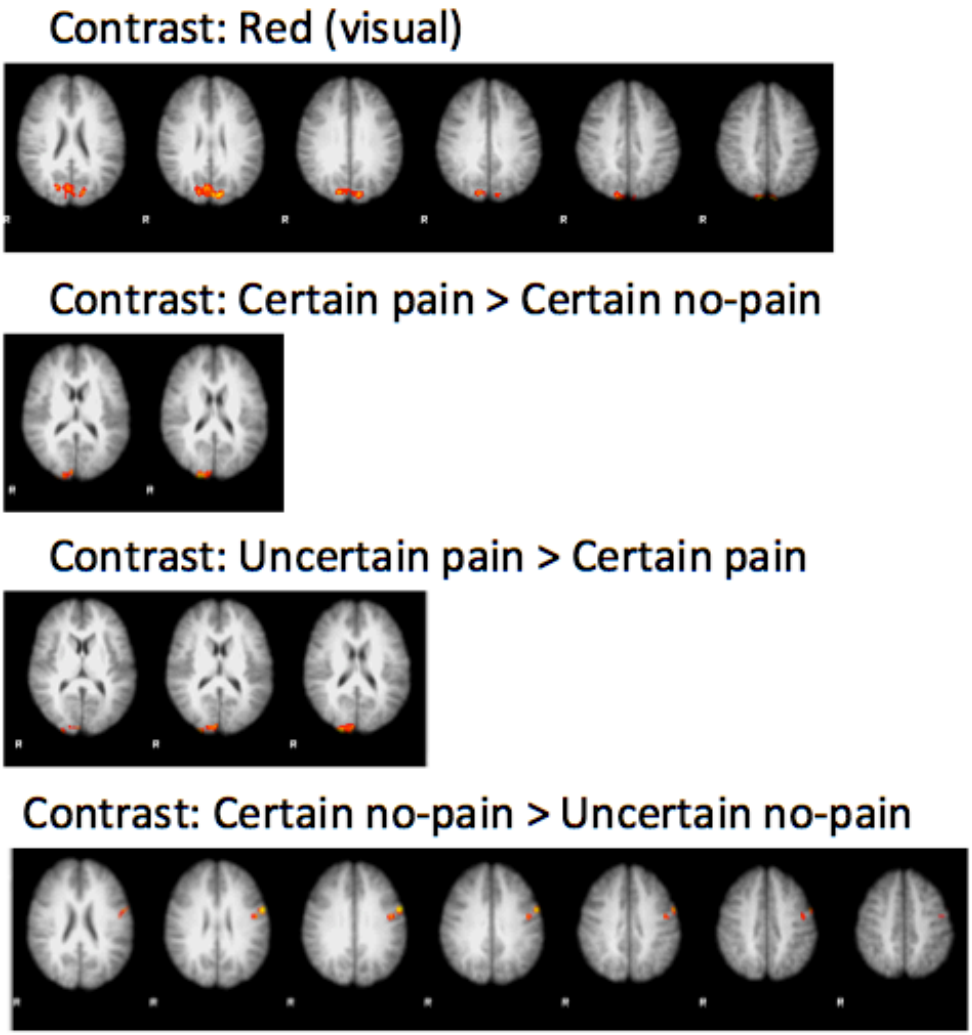


Figure 3.3. Example of smaller clusters found in 2nd level contrasts, including mainly occipital and somatosensory regions.

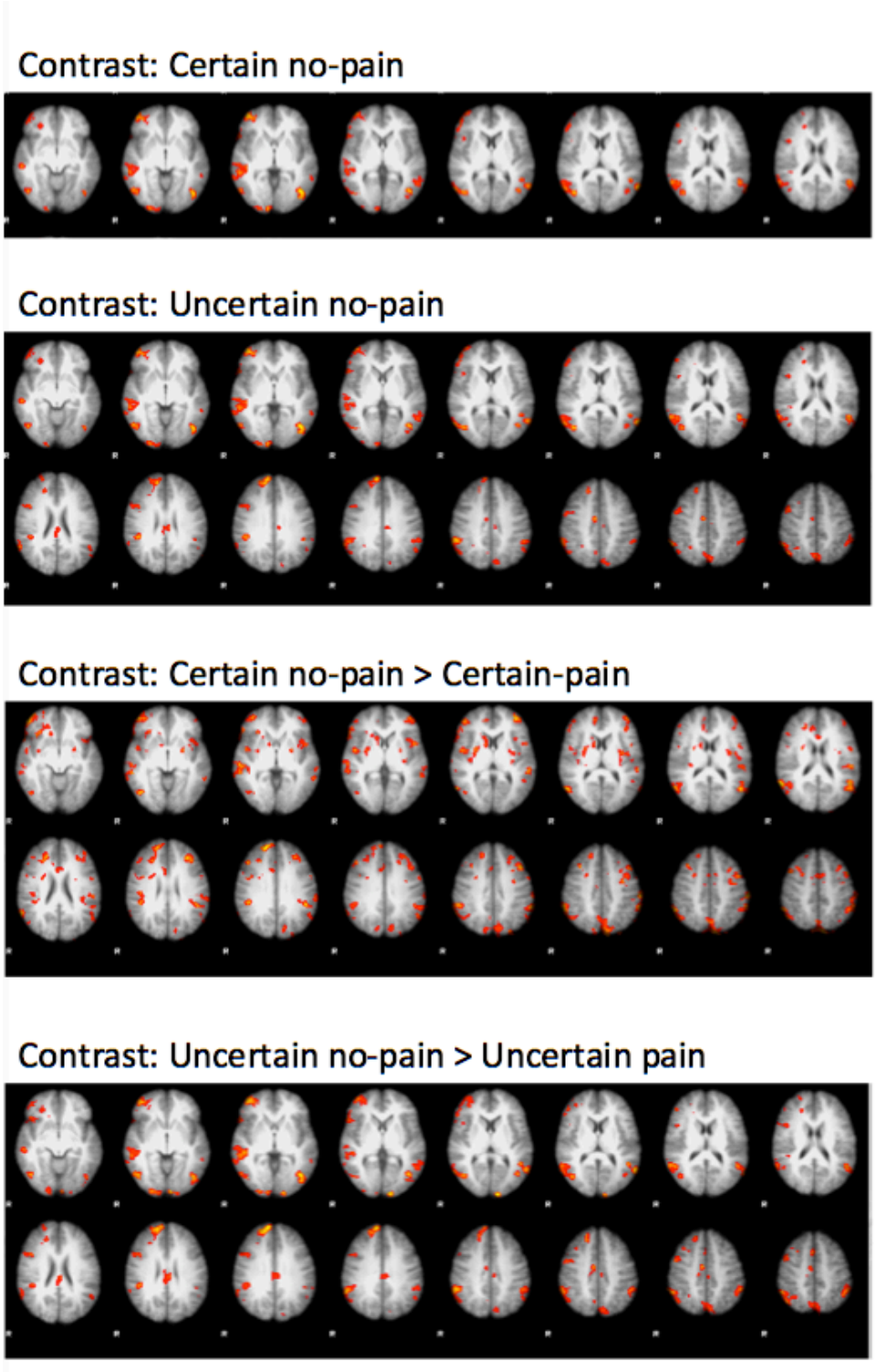


Figure 3.4: Examples of the wider-spread activations found in 2nd level contrasts, incorporating a number of regions including prefrontal, insula, anterior cingulate, intraparietal, posterior parietal, sensorimotor, occipital and mid-temporal regions.

Discussion

Results from the pain ratings show that both the certain and uncertain pain conditions were rated significantly higher than the certain and uncertain no-pain conditions. This finding

was consistent regardless to whether the analysis used all the participants (N=16, $p < .001$) or only the participants that were involved in the fMRI analysis (N=9, $p = .005$). While this does suggest that the stimulus was perceived as noxious (or at least more noxious) compared to the stimuli in the non-pain conditions, this was not the result that we were expecting to find. Previous research has found that anticipation of uncertain noxious stimulation can result in the stimulus being perceived, or at least rated, as more painful or unpleasant than a stimulus that is known to be noxious. This effect has even gone so far as to cause participants to perceive uncertain, non-painful stimuli as painful (Ploghaus et al., 1999; Sawamoto et al., 2000; Porro et al., 2002; Ploghaus et al., 2003). As such, we would have expected that the uncertain painful stimuli to be rated significantly higher than the certain noxious stimuli, and that the uncertain non-painful stimuli may have been rated on par with the certain noxious stimuli. However, there was no significant difference between the certain and uncertain painful stimuli, nor between the certain and uncertain non-painful stimuli. As research into this phenomenon has primarily used mechanically generated thermal heat-pain, and individual thresholds were established prior to testing in "training" phases, our results may be related to the utilization of the cold-pressor task. Due to the use of this task, the onset of stimulus to become being considered as noxious may be longer, as it could take up to 20 seconds for the cold to cross that threshold as opposed to the mechanically administered thermal stimuli, which would have a much more acute onset in comparison. The offset of nociception may have reduced the anticipatory effect, or even just altered the timings so it would have occurred later on in the blocks, to the extent that both certain and uncertain painful and non-painful stimuli were considered as innocuous during the initial application, rendering the effect null. Along these lines, another factor to consider is related to the training prior to scanning that participants received in previous research, and the relative lack of it in the current research. As the BOLD signal in the pACC for the anticipation of pain has been shown to increase over successive trials, indicating an adaptive mechanism (Ploghaus et al. 1999), and considering that participants only had a brief introduction to the stimulus prior to scanning, an initial baseline for the anticipatory process may not have been established. Due to concerns that the equipment was utilized was ineffective in the capacity required, this research was discontinued until a more suitable stimulus was acquired. It is also worth considering that two certain conditions were included in this study, where in other studies there has only been one certain condition (either painful or not painful), and this may have reduced some of the uncertainty as it could become easier to identify stimuli when there are two certain conditions that could be used to draw a direct comparison to.

Initial analysis of the fMRI data also only demonstrated activity in the intraparietal sulcus in one contrast (Uncertain no-pain > uncertain pain), which is involved in numerical magnitude estimation (Sigler & Opfer, 2003). However, upon further examination we found that participants 1 and 2 were unsuitable to be included, due to early issues with the experiment protocol, while participants 4, 8, 9, 10, 11 and 18 had severe movement artefacts, which was assessed using a Matlab code available from mumfordbrainstats.tumblr.com. With the remaining participants, nine of the 17 contrasts resulted in activation of cortical areas (please refer to Table 3.2 for summation). Of the visual (anticipatory) cues, where we would expect to see the ACC anticipatory activation, only the red (certain pain) analysis showed activity, and that was located in the occipital cortex, which we can expect to see during the presentation of visual stimuli. Of the tactile stimuli, only the certain pain condition (Red_p.) failed to illicit a response. The certain and uncertain no-pain conditions (Green_n.p. and Amber_n.p., respectively) showed activity in a number of structures, including right ventromedial prefrontal cortex (VMPFC), bilateral insula, sensorimotor (S1/M1) areas, right mid-temporal, posterior parietal cortex (including intraparietal sulcus), and occipital cortex. In the uncertain no-pain condition there was also activity in the posterior portion of the ACC, which (speculatively speaking) may suggest that the anticipatory part of the blocks was offset from our original projections, but as the ACC is involved in a number of functions this would be difficult to examine further. In the uncertain pain condition there were also a few smaller clusters of activation, which were located in the right VMPFC, bilateral intraparietal sulcus, and left somatosensory (S1). Of the contrasts between conditions, the certain pain vs.(>) certain no-pain, and uncertain pain vs. certain pain only elicited activation in the right occipital cortex, which again we can assume may be due to the presentation of visual stimuli, in this case the VAS. In the certain pain vs. uncertain pain contrast, there was only activation in the left pre-central gyrus, which is part of the primary motor area. As such, this activity may be due to the movement caused by the rating of the stimuli, via the joystick. In the certain no pain vs. certain pain contrast there was widespread activation clusters, including the VMPFC, sensorimotor areas, medial frontal, bilateral mid-temporal cortex, bilateral posterior parietal and intraparietal sulcus, parietal operculum, and bilateral occipital. The final contrast, uncertain no-pain vs. uncertain pain, showed activity in the VMPFC, right mid-temporal, bilateral posterior parietal and intraparietal sulcus, occipital, somatosensory and posteriorACC/mid-ACC. A majority of these activations overlap between the contrast, particularly the occipital, somatosensory, prefrontal areas, and posterior parietal/intraparietal sulcus, while sections of the cingulate cortex and insula only showing activity in a handful of

the contrasts. As previously mentioned, areas of the occipital cortex, and the somatosensory, can be due to the stimulus and task (i.e. the tactile stimulation of the cold-pressor/control and VAS). A majority of the other activations that were more widespread and incorporated an assortment of cortical areas can also be explained by the task, but not in a fashion that fits our hypothesis.

While most of these areas are involved in pain perception, it is worth noting that none of these structures are dedicated or specifically involved in nociception. Individually, these areas are synonymous with a variety of functions; the VMPFC has been linked to attributing task stimuli with affective meaning, including pain (Roy, Shohamy & Wager, 2012), as well as an association with generating or integrating coping mechanisms (Salomons, Johnstone, Backonja, Shackman & Davidson, 2007). The intraparietal sulcus is typically shown to be involved in magnitude estimation (Sigler & Opfer, 2003), as well as estimations of the spatial representation of both numerical figures and symbolic representations of number (Vogel, Grabner, Schneider, Siegler & Ansari, 2013). The posterior parietal cortex has been linked to a network of structures (which includes the bilateral insula and portions of the premotor cortex) that has been demonstrated to be involved in tactile discrimination tasks (Ploger, Ruff, Blankenburg, Bestmann, Wiech, Stephan, Capilla, Friston & Dolan, 2006), and the generation of subjective perceptions from sensory stimuli (de Lafuente & Romo, 2006). The mid (ventral) temporal cortex is also involved in a number of different tasks, including memory and the storing of semantic object information, i.e. the attributes of specific objects (Chao, Haxby and Martin, 1999). The final two regions that demonstrated activity in multiple of the presented contrasts are the ACC and insula cortex, both of which are highly interconnected both functionally and structurally with a number of other cortices, to the extent that portions of the insula have been speculated to act as a central processing hub in several theories of nociceptive perception (Baliki, Geha & Apkarian, 2008; Morrison, Perini & Dunham, 2013).

Baliki, Geha and Apkarian (2008) investigated the relationship between pain perception, its neurological representation and subsequent magnitude estimation; in other words how nociception is translated into a numerical rating on a VAS. They speculated that since pain perception is essentially a form of stimulus intensity assessment, it might require a central module similar to those observed in other sensory discrimination tasks. By examining participants' responses and neurological activity during a pain-rating task and a visual rating task (where participants were required to rate the length of a bar without the application of noxious stimulation) and comparing the two, they found a network of structures exhibiting

overlapping activity. This network included the bilateral insula, dorsal and ventral premotor cortex, posterior parietal cortex, and the mid temporal cortex. Additionally, during the pain rating task there was an increased response in the left primary sensorimotor cortex, thalamus, putamen, caudate and cerebellum, as well as in the supplementary motor area, middle portions of the ACC, and areas of the dorsolateral prefrontal cortex. Further examinations led them to discover two distinct subdivisions in the insula, each with its own separate functionally-connected networks to different structures, and each selectively active for either pain or magnitude rating. First, the magnitude rating-related insula correlated only with areas dominant in capturing task-variance, including posterior parietal cortex, dorsal and ventral premotor cortices, and supplementary motor area. Next there is the nociceptive-associated insula, which had extensive connections to areas involved in pain perception, including bilateral thalamus, basil ganglia, amygdala, anterior ACC and ventral striatum. This suggested that sensory magnitude rating tasks involve two distinct functional processes; one which is autonomous of sensory modality and associated with rating performance, while the other appears to be specific for pain modality. Essentially it appears that this network of structures, sensory information is processed and moderated by inputs from structures involved in higher cognitive function, which is then processed into qualitative perception, which in turn is translated into quantitative, numerical-magnitude reports. In a similar investigation, it was found that conscious cognitive evaluation of noxious stimulation in the absence of sensory stimulation involved activity in the bilateral anterior insula/frontal operculum, dorsolateral prefrontal cortex, bilateral medial prefrontal cortex/ACC, right superior parietal cortex, inferior parietal lobule, orbital prefrontal cortex, and left occipital cortex (Kong, White, Kwong, Vangel, Rosemann, Gracely & Gollub, 2006). When discussing the properties exhibited by the magnitude-related portion of the insula, they found a resemblance to a previously outlined general task-related network. These properties included reflecting task variance, functional correlations with task-activated regions, better correlations over the time-course of pain ratings, and exhibiting BOLD activity delayed from stimulus peak and preceding pain-perception peak. The final property there does seem to match with previous research where the ACC and insula/parietal operculum activity in anticipation of noxious stimuli (Ploghaus et al, 1999; Sawamoto et al, 2000). Baliki, Geha and Apkarian (2008) also discussed how frontal and parietal regions play a key role in the formation of subjective perceptions and experiences from sensory stimuli, and how the networked structures observed in their research bore a close resemblance to those identified during a tactile discrimination task (de Lafuente & Romo, 2006; Pleger et al, 2006). The

authors concluded that, as well as the visual dorsal "where" stream and the ventral "what" stream, there is also a central neurological network dedicated to quantitative assessment, which they refer to as a "how-much" system. In relation to nociceptive perception, they speculated that due to the high saliency of painful stimuli necessitates the activation of this system, which in turn receives sensory and neurological inputs from a number of connected structures into the central hub, in this case the insula. In relation to the contrasts presented in our experiment, four of the nine contrasts show activity in the areas outlined in the previous research examining functional networks involved in tactile and painful assessment in relation to magnitude estimation, as mentioned above. Three of these contrasts (Uncertain no-pain, certain no-pain > certain pain, and uncertain no-pain > uncertain pain) demonstrate activity in the ACC, and only two (certain no-pain and certain no-pain > certain pain) involve the insula. The interesting point is that almost all of these activations prove to be higher in the innocuous stimuli compared to the noxious. The painful stimulus was rated as significantly more painful that the non-painful, but on average only around the median of the VAS, where the stimulus would be beginning to be regarded to as painful. In part, this activity may be reflective of the continuous assessment of the stimuli. As mentioned, the cold pressor would gradually become more painful as opposed to the more acute onset associated with thermal-heat pain. As such, this activity could be due to the continuous assessment and rating of the stimuli, demonstrating a gradual assessment-and-decision making process. Another factor to consider may be the inclusion of two certain conditions, as opposed to the previous research, which has predominantly only incorporated one certain condition; a certain no-pain or a certain pain condition. By including both a certain pain and a certain no-pain, any ambiguity between the noxious and innocuous stimuli may have been erased, as participant would then have a stimuli to directly compare both of the uncertain stimuli, and which would constitute as painful or not (at least, in this context). Coupling the removal of ambiguity and the more gradual onset of noxious stimulation, we may be developing a clearer picture as to why previous fMRI results have not been replicated; though the design has largely been kept the same, the change in stimuli and inclusion of an extra condition has rendered it ineffective at examining the uncertain, anticipatory effect.

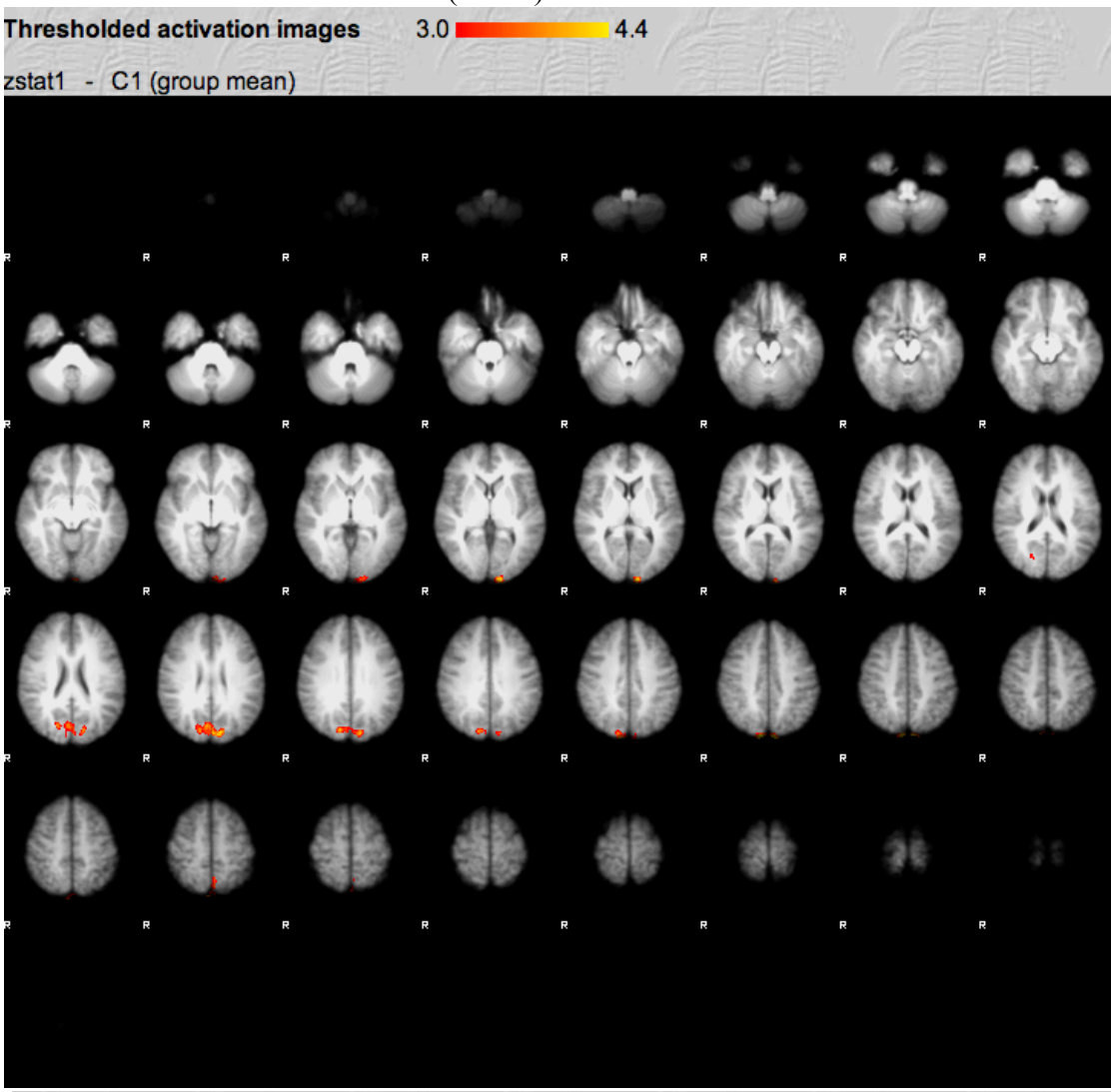
To better follow this avenue of investigation in future, it would probably be best to utilise a method of stimulation with a less gradual onset, and possibly thoroughly train the participants in the stimuli prior to scanning. While thermal pain is traditionally used stimuli of choice, it may be possible to elicit the reaction using pressure-pain; this was a consideration that was put forward after initial analysis of the fMRI results, which went so far

as to examining the use of Von Frey hairs, as they would be scanner-compatible. Unfortunately, this too seemed ineffective to elicit the appropriate response outside of clinical or chronic-pain populations. Removal of the secondary certain condition would also be required, as it may prove more successful at generating or enhancing the uncertainty necessary, as participants would be unable to consciously or unconsciously draw a direct comparison between the painful and non-painful stimuli. Taking into consideration these factors and combining it with the activity observed in the fMRI results, it appears safe to conclude that rather than producing a certain/uncertain anticipatory effect, all that we were able to do was generate a task associated with the assessment of the intensity of tactile stimulation, which may occasionally have been painful. As such, we are forced to reject our hypothesis, and accept that in our hands cold-pain stimuli cannot produce the anticipatory effect found in previous research. This lead to a decision to post pone the previously proposed fMRS research until a better paradigm could be established.

Chapter 3: Supplementary Results

Output from fMRI FSL 2nd level contrasts

SS0034 2nd Level Contrast 3: Red (visual)

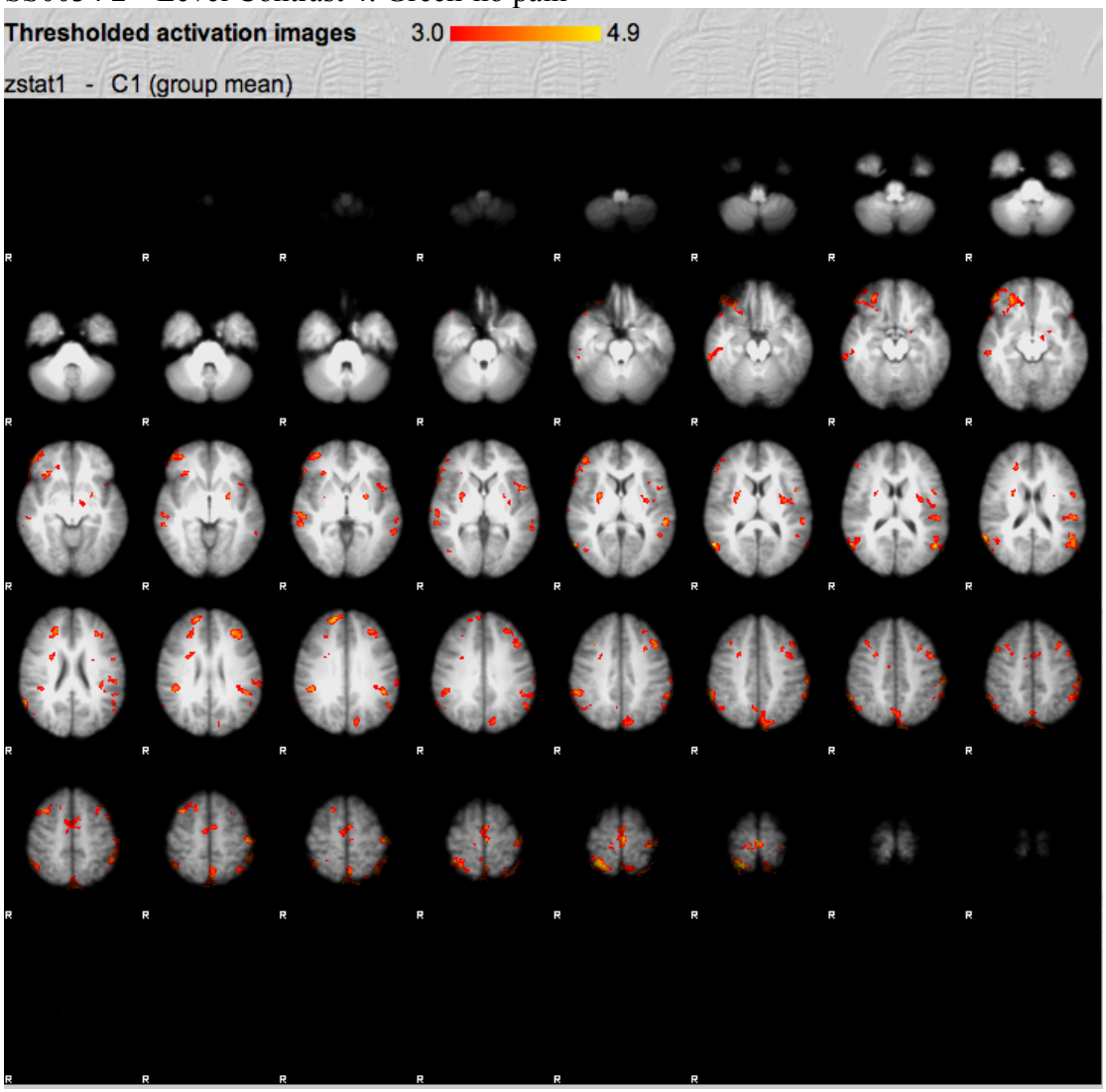


Co-ordinate information for cluster_zstat1 - back to main FEAT report

Cluster List

Cluster Index	Voxels	P	-log10(P)	Z-MAX	Z-MAX X (mm)	Z-MAX Y (mm)	Z-MAX Z (mm)	Z-COG X (mm)	Z-COG Y (mm)	Z-COG Z (mm)	COPE-MAX	COPE-MAX X (mm)	COPE-MAX Y (mm)	COPE-MAX Z (mm)	COPE-MEAN
2	863	5.96e-08	7.22	4.46	-8	-92	46	2.57	-84	35.2	633	-4	-70	60	264
1	216	0.0087	2.06	4.27	-10	-106	6	-10.4	-105	1.85	330	-6	-100	-4	179

SS0034 2nd Level Contrast 4: Green-no pain

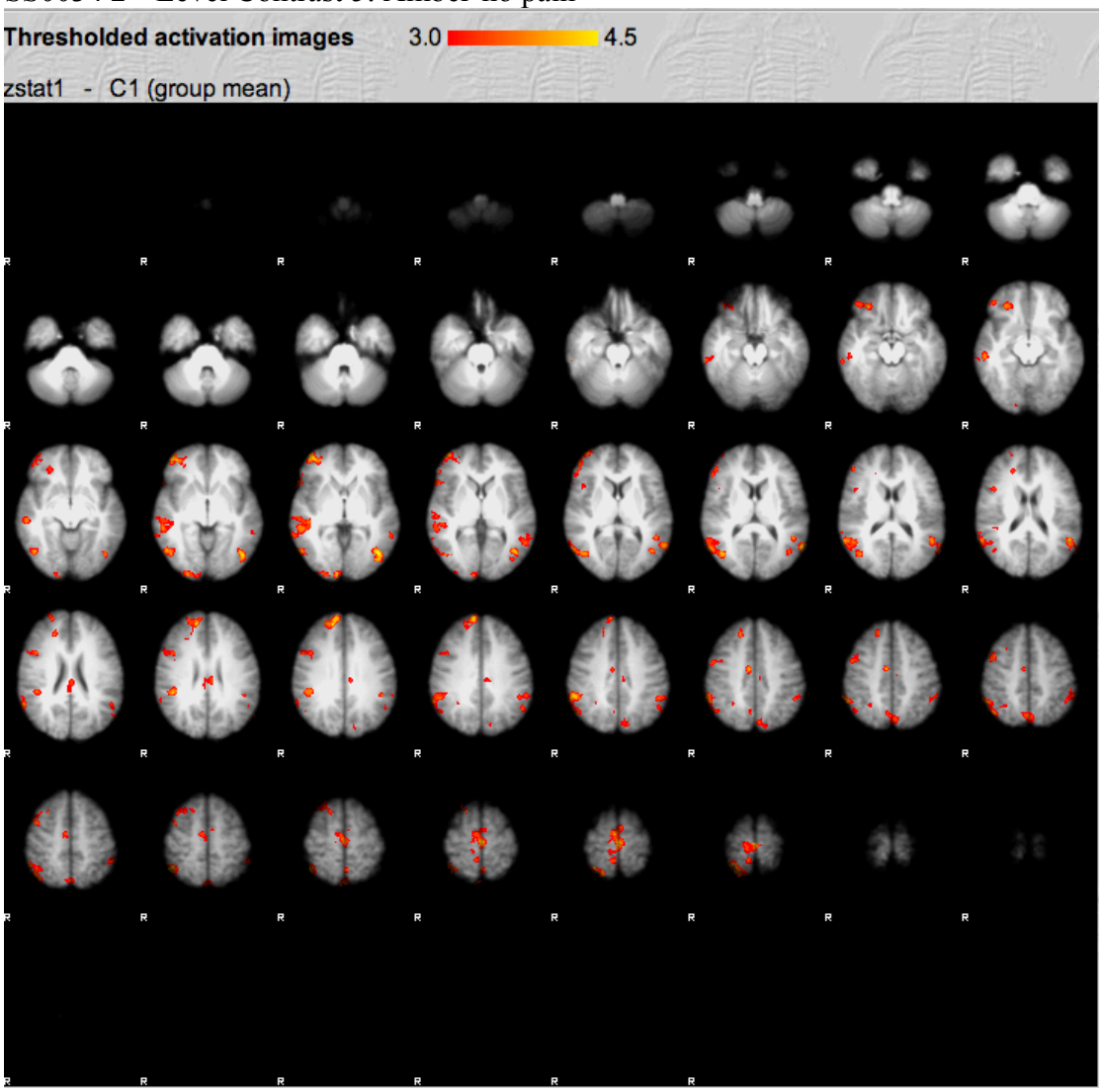


Co-ordinate information for cluster_zstat1 - back to main FEAT report

Cluster List

Cluster Index	Voxels	P	-log10(P)	Z-MAX	Z-MAX X (mm)	Z-MAX Y (mm)	Z-MAX Z (mm)	Z-COG X (mm)	Z-COG Y (mm)	Z-COG Z (mm)	COPE-MAX	COPE-MAX X (mm)	COPE-MAX Y (mm)	COPE-MAX Z (mm)	COPE-MEAN
14	3154	3.67e-21	20.4	4.46	-62	-26	48	-34.2	-52.1	49.8	3.47e+03	-4	-70	60	1.46e+03
13	1275	2.91e-11	10.5	4.77	30	42	-14	43.1	39.9	-8.56	3.44e+03	46	54	0	1.22e+03
12	1226	5.91e-11	10.2	4.94	30	-54	72	44.3	-51.6	55.1	4.64e+03	44	-54	64	1.79e+03
11	620	1.07e-06	5.97	4.48	-2	-20	74	0.911	-11.4	66	3.11e+03	0	-6	74	1.32e+03
10	435	3.75e-05	4.43	4.39	-54	-68	16	-55.3	-62.9	22.6	1.99e+03	-56	-64	38	1.09e+03
9	375	0.000133	3.88	4.19	56	-28	0	61.6	-30.2	-7.1	1.34e+03	66	-38	0	849
8	370	0.000148	3.83	4.22	-32	36	28	-38	30.3	33.7	2.43e+03	-34	34	28	1.3e+03
7	348	0.00024	3.62	4.83	62	-66	10	57.8	-62.4	16.8	2.13e+03	58	-68	10	1.15e+03
6	318	0.00047	3.33	4.54	18	52	32	20	45.7	28.6	1.89e+03	22	52	32	981
5	316	0.000492	3.31	4.1	-26	0	-2	-29.5	-5.12	4.93	1.42e+03	-44	-6	12	832
4	293	0.000839	3.08	4.09	28	0	8	27.9	2.59	21.6	1.24e+03	28	2	8	720
3	272	0.00138	2.86	4.07	38	18	58	33.8	19.1	55.4	2.46e+03	26	28	64	1.23e+03
2	270	0.00145	2.84	3.91	-48	6	50	-40.2	11.6	49.7	1.91e+03	-50	8	50	952
1	224	0.00454	2.34	3.95	-50	10	4	-48.5	10.1	5.53	2.78e+03	-50	16	-10	1.45e+03

SS0034 2nd Level Contrast 5: Amber-no pain

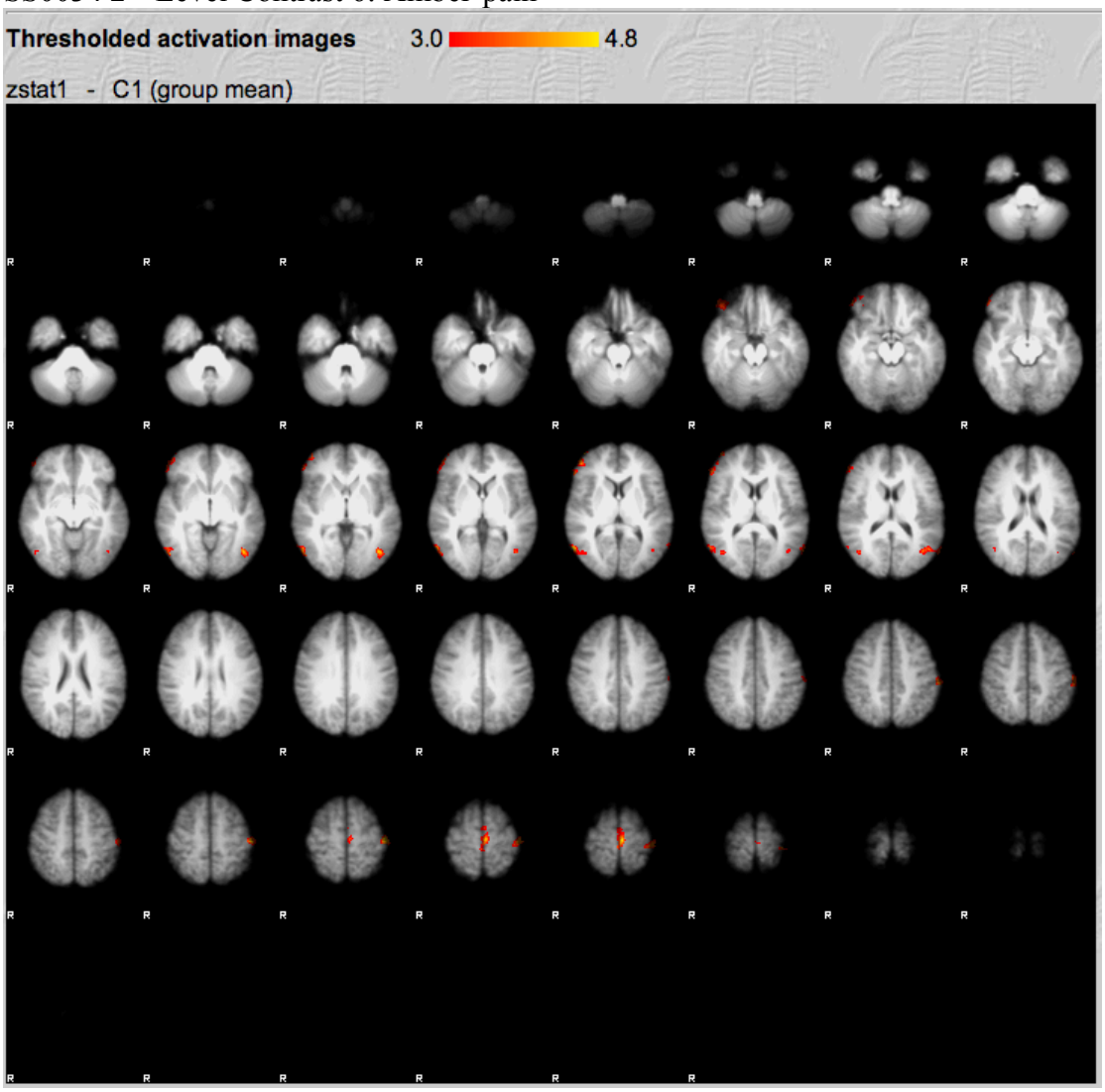


Co-ordinate information for cluster_zstat1 - back to main FEAT report

Cluster List

Cluster Index	Voxels	P	-log10(P)	Z-MAX	Z-MAX X (mm)	Z-MAX Y (mm)	Z-MAX Z (mm)	Z-COG X (mm)	Z-COG Y (mm)	Z-COG Z (mm)	COPE-MAX	COPE-MAX X (mm)	COPE-MAX Y (mm)	COPE-MAX Z (mm)	COPE-MEAN
12	2907	1.39e-20	19.9	4.23	62	-66	10	51.4	-51.8	26.1	3.75e+03	44	-54	64	1.16e+03
11	1010	8.05e-10	9.09	4.13	0	-22	76	3.78	-21.1	67.1	2.26e+03	0	-6	74	935
10	951	2.09e-09	8.68	4.22	46	56	0	43.4	43.5	-2.31	2.87e+03	46	54	0	1.11e+03
9	601	9.54e-07	6.02	4.16	-62	-64	12	-57.5	-58.6	15	1.99e+03	-54	-62	38	1.01e+03
8	527	4.05e-06	5.39	4.6	10	60	36	17.8	51.2	32.4	1.82e+03	20	52	32	992
7	446	2.11e-05	4.68	3.67	0	-70	54	-1.85	-74.5	50.9	2.34e+03	4	-76	64	1.23e+03
6	415	4.07e-05	4.39	3.73	46	6	56	38.9	14	56.4	2.17e+03	26	28	64	1.21e+03
5	347	0.000183	3.74	4.52	-44	-72	0	-44.4	-72.9	0.962	2.95e+03	-46	-72	0	1.37e+03
4	346	0.000188	3.73	3.72	-50	-42	32	-54.8	-45.3	45.5	2.09e+03	-52	-46	60	1.03e+03
3	239	0.0025	2.6	4.07	10	-100	2	18.8	-99.7	-2.24	2.68e+03	8	-102	2	1.25e+03
2	230	0.00316	2.5	3.76	46	14	22	49.6	13.1	27.9	2.07e+03	48	12	22	1.39e+03
1	189	0.00954	2.02	3.79	-4	-24	26	-2.57	-25.5	29.7	1.79e+03	0	-34	26	1.01e+03

SS0034 2nd Level Contrast 6: Amber-pain

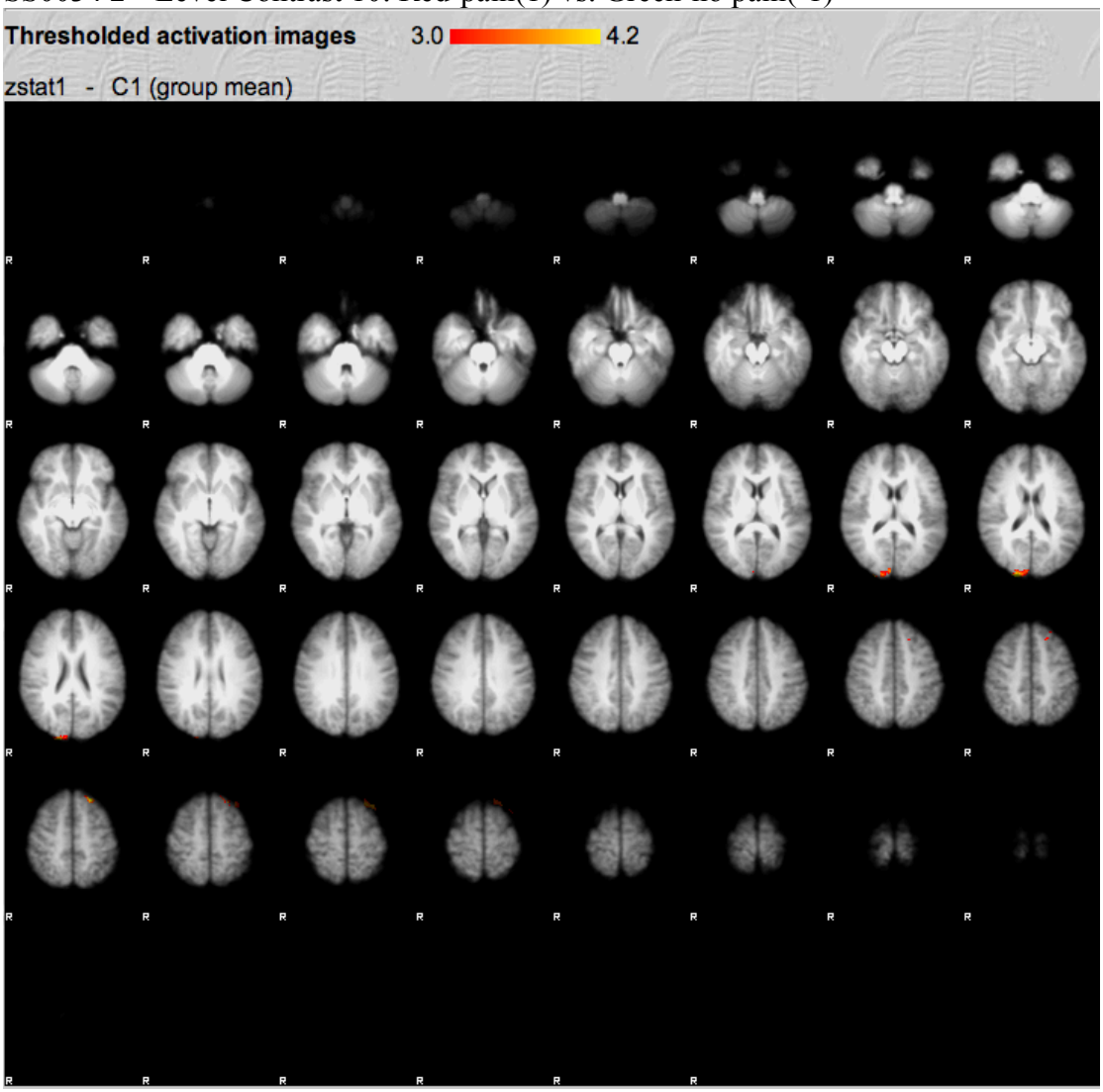


Co-ordinate information for cluster_zstat1 - back to main FEAT report

Cluster List

Cluster Index	Voxels	P	-log10(P)	Z-MAX	Z-MAX X (mm)	Z-MAX Y (mm)	Z-MAX Z (mm)	Z-COG X (mm)	Z-COG Y (mm)	Z-COG Z (mm)	COPE-MAX	COPE-MAX X (mm)	COPE-MAX Y (mm)	COPE-MAX Z (mm)	COPE-MEAN
5	481	0.000266	3.58	4.14	46	60	8	51.1	45.2	-1.77	520	50	48	8	196
4	413	0.000775	3.11	4.48	-52	-20	64	-52.5	-23.7	61.5	522	-62	-24	48	252
3	319	0.00377	2.42	4.82	62	-66	8	55.2	-69	5.6	430	54	-68	-2	199
2	284	0.00704	2.15	4.46	-4	-20	72	-1.94	-17.3	70.2	468	0	-6	74	179
1	284	0.00704	2.15	4.36	-44	-72	-2	-49.2	-69.9	7.25	510	-46	-72	0	249

SS0034 2nd Level Contrast 10: Red-pain(1) vs. Green-no pain(-1)

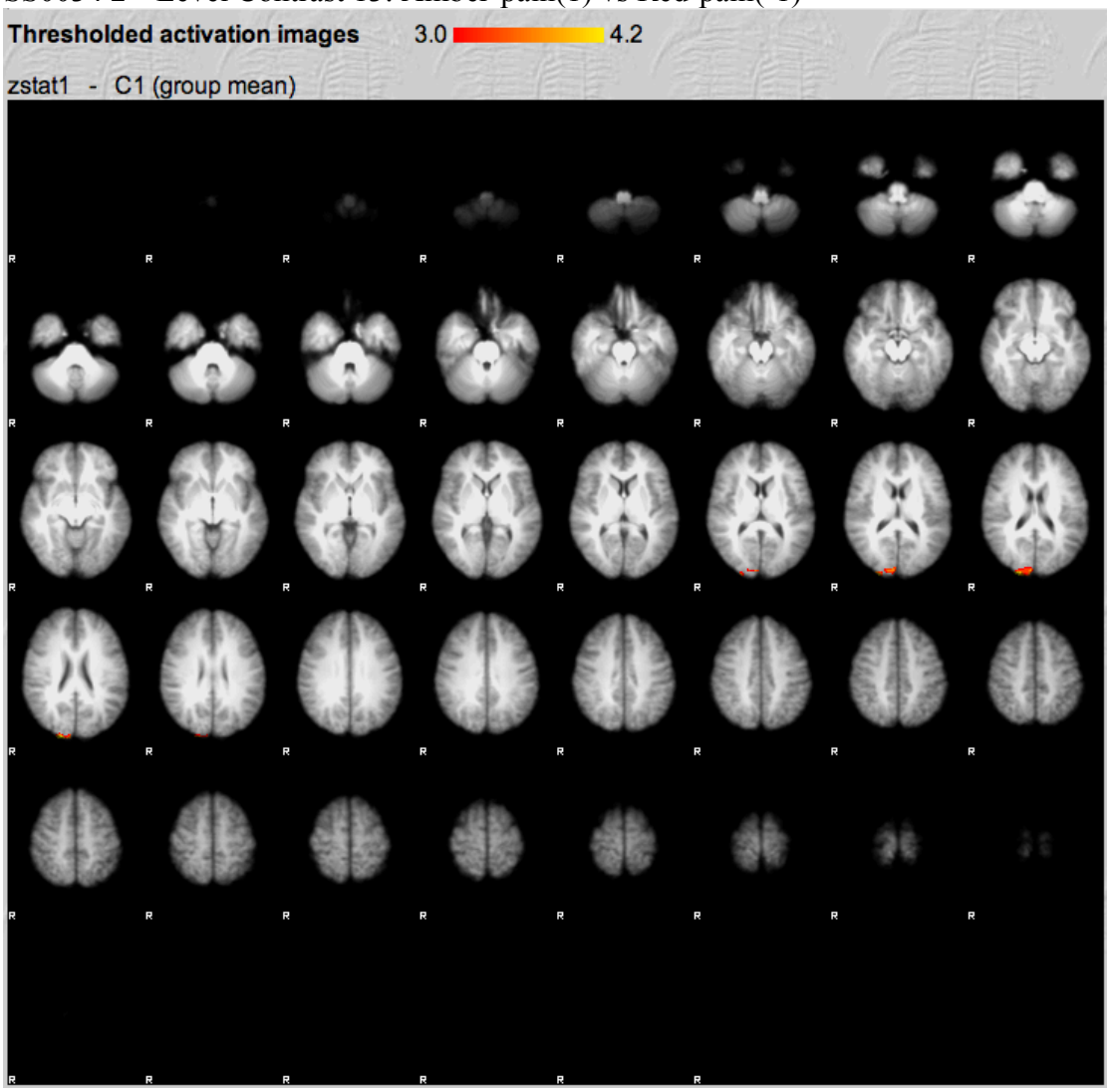


Co-ordinate information for cluster_zstat1 - back to main FEAT report

Cluster List

Cluster Index	Voxels	P	-log10(P)	Z-MAX	Z-MAX X (mm)	Z-MAX Y (mm)	Z-MAX Z (mm)	Z-COG X (mm)	Z-COG Y (mm)	Z-COG Z (mm)	COPE-MAX	COPE-MAX X (mm)	COPE-MAX Y (mm)	COPE-MAX Z (mm)	COPE-MEAN
2	199	0.00553	2.26	4.29	20	-102	22	15.2	-100	19.9	1.69e+03	20	-100	22	1.09e+03
1	188	0.00759	2.12	4.21	-24	34	58	-24.4	31.1	61.8	1.9e+03	-14	28	70	1e+03

SS0034 2nd Level Contrast 13: Amber-pain(1) vs Red-pain(-1)

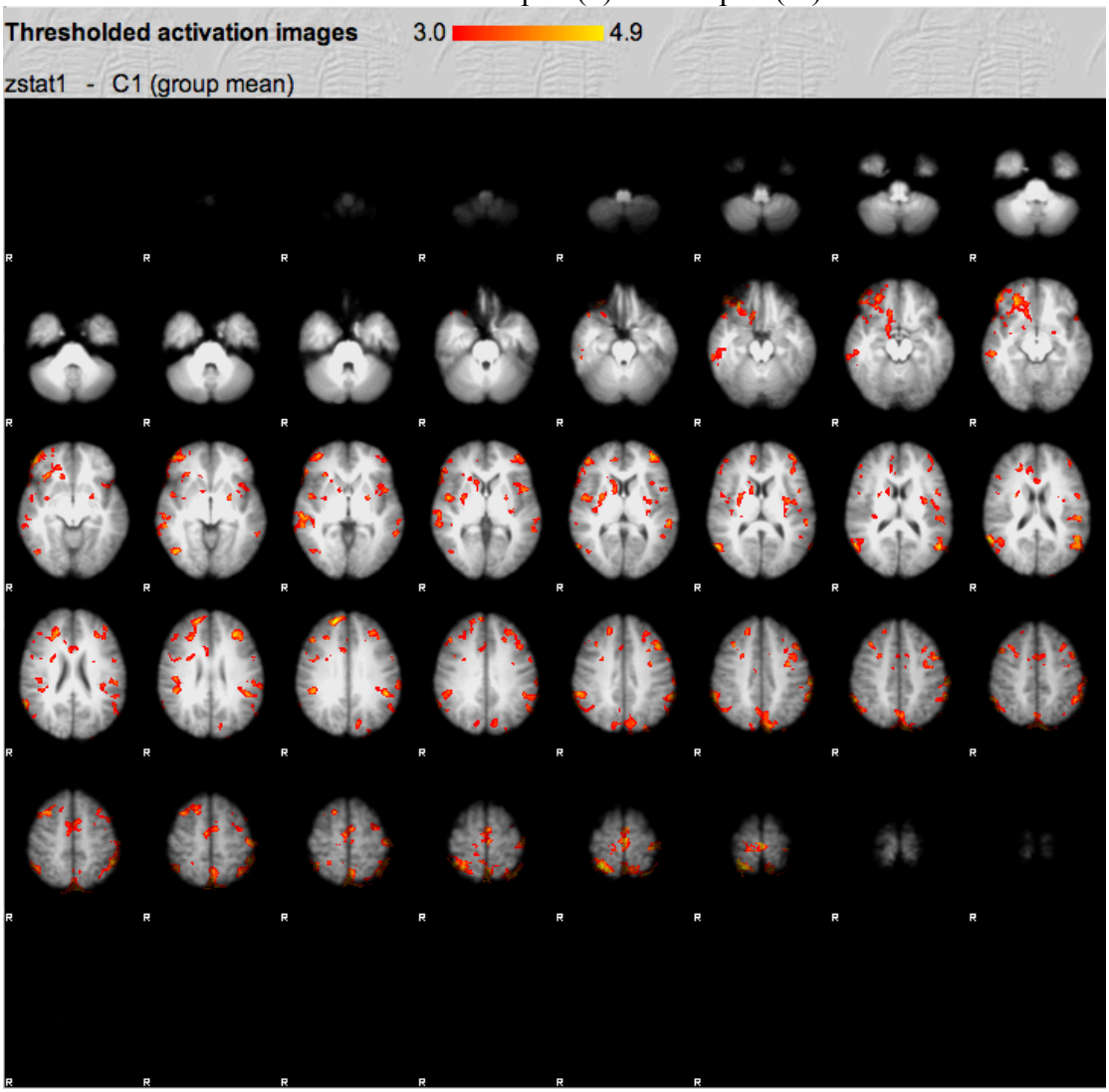


Co-ordinate information for cluster_zstat1 - back to main FEAT report

Cluster List

Cluster Index	Voxels	P	-log10(P)	Z-MAX	Z-MAX X (mm)	Z-MAX Y (mm)	Z-MAX Z (mm)	Z-COG X (mm)	Z-COG Y (mm)	Z-COG Z (mm)	COPE-MAX	COPE-MAX X (mm)	COPE-MAX Y (mm)	COPE-MAX Z (mm)	COPE-MEAN
1	304	0.000126	3.9	4.28	24	-102	22	16	-99.5	19.5	2.03e+03	14	-100	18	1.14e+03

SS0034 2nd Level Contrast 14:Green-no pain(1) vs Red-pain(-1)

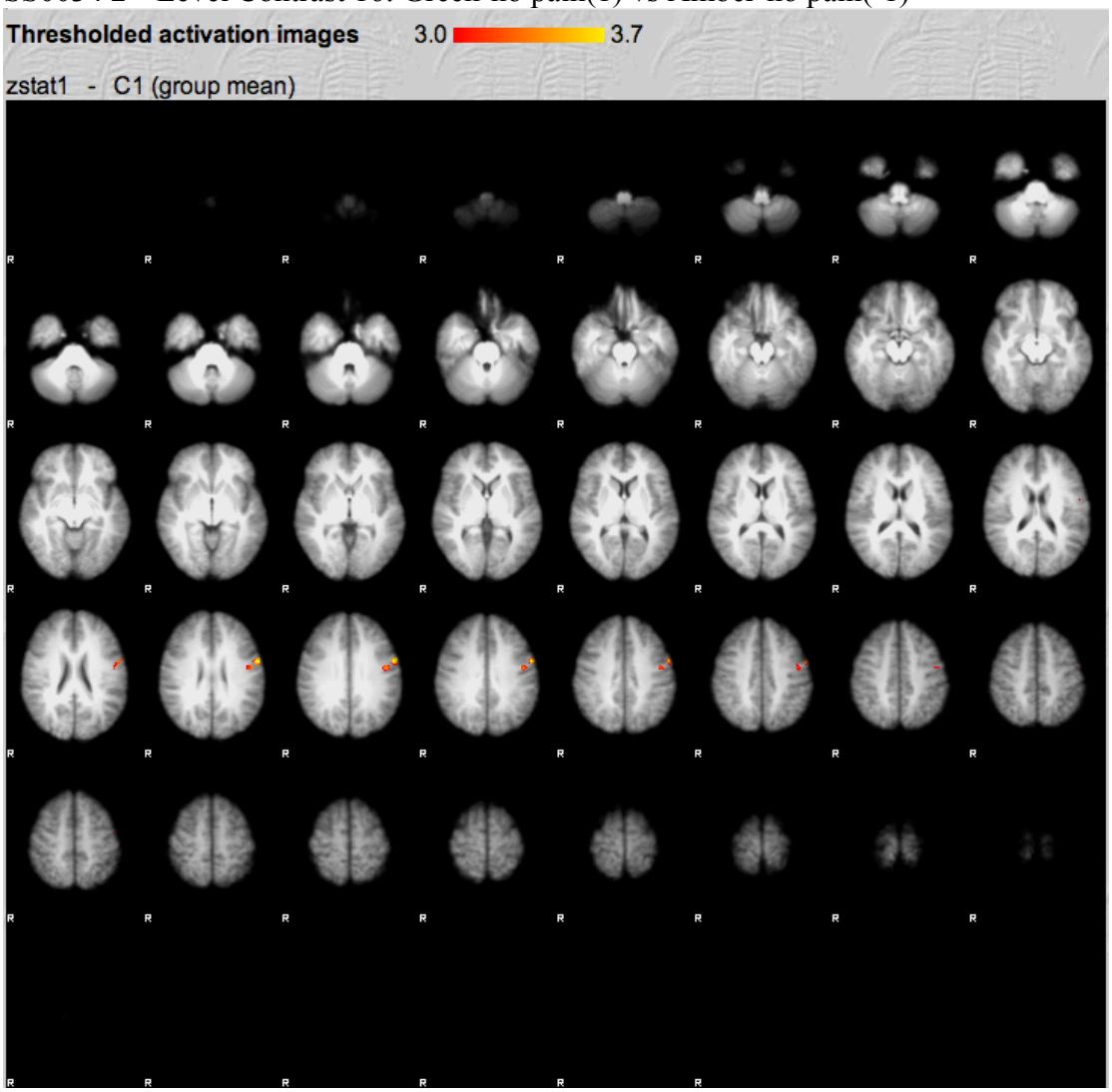


Co-ordinate information for cluster_zstat1 - back to main FEAT report

Cluster List

Cluster Index	Voxels	P	-log10(P)	Z-MAX	Z-MAX X (mm)	Z-MAX Y (mm)	Z-MAX Z (mm)	Z-COG X (mm)	Z-COG Y (mm)	Z-COG Z (mm)	COPE-MAX	COPE-MAX X (mm)	COPE-MAX Y (mm)	COPE-MAX Z (mm)	COPE-MEAN
12	4363	2.75e-28	27.6	4.62	-50	-42	30	-30.9	-55.6	49.8	3.37e+03	-4	-70	60	1.26e+03
11	2625	5.93e-20	19.2	4.54	30	38	-20	39.7	31.5	-5.55	3.11e+03	46	54	0	1.02e+03
10	2132	2.82e-17	16.5	4.91	30	-54	72	46.9	-53.8	42.9	4.29e+03	44	-54	64	1.43e+03
9	1475	2.34e-13	12.6	4.38	-30	36	28	-39	25.2	34	2.26e+03	-34	34	28	978
8	1043	1.88e-10	9.73	4.21	30	20	56	27.8	9.88	30.5	2.05e+03	46	16	54	800
7	911	1.73e-09	8.76	4.55	0	-20	74	1.81	-11.3	64.9	2.81e+03	0	-6	74	1.1e+03
6	680	1.19e-07	6.92	4.21	60	-26	0	61.6	-31.6	-6.22	1.36e+03	68	-40	0	772
5	577	8.34e-07	6.08	4.68	18	54	32	20.9	43.4	31.8	1.75e+03	22	52	32	817
4	517	2.8e-06	5.55	4.37	-54	-66	16	-55.3	-62.9	23.1	1.86e+03	-54	-62	36	962
3	416	2.47e-05	4.61	3.99	-50	10	4	-46.4	11.1	2.48	2.58e+03	-50	16	-8	1.27e+03
2	363	8.3e-05	4.08	4.07	-36	-6	14	-32.8	-6.96	8.08	1.33e+03	-44	-6	12	722
1	328	0.000191	3.72	3.9	12	50	12	7.16	29.4	22.6	2.18e+03	2	22	38	761

SS0034 2nd Level Contrast 16: Green-no pain(1) vs Amber-no pain(-1)

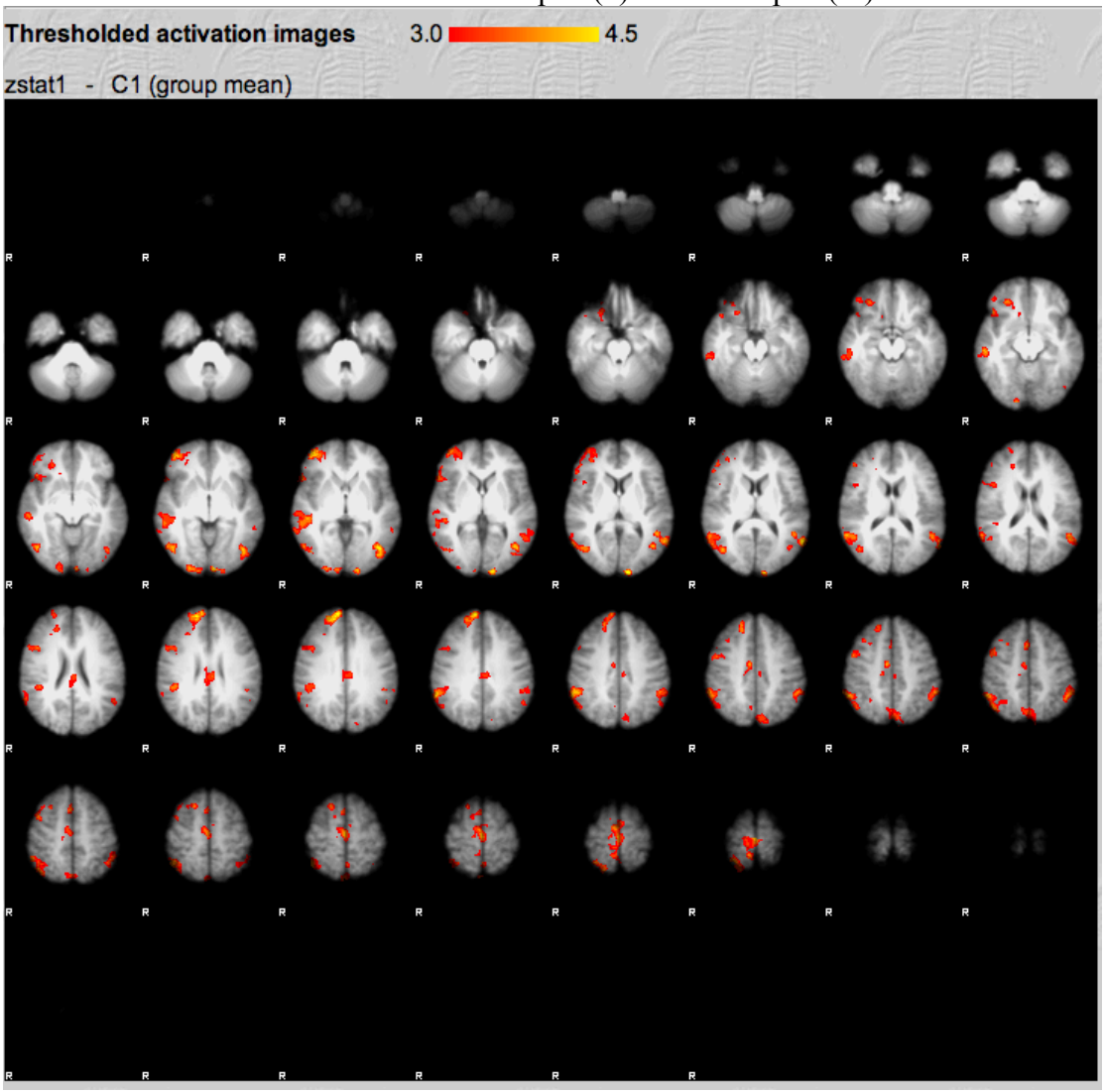


Co-ordinate information for cluster_zstat1 - back to main FEAT report

Cluster List

Cluster Index	Voxels	P	-log10(P)	Z-MAX	Z-MAX X (mm)	Z-MAX Y (mm)	Z-MAX Z (mm)	Z-COG X (mm)	Z-COG Y (mm)	Z-COG Z (mm)	COPE-MAX	COPE-MAX X (mm)	COPE-MAX Y (mm)	COPE-MAX Z (mm)	COPE-MEAN
1	310	0.000192	3.72	3.8	-58	2	32	-54.5	-1.57	34.6	2.06e+03	-58	2	30	1.37e+03

SS0034 2nd Level Contrast 17: Amber-no pain(1) vs Amber-pain(-1)



Co-ordinate information for cluster_zstat1 - back to main FEAT report

Cluster List

Cluster Index	Voxels	P	-log10(P)	Z-MAX	Z-MAX X (mm)	Z-MAX Y (mm)	Z-MAX Z (mm)	Z-COG X (mm)	Z-COG Y (mm)	Z-COG Z (mm)	COPE-MAX	COPE-MAX X (mm)	COPE-MAX Y (mm)	COPE-MAX Z (mm)	COPE-MEAN
13	3135	7.61e-25	24.1	4.23	48	-70	-6	51.6	-51.3	26.2	3.22e+03	44	-54	64	985
12	1581	1.87e-15	14.7	4.23	42	54	0	42.2	35.7	1.82	2.44e+03	46	54	0	973
11	1091	6.76e-12	11.2	4.14	2	-16	64	4.48	-20.8	65.9	1.8e+03	0	-6	74	816
10	719	8.08e-09	8.09	4.43	10	60	36	17.9	50.5	33.1	1.62e+03	20	52	32	840
9	648	5.96e-08	7.22	3.82	-60	-44	48	-53.5	-48.6	46.7	1.86e+03	-54	-62	38	796
8	539	3.58e-07	6.45	4.24	-62	-62	12	-57.6	-57.8	11.8	1.49e+03	-54	-56	18	854
7	509	7.75e-07	6.11	3.57	-6	-80	46	-1.98	-75.5	50.8	2.05e+03	4	-76	64	1.08e+03
6	388	1.39e-05	4.86	4.33	-44	-72	0	-44.6	-73.6	0.298	2.44e+03	-46	-72	0	1.08e+03
5	385	1.51e-05	4.82	3.83	20	22	62	38.5	12.8	53.9	1.64e+03	20	22	68	938
4	346	4.11e-05	4.39	3.84	-2	-24	26	-1.25	-24.5	31.2	1.59e+03	0	-34	26	940
3	265	0.000375	3.43	4.18	26	-98	-2	19.2	-98.2	-4.05	2.15e+03	8	-102	2	1.03e+03
2	214	0.0017	2.77	4.55	-12	-104	10	-11.6	-102	1.91	1.55e+03	-12	-102	6	920
1	203	0.00238	2.62	3.84	2	16	54	4.91	14.5	59.2	1.89e+03	2	16	54	1.01e+03

Chapter 3 References

Alger, J. R. (2010). Quantitative proton magnetic resonance spectroscopy and spectroscopic imaging of the brain: A didactic review. *Topics in Magnetic Resonance Imaging.* 21(2): 115-128.

Baliki, M. N., Geha, P. Y. and Apkarian, A. V. (2008). Parsing Pain Perception Between Nociceptive Representation and Magnitude Estimation. *Journal of Neurophysiology.* 101: 875-887.

Chao, L. L., Haxby, J. V. and Martin, A. (1999). Attribute-based neural substrates in temporal cortex for perceiving and knowing about objects. *Natures Does Neuroscience.* 2(10): 913-919.

de Lafuente, V. and Romo, R. (2006). Neural correlate of subjective sensory experience gradually builds up across cortical areas. *Proceedings of the Natural Academy of Sciences of the United States of America*. 103(39): 14266-14271.

Duncan, J. S. (1996). Magnetic Resonance Spectroscopy. *Epilepsia.* 37(7): 598-605.

Gu, X., Zhou, T. J., Anagnostou, E., Soorya, L., Kolvezon, A., Hof, P. R. and Fan, J. (2017). Heightened brain response to pain anticipation in high-functioning adults with autism spectrum disorder. *European Journal of Neuroscience.* P 1-10. Doi: 10.111/ejn.13598.

Keltner, J. R., Furst, A., Fan, C., Redfurn, R., Inglis, B. and Fields, H. L. (2006). Isolating the modulatory effect of expectation on pain transmission: A functional magnetic resonance imaging study. *The Journal of Neuroscience.* 26(16): 4437-4443.

Kong, J., White, N. S., Kwong, K. K., Vangel, M. G., Rosemann, I. S., Gracely, R. H. and Gollub, R. L. (2006). Using fMRI to dissociate sensory encoding from cognitive evaluation of heat pain intensity. *Human Brain Mapping.* 27:715-721.

Melzack, R. (1999). From the gate to the neuromatrix. *Pain.* 6:121-126.

Morrison, I., Perini, I. and Dunham, J. (2013). Facets and mechanisms of adaptive pain behaviour: predictive regulation and action. *Frontiers in Human Neuroscience.* 7(755): 1-11.

Mullins, P. G., Rowland, L. M., Jung, R. E. and Sibbitt Jr., W. L. (2005). A novel technique to study the brain's response to pain: Proton magnetic resonance spectroscopy. *NeruoImage.* 26: 642-646.

Nielsen, C. S., Staud, R. and Price, D. D. (2009). Individual differences in pain

sensitivity: Measurement, causation, and consequences. *The Journal of Pain.* 10 (3): 231-237.

Pinel, J. P. J. (2003). Biopsychology (5th ed.) pp. 95-98. Boston: Allyn and Bacon

Pleger, B., Ruff, C. C., Blankenburg, F., Bestmann, S., Wiech, K., Stephan, K. E., Capilla, A., Friston, K. J. and Dohn, R. J. (2006). Neural coding of tactile decisions in the human prefrontal cortex. *Journal of Neuroscience.* 26: 12596-12601.

Ploghaus, A., Becerra, L., Borras, C. and Borsook, D. (2003). Neural circuitry underlying pain modulation: expectation, hypnosis, placebo. *Trends in Cognitive Sciences*. 7 (5): 197-200.

Ploghaus, A., Narain, C., Beckmann, C. F., Clare, S., Bantick, S., Wise, R., Matthews, P. M., Rawlins, J. N. P. and Tracey, I. (2001). Exacerbation of pain by anxiety is associated with activity in a hippocampal network. *The Journal of Neuroscience.* 21 (24): 9896-9903.

Ploghaus, A., Tracey, I., Gati, J. S., Clare, S., Menon, R. S., Matthews, P. M. and Rawlins, J. N. P. (1999). Dissociating pain from its anticipation in the human brain. *Science*. 284: 1979-1981.

Roy, M., Shohamy, D. and Wager, T. D. (2012). Ventromedial Prefrontal-Subcortical Systems and the Generation of Affective Meaning. *Trends in Cognitive Sciences.* 16(3): 147-156.

Salomons, T. V., Johnston, T., Backonja, M-M., Shackman, A. J. and Davidson, R. J. (2007). Individual Differences in the Effects of Perceived Controllability on Pain Perception: Critical Role of the Prefrontal Cortex. *Journal of Cognitive Neuroscience.* 19(6): 993-1003.

Sawamoto, N., Honda, M., Okada, T., Hanakawa, T., Kanda. M., Fukuyama, H., Konishi, J. and Shibasaki, H. (2000). Expectation of pain enhances responses to nonpainful somatosensory stimulation in the anterior cingulate cortex and parietal operculum/ posterior insula: an event-related functional magnetic resonance imaging study. *The Journal of Neuroscience.* 20, 19: 7438-7445.

Siegler, R. S. and Opfer, J. E. (2003). The development of numerical estimation: Evidence for multiple representations of numerical quantity. *Psychological Science.* 14: 237-243.

Vogel, S. E., Grabner, R. H., Schneider, M., Siegler, R. S. and Ansari, D. (2013).

Overlapping and distinct brain regions involved in estimating the spatial position of numerical and non-numerical magnitudes: An fMRI study. *Neuropsychologica.* 51: 979-989.

Vogt, B. A. (2005). Pain and emotion interactions in subregions of the cingulate gyrus. *Nature.* 6: 533-544.

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Chapter 4: The effect of experimenter gender, and the inclusion of an observer, on participant pressure-pain threshold ratings.

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Pain is, undoubtedly, a subjective experience and complex to the extent that research into the field is still encountering and exploring new aspects and influential factors. The actual experience of pain is not just as a result of the noxious stimuli, location and intensity, but also arises from the interplay of mental processes, including social factors, which may subconsciously affect the neurological processes that interpret and perceive painful stimuli (Weich, Ploner & Tracey, 2008). A question that arises therefore is; what aspects external to the individual have been found to reliably affect these perceptions, and what processes may be influencing them? Initially the avenue of investigation that preceded this focus was aimed at much broader aspects of biology, such as gender, race and age (Woodrow, Freidman, Siegelaub & Collen, 1972) in order to easily categorise differential responses to pain. However, the development of the theory of a neuromatrix of biological, social and psychological factors that can influence how individuals respond to noxious stimuli led to investigations into how internal and external factors may interact to influence and alter an individuals perception of pain (Melzack, 2005).

The investigation of gender differences has been a predominant field of pain research, especially in the examination of biological differences in pain perception in healthy participants (Fillingim, King, Ribeiro-Sasilva, Rahim-Williams & Riley III, 2009). Reviews examining the differences between genders have noted that although previous research appears to favour the theory that males demonstrate a higher pain tolerance threshold than females, there are a number of contextual factors that can mediate gender-based pain threshold differences, including the method of noxious stimulation utilized. It has been demonstrated that female participants tolerate thermal (heat and cold) and pressure-pain less than males, whereas ischemic pain does not elicit any statistically significant gender disparities (Fillingim et al., 2009; Racine, Tousignant-Laflammem, Kloda, Dion, Dupuis & Choinere, 2012a; 2012b). It has also been shown that influencing participant's gender-role expectations can alter their response to noxious stimuli i.e. by informing the participant how the average man or woman (depending on their own gender) performs during noxious stimulation, researchers were able to eliminate gender-based differences (Robinson, Gagnon, Riley III & Price, 2003). Another established external influence on pain thresholds is that of the experimenter's gender, which appears to act as a psychological or psychosocial mechanism that alters the participants' reports of pain tolerance thresholds.

A number of studies have examined and explored the effect by which experimenter gender has influenced participant's pain tolerance threshold, as well as any underlying mechanisms that may account for it. Initially it was demonstrated that male participants

reported significantly less pain in the presence of a female experimenter compared to a male experimenter, whereas female participants did not appear to exhibit any statistically significant differences between experimenter genders (Levine & DeSimone, 1991; Gijsbers & Nicholson, 2005). Further investigations have also examined apparent professional status, finding that participants tolerated noxious stimulation longer not only when tested by the opposite sex, but also when the experimenter was perceived as being of a higher professional status (Kallai, Barke & Voss, 2004). Participant's autonomic responses (such as heart rate, skin conductance and arousal) have also been recorded and examined in relation to the experimenter gender effect, and it was found that there was a significant effect of experimenter gender on the perceived intensity of noxious stimulation, as well as emotional arousal, but there were no physiological interactions. The researchers also found that males reported lower heat-pain ratings when tested by a female experimenter, while female participants exhibited no significant differences between experimenter genders (Aslasken, Myrbakk, Hoifodt & Flaten, 2007). These results not only supported previous findings, they also indicated through the absence of any physiological changes that psychosocial processes, rather than more direct sexual motives, may elicit the experimenter gender effect.

With the present study we hope to extend these findings and ask the question: "Does the experimenter-gender effect extend beyond the experimenter to the inclusion of an observer (as well as the observer's gender), and if it does, what bio- or psychosocial factors may be involved in eliciting the response?"

The presence of an observer, while not having been examined thoroughly during either clinical or experimental pain trials, has been reported to adversely affect neurophysiological performance during bedside examinations in clinical populations with neurological disorders, particularly if the observer is a significant other (Binder & Johnson-Green, 1995; Kehrer, Sanchez, Habif, Rosenbaum & Townes, 2000; Constantinou, Asgendorf & McCaffrey, 2005). However, previous literature has found that the presence of an observer can cause participants to limit their non-verbal expressions and cause noxious stimuli to be perceived as less intense (Kleck, Vaughan, Cartwright-Smith, Vaughan, Colby & Lanzetta, 1976). This was though to be due to the 'calming effect' the presence of an observer may have. Contrarily, if the participants were focusing their attentional resources on moderating their non-verbal expressions, it may be plausible that their attention was no longer stimuli-orientated, reducing the perceived level of intensity.

The theory of objective self awareness (Duval & Wicklaund, 1972) stipulates that if an individual feels as though they are being evaluated across multiple dimensions, a state of

objective self-awareness may be triggered causing the individual to be more attentive to their physiological and psychological state, in turn altering their perceptions (Duval & Wicklund, 1972). Research supporting this theory has shown that anxiety mediates the process of self-awareness in both social situations and the perception of pain (Spurr & Stopa, 2002), while attentional theories of pain stipulate that it influences cognitive functions by redistributing the attentional focus of the individual from goal-orientated attentive behaviours to stimulus-orientated attentive behaviours, whilst increasing the attentional awareness of the individual to threat-related stimuli (Eyesenk, Derakshan, Santos & Calvo, 2007). Indeed prior studies utilizing fMRI have demonstrated that participants may utilize cognitive strategies, including attentional or cognitive engagement, to modulate the intensity of painful stimuli (Seminowicz, Mikluis & Davis, 2004; Legrain, Van Damme, Eccleston, Davis, Seminowicz & Crombez, 2009). There have been a number of neuroimaging studies that support this theory in relation to noxious stimuli in the form of examining anticipatory anxiety (Ploghaus, Narain, Beckmann, Clare, Bantick, Wise, Matthews, Rawlins & Tracey, 2001; Sawamoto, Honda, Okada, Hanakawa, Kanda, Fukuyama, Konishi & Shibasaki, 2000), as well as the influence of state-specific anxiety (Tsao, Myers, Craske, Bursch, Kim, & Zelter, 2004) measured using the state-trait anxiety inventory (STAI).

Along side anxiety, personality is another biosocial factor that has been proposed to influence pain perception, though the utilization of different methodologies has produced varied results (Jarrett, 2011). For example, using the big five personality inventory (BFI), it has been found that while extroverts are more likely to complain about pain, their thresholds are higher than introverts (Lynn & Eyesenk, 1961). It has also been found that conscientious individuals have a higher pain tolerance compared to non-conscientious individuals (Cray, Springborne, Lotsch, Johnson & Hummel, 2011), and that neuroticism is linked to an increased perception of painful stimuli in women suffering from chronic pain conditions (Malin & Littlejohn, 2012). As such we will use the BFI to investigate possible modulatory effects personality types may have on pain perception.

The aim of our present investigation therefore is to not only replicate the phenomenon of experimenter gender influence on participant's pain reports, but to determine if it extends to the presence and gender of an additional observer. It is expected that the presence of an observer will directly affect pain ratings, and that male participants will give their highest pain pressure threshold (PPTs) when tested by a female experimenter in the presence of a female observer, while female participants may not demonstrate any significant differences.

Measures of STAI and BFI will also be taken to determine subtle interactions between conditions.

Methods

Participants

Fifty-one undergraduate volunteers (30 female) participated in exchange for partial course credit. The study had six different sessions, each completed on different days. Of the 51 participants, 12 (five female) completed fewer than four sessions and were therefore excluded. Of the remaining participants, 33 (24 female) completed all six sessions, three (all male) completed five sessions and three (one female) completed four sessions. Thus, 39 participants were included in the final analysis. Twenty-five of them were female, ages ranged from 18 to 37 (mean=20.68; SD=3.75); the remaining 14 were male, ages ranged from 18 to 23 (mean=20.29; SD=1.33). The gender imbalance between males and females is reflective of the ratio of males to females in the general undergraduate population at Bangor University. Of these 33 participants, 32 were right handed. The Bangor University School of Psychology Ethics Committee approved all study procedures and participants provided written consent.

Observers and Experimenters

Three experimenters collected data during the study. All were post-graduate students. There was one male (age 24-25) and two female experimenters (age 22 and 29). The experimenters had been carefully trained in the acquisition of pain thresholds but were blind to exact study hypotheses. Four post-graduate students served as observers during the data collection sessions; two male observers (aged 40 and 41), and two female observers (aged 22 and 26). The observers were blind to study hypotheses throughout the data collection period.

Materials

A Wagner FPX 50 algometer topped with a round rubber stub 1cm in diameter was used to measure pressure-pain threshold for each participant, which measures in pound-force (lbf; 1 lbf≈4.45 N). This method has proven reliable in previous literature (Chesterton, Sim, Wright & Foster, 2007). The participants also completed four questionnaires. These consisted of a measure of trait anxiety, the State-Trait Anxiety Inventory (STAI-1); a measure of in-the-moment or state anxiety (STAI-2), a five-factor personality inventory, the Big Five personality inventory (BFI); and an experimenter rating questionnaire to examine participants' perceptions of the experimenter in each session.

Procedure

Participants attended the lab in groups ranging between one and eight participants. They were seated in a waiting area and called individually into the test room. Once in the test room, the experimenter used the algometer to assess participants' pressure-pain thresholds (PPT). Participants then returned to the waiting area and completed questionnaires for 10 minutes. They then returned to the test room for a second PPT test, followed by a 10-minute break and a third PPT test. Study sessions lasted approximately 30 minutes.

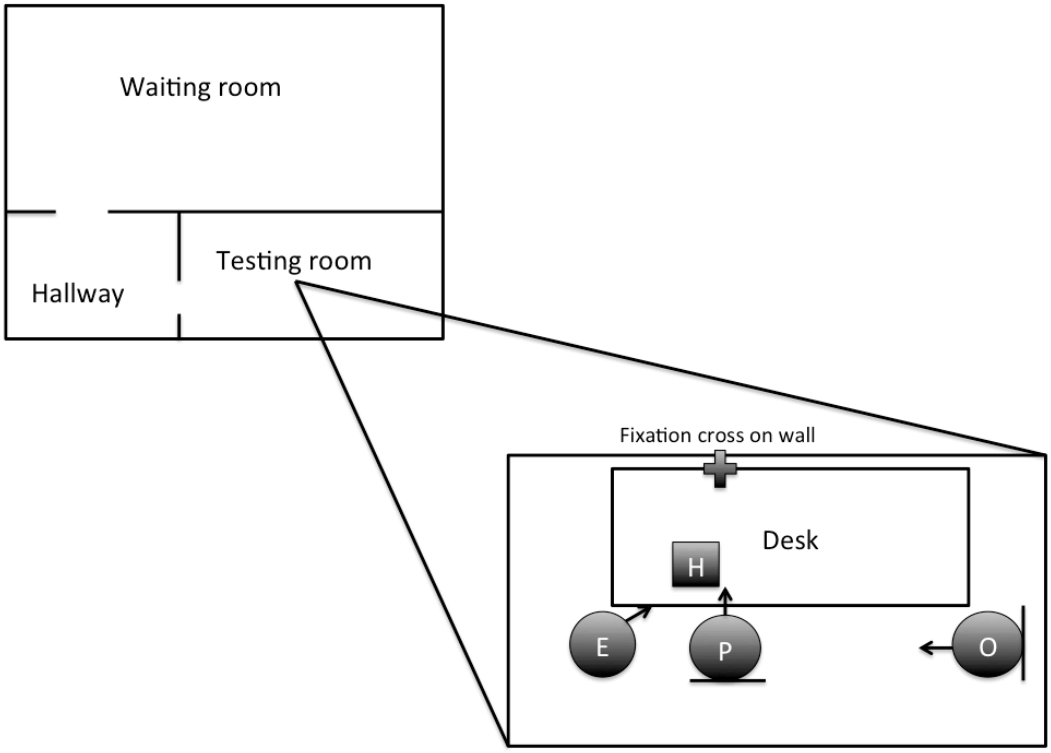

Figure 4.1: Example schematic of testing set up (not to scale). Participants waited and completed questionnaires in the Waiting Room, then completed PPT test in testing room. In the testing room there was a desk and a fixation cross (black on white paper) was affixed to the wall in order to direct participant gaze. Participant's hand was placed on the table in the area marked H, experimenter (E) would take readings while the observer (O) watched. Observer and participant were seated, while experimenter stood. Arrows are representative of gaze orientation.

Each participant completed one session under each of six conditions. In three of the sessions, a male experimenter administered the PPT tests (see Figure 4.1 for an example of the test/waiting room setups). A female experimenter completed the PPT tests in the remaining sessions. In two conditions (one with a female and one with a male experimenter), there was no observer present. In two conditions (one female and one male experimenter), there was a male observer present. In the remaining conditions, the observer was female. The order with which participants completed the study sessions was randomized.

To obtain PPTs, pressure was applied, via the algometer, to the pressure point located in the fleshy webbing between the thumb and index finger of the participant's non-dominant

hand. When participants experienced the sensation shifting from 'uncomfortable' to 'painful', they were instructed to inform the experimenter, who then released the algometer. The experimenters were trained to apply pressure at the rate of approximately 1 lbf (pound-force) per second. While the readings were being taken, participants were instructed to look at a fixation cross on the wall in front of them. This meant that participants were able to view the stimulation site in peripheral vision, without looking directly at it. Research shows that directly viewing the body during painful stimulation can result in an analgesic effect, increasing pain tolerance and threshold (Longo, Betti, Aglioti & Haggard, 2009). However, the anticipation of noxious stimulation may enhance an individual's perception of pain, thereby reducing pain threshold (Sawamoto et al., 2000). This procedure therefore served to minimize both effects. To remind participants of the observer's presence, the observer was seated in a position located such that they were visible in the periphery of participants gaze on the opposite side to the experimenter. Three ratings were collected per session to ensure good reliability of the ratings. Overall, the PPT tests achieved excellent reliability (Cronbach's alpha for each condition: MaleExp, No Obs=.96; MaleExp, MaleObs=.98; MaleExp, FemaleObs=.97; FemaleExp, No Obs=.98; FemaleExp, MaleObs=.99; FemaleExp, FemaleObs=.97).

Between the first and second PPT readings, the participants were asked to complete the questionnaires in the waiting area. In the first test session, the participants were presented with the STAI-1, STAI-2, the BFI, and the experimenter-rating questionnaire. In the subsequent test sessions (2-6), they completed STAI-2 and the experimenter-rating questionnaire following the first PPT test.

The testing took place over two weeks for each participant, with three test conditions per week on each Monday, Wednesday and Friday between 12.00 and 15.00. Some participants missed one or two sessions, and a 'make-up' session was arranged for those who were available and willing. Participants who completed at least four test sessions were included in the dataset. To minimize the effect of this missing data in our analyses, we substituted missed sessions with that participant's grand-average PPT rating. Participants were fully debriefed following their final test condition.

Questionnaires

The State-Trait Anxiety Inventory (STAI) consists of two forms, the Y-1, which instructs the participant to rate how they are feeling right now i.e. state anxiety, and the Y-2, which instructs the participant to indicate how they feel in general i.e. trait anxiety. Both the Y-1 and the Y-2 consist of 20 items, each scored n a four-point Likert scale, wherein 1=

Almost Never, 2= Sometimes, 3= Often, and 4= Almost Always. The Y-1 contains statements such as “I am tense”, “I feel strained” and “I am jittery”, along with 10 reverse-scored questions such as “I feel calm”, “I feel secure” and “I feel comfortable”. The Y-2 contains similar questions, such as “I feel nervous and restless”, “I feel like a failure” and “I lack self-confidence”, along with 7 reverse-scored questions, such as “I feel pleasant”, “I am happy”, and “I am content”. The anxiety score is calculated by adding together the numerical score and reverse-scores for each item, so that a high score is reflective of a high state or trait of anxiety. The STAI was designed to distinguish between anxiety as an emotional state and individual differences in personality traits. It has been demonstrated that the STAI is highly reliable and able to discriminate between high and low stress and anxiety (Spielberger, Gorsuch, Lushene, Vagg & Jacobs, 1983; Metzger, 1976).

The Big Five personality Inventory (BFI) is designed to measure the personality traits of Extroversion, Agreeableness, Conscientiousness, Neuroticism and Openness. It consists of 44 items and participants are instructed to indicate the extent to which they agree or disagree, with each statement beginning with the prefix “I am someone who…” scored on a five-point likert scale, ranging from 1 (Disagree Strongly) to 5 (Agree Strongly), including 16 reverse-scored items. Scale scores for each trait are calculated by averaging scores of the items associated with each trait. Extroversion is calculated using the average of eight items, such as “is talkative” and “is full of energy”, along with reverse scores of items such as “is reserved’ and ‘is sometimes shy, inhibited”. Agreeableness is calculated using the average of nine items, such as “is helpful and useful to others” and “has an assertive personality”, with reverse scored items such as “tends to find fault with others” and “starts quarrels with others”. Conscientiousness is calculated using the averages of nine items such as “does a thorough job” and “is a reliable worker”, with reverse scored items such as “can be somewhat careless” and “tends to be lazy”. Neuroticism is calculated using average scores of eight items such as “is depressed, blue” and “can be tense”, along with reverse scored items such as “is relaxed, handles stress well” and “is emotionally stable, not easily upset”. Finally, Openness is calculated using average scores of seven items such as “is original, comes up with new ideas” and “is curious about many different things”, with reverse scored items such as “prefers work that is routine” and “has few artistic interests”. The BFI has proven to be a very reliable tool for the measuring of, and distinguishing between, these five personality traits (John, Donahue, & Kentle, 1991; John, Naumann & Soto, 2008).

The experimenter-rating questionnaire was designed for the purpose of this study in order to examine whether the participants’ view of the experimenter changes between each

test session, and if it does whether these opinions may influence their PPTs. It consists of six items, each on a seven-point likert scale, where 1= Not at all and 7= Extremely. The questionnaire asked "To what extent did you find the experimenter to be…", with the items "Warm & Friendly", "Attractive", Competent & Professional", "Outgoing", "In Control" and "Trustworthy". Reliability analysis on each item demonstrated high reliability, with each items Chronbach's alpha as follows; Warm and Friendly= .85, Attractive= .95, Competent and Professional= .75, Outgoing= .77, In Control= .70, and Trustworthy= .72. The numerical value for each item was used as that trait's score for each test condition.

Results

PPT analysis

The PPT thresholds for each condition (averaged across the three readings in a session) were run through an independent samples t-test to check whether there were significant differences between males and females, and it was found that in all conditions males had a significantly higher PPT than females. Levene's test of equality of error variance was not violated in five of the six conditions, so it is noted that the test for the female experimenter, control observer condition did violate the assumption of equal variance; Males (M=16.21, SD=7.04), females (M=7.98, SD=4.04), $t(17.905)=-4.021$; $p=.001$. Due to the unequal and sample sizes, the data set was randomly divided into two groups, per sex and condition, and subjected to an independent samples t-test. Of the 48 t-tests, none of them violated the assumption of equal variances, indicating that the results from further analysis may not be due to low power and increasing our confidence in the results. For a full catalogue of the t-test results, please see the supplementary section towards the end of this chapter.

Following the t-tests, the data was subjected to mixed-model ANOVA with experimenter gender (male, female) and observer-level (no observer, male, female) as within-subjects factors, and participant gender (male, female) as the between-subjects factor. There was a significant interaction between experimenter gender and participant gender, $F(1, 37) = .12.77$, $p < .001$, $\eta^2 = .257$, indicating that experimenter gender affected men's and women's pressure-pain thresholds differently. Means and standard deviations appear in Table 4.1. The interaction between observer type and experimenter gender approached significance, $F(2, 36) = 2.97$, $p = .064$, $\eta^2 = .141$, such that the presence , as well as gender, of the observer may have contributed to the experimenter gender effects in each condition, either enhancing the effects or as an extension of them.

There were no other significant interactions (all p-values >.50).

	Male Participants			Female Participants		
Condition	n	M	SD	n	M	SD
Male exp. Male obs.	14	12.77	4.90	25	7.84	3.36
Male exp. No obs.	14	11.81	4.59	25	7.92	3.19
Male exp. Female obs.	14	12.59	4.68	25	8.26	3.13
Female exp. Male obs.	14	15.56	6.90	25	7.74	5.19
Female exp. No obs.	14	16.21	7.04	25	7.98	4.04
Female exp. Female obs.	14	18.18	7.05	25	9.28	5.14

Table 4.1. Means and standard deviations of male and female PPTs in pound-force (lbf) for each condition.

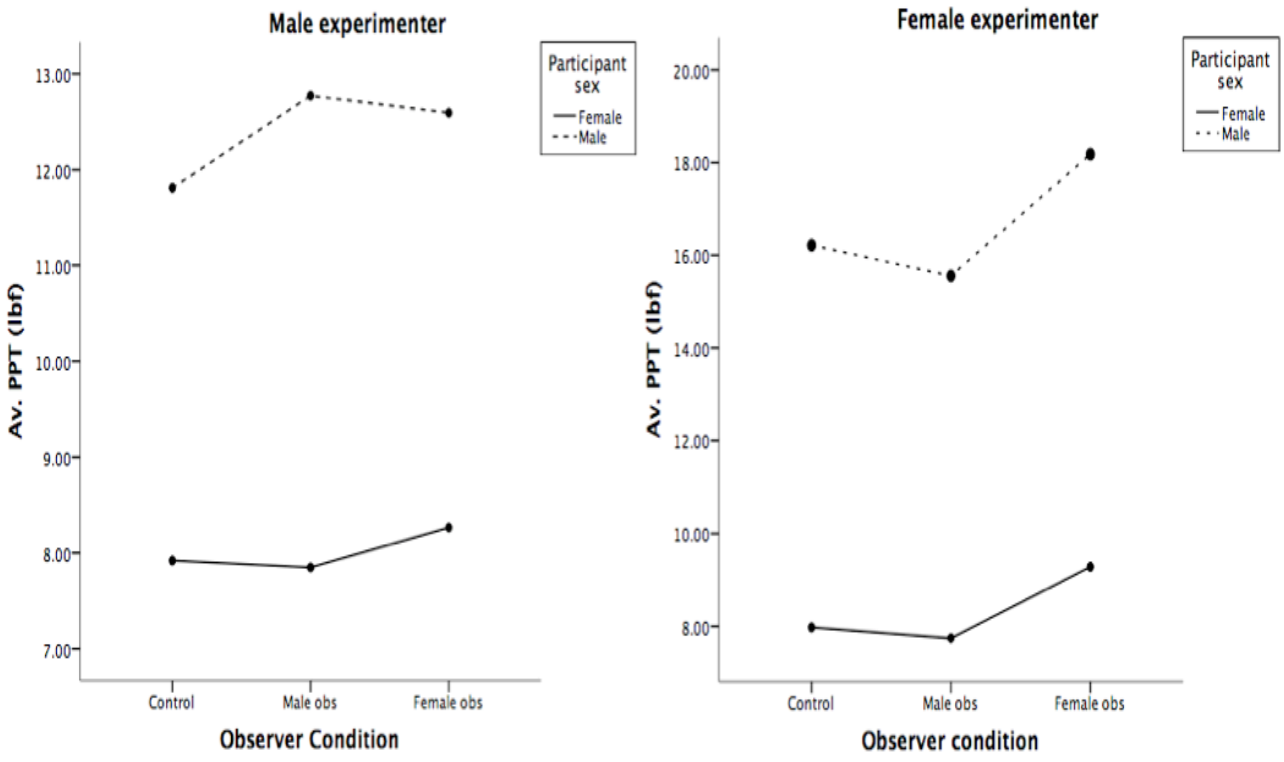

Figure 4.2. Plots from the repeated-measures ANVOA demonstrating the findings for male and female participants' PPT scores in each condition. Results for the male experimenter is on the left, and female experimenter on the right. Each point represents the average scores in each observer (obs) condition.

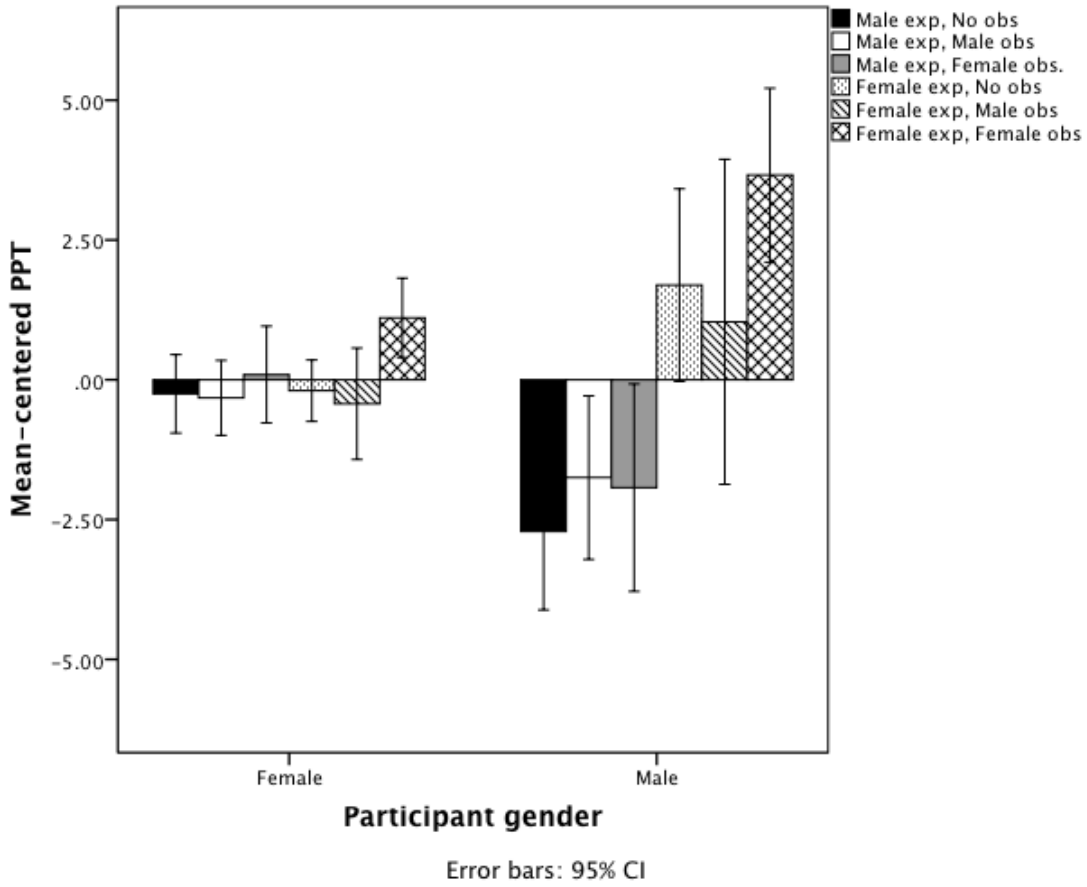

Figure 4.3. Mean-centered data for each condition, calculated by subtracting each participant's overall mean PPT from their mean PPT for each condition. Exp=experimenter, obs=observer.

On average, men tolerated significantly higher levels of pressure pain than did women, $F(1, 37) = 20.71$, $p < .001$, η^2=.359. In addition, there were significant main effects for both experimenter gender, $F(1, 37) = 17.298$, $p < .001$, η^2= .319, and observer-level, $F(1, 37) = 6.273$, p = .005, η^2= .258. Figure 4.3 displays these effects. Specifically, these results show that throughout all conditions, male participants demonstrated a higher PPT than females, regardless of experimenter gender or observer condition.

STAI scores

A similar mixed-model ANOVA (with experimenter gender and observer condition as within-subjects factors, and participant gender as between-subjects factor) was run for the measures of state anxiety taken in each of the six conditions, though no significant interactions or main effects were observed (see Figure 4.4).

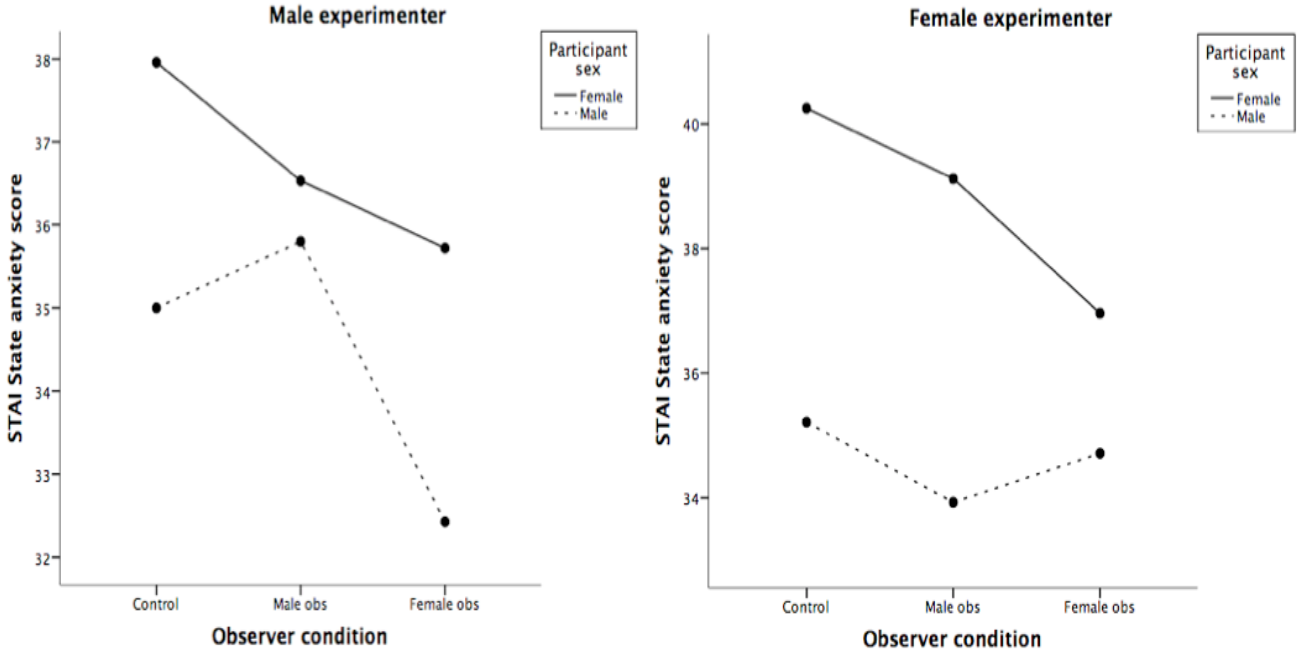

Figure 4.4. Results from state anxiety scores vs. observer condition ANOVA, as with Figure 3, Male experimenter results are on the left while female experimenter results are on the right, with observer (obs) condition displayed along the horizontal axis and the average STAI score per condition is displayed with each point on the charts.

Pearson product-moment correlations were conducted between Overall PPT and Trait anxiety, but the result was not significant overall, r=-.115, n=39, p=.487, r^2=.01, sig.=2-tailed, or when participant genders were analysed separately, males; r=-.096, n=14, p=.774, r^2=.009, sig.=2-tailed; females; r=-.104, n=25, p=.744, r^2=.011, sig.=2-tailed (see Figure 4.5). Similarly, Pearson correlations (2-tailed) were conducted examining the relationships between PPT and state anxiety in each of the six conditions (both with the sample as a whole and when separating by participant gender), but no significant results were found.

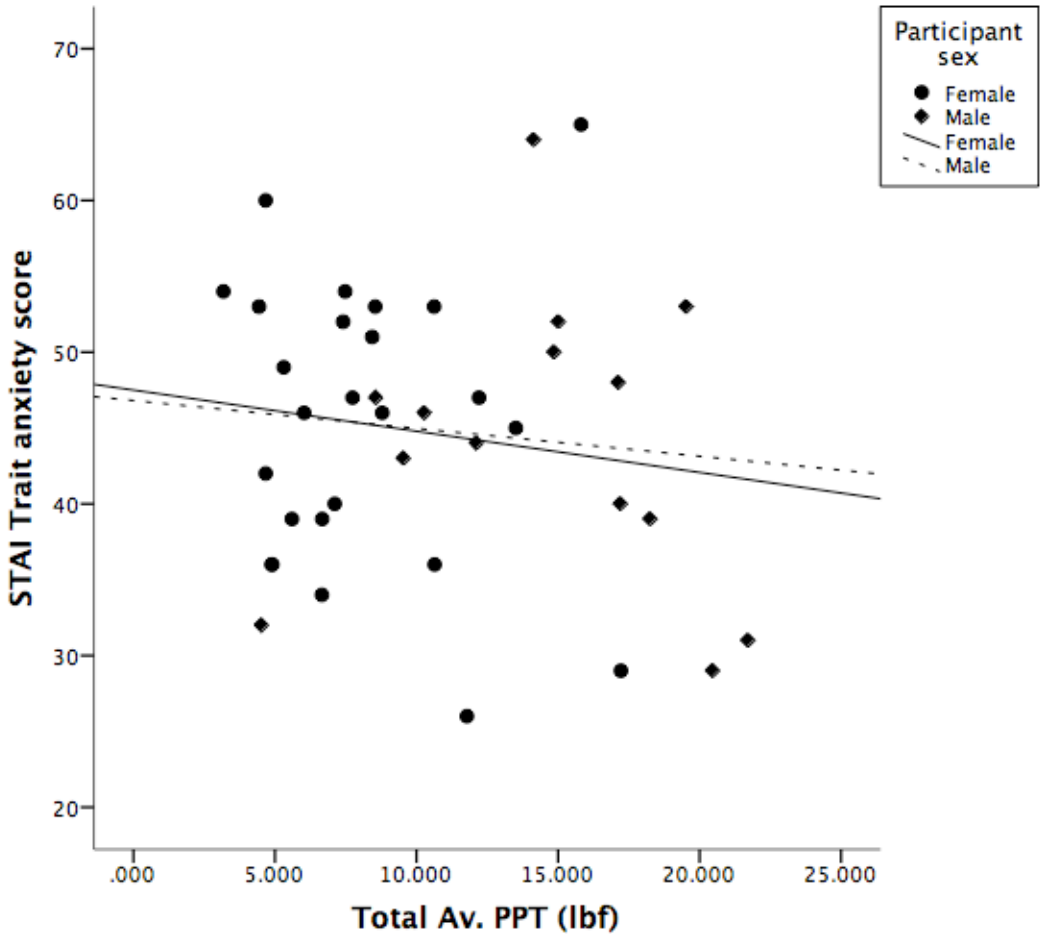

Figure 4.5. Pearsons correlation for male and female Total average PPT (mean of all ratings and all conditions to give participants one score) and Trait anxiety score. No correlation was found.

BFI scores

While exploring the relationships between the overall PPT and results from the BFI, it was found that a significant positive correlation was observed between Overall PPT and Openness, r=.572, n=39, p<.001, r^2=.327, sig.=2-tailed. Further examination revealed that this correlation was only found in females, r=.457, n=25, p=.022, r^2=.208, sig.=2-tailed, and not males, r=.416, n=14, p>.05, r^2=.173, sig.=2-tailed (see Figure 4.6). This would indicate that this is predominantly observed in females, though the increased significance found when analysing the sample as a whole may indicate that males are a contributing factor.

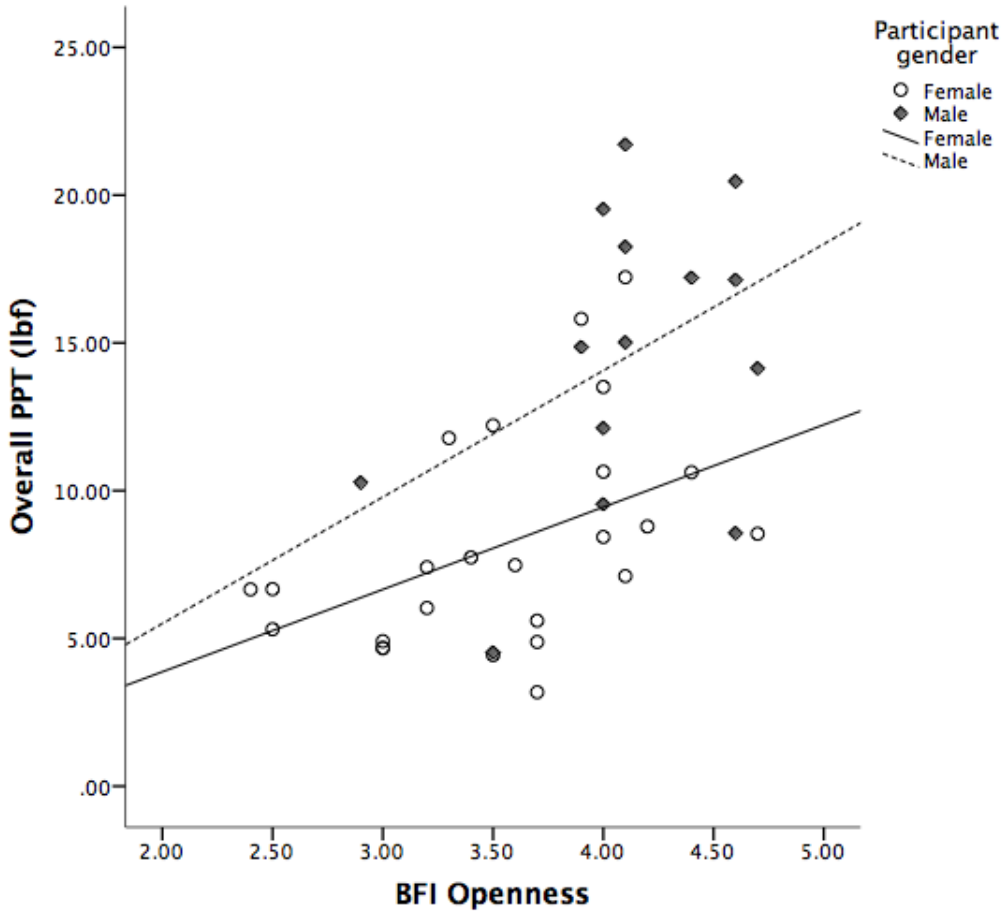

Figure 4.6. Pearson product-moment correlation between average PPT of all conditions (Overall PPT, lbf) and BFI Openness for male and female participants, where only female participants demonstrated a statistically significant correlation.

A MANOVA was conducted in order to examine relationships between the participant's rating of the experimenter and the testing conditions. Using Pillai's trace, there was a significant effect of participant gender on experimenter ratings, V=0.139, F(6, 188)=5.038, p<.001, but there was no effect of either experimenter gender or observer conditions. Separate univariate ANOVAs demonstrated significant effects of participant gender for ratings of 'warm & friendly', F(1,193)=21.09, p<.001, 'attractiveness', F(1, 193)=5.947, p=.016, 'in control', F(1,193)=10.653, p=.001, and 'Trustworthy', F=(1, 193)=10.748, p=.001.

A between-groups ANCOVA was used to examine the effect of experimenter genders and observer condition while controlling for the covariates of state anxiety and for the participants' ratings of experimenter characteristics. The ANCOVA for state anxiety violated the assumption of equality of variance, sig= .02. Adjusting for influences of state anxiety, there were significant main effects of participant gender, F(1, 23.41)=86.937, p>.001,

η^2=.282, and experimenter gender, F(1, 23.41)=12.484, p>.001, η^2=.053, as well as the significant interaction between participant gender and experimenter gender, F(1, 23.41)=8.58, p>.005, η^2=.037.

The ANCOVA for the participants' ratings of the experimenter did not violate any assumptions. Adjusting for influences of the participants' ratings, there was a main effect of participant gender, F(1, 187)=84.82, p>.001, η^2=.312, experimenter gender, F(1, 187)=11.17, p=.001, η^2=.056, and a significant interaction of participant gender and experimenter gender, F(1, 187)=9.86, p>.005, η^2=.050. These indicate that the differences between participant genders and experimenter genders, as well as the effect that experimenter gender has on male and female participants PPT, is not contributable towards state anxiety, or any of the items on the participant rating questionnaire.

Discussion

The results of this experiment reflect previous findings reported in the literature. Male participants demonstrated significantly higher PPTs than females, but this is not surprising due to previously reported findings reviewing gender-based disparities in pressure-pain stimulation (Fillingim et al., 2009; Racine et al., 2012). The significant main effect found for experimenter gender also supports previous literature, especially when taking into consideration the significant interaction between participant gender and experimenter gender. Previous literature has shown that male participants' pain tolerance threshold is higher when tested by a female experimenter, while female participants show no differences when tested by a male or female experimenter, which has been successfully replicated in the current study. Not only that, the results indicate that male participants PPT actually drops below the mean scores when tested by a male experimenter (see Figure 3.1), suggesting that the experimenter gender effect may not only be elicited by female experimenters, and that pain ratings may also be influenced by male experimenters – but in the opposite direction.

The main effect found for the observer conditions supports the hypothesis that the presence of an additional third-party does mediate or influence the participant's reports of pain, though as with the experimenter effect this is predominantly observed in male participants. The interaction between experimenter gender and observer condition did approach significance, which would indicate that the observer effect might act as an extension of the experimenter effect. A number of ways that this finding could be further examined to determine whether the observer effect is an influence or an extension of the experimenter effect. To determine whether it is an extension of the experimenter gender

effect, it could be reduced or removed by eliminating the participants' ability to determine (consciously or subconsciously) the experimenter's gender, by either dressing the experimenter in a style that does not betray their gender. Similarly, by utilising a remote mechanical stimulus to remove the experimenter whilst the observer remains present for the testing, the experimenter gender effect could be eliminated, and it could be assumed that if the presence and gender of the observer still has an effect, it would indicate that it is a similar, yet distinct effect. In either scenario, if there is no observer effect once the experimenter gender effect has been removed it could be deduced that it is an extension of the experimenter gender effect, rather than a contributing factor.

One of the primary limiting factors to consider with these results is that of the sample size, especially due to the disparities between the male and female sample size. While both the lack of variation in the female participants PPTs and large variation of the male participants PPTs between conditions is congruent with previous reports of pain tolerance mediations by experimenter gender, it is plausible this result may be examined more thoroughly with more rigorous testing and by increasing the number of males, though reports from previous literature would support the expectation that more males may enhance this effect. With larger, equal sample sizes a more accurate picture of the effect may be painted. It is of note, however, that when randomly splitting the male and female PPT scores per condition and analysing the differences between groups, there were no significant differences found, which in turn improves our confidence in the results presented here.

The effect the presence of an observer has on pain perception is of interest due the potential implications it may present for clinical environments such as in hospitals where nurses, trainees, staff and other family members may be present during patient assessments, especially in instances of both chronic and acute pain. Further investigation into this observer effect would warrant the implementation of a gender-neutral observer condition, possibly in the form of a camera or other electronic recording equipment such as those utilized in clinical appointments for training purposes or remote assessments. As well as the removal of a physical observer, it may be worth considering the inclusion of more than one observer to the assessment environment. This would allow examination of whether this effect is a simple binary no observer/observer effect, or if it increases incrementally depending on how may observers there are. If the presence of an observer is inducing the theorised state of objective self-awareness, it may indeed be plausible that the inclusion of further observers would proportionately increase this effect, in the similar manner as how it may be triggered when

one addresses a large group compared to a smaller one in those who may fear public speaking.

The lack of effects and correlations for both state and trait anxiety suggest that anxiety is not a determining factor in mediating experimenter effects on pain responses in healthy participants. A majority of studies examining anxiety-related states of objective self-awareness have investigated the effect in patients with either social anxiety or phobia (Spurr & Stopa, 2002), or in patients with chronic pain (Mccraken, Gross, Aikens & Carnrike, 1996; Vaughn, Wichowski & Bosworth, 2007). These findings indicate that although state and trait anxiety may be a contributing factor to pain perception in clinical populations, it may not be a factor in healthy populations, and does not seem to be involved in the well documented experimenter, or "white coat" effect. This can also be seen from the ANCOVA results, which demonstrated that STAI scores were not a confounding variable between conditions in relation to PPT scores.

One potentially relevant theory surrounding the impacts of social aspects on pain perception comes in the form of the brain opioid theory of social attachment. This theory started gaining traction from observed similarities between human social relationships and individuals addicted to opioid-based narcotics. In a discussion on the subject, Machin and Dunbar (2011) outlines three of the stages that are evidenced in parallel between these two populations; First there is an initial phase in which individuals feel euphoric, which is then followed by addiction due to the role that endorphins play on the neurological reward system. Next comes a stage of tolerance-habituation. In social relationships this can manifest in the manner by which relationships evolve from "romantic to companionate love". Similarly in those with opioid addictions they can find that they need more and more opioids to achieve the same high. The final phase outlined by Machin and Dunbar is that of intense withdrawal if the source of addiction is removed, which can be seen as separation distress in a social context. The reason that these social behaviours can be seen as similar to those of opioid addiction is due to the manner in which β-endorphins, a powerful endogenous opioid peptide involved in the modulation of both physical and emotional pain and stress, as well as a role in neurological reward systems, binds to μ-opioid receptor cells in the nervous system as a mechanism of social bonding. The relationship between the β-endorphins and μ-opioid receptors operates in much the same way as opioid-based painkillers (such as morphine) do, including having analgesic properties to reduce the impact of noxious stimulation. Recently, it has been shown that there is a positive correlation between the size of individual human's social networks and pain tolerance, insofar as the more people participants considered as

having within their social network, the higher their pain tolerance (Johnson & Dunbar, 2016). The implications of this study does fit with previous research examining differences in male and female coping strategies, especially considering that females tend to seek out social contact and aid when they are experiencing pain, whereas males tend to focus intrinsically. It may also play a role in the currently proposed observer effect, which could turn a simple assessment of pain tolerance into a more socially perceived affair. While a direct conclusion cannot be drawn in the present study, it may offer an additional factor to examine in the future, especially considering the lack of findings in regards to the STAI scores. By adding in a questionnaire such as the International Personality Item Pool, which was utilised by Johnson and Dunbar (2016), we could gage participants' social networks and see whether there was a relationship between that and the observer conditions. Further questions that could be addressed could include whether the observer effect, if replicable, would be dependent or relative to how comfortable the participants are with strangers, or does it still occur if the observer is familiar to the participants, such as a friend or peer might be. It is worth noting, however, that although the brain opioid theory of social attachment may be able to account for social modulations of pain perception, it does not account for the experimenter gender effect that is also discussed in the present research.

The findings associated with the BFI, that is, the correlation between Openness and overall PPT, are unexpected. A majority of studies examining relationships between personality and pain ratings, in either healthy or clinical populations, have documented effects of extroversion, conscientiousness and neuroticism (Lynn & Eyesenk, 1961; Cray et al., 2011; Malin & Littlejohn, 2012), but there do not appear to be any findings linked to openness. In fact, taking into consideration the examples of pain perception and social networks, it could possibly explain why there has been such a mixed opinion on any correlations with BFI results, particularly when pertaining to extroversion. In theory, one who is more extroverted in nature may find themselves more inclined towards socialisation and develop a more diverse network; however, extroversion is not a direct indication of social life or success, it is merely a factor by which one may be considered outgoing, similarly with conscientiousness. In regards to the results found in this study, as this was a voluntary study it is plausible that those who participated were more open to the experience of participation. Similarly is also possible that the more open to experiences the participant was, the more stimulation they were able to tolerate before experiencing pain. Either way, the lack of literature available on the subject suggests further investigation to examine this result more fully is warranted. Understanding personality factors in pain perception may lead to a more

reliable understanding of how psychosocial factors interact with stimuli and contextual arrangement, especially considering emerging evidence regarding some social factors, which could possibly enable us to assess clinical pain and discomfort with greater precision, in turn allowing us to more accurately evaluate treatment options, and therefore pursue the most effective, or more accurately tailored method of treatment.

When examining the participant's experimenter ratings as covariates, they were not found to affect their PPTs. This lack of effect for the ratings of attractiveness could support results in previous literature, which found that autonomic responses did not relate to experimenter-based variances of pain reports (Aslaken et al., 2007), suggesting a non-sexual motivation behind the effect, but it would be interesting to see how sexual orientation could factor into this effect. However, these findings seem at odds with previous investigations that examined the influence of perceived professionalism (Kallai, Barke & Voss, 2004). This may be due to a lack of intentional manipulation of the perceived levels of professionalism and the lack of variance between professional levels in the experimenters (i.e. postgraduate students). Either way, the lack of effects found of the participants perceptions of the experimenter on their PPTs provides further evidence that the experimenter gender effect (and, by extension, the observer effect) is not mediated by conscious or overt thoughts or perceptions that the participants have of the experimenter.

To address this observer effect from an informal perspective, the presence of an observer effect seems fairly understandable. As previously mentioned, being observed can alter one's attentional focuses to become task-specific focus as opposed to goal orientated, which can be witnessed in day-to-day life. Many people can attest to instances where the achieve some small victory or trick, they go to a peer who may then request verification, and the response can be along the lines of "well, I won't be able to now you're watching!" Another example of this effect can be evidenced from public speaking, as mentioned previously, where an individual can experience anxiety concerning whether they may make a mistake or it would be obvious that they are nervous. Personally, I am usually concerned that the audience will notice my hands are shaking, or that I make phonological errors such as lisping, incorrect pronunciation, stuttering or "ums". Another example could be whilst one is at work, doing the same tasks 100 times a day with no issue, but as soon as an authoritative figure checks in on the quality of work or productivity, that same task suddenly becomes a lot harder to do and one has to focus intently to ensure that no errors, which would normally rarely occur, are made. Of course, these examples are purely from an observational, anecdotal, and informal opinion of social experiences, rather than those backed by scientific

evidence, although there are some relevant articles discussing the role of attentional focus and its affect on pain perception, as outlined in the introductory segment of this chapter. Perhaps a more direct method to enhance or induce an observer effect would be to tell the participant that the observer will be watching and assessing their behaviour/performance/reaction to the pain-rating task, possibly making it appear as a formal capacity by having the observer (seemingly) write notes using, for example, a clipboard to add to the possible sense of being assessed.

It is apparent that the presence of an additional observer influences pain ratings and that it may be an extension of the experiment gender effect. However results also suggest it is possible that the observer effect could be separate and may enhance the experimenter effect, as seen in the female experimenter, female observer conditions where both male and female participants PPTs were at their highest. Given that the implications of the observer effect extend beyond the laboratory and into everyday clinical environments, where it may be commonplace for further third-party staff or family members to be present during patient interviews, we feel a further understanding of the possible mediators on the observer effect is not only of interest to those involved in pain research, but also for clinical practitioners. Pain, especially chronic pain, is not always taken seriously in society, as there are no real quantifiable tests to objectively assess it, or a method to accurately gage how one may feel past verbal descriptions. As such, understanding factors such as these that may influence how one perceives pain are not only of interest for accurate assessments and treatment, they may be deemed crucial in allowing clinicians to properly alleviate patients' suffering. That none of the psychosocial or biosocial measurements taken showed an underlying influence on this effect suggests that there is a factor other then anxiety or social interactions mediating the effect. Further investigation into the observer effect should include the use of electronic equipment to examine whether it is the physical presence (and gender) of the observer or just the concept of being observed that elicits the effect (Duval & Wicklund, 1972), and whether this effect can be incrementally increased as more observers are added into the assessment. Given the subjective nature of pain and pain reporting, it is important that we fully understand all the determinants of this complex process, and that the interplay of experimenter and observers on participant and possibly patient reports should be considered when investigating or asking about pain. To be able to relieve the suffering of those in pain by establishing the cause, what may influence perceptions and even whether the pain they experience is a symptom of a condition or a condition itself is, or should be, of the utmost importance. The impacts of this avenue of research may not change the whole world, but for

those suffering from acute or chronic pain it may change their world, or at least how they perceive it.

Chapter 4: Supplementary results

Initial t-tests refer to the undivided analysis, while Split 1-4 refers to each of the four split-file analyses.

Initial t-test results

- Male Experimenter, Observer Control

Males (M=11.81, SD=4.52) vs. females (M=7.92, SD=3.19), $t(37)= -3.11$; $p=.004$.

- Male Experimenter, Male Observer

Males (M=12.77, SD=4.89) vs. females (M=7.85, SD=3.36), $t(37)= -3.178$; $p=.001$.

- Male Experimenter, Female Observer

Males (M=12.59, SD=4.78) vs. females (M=8.26, SD=3.13), $t(37)= -3.463$; $p=.001$.

- Female Experimenter, Observer Control

Males (M=16.21, SD=7.04) vs. females (M=7.98, SD=4.04), $t(17.905)= -4.021$; $p=.001$.

- Female Experimenter, Male Observer

Males (M=15.56, SD=6.90) vs. females (M=7.74, SD=5.19), $t(37)= -4.001$; $p<.001$.

- Female Experimenter, Female Observer

Males (M=18.18, SD=7.05) vs. females (M=9.28, SD=5.14), $t(37)= -4.533$; $p<.001$.

Split 1; Female participants

- Male Experimenter, Observer Control

Group 1 (n=13, M=7.91, SD=2.76) vs. Group 2 (n= 12, M=7.93, SD=3.73), $t(23)= -.010$; $p=.992$.

- Male Experimenter, Male Observer

Group 1 (n=13, M=8.23, SD=3.23) vs. Group 2 (n= 12, M=7.44, SD=3.59), $t(23)= .577$; $p=.57$.

- Male Experimenter, Female Observer

Group 1 (n=13, M=8.44, SD=3.21) vs. Group 2 (n= 12, M=8.07, SD=3.16), $t(23)= 292$; $p=.773$.

- Female Experimenter, Observer Control

Group 1 (n=13, M=8.33, SD=4.92) vs. Group 2 (n= 12, M=7.60, SD=3.00), $t(23)= .446$; $p=.660$.

- Female Experimenter, Male Observer

Group 1 (n=13, M=7.97, SD=6.25) vs. Group 2 (n= 12, M=7.49, SD=4.01), $t(23)= .227$; $p=.823$.

- Female Experimenter, Female Observer

Group 1 (n=13, M=9.21, SD=4.96) vs. Group 2 (n= 12, M=9.36, SD=4.83), *t*(23)= -.074; *p*=.942.

Split 1; Male participants

- Male Experimenter, Observer Control

Group 1 (n=7, M=13.04, SD=4.96) vs. Group 2 (n= 7, M=10.58, SD=4.20), *t*(12)= .999; *p*=.338.

- Male Experimenter, Male Observer

Group 1 (n=7, M=12.03, SD=4.60) vs. Group 2 (n= 7, M=13.52, SD=5.43), *t*(12)= -.555; *p*=.589.

- Male Experimenter, Female Observer

Group 1 (n=7, M=13.13, SD=4.66) vs. Group 2 (n= 7, M=12.06, SD=5.00), *t*(12)= .416; *p*=.685.

- Female Experimenter, Observer Control

Group 1 (n=7, M=17.18, SD=7.17) vs. Group 2 (n= 7, M=15.25, SD=7.35), *t*(12)= .499; *p*=.627.

- Female Experimenter, Male Observer

Group 1 (n=7, M=14.45, SD=7.75) vs. Group 2 (n= 7, M=16.67, SD=6.35), *t*(12)= -.587; *p*=.568.

- Female Experimenter, Female Observer

Group 1 (n=7, M=16.91, SD=7.56) vs. Group 2 (n= 7, M=19.56, SD=6.84), *t*(12)= -.661; *p*=.521.

Split 2; Female participants

- Male Experimenter, Observer Control

Group 1 (n=13, M=7.70, SD=3.39) vs. Group 2 (n= 12, M=8.16, SD=3.28), *t*(23)= -.350; *p*=.730.

- Male Experimenter, Male Observer

Group 1 (n=13, M=7.49, SD=3.39) vs. Group 2 (n= 12, M=8.24, SD=3.43), *t*(23)= -553; *p*=.586.

- Male Experimenter, Female Observer

Group 1 (n=13, M=7.94, SD=3.47) vs. Group 2 (n= 12, M=8.62, SD=2.81), *t*(23)= -.553; *p*=.598.

- Female Experimenter, Observer Control

Group 1 (n=13, M=7.86, SD=4.50) vs. Group 2 (n= 12, M=8.11, SD=3.68), *t*(23)= -.151; *p*=.882.

- Female Experimenter, Male Observer

Group 1 (n=13, M=7.99, SD=5.59) vs. Group 2 (n= 12, M=7.48, SD=4.96), *t*(23)= .240; *p*=.813.

- Female Experimenter, Female Observer

Group 1 (n=13, M=9.18, SD=5.79) vs. Group 2 (n= 12, M=9.39, SD=4.58), *t*(23)= -.102; *p*=.919.

Split 2; Male participants

- Male Experimenter, Observer Control

Group 1 (n=7, M=12.13, SD=5.38) vs. Group 2 (n= 7, M=11.49, SD=4.06), *t*(12)= .253; *p*=.805.

- Male Experimenter, Male Observer

Group 1 (n=7, M=13.41, SD=4.90) vs. Group 2 (n= 7, M=12.14, SD=5.20), *t*(12)= .472; *p*=.646.

- Male Experimenter, Female Observer

Group 1 (n=7, M=14.06, SD=5.30) vs. Group 2 (n= 7, M=11.13, SD=5.20), *t*(12)= 1.191; *p*=.256.

- Female Experimenter, Observer Control

Group 1 (n=7, M=16.12, SD=6.84) vs. Group 2 (n= 7, M=16.31, SD=7.80), *t*(12)= -.046; *p*=.964.

- Female Experimenter, Male Observer

Group 1 (n=7, M=16.76, SD=5.52) vs. Group 2 (n= 7, M=14.36, SD=8.33), *t*(12)= .636; *p*=.537.

- Female Experimenter, Female Observer

Group 1 (n=7, M=20.32, SD=6.12) vs. Group 2 (n= 7, M=16.05, SD=7.72), *t*(12)= 1.147; *p*=.274.

Split 3; Female participants

- Male Experimenter, Observer Control

Group 1 (n=14, M=8.27, SD=2.61) vs. Group 2 (n= 11, M=7.48, SD=3.90), *t*(23)= .608; *p*=.549.

- Male Experimenter, Male Observer

Group 1 (n=14, M=8.67, SD=3.43) vs. Group 2 (n= 11, M=6.80, SD=3.09), *t*(23)= 1.408; *p*=.173.

- Male Experimenter, Female Observer

Group 1 (n=14, M=8.89, SD=2.89) vs. Group 2 (n= 11, M=7.46, SD=3.37), *t*(23)=-4.141; *p*=.265.

- Female Experimenter, Observer Control

Group 1 (n=14, M=9.26, SD=4.49) vs. Group 2 (n= 11, M=6.34, SD=2.79), *t*(23)= 1.890; *p*=.071.

- Female Experimenter, Male Observer

Group 1 (n=14, M=9.16, SD=6.01) vs. Group 2 (n= 11, M=5.95, SD=3.39), *t*(23)= 1.581; *p*=.128.

- Female Experimenter, Female Observer

Group 1 (n=14, M=10.31, SD=5.34) vs. Group 2 (n= 11, M=7.97, SD=4.79), *t*(23)= 1.133; *p*=.269.

Split 3; Male participants

- Male Experimenter, Observer Control

Group 1 (n=7, M=10.52, SD=3.97) vs. Group 2 (n= 7, M=13.10, SD=5.11), *t*(12)= -1.057; *p*=.311.

- Male Experimenter, Male Observer

Group 1 (n=7, M=11.53, SD=5.42) vs. Group 2 (n= 7, M=14.02, SD=4.37), *t*(12)= -.945; *p*=.363.

- Male Experimenter, Female Observer

Group 1 (n=7, M=12.26, SD=5.99) vs. Group 2 (n= 7, M=12.93, SD=3.38), *t*(12)= -.260; *p*=.799.

- Female Experimenter, Observer Control

Group 1 (n=7, M=15.11, SD=6.62) vs. Group 2 (n= 7, M=17.32, SD=7.80), *t*(12)= -.571; *p*=.578.

- Female Experimenter, Male Observer

Group 1 (n=7, M=13.28, SD=6.34) vs. Group 2 (n= 7, M=17.84, SD=7.13), *t*(12)= -1.265; *p*=.230.

- Female Experimenter, Female Observer

Group 1 (n=7, M=16.31, SD=8.80) vs. Group 2 (n= 7, M=20.05, SD=4.72), *t*(12)= -.991; *p*=.341.

Split 4; Female participants

- Male Experimenter, Observer Control

Group 1 (n=12, M=7.61, SD=4.06) vs. Group 2 (n= 13, M=8.21, SD=2.26), *t*(23)= -.464; *p*=.647.

- Male Experimenter, Male Observer

Group 1 (n=12, M=7.32, SD=3.53) vs. Group 2 (n= 13, M=8.34, SD=3.25), *t*(23)= -.752; *p*=.460.

- Male Experimenter, Female Observer

Group 1 (n=12, M=8.45, SD=3.43) vs. Group 2 (n= 13, M=8.09, SD=2.94), *t*(23)= .285; *p*=.778.

- Female Experimenter, Observer Control

Group 1 (n=12, M=7.79, SD=4.83) vs. Group 2 (n= 13, M=8.15, SD=3.35), *t*(23)= -.217; *p*=.830.

- Female Experimenter, Male Observer

Group 1 (n=12, M=7.57, SD=5.74) vs. Group 2 (n= 13, M=7.90, SD=4.87), *t*(23)= -.155; *p*=.878.

- Female Experimenter, Female Observer

Group 1 (n=12, M=9.51, SD=6.15) vs. Group 2 (n= 13, M=9.07, SD=4.25), *t*(23)= .211; *p*=.835.

Split 4; Male participants

- Male Experimenter, Observer Control

Group 1 (n=8, M=11.77, SD=3.99) vs. Group 2 (n= 6, M=11.87, SD=5.71), *t*(12)= -.039; *p*=.526.

- Male Experimenter, Male Observer

Group 1 (n=8, M=13.53, SD=3.84) vs. Group 2 (n= 6, M=11.76, SD=6.30), *t*(12)= .653; *p*=.526.

- Male Experimenter, Female Observer

Group 1 (n=8, M=12.42, SD=3.71) vs. Group 2 (n= 6, M=12.82, SD=6.13), *t*(12)= -.151; *p*=.883.

- Female Experimenter, Observer Control

Group 1 (n=8, M=16.80, SD=5.01) vs. Group 2 (n= 6, M=15.44, SD=9.63), *t*(12)= .344; *p*=.737.

- Female Experimenter, Male Observer

Group 1 (n=8, M=16.22, SD=7.08) vs. Group 2 (n= 6, M=14.68, SD=7.21), *t*(12)= .400; *p*=.696.

- Female Experimenter, Female Observer

Group 1 (n=8, M=17.91, SD=5.54) vs. Group 2 (n= 6, M=18.54, SD=9.28), *t*(12)= -.158; *p*=.877.

Chapter 4 References

Aslaken, P. M, Myrbakk, I., N., Hoifodt, R. S. and Flaten, M. A. (2007). The effect of experimenter gender on autonomic and subjective responses to pain stimuli. *Pain*. 129: 260-268.

Binder, L. M. and Johnson-Green, D. (1995). Observer effects on neuropsychological performance: A case report. *Clinical Neuropsychologist.* 9 (1): 74-78.

Chesterton, L. S., Sim, J., Wright, C. C. and Foster, N. E. (2007)Interrater reliability of Algometry in measuring pressure pain thresholds in healthy humans, using multiple raters. *Clinical Journal of Pain*. 23(9), pp 760-766.

Constantinou, M., Asgendorf, L. and McCaffrey, R. J. (2005). Effects of a third party observer during neuropsychological assessment: when the observer is a video camera. *Journal of Forensic Neuropsychology.* 4 (2): 39-47.

Cray, I., Springborn, M., Lotsch, J., Johnson, A. N. B. and Hummel, T. (2011). Agreeable smellers and sensitive neurotics - correlations among personality traits and sensory thresholds. *PLOS ONE.* 6(4).

Duval, S. and Wicklund, R. A. (1972). A theory of objective self-awareness. London: Academic Press.

Eyesenck, M. W., Derakshan, N., Santos, R. and Calvo, M. G. (2007). Anxiety and cognitive performance: Attentional control theory. *Emotion.* 7(2): 336-353.

Fillingim, R. B., King, C. D., Ribeiro-Sasilva, M. C., Rahim-Williams, B. and Riley III, J. L. (2009). Sex, gender and pain: A review of recent clinical and experimental findings. *The Journal of Pain*. 10 (5): 447-485.

Gijsbers, K. and Nicholson, F. (2005). Experimental pain thresholds influenced by sex of the experimenter. *Perceptual Motor Skills*. 101: 803-807.

Jarrett, C. (2011). Ouch! The different ways people experience pain. *The Psychologist.* 24(6): 416-420.

John, O. P., Donahue, E. M., and Kentle, R. L. (1991). The Big Five Inventory—Versions 4a and 54. Berkeley, CA: University of California, Berkeley, Institute of Personality and Social Research.

John, O. P., Naumann, L. P., and Soto, C. J. (2008). Paradigm shift to the integrative Big Five trait taxonomy: History, measurement, and conceptual issues. In O. P. John, R. W. Robins, & L. A. Pervin (Eds.), Handbook of personality: Theory and research (pp. 114-158). New York, NY: Guilford Press.

Johnson, K. V. A, and Dunbar, R. I. M. (2016). Pain tolerance predicts human social network size. *Scientific Report.* 6(25267): 1-5.

Kallai, I., Barke, A. and Voss, U. (2004). The effects of experimenter characteristics on pain reports in women and men. *Pain*. 112: 142-147.

Kehrer, C. A., Sanchez, P. N., Habif, U., Rosenbaum, G. J. and Townes, B. D. (2000). Effects of significant-other observer on neuropsychological test performance. *The Clinical Neuropsychologist*. 14 (1): 67-71.

Kleck, R. E., Vaughan, R. C., Cartwright-Smith, J., Vaughan, K. B., Colby, C. Z. and Lanzetta, J. T. (1976). Effects of being observed on expressive, subjective, and physiological responses to painful stimuli. *Journal of Personality and Social Psychology*. 34 (6): 1211-1218.

Legrain, V., Van Damme, S., Eccleston, C., Davis, K. D., Seminowicz, D. A. and Crombez G. (2009). A neurocognitive model of attention to pain: behavioural and neuroimaging evidence. *Pain*. 144(3). Pp230-232.

Levine, F. M. and De Simone, L. L. (1991). The effects of experimenter gender on pain reports in male and female subjects. *Pain*. 44: 69-72.

Longo, M. R., Betti, V., Aglioti, S. M. and Haggard, P. (2009). Visually induced analgesia: seeing the body reduces pain. *The Journal of Neuroscience.* 29 (39): 12125-12130.

Lynn, R. and Eyesenk, H. J. (1961). Tolerance for pain, extraversion and neuroticism. *Perceptual Motor Skills.* 12: 161-162.

Machin, A. J. and Dunbar, R. I. M. (2011). The Brain Opioid Theory of Social Attachment: A Review of the Evidence. *Behaviour.* 148(9-10): 985-1025.

Malin, K. and Littlejohn, G. O. (2012). Neuroticism in young women with fibromyalgia links to key clinical features. *Pain Research and Treatment*.

McCracken, L. M., Gross, R. T., Aikens, J. and Carnrike, Jr., C. L. M. (1996). The assessment of anxiety and fear in persons with chronic pain: A comparison og instruments. *Behaviour Research and Therapy.*34(11-12). Pp 927-933.

Melzack, R. (2005). Evolution of the neuromatrix theory of pain. The Prithvi Lecture: Presented at the third Wold Congress of World Institute of Pain. Barcelona 2004. *Pain Prac.* 5:85-94.

Metzger, R. L. (1976), A reliability and validity study of the State-Trait Anxiety Inventory. *Journal of Clinical Psychology.* 32(2). Pp 276-278.

Ploghaus, A., Narain, C., Beckmann, C. F., Clare, S., Bantick, S., Wise, R.,

Matthews, P. M., Rawlins, J. N. P. and Tracey, I. (2001). Exacerbation of pain by anxiety is associated with activity in a hippocampal network. *The Journal of Neuroscience.* 21(24): 9896-9903.

Racine, M., Tousignant-Laflamme, Y., Kloda, L. A., Dion, D., Dupuis, G. and Choinere, M. (2012). A systematic literature review of 10 years of research of sex/gender and experimental pain perception – part 1: Are there really differences between women and men? *Pain.* 153, pp 602-618.

Robinson, M. E., Gagnon, C. M., Riley III, J. L and Price, D. D. (2003). Altering gender role expectations:effects on pain tolerance, pain threshold, and pain ratings. *The Journal of Pain*. 4 (5): 284-288.

Sawamoto, N., Honda, M., Okada, T., Hanakawa, T., Kanda. M., Fukuyama, H., Konishi, J. and Shibasaki, H. (2000). Expectation of pain enhances responses to nonpainful somatosensory stimulation in the anterior cingulate cortex and parietal operculum/ posterior insula: an event-related functional magnetic resonance imaging study. *The Journal of Neuroscience.* 20, 19: 7438-7445.

Seminowicz, D. A., Mikulis, D. J. and Davis, K. D. (2004). Cognitive modulation of pain-related brain responses depends on behavioural strategy. *Pain.* 12(1-2). Pp. 48-58.

Spielberger, C. D., Gorsuch, R. L., Lushene, R., Vagg, P. R., and Jacobs, G. A. (1983). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press.

Spurr, J. M. and Stopa, L. (2002). The observer perspective: effects on social anxiety and performance. *Behaviour Research and Therapy.* 40, Pp 1009-1028.

Tsao, J. C. I., Myers, C. D., Craske, M. G., Bursch, B., Kim, S. C. and Zeltzer, L. K. (2004). Role of anticipatory anxiety and anxiety sensitivity in children's and andolescent's laboratory pain responses. *Journal of Pediatric Psychology.* 29(5): 379-388.

Vaughn, F., Wichowski, H. and Bosworth, G. (2007). Does preoperative anxiety level predict postoperative pain? *AORN Journal.* 85(3). Pp 589-594, 597-604.

Wiech, K., Ploner, M. and Tracey, I. (2008). Neurocognitive aspects of pain perception. *Trends in Cognitive Sciences.* 12(8): 206-313

Woodrow, K. M., Freidman, G. D., Siegelaub, A. B. and Collen, M. F. (1972). Pain tolerance: differences according to age, sex and race. *Psychosomatic Medicine.* 34: 548-556.

Chapter 5: Evidence for regional specificity of pain sensitivity in the Insula: A ^{1}H-MRS examination of Glutamate and GABA.

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It has been widely accepted that pain has developed as an evolutionary response to assess and recognise any stimuli that may lead to actual or potential tissue damage of an organism (Lynn, 1984; IASP, 1994), though it is of interest that, in humans, it is a subjective experience. With the advent of non-invasive neuroimaging techniques such as fMRI, several structures have been identified to be reliably active and associated with varying aspects of pain processing. Among these structures are the anterior cingulate, ventrolateral prefrontal cortex, amygdala and both anterior and posterior insula, forming a core network of structures, which the exact nature of is still under some debate (Tracey & Mantyh, 2007; Wagner, Atlas, Lindquist, Roy, Woo & Kross, 2013; Ingvar, 1999; Iannetti & Mouraux, 2010). The anterior and posterior insula are of particular interest as although they are nominally parts of the same anatomical structure, they are both shown to be active in different aspects of nociceptive processing (Augustine, 1996). While the posterior insula is shown to be involved in sensory aspects of pain perception, the anterior portion is shown to be involved in affective aspects and emotional processing of noxious stimuli (Tracey & Mantyh, 2007; Craig, Chen, Bandy & Reiman, 2000; Singer, Seymour, O'Doherty, Kaube, Dolan & Frith, 2004).

As well as producing reliable fMRI activations, noxious stimuli have been observed to produce changes in neurotransmitter levels through the use of proton MRS. (Mullins, Rowland, Jung & Sibbitt, 2005; Gussew, Rzanny, Erdtel, Scholle, Kaiser, Mentzel & Reichenbach, 2010; Cleve, Gussew & Reichenbach, 2015). Mullins et al. (2005) was among the first to examine and demonstrate functional fluctuations in the concentration of the predominant excitatory neurotransmitters, Glutamate (Glu) and Glutamine (Gln), during the application of noxious stimulation in specific brain regions. While most neuroimaging techniques focus on obtaining a spatial representation of cortical areas and the activity within structures, ^{1}H-MRS is used to quantify metabolic concentrations within a selected voxel of interest (VOI) by exciting protons with specific resonance frequencies, resulting in a chemical specific spectrum (Alger, 2010). At 3T, Glu and Gln can be difficult to examine independently due to potential overlap of their signal peaks. As such they may be reported together, and the product of Glutamate and Glutamine is commonly reported as Glx (Feraco, Bacci, Pedrabissi, Passamonti, Zompogna, Malatolta & Leonardi, 2011).

In addition to the use of 1H-MRS to investigate dynamic neural responses to pain, several studies have emerged examining differences between healthy individuals and those suffering from neuropathic chronic pain syndromes, particularly fibromyalgia (FM) due to it's high prevalence rate in the population (Breivik, Collett, Ventrafridda, Cohen & Gallacher, 2006; Neumann & Buskila, 2003). These studies have demonstrated increases in

Glu and Glx in several of the structures involved in pain processing in patients with FM compared to healthy controls (Valdes, Collado, Bargallo, Vasquez, Rami, Gomez & Salamero, 2010; Feraco et al., 2011; Fayed, Garcia-Campayo, Magallon, Andres-Bergareche, Luciano, Andres & Beltran, 2010), particularly in the posterior insula (Harris, Sundgren, Pang, Hsu, Petrou, Kim, McLean, Gracely & Clauw, 2008; Harris, Sundgren, Craig, Kirshenbaum, Senm Napadow & Clauw, 2009; Harris & Clauw, 2012). Patients with FM have also been found to have decreased levels of GABA in the anterior insula, and that decreased levels in the posterior insula correlated with an increased sensitivity to pressure-pain stimulus (Harris & Clauw, 2012; Foerster, Petrou, Edden, Sundgren, Schmidt-Wilcke, Lowe, Harte, Clauw & Harris, 2012). Due to these findings, we are particularly interested in examining levels of both the excitatory and inhibitory neurotransmitters in the anterior and posterior sections of the insula cortex in healthy individuals, particularly as it was noted in Harris et al (2009) that a significant negative correlation between pressure-pain thresholds (PPTs) and Glu and Glx was observable across both FM patient and healthy control groups. These findings may indicate that patients with chronic pain conditions have enhanced reactive neurometabolic processes, essentially reflecting a standard neurometabolic reaction to pain thresholds, as observed in non-clinical participants, but in the extreme.

In the present study, we intend to examine the PPTs of healthy individuals in relation to their levels of Glu, Glx and GABA in both the anterior and posterior sections of the Insula cortex. As a majority of the previous research has been conducted primarily using females (both FM patients and healthy controls) we are interested to see whether these effects are both replicable and generalizable to the population at large. As such we have developed several hypotheses that we intend to test. Based on the literature, we hypothesize that excitatory neurotransmitters (Glu/ Glx) will have a negative correlation with PPT in the posterior insula, and that insular GABA may have a positive correlation with PPT. From further exploratory analysis, we expect to see a difference between male and female regional neurometabolic concentrations, as well as regional concentration differences between the anterior and posterior insula. These results would indicate that baseline levels of excitatory and inhibitory neurotransmitters truly reflect pressure-pain threshold.

Methods

Participants

Eighteen healthy participants were recruited for this study; 11 male, ranged 20-29 (mean ± SD age 22.5 ± 2.8 years) and 7 females, ranged 19-27 (mean ± SD age 21.4 ± 2.9 years) with an overall range of 19-29 (mean ± SD age 22.06 ± 2.9 years). Bangor University

Ethics Committee granted ethical approval to the experiment protocol prior to commencement, and written informed consent was gained from each participant. All participants met the following self-reported inclusion criteria 1) Not to suffer from any chronic pain condition or be on medication for such a condition; 2) Not to have been subjected to or experienced a condition that may have induced chronic pain and/or have been on medication for such a condition within 3-6 months prior to the experimental session; 3) Not to suffer from a mental health condition and/or be on medication for such a condition that may be believed to have an impact on neurochemistry; and 4) no safety contradictions for MRI.

Experimental Pain Pressure Threshold

Experimental pain pressure threshold was measured with the use of a Wagner Force Ten FDX Digital Force Gage algometer. The algometer was applied to the first dorsal interosseous muscle pressure point in the left hand of the participant at a consistent rate of 1 pound-force (lbf) / sec by an experimenter trained in the use of the equipment and methodology. Measurement of pain thresholds was conducted by instructing the participant that they should inform the experimenter as soon as the sensation started to become painful, and not to continue until the pain became unbearable. Pressure-pain thresholds (PPTs) were measured twice during the experimental session; once before scanning and once afterwards, with the mean of the ratings being used in order to ascertain participant's PPT's, and has been demonstrated to be a reliable method to do so (Chesterton, Sim, Wright & Foster, 2007).

^{1}H-MRS

Magnetic resonance spectroscopic scanning was conducted on a Phillips Achieva 3.0T MRI equipped with spectroscopic capabilities and a 32-channel head coil. Routine MR images were collected for reference, followed by T1-weighted images (FOV= 240x240x150, 214 slices 0.7mm thick, TR – 11 ms, TE = 2.2 ms) used for voxel placement. Spectroscopic voxels were placed on the right Anterior Insula (Broca's area 13, contralateral to stimulation), right Posterior Insula (Broca's 14, again, contralateral), and on an area that encompassed bilateral occipital cortex, used in the capacity of a control measurement.

Single voxel, water suppressed macro-molecule edited MEGA-PRESS Spectroscopy (25, 26) (voxel size = 25 x 25 x 30 mm, TR = 2000ms, TE = 80ms) during a 10-minute rest period was acquired from each area of interest as a baseline static measurement of neurometabolic concentrations, followed by an acquisition of unsuppressed water measurement for each area. Participants were not required to complete any tasks during scanning. MEGA-PRESS is a difference edited technique that involves the collection of both

edit ON and edit OFF spectra, and then subsequent subtraction of the edit ON from the edit OFF spectrum. The edit OFF spectrum is essentially a normal PRESS sequence, and so while the difference spectrum from MEGA-PRESS sequence allows measurement of GABA, the edited OFF spectrum can be used for determination of concentrations of the other metabolites, Glutamate included. (For a review see Mullins, McGonigle, O'Gorman, Puts, Vidyasagar, Evans & Edden, 2014)

^{1}H-MRS Analysis

Raw data was analysed and fit using Tarquin V. 4.3 (http://tarquin.sourceforge.net/; Wilson, Reynolds, Kauppinen, Arvanities & Peet, 2011), wherein metabolic signals were fit using a simulated basis set for both the PRESS and MEGA-PRESS acquisitions. For GABA the result of the subtraction of the "ON" edited spectrum from the "OFF" edited spectrum of the MEGA-PRESS acquisition is fit, while for all other metabolites the average of the "OFF" edited spectrums is fit as a simple PRESS acquisition. The MEGA-PRESS acquisition was fit using the "1H MEGA-PRESS GABA" basis set, while the PRESS acquisition was fit using the "1H Brain full" basis set, (the following fitting parameters were used for both fits: starting point = 10; end point =2048; dynamic frequency correct on using NAA, Cho and Cre) to obtain Glu, Gln and Glx concentrations.

By default TARQUIN assumes a relaxation correction factor of 0.7 (water attenuation at 30 ms at 1.5 T) and tissue composition of the voxel to be mostly white matter giving a water concentration of 35880 mM. As the tissue content of our voxels actually contained CSF, grey matter and white matter, default values for water attenuation and water concentration in TARQUIN were set to 1 and 55550 mM respectively. MATLAB and SPM8 were then used in conjunction with a Partial Volume Correction programme (available at: http://biu.bangor.ac.uk/projects.php.en) to calculate actual tissue composition in the voxel and compensate for water content and relaxation difference following the method of Gasparovic, Song, Devier, Blockholt, Caprihan, Mullins, Posse, Jung and Morrison (2006), assuming water concentrations of 35880 mM for white matter, 43300 mM for grey matter and 53888 mM for CSF. Relaxation values for tissue water and metabolite concentration were taken from the literature (Choi & Frahm, 1999; Choi, Coupland, Bhardwaj, Kaira, Casault, Reid & Allen, 2006; Kreis, Slotboom, Hofmann & Boesch, 2005; Traber, Block, Lamerichs, Gleseke & Schild, 2004). Tissue and relaxation corrected concentrations were then used to assess the respective metabolite levels for each region of interest (ROI).

Statistics

Corrected metabolite levels along with individual and mean PPT's were analysed using SPSS V.20. As a relationship between metabolite levels and PPT's had been previously hypothesised, Pearson product-moment correlations were conducted between corrected metabolite concentrations (Glu, Gln, Glx and GABA) in each ROI (Anterior Insula, Posterior Insula and Occipital) and mean PPTs. Descriptive statistics were also ascertained for relevant participant data, and a T-test was conducted between PPT 1 and PPT 2 to check for any significant differences or variability. As well as conducting analyses on the group as a whole, the data was split in order to examine any differences between male and female participants.

Post-hoc measurement of Sensitivity

The measurement of sensitivity was calculated by subtracting PPT1 from PPT2 in order to assess what effect repeated stimulation of the site used to measure PPT had on participants i.e. hyperalgesia or hypoalgesia. A negative sensitivity score indicates increased sensitisation to the stimuli, while a positive number indicates a decreased sensitivity to the stimuli.

Results

PPT and sensitivity

A paired-samples T-test between PPT rating 1 and PPT rating 2 demonstrated a statistically significant decrease from PPT 1 (M=9.86, SD= 4.48) to PPT 2 (M= 8.64, SD= 3.95), $t(32)= 3.903$, $p <.001$ (two-tailed). The mean decrease in PPT was 1.22 with a 95% confidence interval ranging from .584 to 1.859 (Figure 5.1). The eta-squared statistic (.32) indicates a large effect size.

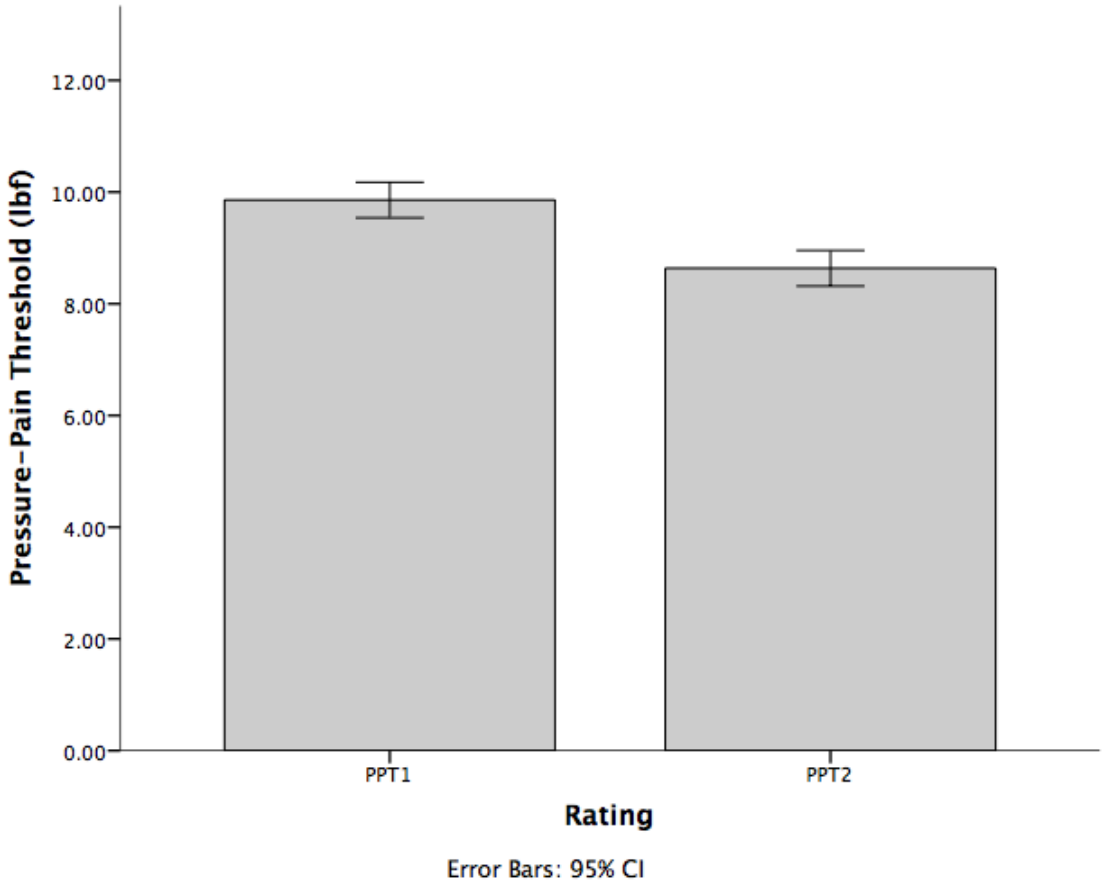

Figure 5.1: Results from t-test between overall PPT1 and PPT2, demonstrating the drop in threshold.

To examine differences between genders, several paired-samples t-tests were conducted, the results from which can be seen in Table 5.1. Analysis showed that in females there was a significant decrease in threshold from PPT1 (M=8.73, SD=3.79) to PPT2 (M=7.09, SD=2.43), $t(15)=3.78$, $p<.005$. The mean decrease in pressure pain threshold was 1.64 with a 95% CI ranging from .715 to 2.56, with an eta-squared of .49. In contrast, males demonstrated no significant difference between PPT1 (M=10.92, SD=4.92) and PPT2 (M=10.09, SD=4.59), $t(16)=1.878$, $p>.05$. There were no significant differences between male and female AvPPT or PPT1, but there was a significant difference for PPT2, with females demonstrating a lower PPT (M=7.09, SD =2.43) than males (M10.09, SD=4.59, $t(24.61)$ -2.373, $p<.05$ (two-tailed, equal variances not assumed). The magnitude for the differences in means (mean difference= -3.01, 95% CI: -5.62 to-0.395) was quite large (eta-squared=-.18).

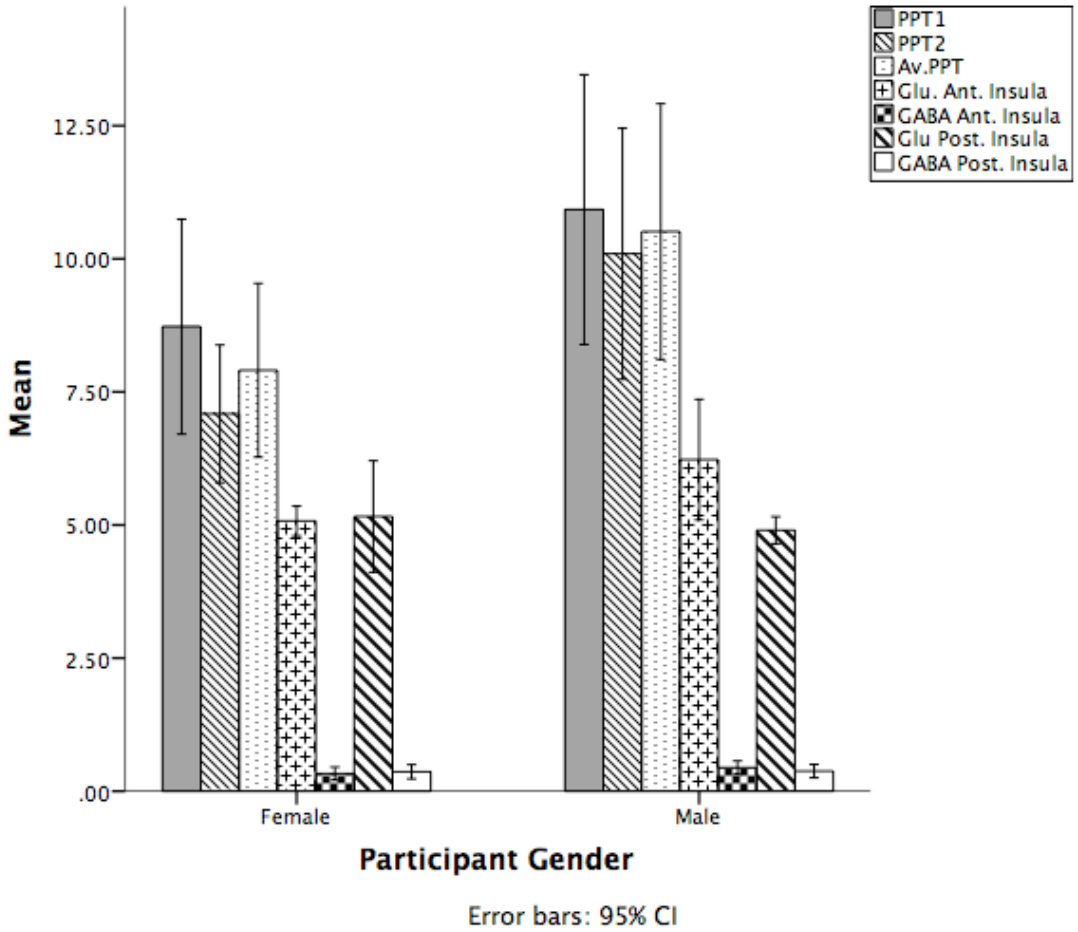

Figure 5.2. Mean scores for male and female participants' PPT1, PPT2, Average PPT, Anterior Insula Glutamate and GABA, Posterior Insula Glutamate and GABA. Significant differences were found between male and females' PPT2 and concentrations of Glutamate in the Anterior Insula. No other significant differences were found.

There was no significant difference in sensitivity between males (M=-0.83, SD=1.82) and females (M=-1.64, SD=1.73), $t(31)=-1.499$, $p > .05$ (two-tailed, equal variances assumed), but there was an observable negative correlation between sensitivity and Av.PPT solely in females, r=-.799, n=16, $p < .001$, $r^2=0.64$, indicating that higher PPT was related to increased sensitisation to the stimuli.

Location; metabolite; variable	Males	Females	*P*
Anterior Insula			
Glutamate	6.23 ± 2.20	5.08 ± 0.52	.05
GABA	0.44 ± 0.24	0.33 ± 0.23	.19
Posterior Insula			
Glutamate	4.90 ± 0.50	5.16 ± 1.96	.60
GABA	0.38 ± 0.24	0.37 ± 0.25	.60
Occipital			

Glutamate	4.88 ± 0.45	5.20 ± 1.60	.26
GABA	0.55 ± 0.31	0.42 ± 0.16	.14
Other Variables			
PPT1	10.92 ± 4.67	8.73 ± 3.79	.16
PPT2	10.09 ± 4.59	7.09 ± 2.43	.03
Av.PPT	10.51 ± 4.67	7.90 ± 3.06	.69
Sensitivity	-0.83 ± 1.82	-1.64 ± 1.73	.20
State Anxiety	33.33 ± 10.38	40.22 ± 13.88	.32
Trait Anxiety	37.50 ± 7.64	38.67 ± 9.99	.81

Table 5.1. Results from paired-samples t-tests between males and females, showing mean and standard deviation (M ± SD) for each variable. Significance (*P)* values of comparisons shown in far right column

Neurometabolic differences & correlations

Independent-samples t-tests showed that there was a significant difference in Glu concentration in the anterior insula between males (M=6.23, SD=2.20) and females (M=5.08, SD=0.52), $t(17.90)=-2.096$, $p=.05$ (two-tailed, equal variances not assumed). The magnitude for the differences in means (mean difference =-1.15, 95% CI:-2.31 to 0.003) was quite large (eta squared = -.16). There were no other statistical differences between male and female neurometabolic concentrations.

Pearson product-moment correlations were used to analyse the relationships between neurometabolites and PPT, among other variables. It was found that, in males but not females, AvPPT correlated with Glu concentrations in the anterior insula (r=.615, n=17, $p=.009$, $r^2=0.38$). It was also shown that Glu in the posterior insula correlated negatively with GABA in the posterior insula (r=-.553, n=17, $p=.021$, $r^2=0.31$). There were no significant correlations between regional metabolic concentrations and PPT in females.

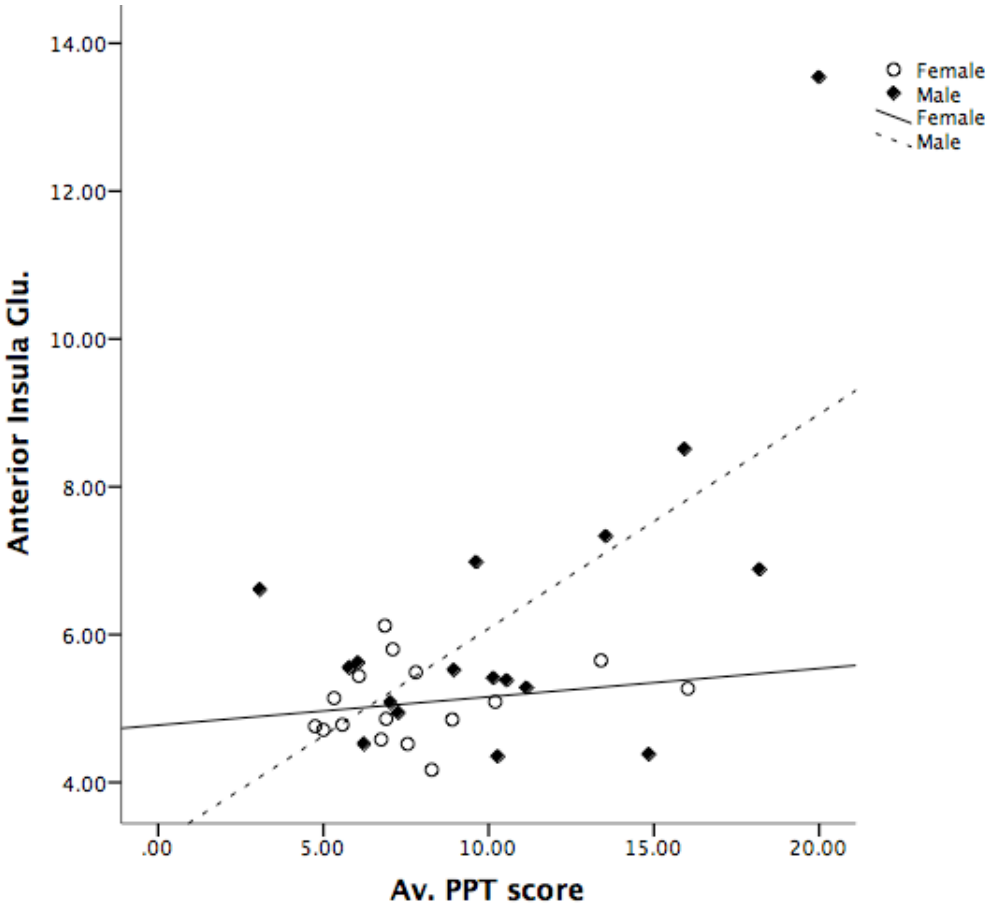

Figure 5.3. Results of the correlation between Average PPT scores and Glutamate concentration in the anterior Insula. There was a significant correlation between the two in males, but not in females.

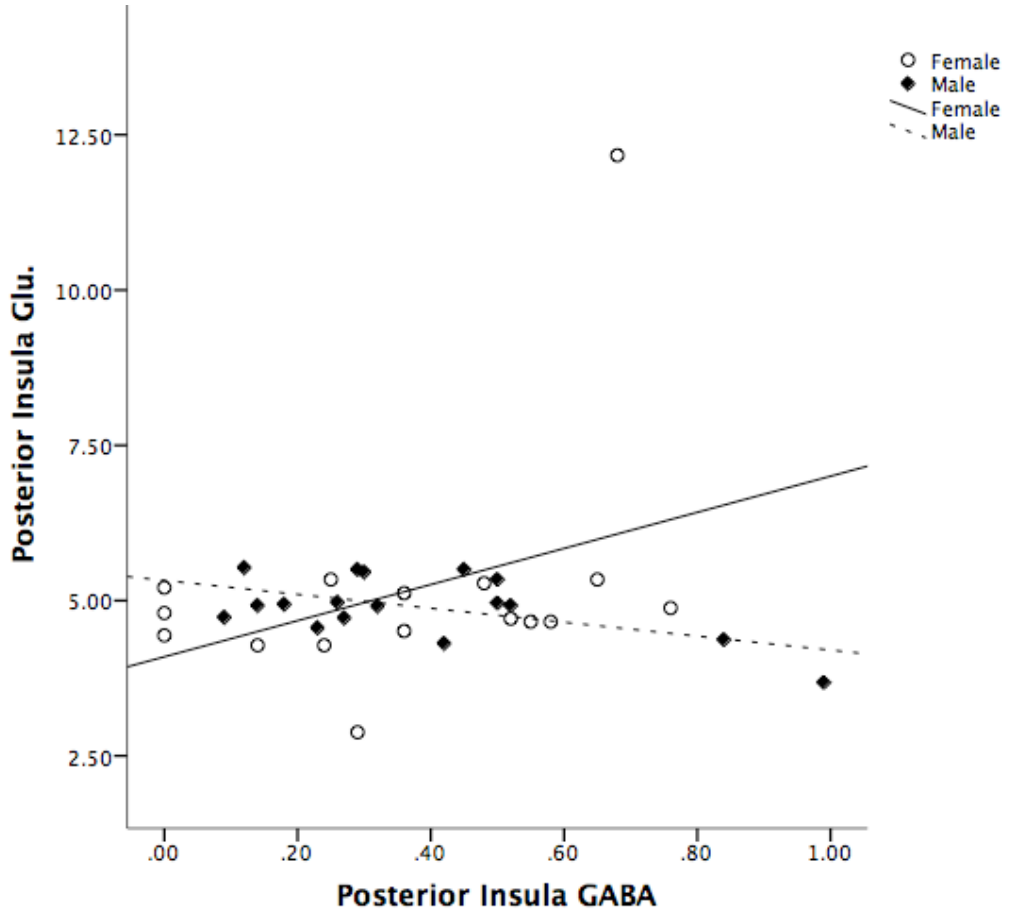

Figure 5.4. Results of the correlation between concentrations of Glutamate and GABA in the posterior Insula. A significant negative correlation was observed in males, but no correlation was found for females.

Grey and white matter concentrations were also examined in conjunction with PPT and STAI values as there have been noted differences between patients with chronic pain and healthy individuals. There were no observed significant correlations between brain matter density and PPT, STAI or neurometabolic concentrations.

Discussion

The analysis of grey and white matter concentrations was conducted in order to establish whether brain matter density had any impact upon perception of noxious stimuli, and the lack of observed correlations supports previous evidence that suggests that changes in brain matter density are facilitated by, not the cause of, chronic pain (Rodriguez-Raecke, Niemeier, Ihle, Ruether & May, 2009). With regards to the PPT reports, it is interesting that there were no overall significant differences between males and females, especially considering that experimentally induced pressure-pain has been reliably reported to account for a statistically significant disparity between genders, with females having a lower threshold than males (Fillingim, King, Ribeiro-Sasilva, Rahim-Williams & Riley, 2009). However, it is worth noting that females displayed a significant correlation between high PPT

and the calculated measure of sensitivity, which accounted for the significant decrease in female's threshold from PPT1 to PPT2. This may be consistent with previous literature that has indicated a high variance between male and female pressure-pain reports. Studies examining test-retest reliability of PPTs have reported that, certainly in females, there is a significant lowering of PPT values over consecutive days in comparison to day-one baseline (Jones, Kilgour & Contois, 2007). It was argued by Jones, Kilgour and Contois (2007) that their results had not previously been observed as researchers had tested males and females indiscriminately, without accounting for factors such as stress, hormonal or metabolic fluctuations that may influence perceptions of noxious stimuli. The results of the present study showed a drop from PPT1 to PPT2, one that was statistically significant in females to the extent that the female's PPT was then significantly lower than the males. These results would be consistent with previous findings (Jones, Kilgour & Contois, 2007), though the temporal difference between PPT measurements in the current study was shorter; an hour compared to a day. These results may indicate a form of behavioural adaptive response to pressure-pain, in that previous experiences are taken into account and used as a predictive measure, which then instigates anticipatory processes regarding potentially noxious stimuli that in turn either initiates aversive behaviour to prevent tissue damage, or lowers the pain threshold, facilitating withdrawal behaviours. This could account for why it has been shown that females' PPT seems to continually lower over periods of time.

There have been no known studies reporting significant differences between male and female regional baseline neurometabolic concentrations, at least in healthy populations. As such it is interest that males demonstrated a significantly higher concentration of Glu in the anterior insula when compared with females. It is of further interest that, again in the male participants, that Glu in the same region correlated with Av.PPT, which has been previously unreported in relation to baseline measurements. Due to the anterior insula's role in the processing of emotional aspects of pain perception, i.e. unpleasantness rather than intensity, this correlation could indicate either that male participants with a higher PPT perceive noxious stimuli as more unpleasant, or that due to the subjective nature of pain perception and individual thresholds that the participants who reported as having a higher PPT merely waited until they attributed more unpleasantness to the sensation before regarding it as painful. It was initially hypothesised that the opposite relationship would be observed between baseline Glu and Av.PPT in the posterior insula, based on previous literature (Harris et al., 2009; Harris & Clauw, 2012). When taking into account that the effect was originally observed in females with chronic pain disorder, and that the previous findings were not

replicated in the current experiment in either males or females, there are a couple of considerations that may be made. Firstly, it may be possible that the baseline concentrations observed in the chronic pain patients may have been more reflective of dynamic alterations in excitatory neurometabolism based on the nature of the patients' condition than on steady state levels. The current lack of correlations may also be an indication that research approaches and considerations of exactly what baseline neurometabolic concentrations may indicate, or the process they may reflect, particularly in relation to potential reactions to stimuli; in this instance that a higher concentration of baseline Glu in an area related to pain tolerance is indicative of sensitivity to noxious stimuli, at least in a healthy sample. If some of the structures being examined are likely to be more functional, they may have a higher level of at-rest glutamatergic excitatory activity that, in turn, may have a higher level of concentration variability, making it difficult to attribute indirect, non-dynamic measurements (such as pain tolerance) to baseline neurometabolic concentrations reliably (Duncan, Wiebking & Northoff, 2014). Further investigations may wish to either replicate the methodologies utilised in this experiment in order to determine whether this relationship is reliably observed in healthy participants, or examine direct comparisons with patient cohorts to further our understandings of what differences there are between healthy and patient male populations. Another possible avenue of investigation could examine the relationship between baseline measurements and pain tolerance threshold in a functional experiment. This would enable us to assess dynamic changes and whether baseline concentrations can be utilised to predict biological reactions to stimuli. For example, does a high PPT and lower Glu levels result in an exponentially larger increase in glutamatergic activity, or would Glu concentrations alter proportionately to PPT? Glu concentrations in the left anterior insula have been reliably demonstrated to increase up to 18% with the application of acute heat stimulation (Gussew et al. 2010), so it would be of interest to examine proportional changes in further detail, i.e. does unpleasantness or intensity increase or decrease the concentrations of Glu? In either case these findings, along with the observed negative correlation between Glu and GABA in the anterior insula support theories that there may be an excitatory-driven mechanism to the processing and perception of information regarding noxious stimulation. Further research could examine the participant's measure of unpleasantness, as well as whether this correlation between Av.PPT and baseline Glu in the anterior insula of males.

The results found in this experiment do not support the originally proposed hypothesis; and yet some of these results are of interest and follow findings of previous literature. The anterior insula has been proven in both dynamic ^{1}H-MRS examinations and

fMRI experiments to be active during the application of experimentally induced pain, and the correlation between baseline Glu and Av.PPT may lead to further examinations into the role that this region may play in neurometabolic processes. It is possible that this finding may support a relatively recently developed model of pain, called the predictive regulation and action model (PRA, Morrison, Perini & Dunham, 2013), which is partially based around findings that indicate that certain cortices have seeming developed adaptive anticipatory processes in relation to pain perception in order to facilitate aversive behavioural actions and limit potential tissue damage. In this instance, the increased concentration of Glu in the anterior insula may reflect an adaptive response; due to the anterior insula's role in the subjective processing of pain, those with high PPTs may attribute a higher level of unpleasantness to pain in order to prevent continual subjection to noxious stimuli, especially if it is based on previous experiences. In other words, the brain may be taking into account that although the stimuli may not necessarily be painful enough to cause tissue damage, continual exposure may prove detrimental and as such by assigning a higher level of unpleasantness to the stimuli it is able to produce withdrawal or other adaptive behaviour as a preventative measure. Taking ratings of stimuli unpleasantness and examining them along with PPT and neurometabolic concentrations of Glu in the anterior insula could examine this further. Doing so may paint a more representative picture of pain processing and adaptive anticipatory behaviour in healthy males. There are a few considerations as to why this relationship was only found in males and not females; it may reflect that this is related to a form of adaptive behaviour exclusive to males, or it may reflect comments made by previous research into test-retest reliability of testing PPT, which postulated that as males and females were tested indiscriminately without allowing for regular hormonal or metabolic fluctuations that may impact processes, these findings are not fully representative (Jones, Kilgour & Contois, 2007). This could be examined more rigorously by measuring PPT and metabolic concentrations over a larger period of time in order to allow for any potential fluctuations, and if there are regular fluctuations, it could be examined to determine what impact upon neurometabolic differences between genders, as well as their relationship to PPT. The negative correlation between Glu and GABA in the same region (again in males and not females) may also support the previous research examining dynamic changes in glutamatergic neurotransmission, and the finding of excitatory-driven process (Gussew et al., 2010). With a more extensive examination that accounts for subjective perceptions, the relationships between pressure-pain threshold and baseline neurometabolic concentrations could be better understood.

As this experiment was not able to replicate the previous findings from female chronic pain patients, a number of implications may be drawn. Firstly, it may indicate that the findings from chronic pain patients may have been more of a reflection of functional changes in neurochemistry due to the nature of the condition, rather than a true baseline or resting measure of concentration. Another consideration that may be made is that our current understanding of the relationship between baseline neurometabolic concentrations and neurological processes may not be completely accurate. If this is the case, then research into baseline concentrations may need further scrutiny, taking into account the roles of the structures of interest, as well as whether they may exhibit activity, even at baseline. Regions involved, or sharing functional connectivity, with cortices that may have a high level of activity whilst at rest, may reflect this activity, however minute, in the concentrations of neurotransmitters. Examinations into resting state functional connectivity of the insula found two separate and distinct networks, one from the anterior insula, and one from the posterior insula. The anterior network extended from the ventral portion of the AI to the rostral portion of the ACC, middle and inferior frontal cortex, as well as the temporal-parietal cortex; areas involved in attention control and saliency-detection, not to mention the integration of multimodal signals into an interospective representation and subsequent awareness. In regards to the posterior region, the network extended from the dorsal portion of the PI (which has been linked to pain perception during electrode stimulation; Stephani, Vaca, Maciuna, Koubeissi & Lüders, 2011), to the dorsal-posterior cingulate, sensori-, pre-, and supplementary motor areas, temporal cortex, and occipital areas; structures that have been related to response-selection, skeletomotor orientation, and pain (Cauda, D'Agata, Sacco, Duca, Geminiani & Vercelli, 2011). In direct contrast to the connectivity observed in non-clinical participants, there appears to be alterations in insular resting-state functional connectivity in patients with chronic pain. Research has shown that patients with fibromyalgia demonstrated lower levels of functional connectivity between the left AI and both left and right frontal gyri, as well as between the left PI and the right superior frontal gyrus when compared to healthy controls. It was also found that patients had greater functional connectivity between sections of the right mid- and posterior insula and the right mid- and posterior cingulate, and also between the right AI and the left superior temporal gyrus. The researchers also found a correlation between higher functional connectivity between the insula and cingulate cortex and lower PPT, indicating that the lower pain thresholds exhibited by chronic pain patients may be due to this increased resting-state functional connectivity. (Ichesco, Schmidt-Wilke, Bhavsar, Clauw, Peltier, Kim, Napadow,

Hampson, Kairys, Williams & Harris, 2014). Factoring these differences between healthy and clinical populations found in fMRI, these differences might account for discrepancies found between previously reported baseline concentrations in patients with fibromyalgia and this study's examination in healthy participants. That being said, it is difficult to accurately gage due to the broad nature of ^{1}HMRS measurements, i.e. the measurements account for the given concentration of neurometabolites within a voxel, regardless as to whether they are presynaptic/postsynaptic, grey matter/white matter, intercellular/extracellular etc.

The results of the research do not support the original hypotheses, with negative results being the predominant finding. As such the results suggest the relationship between the MRS measurable neurotransmitters may be more complex then originally expressed. However these "negative" results still have value, as some of the unexpected results have lead to the suggestion of other avenues and additional areas of research to follow. Doing so may help us to fully understand the relationship between neurotransmitter levels and pain perception.

Chapter 5: Supplementary material

STAI inclusion.

The State-Trait Anxiety Inventory (STAI) was included mid-sample as an additional measure to examine any relationships between anxiety and PPT, as well as anxiety and neurometabolic measurements of the Insula, an area previously shown to demonstrate increased BOLD activation in response to aversive stimuli in participants that score higher on measures assessing anxiety proneness i.e. STAI, BFI etc. (Stein, Simmons, Feinstein & Paulus, 2007). STAI was administered to 15 participants (9 females) after gaining informed consent and prior to the first PPT was taken before scanning. STAI score were analysed using t-tests, to assess differences between males and females, and they were correlated with PPT and neurometabolic concentrations of both the anterior and posterior insula.

State and trait anxiety: Results.

State and trait anxiety results are being considered separately in this study due to its mid-sample inclusion and the resulting small n (15; 9 females).

In males, state and trait anxiety only correlated with each other (r=.996, n=6, p=.000, r^2=0.992) and there were no differences between male and females state and trait. In females, state and trait also correlated highly with each other (r=.975, n=9, p=.000, r^2=0.95). In addition, state and trait both correlated with sensitisation (state anxiety: r=.784, n=9, p=.012, r^2=0.61; Trait anxiety: r=.793, n=9, p=.011, r^2=0.63.), indicating lower STAI scores were related to increased sensitisation. Glu in the anterior insula correlated negatively with both state anxiety and trait anxiety (State: r=-.785, n=9, p=.012, r^2=0.62; Trait anxiety r=-.789, n=9, p=.012, r^2=0.62.). GABA in the anterior insula also correlated negatively with both state and trait anxiety (State: r=-.780, n=9, p=.013, r^2=0.60; Trait: r=-.713, n=9, p=.031, r^2=0.51). Correlations with Glu and GABA indicate that the higher the STAI scores, the lower the concentrations of neurotransmitter in the anterior insula.

State and trait anxiety: Discussion.

With regards to the STAI results, there was certainly no intended manipulation of state anxiety in the present study. Anxiety is often discussed in relation to chronic pain, with evidence to suggest it correlates with an increase in intensity (Tang & Gibson, 2005), though some argue that this is a chicken-and-egg scenario; does the anxiety enhance the chronic pain, or does the chronic pain increase anxiety and affect emotional state? It is unsurprising that the area in which correlations between neurometabolites and STAI scores were found was the anterior insula, as anterior insula functionality is typically associated with the emotional processing of noxious stimulation, though most research into anxiety disorders

have found that N-acytalaspartate (NAA) and Creatine typically play a role in anxiety disorders (Trzesnak, Arujo & Crippa, 2008). However, it may be difficult to draw solid conclusions from these findings due to the small sample and disproportionate male-to-female ratio.

Chapter 5 References

Alger, J. R. (2010) Quantitative Proton Magnetic Resonance Spectroscopy and Spectroscopic Imaging of the Brain: A Didactic Review. *Topics in Magnetic Resonance Imaging.* 21(2):115-128.

Augustine, J. R. (1996) Circuitry and functional aspects of the insular lobe in primates including humans. *Brain Research Revie*ws. 22, pp 229-244.

Breivik, H., Collett, B., Ventafridda, V., Cohen, R. and Gallacher, D. (2006). Survey of chronic pain in Europe: Prevalence, impact on daily life, and treatment. European Journal of Pain. 10. Pp 287-333.

Chesterton, L. S., Sim, J., Wright, C. C. and Foster, N. E. (2007)Interrater reliability of Algometry in measuring pressure pain thresholds in healthy humans, using multiple raters. Clinical Journal of Pain. 23(9), pp 760-766.

Choi, C., Coupland, N. J., Bhardwaj, P. P., Kaira, S., Casault, C. A., Reid, K. and Allen, P. S. (2006). T2 measurement and quantification of glutamatein human brain in-vivo. *Magnetic Resonance in Medicine.* 56(5), pp 971-977.

Choi, C. G. and Frahm, J. (1999) Localised proton MRS of the human hippocampus: metabolite concentrations and relaxation times. *Magnetic Resonance in Medicine.* 41(1), pp 204-207.

Cleve, M., Gussew, A. and Reichenbach, J. R. (2015) In vivo detection of acute pain-induced changes of GABA+ and Glx in the human brain by using functional 1H-MEGAPRESS MR spectroscopy. NeuroImage. 105. Pp. 67-75.

Craig, A. D., Chen, K., Bandy, D. and Reiman, E. M. (2000) Thermosensory activation of the Insula cortex. Natural Neuroscience. 3, pp 184-189.

Cauda, F., D'Agata, F., Sacco, K., Duca, S., Geminiani, G. and Vercelli, A. (2011). Functional connectivity of the insula in the resting brain. *Neuroimage*. 55: 8-23.

Donahue, M. J., Near, J., Blicher, J. U. and Jezzard, P. (2010) Baseline GABA concentration and fMRI response. *NeuroImage*. 53(2), pp 392-398.

Duncan, N. W., Wiebking, C. and Northoff, G. (2014). Associations of regional GABA and glutamate with intrinsic and extrinsic neural activity in humans – A review of multimodal imaging studies. *Neuroscience and Biobehavioral Reviews.* 47, pp 36-52.

Fayed, N., Garcia-Campayo, J., Magallon, R., Andres-Bergareche, H., Luciano, J. V.,

Andres, E. and Beltran, J. (2010) Localized 1H-NMR spectroscopy in patients with fibromyalgia: a controlled study of changes in cerebral glutamate/glutamine, inositol, choline, and N-acetylaspartate. Arthritis Research and Therapy. 12. R134.

Feraco, P., Bacci, A., Pedrabissi, F., Passamonti, L., Zompogna, G., Malavolta, N. and Leonardi, M. (2011) Metabolic abnormalities in pain-processing regions of patients with fibromyalgia: a 3T MR spectroscopy study. *American Journal of Neuroradiology.*

Fillingim, R. B., King, C. D., Ribeiro-Sasilva, M. C., Rahim-Williams, B. and Riley III, J. L. (2009). Sex, gender and pain: A review of recent clinical and experimental findings. *The Journal of Pain*. 10 (5): 447-485.

Foerster, B. R., Petrou, M., Edden, R. A. E., Sundgren, P. C., Schmidt-Wilcke, T., Lowe, S. E., Harte, S. E., Clauw, D. J. and Harris, R. E. (2012). Reduced Insular γ-Aminobutyic Acid in Fibromyalgia. Arthritis and Rheumatism. 64(2), pp 579-583.

Gasprovic, C., Song, T., Devier, D., Blockholt, H. J., Caprihan, A., Mullins, P. G., Posse, S., Jung, R.E., and Morrison, L. A. (2006) Use of tissue water as a concentration reference for proton spectroscopic imaging. *Magnetic Resonance in Medicine.* 55(6), pp 1219-1226.

Gussew, A., Rzanny, R., Erdtel, M., Scholle, H. C., Kaiser, W. A., Mentzel, H. J. and Reichenbach, J. R. (2010) Time-resolved functional 1H MR spectroscopic detection of glutamate concentration changes in the brain during acute heat pain stimulation. *NeuroImage*. 49(2). Pp. 1895-1902.

Harris, R. E. and Clauw, D. J. (2012) Imaging central neurochemical alterations in chronic pain with proton magnetic resonance spectroscopy. *Neuroscience Letter*. 520, pp 192-192.

Harris, R. E., Sundgren, P. C., Craig, A. D., Kirshenbaum, E., Sen, A., Napadow, V. and Clauw, D. J. (2009) Elevated Insular Glutamate in Fibromyalgia Is Associated With Experimental Pain. Arthritis and Rheumatism. 60(10), pp 3146-3152.

Harris, R. E., Sundgren, P. C., Pang, Y., Hsu, M., Petrou, M., Kim, S. H., McLean, S. A., Gracely, R. H. and Clauw, D. J. (2008) Dynamic Levels of Glutamate Within the Insula Are Associated with Improvements in Multiple Pain Domains in Fibromyalgia. *Arthritis and Rheumatism*. 58(3), pp 903-907.

Iannetti, G. D. and Mouraux, A. (2010) From the neuromatrix to the pain matrix (and back). Experimental Brain Research. 205, pp 1-12.

Ichesco, E., Schmidt-Wilke, T., Bhavsar, R., Clauw, D. J., Peltier, S. J., Kim, J.,

Napadow, V., Hampson, J. P., Kairys, A. E., Williams, D. A. and Harris, R. E. (2014) Altered resting state connectivity of the insular cortex in individuals with fibromyalgia. *Journal of Pain*. 15(8): 815-826.

International Association for the Study of Pain (1994). Classification of chronic pain: descriptions of chronic pain syndromes and definitions of pain terms. Task Force on Taxonomy. Vol. suppl. 3, p. S215. Seattle: IASP Press.

Ingvar, M. (1999) Pain and functional imaging. *Philosophical Transactions of the Royal Society*. 354: 1347-1358.

Jones, D. H., Kilgour, R. D. and Contois, A. S. (2007). Test-Retest Reliability of Pressure-Pain Threshold Measurements of the Upper Limb and Torso in Young Healthy Women. *The Journal of Pain*. 8(8), pp 650-656.

Kreis, R., Slotboom, J., Hofmann, L. and Boesch, C. (2005) Integrated data acquisition and processing to determine metabolic contents, relaxation times, and macromolecule baseline in single examinations of individual subjects. *Magnetic Resonance in Medicine.* 54(4), pp 761-768.

Lynn, B. (1984) Cutaneous Nociceptors. In Holden, A., V. and Winlow, W. The Neurobiology of pain. Manchester, UK: Manchester University Press. P. 106.

Maddock, R. J. and Buonocore, M. H. (2012). MR spectroscopic studies of the brain in psychiatric disorders. *Current Topics in Behavioural Neuroscience.* 11. Pp. 199-251.

Mescher, M., Tannus, A., Johnson, M. O., Garwood, M. and Gruetter, R. (1998). Simultaneous in vivo spectral editing and water suppression. *NMR Biomedicine.* 11(6), pp 266-272.

Morrison, I., Perini, I. and Dunham, J. (2013). Facets and mechanisms of adaptive pain behavior: predictive regulation and action. *Frontiers in Human Neuroscience*. 7(755): 1-11.

Mullins, P. G., McGonigle, D. J., O'Gorman, R. L., Puts, N. A. J., Vidyasagar, R., Evans, C. J., and Edden, R. A. E. (2014) Current practice in the use of MEGA-PRESS spectroscopy for the detection of GABA. *NeuroImage*. 86, pp 43-52.

Mullins, P. G., Rowland, L. M. Jung, R. E. and Sibbitt Jr., W. L. (2005). A novel technique to study the brain's response to pain: Proton Magnetic Resonance Spectroscopy. *NeuroImage.* 26. Pp 642-646.

Neumann, L. and Buskila, D. (2003) Epidemiology of Fibromyalgia. *Current Pain and Headache Reports.* 7. Pp 362-368.

Rodriguez-Raecke, R, Niemeier, A., Ihle, K., Ruether, W. and May, A. (2009). Brain Gray Matter Decrease in Chronic Pain is the Consequence and Not the Cause of Pain. *The Journal of Neuroscience.* 29(44), pp 12746-13750.

Singer, T., Seymour, B., O'Doherty, J., Kaube, H., Dolan, R. J. and Frith, C. D. (2004). Empathy for pain involves the affective but not sensory components of pain. *Science*. 303, pp 1157-1162.

Stein, M. B., Simmons, A. N., Feinstein, J. S. and Paulus, M. P. (2007) Increased amygdala and insula activation during emotional processing in anxiety-prone subjects. *American Journal of Psychiatry.* 164(2), pp 318-327.

Stephani, C., Vaca, F-B., Maciunas, R., Koubeissi, M. and Lüders, H. O. (2011). Functional neuroanatomy of the Insular lobe. *Brain Structure and Function*. 216: 137-149.

Tang, J. and Gibson, S. J. (2005). A psychophysical Evaluation of the Relationship Between Trait Anxiety, Pain Perception and Induced State Anxiety. *The Journal of Pain.* 6(9), pp 612-619.

Tracey, I. and Mantyh, P. W. (2007) The Cerebral Signature for Pain Perception and Its Modulation. *Neuron.* 55, pp 377-391.

Träber, F., Block, W., Lamerichs, R., Gieseke, J. ad Schild, H. H. (2004) 1H metabolite relaxation times at 3.0 tesla: Measurements of T1 and T2 values in normal brain and determination of regional differences in transverse relaxation. *Journal of Magnetic Resonance Imaging.* 19(5), pp 537-545.

Trzesniak, C., Arujo, D. and Crippa, J. A. S. (2008). Magnetic Resonance Spectroscopy in Anxiety Disorders. *Acta Neuropsychiatrica.* 20(2), pp 56-71.

Valdes, M., Collado, A., Bargallo, N., Vazquez, M., Rami, L., Gomez, E. and Salamero, M. (2010) Increased glutamate/glutamine compounds in the brains of patients with fibromyalgia: a magnetic resonance spectroscopy study. Arthritis and Rheumatism. 62. Pp. 1829-1836.

Wagner, T. D., Atlas, L. Y., Lindquist, M. A., Roy, M., Woo, C. W. and Kross, E. (2013). An fMRI-Based Neurologic Signature of Physical Pain. *The New England Journal of Medicine.* 368, pp1388-1397.

Wilson, M., Reynolds, G., Kauppinen, R. A., Arvanities, T. N., and Peet, A. C. (2011). A constrained least-squares approach to the automated quantification of in-vivo 1H magnetic resonance spectroscopy data. *Magnetic Resonance in Medicine*. 65(1), pp 1-12.

Chapter 6: Discussion

While initially this thesis began as an investigation of neurological activity of anticipation of uncertain stimuli, it has developed with the aim of examining biological and social mechanisms that may be accountable for the generalized differences observed between the pain reports of males and females. Within the broadly titled field of "Individual differences", the importance of understanding how sex differences may interact with pain perception was highlighted in the early 1990's in two reviews that brought attention to disparities reported in a number of articles from a variety of approaches (Berkley, 1992; Ruda, 1993). As a consequence of these two articles, further research has been conducted aimed specifically at examining gender differences, which, in turn, has then been reviewed extensively in order to ascertain the degree to which these differences may account for previous findings in the literature, as well as why they appear so prominently (Fillingim, King, Ribeiro-Dasilva, Rahim-Williams & Riley, 2009; Racine, Tousignant-Laflamme, Kloda, Dion, Dupuis & Choinere, 2012a; 2012b). The implication of these findings is particularly of importance considering the one of the main, current theoretical models. The Neuromatrix of pain (Melzack, 1999; 2001), consists of an extensive, multimodal framework of inputs and mediators that can alter how noxious stimuli is perceived; none of which appear to directly identify sex or gender as explicit factors. It is also noted in Fillingim et al. (2009) and Racine et al. (2012a) that despite evidence in support of sex differences in pain perception, a number of articles have been published that either test males and females indiscriminately, or fail to report the gender distribution within their samples. Had research focused on the differences between male and females perceptions of pain, as well as factors that may influence perceptions, we may have had a better insight into how or why pain is such a subjective sensory phenomenon, in turn improving further research and treatments of conditions. This statement is not aimed at attacking, invalidating or devaluating the conduct, procedure or findings of previous research in the field of pain research; it is merely meant to offer an alternate perspective and approach to the field. By understanding how the proverbial cogs fit into the machine, we may be able to advance knowledge and research into building a more complete theory of perception, in turn improving treatments and the quality of life experienced by those most affected within society.

The specific objectives of this thesis has been aimed at addressing what biological and psychosocial factors may act as an underlying mechanism behind some of these observed differences between males and females pain reports, and the experiments have taken alternate approaches and perspectives to answering these objectives, before converging on a core aspect of pain perception, which is associated with sensitivity. From a neurobiological

perspective, the question was aimed at answering whether the regions baseline neurometabolic concentrations of a core structure within the brain, the Insula, plays a key role in the pain thresholds or sensitivity of healthy participants in the same manner it has been demonstrated to in the pain thresholds of fibromyalgia (FM) patients. From a psychosocial approach, the objective has been address by examining what psychological factors may influence changes sensitivity in regards to the well-established experimenter gender effect, and whether this effect can either extend to the inclusion of an additional observer, or whether the presence of an additional observer, as well as their gender, might enhance the experimenter gender effect. While the results of these experiments may not have answered questions in the way that the initial hypotheses expected, the results certainly contribute to the growing literature aimed at examining both gender and individual differences related to pain perception, and offer perspectives that may lead to further opportunities for advancement in the field.

As previously stated, this thesis originated as an examination of functional neurometabolic concentrations in the anterior cingulate cortex (ACC) in anticipation of potentially noxious stimulation, as it had been previously reported in fMRI experiments (Ploghaus, Tracey, Gati, Clare, Menon, Matthews & Rawlins, 1999; Sawamoto, Honda, Okada, Hanakawa, Kanda, Fukuyama, Konishi & Shibasaki, 2000), with the investigation reported in chapter three forming a pilot assessment for the development of a suitable scanning protocol. However, the use of cold-pressor did not appear to perform sufficiently in the capacity of a noxious stimuli, possibly due to the larger onset time of pain compared to the previously utlised mechanical thermal pain. In our pilot, participants were presented with three visual stimuli, in the forms of a green, amber, or red circle, followed by a paired tactile stimuli; certain no-pain, uncertain no-pain, uncertain pain, and certain pain. Participants were aware that the green circle was paired to no-pain and that the red circle was paired to pain, rendering them the certain conditions, but they were unaware as to whether the amber circle proceeded pain or no-pain as there was a 50/50 chance of either, thus rendering the amber circle the uncertain condition. This formed seven conditions in total for scanning; the three visual conditions (green/amber/red) establishing the anticipatory conditions, where we would expect to see activity such as that observed in the ACC previously, and four tactile conditions (certain no-pain/uncertain no-pain/uncertain pain/certain pain), where we would expect to see pain-related neuronal activity in at least two of the conditions, which may be modulated by uncertainty. Participants were also asked to continuously rate the intensity of the stimuli on a standarised visual analogue

scale and manually log their responses by pressing a button on a joystick interface. We expected to find that the uncertain conditions would magnify the participants' ratings of the stimuli, resulting in the uncertain pain condition being rated higher than the certain pain, and the uncertain no-pain condition being rated higher than the certain no-pain, if not as high as the certain pain condition. From a neuroimaging perspective, we expected to see increased activity in the ACC in the anticipatory conditions of certain pain (i.e. red circle) and in the uncertain condition (amber circle), possibly with the uncertain anticipation of stimuli resulting in a higher level of activity. This would then be followed by pain-related activity in the associated structures in all but the certain no-pain condition. It was expected that increased activity in the ACC, both prior to and during tactile stimuli, would correlate with higher pain ratings, with the results from this pilot justifying an examination with functional spectroscopy. Unfortunately that was not to be the case. We found that both certain and uncertain pain were rated significantly higher that both certain and uncertain no-pain, while there were no differences between certain and uncertain pain or certain and uncertain no-pain. These findings indicate that while the painful stimulation was rated higher than the non-painful stimuli, it does not necessarily reflect that the painful stimuli was regarded to as noxious due to the ratings being around the median of the scale. In regards to the neuroimaging results only one anticipatory condition exhibited activity (red circle), and that was confined to the occipital cortex. Of the tactile conditions only the certain-pain condition did not result in any activity, while the activity observed in the other three conditions were not quite what we expected. The certain no-pain condition showed activation in the occipital cortex, insula, interparietal sulcus and posterior parietal cortex, S1/M1, and the ventromedial prefrontal cortex. The uncertain pain condition showed some similar activity, albeit on a smaller scale, located in the interparietal sulcus and somatosensory cortex. Of all the tactile conditions, only the uncertain no-pain condition demonstrated activity in the mid-ACC, as well as in the interparietal sulcus, somatosensory cortex, and ventromedial prefrontal cortex, which again was similar to that observed in the certain no-pain condition with additional activity in the mid-temporal cortex. Ten additional contrasts were included as a follow up to these results, and of these ten, five showed activity. Contrasts 10 (certain pain > certain no-pain) and 13 (uncertain pain > certain pain) demonstrated activity in the occipital cortex, while contrast 16 (certain no-pain > uncertain no-pain) showed activity in the somatosensory cortex. The remaining two contrasts, 14 (certain no-pain > certain pain) and 17 (uncertain no-pain > uncertain pain) contained widespread activation in a number of structures, and those common to both contrasts included the occipital cortex, dorsolateral prefrontal cortex,

temporal cortex, sensorimotor/somatosensory cortex, and anterior cingulate. Additional activity in contrast 14 included the posterior portion of the parietal cortex, and the interparietal sulcus. While some of this activity can be associated with the processing of noxious or potentially noxious stimuli, these results were unexpected and warranted further investigation.

While investigating potential explanations for the neuroimaging results of this experiment, several results and account seemed to match the criteria. Baliki, Geha and Apkarian (2008) examined the process by which noxious stimuli is represented in a neural network, assessed and (essentially) translated into a numerical magnitude representation of intensity, such as those used o visual analogue scales. In other words the process by which human quantitatively rate noxious stimuli. They stipulated that pain is essentially an assessment of stimulus intensity and as such, engage a central module. In this instance the researchers assessed neurological activity and evidence that suggested the insular cortex was such a module. By examining the BOLD signal during a pain-rating task and visual-magnitude rating task (i.e. the lengths of lines), then conducting conjunction analysis between the two tasks, they found that visual rating tasks elicit similar activity in areas associated with nociceptive processing, as well as greater activity in visual processing areas. Structures found common to both nociception and visual processing included bilateral premotor cortex, posterior parietal cortex, the insular cortex, supplementary motor area and mid-temporal cortex. They found areas that had greater activation in pain compared to visual-magnitude estimation included the bilateral AI, amygdala, thalamus, basal ganglia, ventral striatum, and anterior ACC, while the pain-rating task itself was associated with activity in these areas as well as the left primary sensorimotor (S1/M1), bilateral posterior parietal cortex, mid-temporal cortex, dorsal and ventral premotor corticies. It was noted that none of these structures activated more for the visual-rating task compared to the pain-rating task. Similar neural responses have been observed in previous research focused on aspects of this process, such as the assessment of decisions about the sizes of tactile stimuli (Pleger, Ruff, Blankenburg, Bestmann, Wiech, Stephan, Capilla, Friston & Dohn, 2006), cognitive evaluation of pain in the absence of noxious stimuli (Kong, White, Kwong, Vangel, Rosemann, Gracely & Gollub, 2006), and the coding of numerical magnitudes of symbolic and non-symbolic representations of numbers (Piazza, Pinel, Le Bihan & Dehaene, 2007). The authors were able to establish two distinct, non-overlapping networks that accounted for these processes as well as being involved in the magnitude estimation of painful stimuli. The first network consisted of the insula, posterior parietal cortex, dorsal and ventral premotor

corticies, and the supplementary motor area; areas which task-variance that they dubbed the magnitude-insula (mag-ins). The second network consisted of structures involved in pain perception, such as the insula, basal ganglia, thalamus, amygdala, anterior portion of the ACC, and ventral striate. This network was referred to as the nociceptive-insula (noci-ins). While these two networks overlapped, their close proximity to one another within the insula suggested that the area served as an interface between nociceptive representation and subjective pain perception, as well as the proposal that as well as the ventral "what" and dorsal "where" visual pathways, these networks may represent a central sensory "how much" network localized to the insula and projecting to the lateral prefrontal cortex.

The results of the experiment in chapter three appear to closely parallel the findings observed by Baliki, Geha and Apkarian (2008), leading us to conclude that our experimental design was insufficient in its aims to elicit an anticipatory effect, and instead became a quantitative magnitude estimation/rating task of the stimuli. There are two main considerations relating to the stimuli and conditions that may have caused this transition. The first is that the nature of the stimulation, the cold-pressor task, was not immediate or intense enough to produce the anticipatory or noxious effect, as we had originally believed it would. While the cold-pressor has been shown to operate as a painful stimulus in previous functional imaging studies, in the context of this experiment it may not have been cold enough for it to be considered as noxious, or the gradual nature by which it comes to become painful may have offset the timings and caused the reactions we had aimed for much later in each block. This could be remedied by utilizing a method of stimulation that had a more acute nociceptive onset, such as the mechanically induced thermal heat-pain shown to be effective in previous studies. The second main issue could be related to the incorporation of a second certain condition; in most other studies into the phenomena, researchers used either a certain pain condition or a certain no-pain condition, not both. By introducing a second certain condition, participants had a direct comparison between pain and no-pain, potentially reducing the uncertainty of the stimulus through recognition and comparison to the certain conditions. In reducing the ambiguity of the stimuli, we could have reduced this uncertain anticipatory effect, or systematically eliminated it. Between these two main concerns with the experimental design, it appears the study was ineffective in the capacity it was initially intended for. Even so, mistakes and failures can be just as important as successes, insofar as they give us something to learn from and highlight issues we may not even be aware of during the inception of research projects. Experience is the one thing you gain after you need it.

Progressing forward from the issues with the pilot study, there are a number of factors that could be improved upon if it were to be reattempted. Identifying a new stimulus and removing one of the certain conditions would be a start, as well as a few other adaptations to form a more robust investigation. While we had no apparent issue with the visual stimuli, we did not take into account the possibility of colour-blindness when we were planning it. This could easily be remedied by using a tick or cross for the certain no-pain or certain pain stimuli, or something positive or negative symbols such as happy or sad faces. For the uncertain condition, we could use a neural symbol or face, or a negative one could be used to enhance an aversive response. Fully training the participants in the pain-rating task prior to scanning may also be a factor to implement, as previous research has found that the ACC BOLD signal can enhance in anticipation of potentially noxious stimuli over successive trials (Ploghaus et al., 1999). While training the participants in the task may increase their BOLD response, if not done correctly it could generate a similar issue with the recognition of stimulus type in the uncertain condition, allowing them to ascertain whether it is painful or not. Finally, in the future it may be best to not solely focus on the ACC in the context of this effect, and examine the insula as a potential ROI as well due to the relationship that has been observed between these two regions in anticipation of stimuli, as evidence by the increased functional connectivity found between the ACC and insula in expectation of actual or potentially noxious stimuli (Menon & Uddin, 2010). The results found in our fMRI analysis pertaining to insula activation may serve to highlight the importance of this cortex in research relating to pain ratings and tactile assessments.

In light of the results from this pilot, it seems pertinent to continue this discussion of insular functions and delve into our examination of baseline neurometabolic concentrations in relation to participant pressure-pain threshold. There have been a number of examinations over the years into how individual pain tolerances can be established, as well as what can cause variations in them, such as those observed in differential social and testing environments. Examining such accounts on pain tolerances, we could not help but wonder whether there may be a simple neurological factor to consider, especially following on previous research involving FM patients (Harris, Sundgren, Craig, Kirshenbaum, Napadow, & Clauw, 2009; Harris & Clauw, 2012). Both the anterior and posterior portions of the insula have repeatedly been demonstrated to be involved in different aspects of nociceptive processing, with the posterior insula being involved in sensory aspects, and the anterior involved in affective aspects and emotional processing (Augustine, 1996; Craig, Chen, Bandy & Reiman, 2000; Singer, Seymour, O'Doherty, Kaube, Dolan & Frith, 2004; Tracey &

Mantyh, 2007). Research utilizing proton magnetic resonance spectroscopy (^{1}H-MRS) found that Glutamate (Glu) in the posterior insula of FM patients correlated negatively with PPT, which was also observable in the healthy group as well (Harris et al., 2009). It has also been reported that decreased levels of gamma-aminobutyric acid (GABA) in the anterior insula is linked to increased sensitization to noxious stimulation (Foerster, Petrou, Edden, Sundgren, Schmidt-Wilcke, Lowe, Harte, Clauw & Harris, 2012; Harris & Clauw, 2012). In our examination of baseline neurometabolic concentrations in the insula, we took two PPT ratings from the participants, one before and one after scanning, and correlated them with spectroscopic scans of their insula cortex at rest in order to determine whether the results found in clinical populations may have been reflective of similar relationships in healthy participants. Unfortunately, we failed to replicate these findings in our sample, i.e. a negative correlation between PPT and Glu in the posterior insula, indicating that higher baseline levels of excitatory neurotransmitters reflects a lower pain threshold as it requires less stimulation before a stimuli is regarded to as painful; but the results are interesting nonetheless. The previous literature focused on FM patients, and as such a majority of the sample, including the healthy controls, were females. As such, there was no indication as to the relationship that males would demonstrate. In the present research, it was found that, although there were no observable correlations in females as initially expected, an alternative relationship was observed in male participants in the form of a positive correlation between male PPT and Glu in the anterior insula, as well as a significant difference in the concentrations of anterior insula Glu between males and females. Both of these results do not appear to have been reported in the previous literature, and as such may open up new lines of enquiry.

As mentioned previously, the anterior insula is typically associated with the processing of affective aspects of noxious stimuli as well as interospective regulation, which is understood as forming a representation of "unpleasantness", whereas the subjective is related to intensity, location etc. With our current understanding of how excitatory neurometabolites may function, a higher regional baseline concentration would be indicative of less stimuli being required to illicit a reaction. In short, higher concentrations of excitatory neurometabolites may facilitate processing, which is why a negative correlation between PPT and Glu was expected. The fact that a positive correlation is observed in an area associated with affective processing would indicate that males with a higher PPT may attribute the stimuli with a higher regard of unpleasantness, which could blur the lines between pain threshold and pain tolerance. Pain threshold is typically defined as being the point at which an innocuous stimuli becomes regarded to as noxious, whereas pain tolerance is understood

as being either the most amount of pain one can endure for the longest period of time, which would be associated with unpleasantness as well as the brain's intention to limit the possibility of tissue damage. The fact that this was only observed in males, as well as the significant differences observed between males and females in the same region may be an indication of differential processing of noxious stimulation, in that the anterior insula in males geared to process the affective component of pain prior to the subjective, acting as an estimative process in order to elicit a behavioural change before any tissue becomes damaged. This perspective could fit into previously examined relationships between insular activity and detection of salient stimuli (of which pain is highly salient; Menon & Uddin, 2010), as well as in the context of a comparatively recent model of pain, called the predicative regulation and action model (PRA; Morrison, Perini & Dunham, 2013). This model is based around behavioural adjustment, in that the nervous system and body have developed in a manner that is designed to anticipate and predict the outcome of potentially noxious stimulation, and adjust behaviour accordingly as an almost preventative measure. It is proposed that the anterior and posterior insula are responsible for weighting the stimuli, and that the anterior insula play an active role in predictive behaviour aimed at determining whether the stimulus is painful (Wiech, Lin, Brodersen, Bingel, Ploner & Tracey, 2010; as cited in Morrison, Perini & Dunham, 2013). If this is the case, it is interesting that this was only observed in males, but then it could be one possible neurological difference between male and female pain perception. As the previous research examining the role of regional neurometabolic concentrations was focused on examining correlations in FM patients, it could be considered that the concentrations observed in the posterior insula may be more reflective of either adaptive or functional changes associated with the condition, which would account for why these findings were not replicable. Further research into this observation could include a larger sample size to ensure reliability, test with a number of different forms of noxious stimuli to ascertain whether these findings are just reflective of pressure-pain, and also examine healthy males in comparison to male patients with chronic pain disorders. Further information could also be collected pertaining to unpleasantness in order to deepen our understanding of this finding, both in males and females. If the role of the anterior insula is different in males compared to females, we may see males rating threshold-stimuli as more unpleasant than females do, regardless to differences in thresholds between participants.

With regards to the other findings, it is firstly interesting that there was no significant difference between male and female PPT, as pressure-pain has shown to reliably elicit a distinct gender difference. This may be due to the small sample size compared to larger

behavioural studies, though it is worth noting that there was a disparity between PPT2 (i.e. the second PPT rating measured) of males and females, as well as a significant decrease in the PPT of females from PPT1 to PPT2. This would support previous literature that has examined the PPT of females over an extended period of time, which showed that females PPT does typically decrease over time, though the temporal difference in that research was over the course of days rather than hours (Jones, Kilgour & Contis, 2007). The observed correlation between females PPT and the post-hoc measure of sensitivity is also interesting. It shows that the higher PPT is associated with increased sensitivity, so that the higher a female participant's initial PPT was, the further it appeared to drop upon re-testing. This would also be congruent with previous literature examining the temporal decline in female PPT, and may actually reflect a similar adaptive/predictive behavioural mechanism as previously discussed. If females take prior knowledge of a stimulus and apply it when inserted in the same context, this would form an anticipatory behaviour designed to limit the severity of any potential damage, essentially acting as an early warning sign that an innocuous stimuli is verging on noxious. Further investigation could examine this pattern of behaviour in depth, collecting additional information related to perceived intensity and unpleasantness; if the threshold changes but the rating of intensity and unpleasantness do not, it could indicate an adaptive anticipatory response to limit any potential tissue damage.

Both of the investigations outlined in Chapters three and five serve to highlight the importance of the insular cortex in nociception and the perception of pain, which is something that I will admit I may not have fully appreciated at the beginning of these experiments. While the variety of neuroimaging techniques available has permitted in-depth examinations of this cortex's functions, advances in functional parcellation has allowed further detailed examinations into the insula. Parcellation is a method of identifying or defining structures within the brain either anatomically, using probabilistic atlases or prior knowledge, or functionally using functional clustering algorithms (Maggioni, Tana, Arrigoni, Zucca & Bianchi, 2014). Rather than the two individual networks of the AI and PI typically discussed in fMRI research (Baliki, Geha & Apkarian, 2008; Menon & Uddin, 2010), especially in resting state connectivity (Cauda, D'Agata, Sacco, Duca, Geminiani & Vercelli, 2011), functional parcellation has revealed three distinct networks, as well as interhemispheric differences between the left and right insula (Chang, Yarkoni, Khaw & Sanfey, 2013; Jakab, Molnár, Bogner, Béres & Berényа, 2012). These three networks were localized to three separate subdivisions of the insula, the first of which was found in the ventroanterior portion. This network was found to encompass areas that included gustation,

emotional regulation and anxiety, such as the amygdala, superior temporal sulcus, ventral tegmental area, and posterolateral orbitofrontal cortex. The second network was localized to the dorsoanterior section of the insula and was functionally connected to the ACC and dorsolateral prefrontal cortex, areas involved in attention, inhibition, task switching, error processing, and other aspects of higher cognitive function. These first two networks support findings from previous literature that have examined functions of the AI using both functional imaging techniques and electrode stimulation, which have been linked to interoceptive representation, gustation, viscerosensory processing, predicting physiological and psychological states, and anxiety (Paulus & Stein, 2006; Singer, Critchley & Preuschoff, 2009; Menon & Uddin, 2010; Stephani, Vaca, Maciunas, Koubeissi & Lüders, 2011; Morrison, Perini & Dunham, 2013). The third and final network observed was in the posterior insula and projected to structures that included the somatosensory cortex and supplementary motor area, while its functions were related to somatosensory stimulation, sensorimotor processes, and nociception. This third network supports findings from previous literature, especially those focused on nociception and pain perception (Baliki, Geha & Apkarian, 2008; Menon & Uddin, 2010; Cauda et al., 2011). In regards to interhemispheric differences, it was found that there were no differences between grey matter volume of the left and right insula, but the anterior portion of the left insula was significantly larger than the posterior on the same side, while in the right insula there were no significant differences between the AI and PI. In relation to projections and connections, both left and right AI connected to the opercula and orbitofrontal cortex, temporal and occipital corticies, and parts of the inferiorfrontal gyrus, with an increased density in the fibres connecting the AI to the orbitofrontal area and inferior frontal gyrus in the left hemisphere. There were no apparent differences between the connections of the left and right PI (Jakab et al., 2012).

Researchers investigating the parcellation of insular functions have noted two main caveats with mapping out the functional anatomy, in the forms of the limitations of assessing resting-state functions (without manipulation or intervention) and the disproportionate examinations of functions. In other words, it can be difficult to accurately gage the extent of resting state functions as most examinations of neurological activity analyze the relationship between the activity and stimuli, or task performance. We could attempt to amplify the activity at baseline to gain a clearer perspective, but that would then be due to cognitive manipulation and not be an accurate reflection of the structure at-rest. In terms of mapping out insular functions, the process can be biased due to the focuses of previous research, where there may be more investigations into nociception and interospective awareness than

gustation and auditory processing. As such, the mapping of functions can be skewed towards the functions that may have more abundance in the literature. Our examination into resting state concentrations and individual PPT could fall into at least one of these categories, if not both. Throughout this thesis we have discussed the plethora of roles attributable to the insula, proving that this is a highly interconnected and complex structure. While previous research had found relationships between baseline neurometabolic concentrations and pain tolerance in a clinical population, to examine the same in a selection of healthy participants may have been too reductionist regarding insular functions. With the implementation of more stringent methods or examining baseline verses functional concentrations, we may gain better insight into this cortical structure.

As a contrast to the insula-centric views of saliency and the neurological functions of predictive states, the neuromatrix theory of pain consists of a framework that includes mediating factors that are biological, psychological and social in nature (among others), emerging research aimed at examining and developing an understanding of sex differences in pain perception is not just limited to the neurological. This seems to be as a result of findings within the literature that have noted that although there are distinct biological differences between males and females in regards to pain perception, there are also psychosocial factors to consider as well. Influences such as state and trait anxiety, and personality traits have been examined in depth in the field of pain research, but it has also been demonstrated that males and females exhibit different behaviours in regards to pain catastrophising, healthcare seeking, and coping strategies (Melzack, 2005; Lynch, Kashikar-Zack, Goldschneider & Jones, 2007; Paller, Campbell, Edwards & Dobs, 2009; Mathna, 2015). As such, it may be advisable for future research to collect data on gender identity or gender indicators and integrate it into perceptual models when comparisons between participants. This may be a method that would allow for ease of testing psychological aspects associated with gender differences in regards to nociceptive processing, especially when taking into consideration the variation in the results of investigations into social aspects such as personality traits.

In our social-based experiment, we examined the mechanisms behind the experimenter effect, along with the inclusion of an observer over the course of six different test sessions, each one forming an individual condition. The conditions were based around experimenter gender and observer type, resulting in the following six: Male experimenter, no observer (control); male experimenter, male observer; male experimenter, female observer; female experimenter, no observer (control); female experimenter, male observer; female experimenter, female observer. To examine potential mechanisms, a selection of self-report

questionnaires was included in each condition, which included the STAI-state anxiety questionnaire and a questionnaire designed to rate participants' impressions or perceptions of the experimenter. In the first testing session, participants also completed the STAI-trait anxiety questionnaire, and the Big Five Personality Inventory (BFI). Based on previous findings, we expected that the gender of the experimenter would affect at least the male participants' PPT, as males had been reported as demonstrating a higher pain threshold when tested by a female as opposed to a male experimenter, while female participants might not show any difference in PPT when tested by a male or female. The questionnaires were included in order to establish wether there were any correlations between personalities or emotional states and pain tolerance, while the observers were included to examine whether this experimenter-gender effect is either enhanced by the observer, or could transfer onto it. Our results did indeed reflected previous findings within the literature, demonstrating males as having a higher pressure-pain threshold (PPT) than females (Fillingim et al., 2009; Racine et al., 2012a), and that males demonstrated a higher pain threshold when tested by a female experimenter compared to a male, whereas female participants did not demonstrate any significant difference in thresholds between male and female experimenters (Levine & DeSimone, 1991; Kallai, Barke & Voss, 2004; Gijsbers & Nicholson, 2007; Aslasken, Myrbakk, Hoifodt & Flaten, 2007). However, while the experiment demonstrated a significant main effect of experimenter, there was also one for the observer, indicating that the observer does indeed affect pain thresholds in an experimental environment. An overall interaction significance between experimenter gender and observer level, indicating that the presence of the observer, as well as their gender, may either enhance the experimenter gender effect, or act as an extension of it. This would warrant further examination with a larger sample size to fully prove or refute whether this interaction is an indication of the proposed effect. The surprising result came from an examination of PPT mean-centred conditional results. It may not just be female experimenters that influence male participant's pain ratings, as it was demonstrated that their PPT dropped below the medium they when tested by a male experimenter, indicating that male experimenters illicit a similar effect as female experimenters, but in the opposite direction. These results have a number of implications in not only an experimental context, but also a clinical one, especially when taking into consideration that clinical examinations and assessments of pain or physiological conditions may involve more than just the assessor.

There are a number of ways in which research could be conducted to examine and explain these findings further. The implication that male experimenters may influence male

participants' pain threshold could be examined in a similar manner as the observer experiment was conducted in this thesis. As well as having a male experimenter condition and a female experimenter condition, by including a third condition in which either the participants administer the test to themselves, or the testing is conducted remotely, it would be possible to ascertain whether there is a "reverse" experimenter effect. If so, it would be expected that male participants' pain thresholds would fall between that of those collected by the male and female experimenters. Similarly, it would be interesting to see how female participants' ratings may or may not change, as typically there is no significant difference between the rating collected by male and female experimenters. Either there could be no significant difference, indicating that females pain ratings are the least affected by experimenter characteristics, or if there is a significant difference is could indicate that females are affected by the experimenter, just not by their gender. In a similar line of enquiry, the observer effect could be tested in much the same way. It would be of interest to ascertain whether the presence or gender of the observer affects pain ratings, by including either an individual whose gender is difficult to determine and utilizing non-gender specific clothing and styles, or if merely the concept of an observer can elicit the effect, utilizing electronic recording equipment or one-way glass window. As mentioned previously, the findings of experiments such as these could have a large impact upon clinical practices, and by finding the closest manner by which to quantify a "true" measure of a patient's pain or severity of condition, it may be possible to implement the best possible form, or course, of treatment. As it is mentioned in Moller (2014) pain is mostly disregarded in medical education, despite it being responsible for why a number of patients seek medical help, merely because it is not quantifiable. It is almost entirely a sensory phenomena that may not be attributable to any external stimuli. As such it can be difficult to examine and even treat properly.

In an effort to explain possible factors or individual traits that account for the experimenter effect, measures of personality (in the form of the big five inventory; BFI), state and trait anxiety (STAI), and experimenter ratings were taken. The initial theory proposed for a possible observer effect at the start of the study followed research from examinations of anxiety, which followed the theory that when an individual considers themselves to being evaluated across one or more domains of performance, a state of introspective awareness is induced that would, in turn, result in an increased sensitivity to noxious stimulation (hyperalgesia; Duval & Wicklund, 1972). This proposition was also following research examining state anxiety, as well as theories that stipulate that increased anxiety would change the focus of attention from goal-orientated attentive behaviours, to stimulus-orientated

attentive behaviours (Spurr & Stopa, 2002; Eyesenk, Derakshan, Santos & Calvo, 2007). However, no significant effects or correlations were observed between PPT and STAI state or trait anxiety. This could indicate either that the changes in anxiety were minute and the STAI was not sensitive enough to detect them; that a more subtle form of biological stress or anxiety is responsible; or that anxiety does not play a part in changes in pain threshold in the experimenter, and now observer, effect. There were also no indications that opinions or ratings of the experimenter are responsible for changes in pain threshold. These findings (and to an extent, the findings of the STAI) are not totally surprising, considering previous research measuring autonomic (i.e. heart rate, skin conductance) during testing of the experimenter effect, which found no correlations between pain ratings and physiological responses, regardless of participant and experimenter gender (Aslasken et al., 2007). The results from the BFI are perhaps of most interest, as there was a significant positive correlation between PPT and Openness, which was initially observed overall, and then only found significant in female participants, though as the level of significance was higher in males and females vs. females alone, it does indicate that males are a contributing factor. Based on previous literature, it was initially expected that Extroversion (Lynn & Eyesenk, 1961), Conscientiousness (Cray, Springborne, Lotsch, Johnson & Hummel, 2011) and Neuroticism (Malin & Littlejohn, 2012) would correlate with PPT in one form or another, especially as Openness has not previously been linked with pain thresholds. However, this finding does make sense when considering the previously mentioned differences in male and female coping strategies in regards to pain, in that males typically adapt using behavioural distractions, whereas females seek comfort from social connections. It may be plausible that females who are more open to social situations, in turn resulting in a healthier social attitude and life. If this is the case, it may indicate that females with (for lack of a better term) "better" social lives may be able to withstand a higher amount of pain due to the comfort they have gained from their social links. This would warrant further investigation, particularly as within the last decade, the understanding of the population as to what constitutes socializing has dramatically changed thanks to the advent of large-scale social networking websites. If this were the case, further research could be conducted into examining how social networking, as well as how much value an individual places on social networking sites, may impact upon the coping mechanisms or quality of life that females with chronic pain disorders experience. For example; how does someone who has 1000 followers and rates the importance or impact of Twitter on their lives as "high" cope better with their condition compared to someone with 1500 followers that rates the importance as "moderate"? Further

quantification of social lives and activities may also shed more light upon how Openness may interact or mediate pain perception. However, this finding does make sense when considering the previously mentioned differences in male and female coping strategies in regards to pain, in that males typically adapt using behavioural distractions, whereas females seek comfort from social connections. It may be plausible that females who are more open to social situations, in turn resulting in a healthier social attitude and life. In fact, a recent study examining human social networks and pain tolerance has found that those with larger social groups actually have higher pain tolerance compared to those with fewer social connections (Johnson & Dunbar, 2016). While it has been previously been suggested that this sort of phenomena might be due to comfort gained from social interaction, the most plausible reason for this relationship comes in the form of the social theory of opioid attachment. Machin and Dunbar (2011) drew parallels between the stages of opioid addition and the development of social relationships due to the manner in which β-endorphins binds to μ-opioid receptor cells in the nervous system as a socially-evolved reward-based mechanism of human social bonding, similarly to the manner in which opioid-based painkillers do when causing alleviation from pain. As a direct result of this, social bonding can become highly addictive and does appear to have analgesic effects, explain the results found by Johnson and Dunbar (2016). While we may not be able to directly link this to our results, it may give an explanation to the relationship between PPT and openness, and could also form the basis for further examination in future; while we examined anxiety as possible driving effect, it could actually be related to participants' adaptability and comfort in new social situations. There are a number of implications for the link between the social opioid theory, and each one could lead to any number of new avenues of examination, especially considering the manner in which online social networking has prospered over the last decade or so. The understanding and definition as to what constitutes socializing has dramatically changed thanks to the advent of large-scale social networking websites. If this were the case, further research could be conducted into examining how social networking, as well as how much value an individual places on social networking sites, may impact upon the pain tolerance of participants, and is the same effect found in clinical populations? How does online social networking, which can be done from almost anywhere, affect the quality of patients with chronic pain disorders? For example; how does someone who has 1000 followers and rates the importance or impact of Twitter on their lives as "high" cope better with their condition compared to someone with 1500 followers that rates the importance as "moderate"? Or does these analgesic effects from large social networks on pertain to actual physical social

interaction? It could also be possible that older generations may benefit more from physical social interaction, while younger generations, who have grown up with social media and are more familiar or dependent on it, may receive the same benefits from online interactions. As society is gravitating more and more towards internet-centric businesses, learning, and socialising, it would be of great interest to examining how this is impacting upon the population psychologically in a variety of domains, not to mention the possible implications this is now appearing to have on aspects such as nociception.

Though these experiments do have their applications and merits, they are not without their limitations. The main concern for both would be their sample sizes. A criterion was proposed by Riley, Robinson, Wise, Myers and Fillingim (1998), which stipulated that any research aimed at examining differences in pain perception should aim to include a minimum of 41 participants per group in order to attain sufficient statistical power. The social experiment examining the experimenter and observer gender effects initially intended to examine 40-50 males and females i.e. a total of 80-100 participants altogether, but due to time constraints and subject attrition rates only 30 female and 21 male participants attended enough sessions to be included in the study. Although the sample sizes fail to meet the criterion put forth by Riley et al. (1998), they are still fairly substantial. Even if it is argued that they are not as generalizable to the population or lack the statistical power, it cannot be denied that they certainly give pause for thought. As such, at the very least this data could be considered a pilot in proposition of a much larger study that would attain sufficient statistical power. Another potential limitation in the social experiment could be the employment of different male observers, and female experimenters and observers. This was again due to time constraints, as the research was carried over from one academic year into another (again due to constraints and subject attrition), alternate postgraduate students were employed to participate in the roles, though their training and performances were kept as identical to the initial experimenter and observer as possible. It is also possible that as the participants were informed that the observers in the experiment were included to evaluate the conduction of the research due to it's nature (i.e. examination of pain); this may have reduced the impact that the observers would have had on the participants. If they were to induce this state of objective self-awareness, influencing the attentive behaviours of the participants, by informing the participants that the observer was evaluating the experimenters, then the effect that the observer had upon the participants state anxiety may have been significantly reduced. Further research may wish to not directly explain the role of the observer unless asked, in order to

keep the uncertainty of their inclusion and see whether that impacts upon participant pain ratings.

In regards to the ^{1}H-MRS experiment, due to the unexpected results there may be a number of limitations to consider before drawing any solid conclusions. Firstly, the sample size is less of a limitation here in comparison to the social study. A majority of imaging studies consists of 10-20 participants per group typically, in part due to the cost of scanning a participant with the use of an MRI. As such, the sample of 11 males and 7 females is relatively sufficient, though larger samples in further investigations would certainly not hurt. One limitation to consider is that of the number of PPT measurements that was taken. It has been established that for the greatest reliability, three pressure-pain ratings should be taken, and the average of those three should form the rating for that participant or condition (Chesterton, Sim, Wright & Foster, 2007). Again, due to time constraints only two PPT ratings were taken, and as such further investigations should aim to acquire at least 3 PPT measurements if possible. As this experiment was focused on examining baseline concentrations, it can be harder to draw direct conclusions compared to in a functional experiment, purely due to possible confounding factors that we may be unaware of. That being said, these results still hold merit as tests of statistical significance, by definition, remove the possibility of a "chance" finding. As previously mentioned, it would also be worthwhile collecting data on participants' perceived unpleasantness and intensity, in order to understand what these results truly reflect, and whether it reflects a behavioural component.

Over the course of this thesis, examinations and speculations have been conducted and composed aimed at understand how and why gender affects pain perception, and where this might fit in with contending theories. It has been established that external factors relating to experimenter characteristics as well as the involvement of others, can influence how males experience pain, and that they do so differently than females do. Although state and trait anxiety does not appear to be a major influence on the sensory perceptions of healthy participants, there is evidence that personality may do, particularly if this is related to typical coping strategies that people are understood as employing. This was established by the findings in the social experiment, which found that the PPT of males alters depending not only on the gender of the experimenter, but the presence and even gender of an additional observer, while females' do not seem to be altered as significantly. Though there were no findings relating to the STAI, the personality trait of Openness (as tested in the BFI) does to correlate with PPT, which may be a reflection of coping strategies that females have been

shown to employ. From the biological approach, it appears that there are differences between the regional neurometabolic concentrations of males and females, and that this may reflect adaptive anticipatory responses to potentially noxious stimulation in males, whereas females may utilize a similar behavioural anticipatory response. From these findings, we may be able to contribute to the ever-expanding knowledge of pain research, and open up new avenues of investigation to further our understanding of what pain is and how to treat it in those who most need respite. One day we may even come close to virtually eliminating pain, which may be considered detrimental to the population at large, but it could have drastic implications for those suffering from unnecessary pain in their day-to-day lives, which would afford them better opportunities in both their personal lives and professions. There is, of course, a long way to go, but as they say; “the journey of 1000 miles begins with a single step.”

Chapter 6 References

Aslaken, P. M, Myrbakk, I. N., Hoifodt, R. S. and Flaten, M. A. (2007). The effect of experimenter gender on autonomic and subjective responses to pain stimuli. *Pain*. 129: 260-268.

Augustine, J. R. (1996) Circuitry and functional aspects of the insular lobe in primates including humans. *Brain Research Revie*ws. 22, pp 229-244.

Baliki, M. N., Geha, P. Y. and Apkarian, A. V. (2008). Parsing Pain Perception Between Nociceptive Representation and Magnitude Estimation. *Journal of Neurophysiology.* 101: 875-887.

Berkley, K. J. (1992). Vive la difference. *Trends in Neurosciences.* 5: 331-332.

Cauda, F., D'Agata, F., Sacco, K., Duca, S., Geminiani, G. and Vercelli, A. (2011). Functional connectivity of the insula in the resting brain. *Neuroimage*. 55: 8-23.

Chang, L. J., Yarkoni, T., Khaw, M. W. and Sanfey, A. G. (2013). Decoding the Role of the Insula in Human Cognition: Functional Parcellation and Large-Scale Reverse Inference. *Cerebral Cortex*. 23: 739-749.

Chesterton, L. S., Sim, J., Wright, C. C. and Foster, N. E. (2007)Interrater reliability of Algometry in measuring pressure pain thresholds in healthy humans, using multiple raters. Clinical Journal of Pain. 23(9), pp 760-766.

Craig, A. D., Chen, K., Bandy, D. and Reiman, E. M. (2000) Thermosensory activation of the Insula cortex. Natural Neuroscience. 3, pp 184-189.

Cray, I., Springborn, M., Lotsch, J., Johnson, A. N. B. and Hummel, T. (2011). Agreeable smellers and sensitive neurotics - correlations among personality traits and sensory thresholds. *PLOS ONE.* 6(4).

Duval, S. and Wicklund, R. A. (1972). A theory of objective self-awareness. London: Academic Press.

Eyesenk, M. W., Derakshan, N., Santos, R. and Calvo, M. G. (2007). Anxiety and cognitive performance: Attentional control theory. *Emotion.* 7(2): 336-353.

Fillingim, R. B., King, C. D., Ribeiro-Sasilva, M. C., Rahim-Williams, B. and Riley III, J. L. (2009). Sex, gender and pain: A review of recent clinical and experimental findings. *The Journal of Pain*. 10 (5): 447-485.

Foerster, B. R., Petrou, M., Edden, R. A. E., Sundgren, P. C., Schmidt-Wilcke, T., Lowe, S. E., Harte, S. E., Clauw, D. J. and Harris, R. E. (2012). Reduced Insular γ-Aminobutyic Acid in Fibromyalgia. Arthritis and Rheumatism. 64(2), pp 579-583.

Gijsbers, K. and Nicholson, F. (2005). Experimental pain thresholds influenced by

sex of the experimenter. *Perceptual Motor Skills*. 101: 803-807.

Harris, R. E. and Clauw, D. J. (2012) Imaging central neurochemical alterations in chronic pain with proton magnetic resonance spectroscopy. *Neuroscience Letter*. 520: 192-192.

Harris, R. E., Sundgren, P. C., Craig, A. D., Kirshenbaum, E., Sen, A., Napadow, V. and Clauw, D. J. (2009) Elevated Insular Glutamate in Fibromyalgia Is Associated With Experimental Pain. Arthritis and Rheumatism. 60(10), pp 3146-3152.

Jakab, A., Molnár, P. P., Bogner, P., Béres, M. and Berényа, E. L. (2012) Connectivity-based parcellation reveals interhemispheric differences in the insula. *Brain Topography.* 25: 264-271.

Johnson, K. V. A, and Dunbar, R. I. M. (2016). Pain tolerance predicts human social network size. *Scientific Report.* 6(25267): 1-5.

Jones, D. H., Kilgour, R. D. and Contois, A. S. (2007). Test-Retest Reliability of Pressure-Pain Threshold Measurements of the Upper Limb and Torso in Young Healthy Women. *The Journal of Pain*. 8(8), pp 650-656.

Kallai, I., Barke, A. and Voss, U. (2004). The effects of experimenter characteristics on pain reports in women and men. *Pain*. 112: 142-147.

Kong, J., White, N. S., Kwong, K. K., Vangel, M. G., Rosemann, I. S., Gracely, R. H. and Gollub, R. L. (2006). Using fMRI to dissociate sensory encoding from cognitive evaluation of heat pain intensity. *Human Brain Mapping.* 27:715-721.

Levine, F. M. and DeSimone, L. L. (1991). The effects of experimenter gender on pain reports in male and female subjects. *Pain*. 44: 69-72.

Lynch, A.M., Kashikar-Zuck, S., Goldschneider, K.R., and Jones, BA. (2007). Sex and age differences in coping styles among children with chronic pain *Journal of Pain Symptom Management*. 33: 208-216.

Lynn, R. and Eyesenk, H. J. (1961). Tolerance for pain, extraversion and neuroticism. *Perceptual Motor Skills.* 12: 161-162.

Machin, A. J. and Dunbar, R. I. M. (2011). The Brain Opioid Theory of Social Attachment: A Review of the Evidence. *Behaviour.* 148(9-10): 985-1025.

Maggioni, E., Tana, M. G., Arrigoni, F., Zucca, C. and Bianchi, A. M. (2014) Constructing fMRI connectivity networks: whole brain functional parcellation method for node definition. *Journal of Neuroscience.* 228: 86-99.

Malin, K. and Littlejohn, G. O. (2012). Neuroticism in young women with fibromyalgia link s to key clinical features. *Pain Research and Treatment*.

Mantha, M. H. (2015). Application of the Neuromatrix Pain Theory to Understand Sex and Gender Differences in Chronic Pain. *AASCIT Journal of Medicine.* 1(5): 67-73.

Melzack, R. (1999). From the gate to the neuromatrix. *Pain.* 6: 121-126.

Melzack, R. (2001). Pain and the neuromatrix in the brain. *Journal of Dental Education.* 65(12):1378-1382.

Melzack, R. (2005). Evolution of the neuromatrix theory of pain. The Prithvi Raj Lecture: presented at the third World Congress of World Institute of Pain, Barcelona 2004. *Pain Practice.* 5(2): 85-94.

Menon, V. and Uddin, L. Q. (2010). Saliency, switching attention and control: a network model of insula function. *Brain Structure and Function*. 214: 655-667.

Moller, A. R. (2014). Pain: it's anatomy, physiology and treatment (second ed.) Aage R Moller Publishing. Richardson, TX.

Morrison, I., Perini, I. and Dunham, J. (2013). Facets and mechanisms of adaptive pain behavior: predictive regulation and action. *Frontiers in Human Neuroscience*. 7(755): 1-11.

Paller, C. J., Campbell, C. M, Edwards, R. R. and Dobs, A. S. (2009). Sex-Based Differences in Pain Perception and Treatment. *Pain Medicine.* 10(2): 289-299.

Paulus, M. P. and Stein, M. B. (2006) An Insular view of anxiety. *Biological Psychiatry*. 60: 383-340.

Piazza, M., Pinel, P., Le Bihan, D. and Dehaene, S. (2007) Amagnitude code common to numerosities and number symbols in human interparietal cortex. *Neuron.* 53: 293-305.

Pleger, B., Ruff, C. C., Blankenburg, F., Bestmann, S., Wiech, K., Stephan, K. E., Capilla, A., Friston, K. J. and Dohn, R. J. (2006). Neural coding of tactile decisions in the human prefrontal cortex. *Journal of Neuroscience.* 26: 12596-12601.

Ploghaus, A., Tracey, I., Gati, J. S., Clare, S., Menon, R. S., Matthews, P. M. and Rawlins, J. N. P. (1999). Dissociating pain from its anticipation in the human brain. *Science*. 284: 1979-1981.

Ploner, M., Lee, M. C., Wiech, K., Bingel, U. and Tracey, I. (2010). Prestimulus functional connectivity determines pain perception in humans. *Proceedings of the National Academy of Science of the United States of America.* 107: 355-360.

Racine, M., Tousignant-Laflamme, Y., Kloda, L. A., Dion, D., Dupuis, G. and

Choinere, M. (2012a). A systematic literature review of 10 years of research of sex/gender and experimental pain perception – Part 1: Are there really differences between women and men? *Pain.* 153: 602-618.

Racine, M., Tousignant-Laflamme, Y., Kloda, L. A., Dion, D., Dupuis, G. and Choinere, M. (2012b). A systematic literature review of 10 years of research of sex/gender and experimental pain perception – Part 2: Do biosocial factors alter pain sensitivity differently in women and men? *Pain.* 153: 619-635.

Riley III, J. L., Robinson, M. E., Wise, E. A. Wyers, C. D and Fillingim, R. B. (1998). Sex differences in the perception of noxious experimental stimuli: a meta-analysis. *Pain.* 74(2-3): 181-187.

Ruda, M. A. (1993). Gender and pain [editorial]. *Pain.* 53: 1-2.

Sawamoto, N., Honda, M., Okada, T., Hanakawa, T., Kanda. M., Fukuyama, H., Konishi, J. and Shibasaki, H. (2000). Expectation of pain enhances responses to nonpainful somatosensory stimulation in the anterior cingulate cortex and parietal operculum/ posterior insula: an event-related functional magnetic resonance imaging study. *The Journal of Neuroscience.* 20, 19: 7438-7445.

Siegler, R. S. and Opfer, J. E. (2003). The development of numerical estimation: Evidence for multiple representations of numerical quantity. *Psychological Science.* 14: 237-243.

Singer, T., Critchley, H. D. and Preuschoff, K. (2009) A common role of insula in feelings, empathy and uncertainty. *Trends in Cognitive Sciences*. 13(8): 334-340.

Singer, T., Seymour, B., O'Doherty, J., Kaube, H., Dolan, R. J. and Frith, C. D. (2004). Empathy for pain involves the affective but not sensory components of pain. *Science*. 303, pp 1157-1162.

Spurr, J. M. and Stopa, L. (2002). The observer perspective: effects on social anxiety and performance. *Behaviour Research and Therapy.* 40, Pp 1009-1028.

Stephani, C., Vaca, F-B., Maciunas, R., Koubeissi, M. and Lüders, H. O. (2011). Functional neuroanatomy of the Insular lobe. *Brain Structure and Function*. 216: 137-149.

Tracey, I. and Mantyh, P. W. (2007) The Cerebral Signature for Pain Perception and Its Modulation. *Neuron.* 55, pp 377-391.

Weich, K., Lin, C. S., Brodersen, K. H., Bingel, U., Ploner, M. and Tracey, I. (2010). Anterior insula integrates information about alience into perceptual decisions about pain. *Journal of Neuroscience.*30: 16324-16311.

Appendices

Contents

Appendix A: Participant Information and Consent Form - Chapter 4

BANGOR BRAIN IMAGING UNIT
Participant Information Sheet

School of Psychology: Bangor University
Information Sheet for Participating in a Research Project

You are being invited to take part in a research study. Before you decide whether to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

TITLE OF STUDY:
A behavioral examination of sex differences and experimenter characteristics in pain sensitivity

INVESTIGATORS:
Dr Paul Mullins, Dr Erin Heerey, Alexander Currie, Karolina Rusiak, Adam Brickley, Jenny Morgan, Pippa Beston

WHAT IS THE PURPOSE OF THE STUDY?

Pain is a universally recognized concept across all cultures, but it is an entirely subjective experience that can be influenced by a number of factors both internal and external relative to the individual (Nielsen, Staud & Price, 2009). These factors can range from individual differences, such as age, gender, ethnicity and life experiences (Woodrow et al., 1972), to differences in the was that the experienter can be perceived (Levine &De Simone, 1991; Gijsbers & Nicholsen, 1995; Fillingham et al, 2009). We are interested in examining how a combination of these factors can influence pain perception over an extended period of time. The results from this experiment could give us further insight into how individual perceptions of pain can be moderated, which could prove to be relevant to both experimental and clinical environments.

WHAT ARE THE PROCEDURES?

Questionnaires
You will be given a series of questionnaires designed to give us more information relating to individual differences between participants. Each questionnaire should take no more then 5 minutes, you will be asked to complete 5 questionnaires in the first test session, and 2 questionnaires during each of the subsequent test sessions. You are welcome to skip any questions you do not feel comfortable answering.

Measurement of pressure pain threshold

Pressure pain threshold will be measured using a device called an algometer. This device measures the amount of pressure applied to a small plastic tip. Using this device we will take readings on the top of the hand, in particular on the fleshy bit of the webbing between the thumb and the index finger. The algometer involves a round stud on the end of a pressure sensitive arm. The round stub is applied to the fleshy bit of the webbing between the thumb and index finger and pressure is applied until you indicate that pressure has become painful. You should inform the researcher as soon as the pressure starts to become painful, do not wait until the pain reaches a certain level, let us know *as soon as it starts to become painful*. At this time pressure will be stopped and the algometer removed. You will be allowed to recover for 10 minutes before a second reading is taken, and the procedure will be followed again, with 10 minutes rest before a third rating is taken. The average of the three readings will then be used as a measure of your pressure pain threshold. Do not try to see how long you can last, or how high you can take the pressure as this would distort our results, and may cause you considerable pain. Remember, you have to let us know as soon as you feel any pain. Pressure pain thresholds will be measured over a total of 6 test sessions during a two-week period.

Follow-up study
You may be contacted in the future with the opportunity to participate in an experiment utilizing the fMRI scanner in this department. If you do not wish to be contacted, please indicate here:

- I would not like to be contacted for further participation involving the scanner

HOW IS CONFIDENTIALITY ENSURED?
The information obtained from the assessments may be published in scientific journals, but your name will not appear in any public document, nor will the results be published in a form that would make it possible for you to be identified

DO I HAVE A RIGHT TO REFUSE OR WITHDRAW?
You may refuse to participate at any time. You may change your mind about being in the study and quit after the study has started, and if you feel, for any reason, uncomfortable, the study will be discontinued.

WHAT WILL HAPPEN TO THE STUDY RESULTS?
They will be kept securely for a minimum of 10 years and possibly indefinitely in the BANGOR BRAIN IMAGING UNIT data archive in accordance with good research practice. Results of the study may be published in a scientific journal or other public format. In this case your data will either be included as part of a group average, or will be anonymised so that no identifying information is given.

WHAT IF I HAVE FURTHER QUESTIONS?
We welcome the opportunity to answer any question you may have about any aspect of this study or your participation in it. Please contact Paul Mullins at the School of Psychology, Bangor University, Gwynedd, LL57 2AS, phone 01248 383631.

ARE THERE COMPENSATION ARRANGEMENTS IF SOMETHING GOES WRONG?
In the unlikely event of anything untoward happening, the University's insurer provides insurance for negligent harm. It does not provide insurance for non-negligent harm but does take a sympathetic view should a claim be made.

WHAT IF I HAVE COMPLAINTS?
This research study has been approved by the School of Psychology Research Ethics and Governance Committee. In the case of any complaints concerning the conduct of research, please address these to Professor C. Leek, Head of School, School of Psychology, Bangor University, Gwynedd, LL57 2AS.

Thank you for considering taking part in this study. Our research depends entirely on the goodwill of potential volunteers such as you. If you require further information, we will be pleased to help you in any way we can.

BANGOR BRAIN IMAGING UNIT
Participant Consent Form

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

TITLE OF STUDY: A behavioral examination of sex differences and experimenter characteristics in pain sensitivity

INVESTIGATORS:
Paul Mullins, Erin Heerey, Alexander Currie

The volunteer should complete this entire sheet himself/herself.
Please circle as appropriate:

Have you read the participant information sheet?

YES / NO

Have you had the opportunity to ask questions and discuss this study?

YES / NO

Have you received enough information about the study?

YES / NO

Do you understand that your participation is voluntary and that you are free to withdraw from the study,

- At any time
- Without having to give a reason
- And without affecting your future medical care?

YES / NO

Do you understand that these are not diagnostic scans?

YES/NO

Do you understand that the Bangor University provides insurance for negligent harm but that it does not provide insurance for non-negligent harm?

YES/NO

Do you understand that the research data may be accessed by researchers working at or in collaboration with the BANGOR BRAIN IMAGING UNIT in similar ethically approved studies, but that at all times your personal data will be kept confidential in accordance with data protection guidelines?

YES/NO

Do you agree to take part in this study?

YES / NO

_____ _____

Date Signature of Participant

_____

Name in block letters

_____ _____

Date Signature of Investigator

_____

Name in block letters

_____ _____

Date Signature of Investigator

_____

Name in block letters

Appendix B: Demographic Questionnaire –Chapter 4

PID:_____ Session:_____ Date:_____ Time:

Demographic Questionnaire

Age:

Gender:

Ethnicity:

First Language:

Year of study in university:

Have you ever suffered from a condition sufficient to require treatment for pain for longer then 3 months (such as physiotherapy, prescription medication or pain management courses)?

If yes to the above question, what has your experience with pain been? (please feel free to skip this question if you do not feel comfortable answering it)

Appendix C: STAI Y1 and Y2 – Chapter 4 (and 5; supplemental)

PID:_____ Session:_____ Date:_____ Time:

SELF-EVALUATION QUESTIONNAIRE

STAI Form Y-1

DIRECTIONS: Please read each statement and circle the appropriate number to indicate how you feel *right* now, that is, *at this moment*.

(1 = ALMOST NEVER, 2 = SOMETIMES, 3 = OFTEN, 4 = ALMOST ALWAYS)

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

PID:_____ Session:_____ Date:_____ Time:

STAI Form Y-2

DIRECTIONS: Please read each statement and then circle the appropriate number to indicate how you would describe how you *generally* feel.

(1 = ALMOST NEVER, 2 = SOMETIMES, 3 = OFTEN, 4 = 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 the 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 disturbed 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

Appendix D: Big Five-Personality Inventory – Chapter 4

PID:_____ Session:_____ Date:_____ Time:

How I am in general

Here are a number of characteristics that may or may not apply to you. For example, do you agree that you are someone who *likes to spend time with others*? Please write a number next to each statement to indicate the extent to which **you agree or disagree with that statement.**

1 Disagree Strongly	2 Disagree a little	3 Neither agree nor disagree	4 Agree a little	5 Agree strongly

I am someone who:

1. _____Is talkative
2. _____Tends to find fault with others
3. _____Does a thorough job
4. _____Is depressed, blue
5. _____Is original, comes up with new ideas
6. _____Is reserved
7. _____Is helpful and unselfish with others
8. _____Can be somewhat careless
9. _____Is relaxed
10. _____ Is curious about many different things
11. _____If full of energy
12. _____Starts quarrels with others
13. _____Is a reliable worker
14. _____Can be tense
15. _____Is ingenious, a deep thinker
16. _____Generates a lot of enthusiasm
17. _____Has a forgiving nature
18. _____Tends to be disorganized
19. _____Worries a lot
20. _____Has an active imagination
21. _____Tends to be quiet
22. ._____Is generally trusting
23. ._____Tends to be lazy
24. ._____Is emotionally stable, not easily upset
25. ._____Is inventive
26. _____Has an assertive personality
27. _____Can be cold and aloof
28. _____Perseveres until the task is finished
29. _____Can be moody
30. _____Values artistic, aesthetic experiences
31. _____Is sometimes shy, inhibited
32. _____Is considerate and kind to almost everyone
33. _____Does things efficiently
34. _____Remains calm in tense situations
35. _____Prefers work that is routine
36. _____Is outgoing, sociable
37. _____Is sometimes rude to others
38. _____Makes plans and follows through with them
39. _____Gets nervous easily
40. _____Likes to reflect, play with ideas
41. . _____Has few artistic interests
42. _____Likes to cooperate with others

PID:_____ Session:_____ Date:_____ Time:

43. _____Is easily distracted

44. . _____Is sophisticated in art, music of literature

Appendix E: Experimenter Rating Questionnaire – Chapter 4

PID:_____ Session:_____ Date:_____ Time:

Please read the statements below circle the appropriate number to indicate how you would describe the experimenter (1= not at all, 7=extremely)

To what extent did you find the experimenter to be:

1)Warm and friendly ……1 2 3 4 5 6 7

2)Attractive……..1 2 3 4 5 6 7

3)Competent and professional……1 2 3 4 5 6 7

4)Outgoing……1 2 3 4 5 6 7

5)In control……..1 2 3 4 5 6 7

6)Trustworthy……1 2 3 4 5 6 7

Appendix F: Participant Debrief Form – Chapter 4

Debrief Form

Thank you for taking part in this study!

It has been shown previously that external factors such as experimenter gender and perceived professional status can alter an individual's perception of noxious stimuli. We are interested in seeing if this gender phenomenon can extend to the presence of an additional observer who is present during test sessions. Based on preliminary results, we expect that the presence of an additional observer will lower or heighten the participant's pain threshold, dependent on the gender of the experimenter and that of the observer. It has also been shown that anxiety and aspects of personality correlate with differences in pain tolerance. We are examining these factors also, to see if they could be a driving force behind any observable differences in pain threshold between the conditions. We also wish to see if there is an overall interaction between personality, anxiety, the presence and gender of an observer, both participant and experimenter gender and pain tolerance threshold for pressure-pain.

If you have any further questions about the experiment, or the results, please contact Alexander Currie at a.g.j.currie@bangor.ac.uk, or Dr. Paul Mullins at p.mullins@bangor.ac.uk if you have any comments or complaints about this procedure of this experiment.

Appendix G: Participant Information, Consent and MRI Screening Form – Chapter 5

BANGOR BRAIN IMAGING UNIT
Participant Information Sheet

School of Psychology: Bangor University
Information Sheet for Participating in a Research Project

You are being invited to take part in a research study. Before you decide whether to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

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WHAT ARE THE PROCEDURES?

Measurement of pressure pain threshold

Pressure pain threshold will be measured using a device called an algometer. This device measures the amount of pressure applied to a small plastic tip. Using this device we will take readings on the top of the hand, in particular on the fleshy bit of the webbing between the thumb and the index finger. The algometer involves a round stud on the end of a pressure sensitive arm. The round stub is applied to the fleshy bit of the webbing between the thumb and index finger and pressure is applied until you indicate that pressure has become painful. You should inform the researcher as soon as the pressure starts to become painful, do not wait until the pain reaches a certain level, let us know *as soon as it starts to become painful*. At this time pressure will be stopped and the algometer removed. You will then be scanned, and once the scanning procedure is completed one final rating will be taken using the algometer. Do not try to see how long you can last, or how high you can take the pressure as this would distort our results, and may cause you considerable pain. Remember, you have to let us know as soon as you feel any pain.

MRI and MRS procedures

The study may involve lying still in the MRI scanner, which resembles a large doughnut in shape, while the MRS and MRI measures are taken. A typical scanner might be 7 feet tall by 7 feet wide by 10 feet long and is mostly comprised of the very large magnet. There is a **horizontal tube** running through the **magnet** from front to back. This tube is known as the **bore**. You will be asked to lie on your back on the scanner patient bed and will have an RF coil placed over your head. The RF coil is what we use to detect

the signal from the hydrogen atoms – no radiation is involved and no dye needs to be injected. The scan is not in any way painful, but the scanner makes a loud noise so we will give you ear plugs as well as headphones to reduce this noise. The patient bed will then move into the bore placing you at the center of the magnet. It is possible that you may feel a little claustrophobic as it is a small confined space however, you will be able to see outside the scanner during the scan and will be able to communicate with the operator at all times. If you find the scan to be uncomfortable in any way, the operator will immediately stop the scan. This study will also include MR measurements of static brain anatomy, and it will also comprise of a study into brain chemistry at rest.
The total session will take a maximum of about one hour.

Because a magnetic field is involved, you cannot be scanned if you have a pacemaker, or metal in your body. We will go through a list of relevant items with you before scanning. Because the scanner is configured as a narrow tube, some individuals with claustrophobia (fear of confined spaces) may find the procedure uncomfortable or intolerable. So, you cannot be scanned if you have a history of claustrophobia. Prior to scanning one of the investigators will go through an MR safety screening survey to ensure your safety.

HOW IS CONFIDENTIALITY ENSURED?
The information obtained from the assessments may be published in scientific journals, but your name will not appear in any public document, nor will the results be published in a form that would make it possible for you to be identified

DO I HAVE A RIGHT TO REFUSE OR WITHDRAW?
You may refuse to participate at any time. You may change your mind about being in the study and quit after the study has started, and if you feel, for any reason, uncomfortable, the study will be discontinued.

WILL MY GP BE INFORMED?
Your GP will not be routinely informed if your participation in this study has been as a normal volunteer.

The MRI scans being done as part of the study you are participating in are designed to answer research questions and not to provide a medical diagnosis. They may not show problems that a ordinary clinical scan would, and since the scientists reviewing the scans are generally not medical doctors, they may fail to notice subtle abnormalities. However, there is the potential that an unexpected abnormality will be found in your scan. The likely hood of such an abnormality being found in a normal volunteer's scan is estimated to be between 2-10%, so you should be aware that such a possibility exists. If this happens with one of your scans, we will ask a neurologist, who is a medical doctor with experience interpreting brain MRI scans and treating brain disorders, to review the images with us. The neurologist will not be told your name, although they may be told your age and gender. If they think there may be an abnormality, we will then contact you. You will be offered the opportunity to meet and have a discussion with the neurologist about the findings and your options. If you have a GP and you agree, we will contact her/him and pass the scans along with the recommendation from the neurologist. We will only contact your GP with your permission and if your brain scans show something of potential medical concern. These scans do not routinely become a part of a medical record, however, if a problem is detected and with your permission, the images are sent to a medic involved in caring for you, they may become part of your medical record. There is also the possibility that you may be unduly worried if a problem is suspected, but is not actually found.

WHAT WILL HAPPEN TO THE STUDY RESULTS?
They will be kept securely for a minimum of 10 years and possibly indefinitely in the BANGOR BRAIN IMAGING UNIT data archive in accordance with good research practice. Results of the study may be published in a scientific journal or other public format. In this case your data will either be included as part of a group average, or will be anonymised so that no identifying information is given.

WHAT IF I HAVE FURTHER QUESTIONS?
We welcome the opportunity to answer any question you may have about any aspect of this study or your participation in it. Please contact Paul Mullins at the School of Psychology, Bangor University, Gwynedd, LL57 2AS, phone 01248 383631.

ARE THERE COMPENSATION ARRANGEMENTS IF SOMETHING GOES WRONG?

In the unlikely event of anything untoward happening, the University's insurer provides insurance for negligent harm. It does not provide insurance for non-negligent harm but does take a sympathetic view should a claim be made.

WHAT IF I HAVE COMPLAINTS?

This research study has been approved by the School of Psychology Research Ethics and Governance Committee. In the case of any complaints concerning the conduct of research, please address these to Mr. Hefin Francis, School Manager, School of Psychology, Bangor University, Gwynedd, LL57 2AS.

Thank you for considering taking part in this study. Our research depends entirely on the goodwill of potential volunteers such as you. If you require further information, we will be pleased to help you in any way we can.

BANGOR BRAIN IMAGING UNIT
Participant Consent Form

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

TITLE OF STUDY: A behavioral examination of sex differences and experimenter characteristics in pain sensitivity.

INVESTIGATORS:
Dr Paul Mullins, Dr Erin Heerey, Alexander Currie.

The volunteer should complete this entire sheet himself/herself.
Please circle as appropriate:

Have you read the participant information sheet?

YES / NO

Have you had the opportunity to ask questions and discuss this study?

YES / NO

Have you received enough information about the study?

YES / NO

Do you understand that your participation is voluntary and that you are free to withdraw from the study,

- At any time
- Without having to give a reason
- And without affecting your future medical care?

YES / NO

Do you understand that these are not diagnostic scans?

YES/NO

Do you understand that the Bangor University provides insurance for negligent harm but that it does not provide insurance for non-negligent harm?

YES/NO

Do you understand that the research data may be accessed by researchers working at or in collaboration with the BANGOR BRAIN IMAGING UNIT in similar ethically approved studies, but that at all times your personal data will be kept confidential in accordance with data protection guidelines?

YES/NO

Do you agree to take part in this study?

YES / NO

_____ _____

Date Signature of Participant

_____

Name in block letters

_____ _____

Date Signature of Investigator

_____

Name in block letters

_____ _____

Date Signature of Investigator

_____

Name in block letters

BANGOR BRAIN IMAGING UNIT
MR Safety Screening Questionnaire

To be completed by ANYONE entering the Magnet Room.
Shaded boxes need to be filled in by participants undergoing a scan only.

Name	BANGOR BRAIN IMAGING UNIT no. (Staff Use Only)
Phone number	Date of Birth
Email address	Weight (kg)

MR scanning uses strong magnetic fields. For your own safety and the safety of others it is **very important** that you do not go into the Scanner Room with any metal in or on your body or clothing.

Please answer the following questions carefully and ask if anything is not clear.

All information is held in the strictest confidence.

Circle one answer for each question.

1. Do you have a pacemaker or artificial heart valve?
Y/N
2. Do you have aneurysm clips (clips put around blood vessels during surgery)?
Y/N
3. Do you have any implants in your body? (e.g., replacement joints, drug pumps, metal pins, plates, coronary stents, breast implants etc.)
Y/N
4. Have you ever had any metal fragments in your eyes?
Y/N
5. Have you ever worked with metal (e.g., grinding, machining, welding) without eye protection?
Y/N
6. Do you have any metal or shrapnel fragments anywhere in your body?
Y/N
7. Do have an indwelling catheter in your body?
Y/N
8. Have you ever had an operation on your head, spine, or chest?
Y/N
9. Have you ever had any surgery (if yes, please give brief details)?
Y/N
   Details _____
10. Do you have any implanted electrical devices (e.g., hearing aid, cochlea implant, nerve stimulator)?
Y/N

11. Have you ever had an MRI scan before?
Y/N
12. Do you wear dentures, a dental plate, or a brace (not fillings)?
Y/N
13. Do you have any transdermal patches? (skin patches)
Y/N
14. Do you have any tattoos or body piercings?
Y/N
15. Is there any possibility that you could be pregnant?
Y/N
16. Are you susceptible to claustrophobia?
Y/N
17. Do you have hypertension (high blood pressure) sufficient to require medication?
Y/N
18. If Yes to 17 above, has your hypertension been adequately treated by medication?
Y/N
19. Have you had or do you have any heart problems?
Y/N
20. Do you have an impaired ability to perspire?
Y/N
21. Do you have reduced thermal regulatory capabilities or an increased sensitivity to raised body temperature?
Y/N
22. Do you suffer from any other medical condition that might be relevant? (e.g., epilepsy, diabetes, asthma)?
Y/N
Details _____

- I confirm that before entering the Magnet Room, I will:
    - remove all metal including coins, keys, lighters, body-piercings, jewellery, watches, wigs/hairpieces, clothing with zips and/or metal buttons, false teeth, hearing aids etc.;
    - remove all cosmetics;
    - remove all prostheses (e.g., prosthetic limbs);
    - turn off and remove mobile phones;
    - ensure that I am not wearing damp clothing
    - conform with the operator's instructions in regard to the above

- I confirm that the above information is accurate to the best of my knowledge. I have read and understood this form and the information sheet and have had the opportunity to ask questions regarding their contents and the MRI procedure that I am about to undergo.
- I acknowledge that BANGOR BRAIN IMAGING UNIT has taken reasonable precautions to screen for potential difficulties and is not liable for any event that might result from incorrect answers to the above.

Signature	Date
Verified by (BANGOR BRAIN IMAGING UNIT Staff Member) Name Signature	Date

Appendix H: Participant Debrief Form – Chapter 5

Debriefing form

In this study, we used MRS to investigate normal levels of neurochemistry and the how they may be different in males and females and how these measures may be related to pain thresholds by measuring your threshold to a pressure pain stimulus. Previous work in this area has found that Glu and Glx levels in women do correlate with pressure pain thresholds, we hope to repeat this finding and see if it also holds true in men, and extend this work to look for correlations with Gamma Amino Butyric Acid (GABA) an inhibitory neurotransmitter. We predict that higher GABA levels will be associated with a higher threshold to pain.

In addition to an interest in the neurochemical basis of pain thresholds, we are also interested in additional factors that may influence pain thresholds. Previous results in this study indicate that participant pressure pain thresholds vary based on the gender of the tester, with all participants demonstrating lower pain threshold when tested by a male then when tested by a female. This is an interesting finding, and while it matches with some literature reports of higher pain thresholds in male participants when tested by a female experimenter, this effect has not previously been shown in females (Levine and De Simone et al 1991, Gijsbers and Nicholson 2005, Fillingham and King et al 2009). We wanted to investigate this experimenter gender effect further, and are curious to see if this effect extends beyond the experimenter, to an additional observer in the room. I.e. does the gender of an observer to the pain pressure test affect the pain pressure threshold reported? This is the real reason for the additional "student" in the testing room during the pressure threshold measurements. We are also interested in what may be a driving factor behind any changes that may be observed, in particular how measures of individual stress levels, aspects of personality and even what your opinion of the tester may be, which was the reason behind the questionnaires you were asked to complete. We thank you for taking part, and apologize for the deception involved in this part of the study, but we are not sure how it might have affected the results if you had been aware of this manipulation.

We hope to report the findings of this research project in the relevant journals when it is finished, if you would like to be informed of the outcome please let us know and we will pass it on to you.