Oscillatory dynamics in the Perception of Pain investigated using Magnetoencephalography

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Thesis Summary

This thesis investigates changes in the oscillatory dynamics in key areas of the pain matrix during different modalities of pain. Gamma oscillations were seen in the primary somatosensory cortex in response to somatic electrical stimulation at painful and non-painful intensities. The strength of the gamma oscillations was found to relate to the intensity of the stimulus. Gamma oscillations were not seen during distal oesophageal electrical stimulation or the cold pressor test. Gamma oscillations were not seen in all participants during somatic electrical stimulation, however clear evoked responses from SI were seen in everyone.

During a train of electrical pulses to the median nerve and the digit, a decrease in the frequency of the gamma oscillations was seen across the duration of the train. During a train of electrical stimuli to the median nerve and the digit, gamma oscillations were seen at ~20-100ms following stimulus onset and at frequencies between 30-100Hz. This gamma response was found to have a strong evoked component. Following a single electrical pulse to the digit, gamma oscillations were seen at 100-250ms and between 60-95Hz and were not temporally coincident with the main components of the evoked response.

These results suggest that gamma oscillations may have an important role in encoding different aspects of sensory stimuli within their characteristics such as strength and frequency. These findings help to elucidate how somatic stimuli are processed within the cortex which in turn may be used to understand abnormal cases of somatosensory processing.

Key words: Magnetoencephalography, Pain, Oscillations, Gamma.

Dedication

I would like to dedicate my thesis to my Grandma, Alice Rossiter as she has always said I would become a scientist and is always fascinated to hear about my experiments. I owe my inquisitive nature to her.

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Abbreviations

ACC = anterior cingulate cortex
AHPs = afterhyperpolarizations
CEPs = cortical evoked potentials
CHEPS = contact heat evoked potential stimulator
CNS = central nervous system
CPT = cold pressor test
DLPFC = dorsolateral prefrontal cortex
EEG = electroencephalography
EMG = electromyography
EPSPs = excitatory post-synaptic potentials
fMRI = functional magnetic resonance imaging
GABA = gamma-aminobutyric acid
ICA = independent component analysis
ISI = inter-stimulus interval
LEFs = laser evoked fields
LEPs = laser evoked potentials
MEG = magnetoencephalography
MRI = magnetic resonance imaging
PET = positron emission tomography
PFC = prefrontal cortex
PT = pain threshold
RMS = root mean square
ROIs = regions of interest
SAM = synthetic aperture magnetometry
SEFs = somatosensory evoked fields
SEPs = somatosensory evoked potentials
SI = primary somatosensory cortex
SII = secondary somatosensory cortex
SnPM = statistical non-parametric mapping
SNR = signal to noise ratio
SPM = statistical parametric mapping
ST = sensory threshold
STT = spinothalamic pathway
VAS = visual analogue scale
VEs = virtual electrodes
VPI = ventral posterior inferior nucleus of the thalamus
VPL = ventral posterior lateral nucleus of the thalamus

1 An Introduction to Pain Literature

1.1 Definition of Pain

Although all of us have an understanding of what pain is, it is a very difficult sensation to accurately define. Many have tried and as the complex relationship between stimulus and perception is explored further, the definition becomes more intricate.

One authoritative definition comes from the International Association for the Study of Pain: “Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage.” (Merskey, 1994). This takes into consideration the unreliable link between pain and injury and how what is perceived can vary so dramatically. Pain can be felt when there is no noxious input, and vice versa (Melzack and Wall, 1965). These issues are what makes pain such a fascinating and complex phenomenon to study. My aim is to try and unravel these factors, may they be psychological, physiological or pathological, that influence how pain is perceived by an individual.

This chapter will give an overview of current pain research, starting with theories and aspects of pain. It will then go on to how the nervous system processes pain at both the peripheral level and centrally in different areas of the cortex. Much of the pain research conducted using electroencephalography (EEG) and magnetoencephalography (MEG) involves evoked potentials/fields, these will be explored as well as the oscillatory dynamics during pain. The psychological modulators of pain will be briefly discussed before concluding with chronic pain syndromes and the treatments currently available for them.

1.2 Theories of Pain

1.2.1 Specificity theory

The first recorded theory of pain was in 1664 by Descartes which was termed specificity theory. This stated that there is a system specifically for pain which carries information from the site of stimulation (e.g. skin) to a pain centre in the brain (see Figure 1.1) (Melzack, 1996).

Figure 1:1 shows an illustration of Descarte's specificity theory. Taken from Melzack and Wall 1965.

1.2.2 Gate-control theory

Gate-control theory formed the foundations for what is known about pain mechanisms today (Dickenson, 2002). Gate-control theory states that there are 3 spinal cord systems involved in pain perception; a gate-control system, a central control trigger

and an action system (Melzack and Wall, 1965) (see Figure 1.2). In the gate-control system, input is received through both large and small diameter fibres; large diameter fibres increase inhibitory controls over the signal whereas small diameter fibres decrease the inhibition, opening the gate and allowing more of the signal through. These inhibitory and excitatory mechanisms are able to control the sensation perceived by the individual. The central control trigger involves higher cognitive processes influencing control over the sensory input, this can be associated with attention, emotion and memories linked to previous experience of the stimulus. The action system controls the behaviour produced in response to the pain, such as a startle reflex, vocalisation etc (Melzack and Wall, 1965). Gate-control theory was the first to mention that pain transmission from peripheral nerves can be modulated by intrinsic nerves and top-down control from the brain which is still valid today (Dickenson, 2002). It drew attention to the key role of the brain and central mechanisms in modulating the pain experience (Melzack, 1996, Wall, 1978, Melzack, 1999).

Figure 1:2 shows a diagram of gate-control theory. The output is controlled by the balance of input from large (L) and small (S) diameter fibres and central control, which then leads to the action system being activated in order to react to the stimulus. SG = substantia gelatinosa, T = central transmission cells. This figure was taken from Melzack and Wall 1965.

1.2.3 Pain Neuromatrix

Melzack and Wall adapted their ideas from the gate-control theory and were the first to use the term ‘pain neuromatrix’ (Melzack, 1999). This concept defines the ‘body-self neuromatrix’ – “a neural network which integrates multiple inputs to produce the output pattern that evokes pain” (Melzack, 1999). This neural network is made up of a number of different cortical areas identified by various studies (Apkarian et al., 2005, Chen, 2001, Derbyshire et al., 1997). There is no single ‘pain centre’ in the brain as was previously thought (see Section 1.2.1).

The ‘neurosignature’ is the output of the neuromatrix (Melzack, 1999) which will determine various properties of the pain experience. This concept encompasses a genetic template built into the body-self; the emotional and cognitive aspects and the influence of the stress system on the pain experience (Melzack, 1999, Melzack, 2001).

1.3 Aspects of Pain

The pain experience has been classified into 3 sections as to the different aspects of pain. The **sensory-discriminative** component encompasses stimulus type, intensity, location, duration etc. The **affective-motivational** component deals with the emotion associated with the pain, linking it to previous experiences and creating the motivation to initiate an action i.e. avoidance behaviour. The last aspect is **cognitive-evaluative** which is involved with understanding the situation, again linking it to past experience and forming new opinions about it (Melzack and Casey, 1968).

The nociceptive system can be separated into two sections – depending on the thalamic nuclei that are involved in each (see Figure 1.3). The lateral nociceptive system involves the lateral thalamic nuclei such as ventral posterior lateral nucleus (VPL), ventral posterior medial nucleus and ventral posterior inferior nucleus (VPI). This system is thought to be responsible for the sensory-discriminative component of pain. The medial nociceptive system includes the medial thalamic nuclei such as the

posterior part of the ventromedial nucleus, ventrocaudal part of medial dorsal nucleus, parafascicular nucleus and centrolateral nucleus and has a role in the affective-motivational aspects of pain (Treede et al., 1999, Price, 2002).

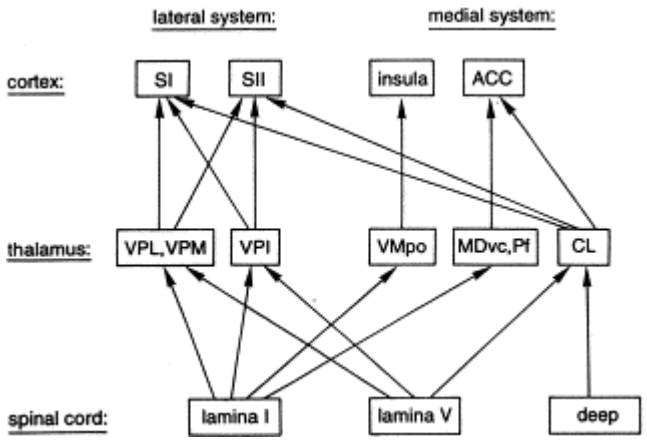


Figure 1:3 diagramatically demonstrates the components of the lateral and medial nociceptive systems. This figure has been taken from Treede et al 1999.

1.4 Peripheral nervous system

1.4.1 Receptors

Information about a sensory stimulus is received on the skin surface by receptors. These can be grouped into different categories according to what stimuli excite them. There are nociceptors which are specific to pain; thermoreceptors which are activated in response to temperature; mechanoreceptors which are sensitive to touch and pressure and chemoreceptors which encode chemical concentrations (Martini, 2001).

Thermoreceptors are free nerve endings and there are four times more that respond to cold than those that respond to heat (Martini, 2001). These are phasic receptors which means that they adapt fast to the environment and are only active when a change in temperature occurs (Martini, 2001).

Nociceptors are slow-adapting (tonic) receptors. They are mostly inactive if there is no painful stimulus but they will stay activated as long as a painful stimulus remains.

Nociceptors are sensitive to extreme temperatures, mechanical damage and chemicals that may be harmful (Martini, 2001).

1.4.2 Peripheral Nerves

Somatosensory information is carried to the central nervous system (CNS) by three types of fibres (see Table 1.1) (Fitzgerald et al., 2007). Aβ fibres are purely sensory and have mechanoreceptors in the skin and there are two types of fibres which are nociceptive specific: Aδ and C fibres. Aδ fibres are myelinated and therefore have a fast conduction velocity of around 5-30m/s (Forss et al., 2005), they encode first pain which is short, sharp, well localized and gives a pricking sensation (Ploner et al., 2002). C fibres are unmyelinated and therefore have a slower conduction velocity (0.5-2m/s) (Forss et al., 2005) encoding second pain which is more sustained and feels more dull and aching than first pain (Ploner et al., 2002). First pain is thought to be necessary for achieving safety from the source of pain by creating a quick behavioural response to avoid it. Second pain initiates different behavioural responses encouraging the individual to rest and enable recuperation from injury (Qiu et al., 2006). Most pain sensations involve both fibre types, however it is possible to selectively activate one or the other experimentally. Using a smaller surface area and lower intensity of laser stimulus preferentially activates C fibres as they have a higher density and lower activation threshold (Raij et al., 2004, Forss et al., 2005).

Name of fibre	**Sensory/Pain specific**	**Myelinated**	**Conduction velocity (m/s)**
Aβ	Sensory	Yes	35-70
Aδ	Pain	Yes	5-30
C	Pain	No	0.5-2

Table 1:1 shows the different types of peripheral fibres that conduct somatosensory information to the CNS.

1.4.3 Neurotransmitters

On the post-synaptic cell membranes of primary afferent nociceptors, three key pharmacological receptors have been found; opiate, gamma-aminobutyric acid

(GABA) and serotonin receptors amongst others (Willis and Westlund, 1997). Excitatory amino acids are found within both peripheral and central nociceptive neurons, primarily glutamate (Whittington et al., 1995) which is known to be an important excitatory influence in pain transmission and sensitization. GABA is thought to be the main inhibitory influence in nociceptive circuits, along with glycine (Willis and Westlund, 1997) although glycine also has an excitatory role within the spinal cord. $GABA_A$ receptors exert an inhibitory effect and AMPA receptors exert an excitatory effect on cells of the cortex which are necessary for high frequency brain oscillations to occur (Cunningham et al., 2004).

1.5 Central Nervous System

1.5.1 Spinal Pathways

Aδ and C fibres carry nociceptive information into the CNS via the dorsal horn of the spinal cord. Lamina II in the dorsal horn is where C fibres reside whereas Aδ fibres can be found in Laminae I and IV (Qiu et al., 2006).

The majority of nociceptive information ascends the spinal cord in the spinothalamic pathway (STT). The lateral STT mediates both noxious and thermal sensations whereas the anterior STT mediates touch (Fitzgerald et al., 2007). Both the anterior and lateral STT are thought to be somatotopically organised, from clinical studies on anterolateral cordotomies (Willis and Westlund, 1997). The second-order neurons of the STT cross the midline in order to reach the contralateral thalamus (Willis and Westlund, 1997). Therefore most somatosensory stimuli will activate the opposite side of the brain to the side the sensation originates.

There are a number of other spinal ascending pathways that transmit nociceptive information. The spinomesencephalic tract contains nociceptive neurons, some of which respond to only noxious, and some to noxious and innocuous stimuli. The spinoreticular tract contains many neurons that respond preferentially to noxious input and may induce homeostatic changes from the brainstem. The spino-limbic tract

carries noxious input to the emotive areas of the brain. The spino-cervicothalamic pathways tends to carry mostly tactile information but also some noxious. The postsynaptic dorsal column pathway responds to mechanical or chemical changes in the viscera (Willis and Westlund, 1997).

1.5.2 Thalamus

The thalamus is the main relay centre for most nociceptive information travelling to the cortex. It receives input from the spinal pathways previously mentioned and then projects to higher cortical areas. It has been found that the VPL nucleus of the thalamus projects to the primary somatosensory cortex (SI) and the VPI nucleus projects to the secondary somatosensory cortex (SII) (see Figure 1.3) (Willis and Westlund, 1997) whereas the medial thalamic nuclei project to the anterior cingulate cortex (ACC) and the insula.

1.6 Cortical areas involved in pain

The cortical areas most frequently mentioned as part of the 'pain neuromatrix' and most commonly seen activated in pain studies are: SI, SII, ACC, insula and the prefrontal cortex (PFC). They all play different roles in the perception of pain and each of them will be explored in turn.

1.6.1 Primary Somatosensory Cortex

SI is located in the post-central gyrus and is involved in the sensory-discriminative aspects of pain, dealing with stimulus intensity, location and duration (Treede et al., 1999). In pain experiments, SI is generally seen activated contralaterally to the stimulus (Ploner et al., 1999, Timmermann et al., 2001, Ploner et al., 2000, Bornhovd et al., 2002). The activity in SI has been found to increase exponentially with increasing stimulus intensity (Coghill et al., 1999, Bornhovd et al., 2002, Della Penna et al., 2004) in some cases even matching the subjects own intensity ratings (Timmermann et al., 2001). There is some controversy about the involvement of SI in

visceral sensation and pain; Schnitzler et al (1999) and Aziz et al (2000a) state that the majority of visceral afferents project to the SII cortex and that there is little or vague representation in SI. Schnitzler et al (1999) hypothesise that this lack of SI representation could explain the poor localization of visceral pain. However, others have found SI activation during visceral stimulation (Hobson et al., 2005, Aziz et al., 2000b, Coen et al., 2007).

1.6.2 Secondary Somatosensory Cortex

SII is generally understood to be located at the upper bank of the sylvian fissure (Frot et al., 1999). This location is very close anatomically to the insula and it is often difficult to separate the two (Peyron et al., 2002). SII is also understood to be part of the lateral nociceptive system alongside SI, these areas are thought to be involved in the sensory-discriminative aspects of pain (Treede et al., 1999). There is some controversy in the literature over whether SII is activated in series (Della Penna et al., 2004) or in parallel (Ploner et al., 1999) with SI. Frot and Mauguiere (1999) believe that it receives its sensory input from SI due to a delay of ~40ms between SI and SII somatosensory evoked potentials (SEPs). However, others find that they are activated at the same time in both somatic (Ploner et al., 1999) and visceral painful stimulation (Hobson et al., 2005) indicating parallel processing. SII is also found to have direct anatomical projections from the VPI nucleus of the thalamus which would suggest a direct path (Willis and Westlund, 1997). Unlike SI, SII is found to be activated bilaterally in the majority of pain studies (Coghill et al., 1999, Ploner et al., 1999, Ploner et al., 2000, Timmermann et al., 2001, Ploner et al., 2002) and may show a left hemisphere dominance (Simoes et al., 2002). In some cases, it has been found to be somatotopically arranged (Mazzola et al., 2006) although some have only found this with innocuous stimuli (Ferretti et al., 2004).

SII is able to process both noxious and innocuous stimuli (Mazzola et al., 2006, Frot et al., 2001) despite having a higher proportion of nociceptive specific neurons (Apkarian and Shi, 1994). The processing of noxious and innocuous stimuli may be

located in different areas of SII; Torquati et al (2005) found posterior SII increased sharply at high intensities whereas anterior SII showed very little increase at higher levels. Different locations of activation in SII have also been seen for visceral and cutaneous with visceral activation being found more lateral to cutaneous (Strigo et al., 2005). SII has been shown to have an S-shaped stimulus response function in relation to increasing intensity (Frot et al., 2007). Unlike SI which increases exponentially with intensity, SII was shown to have a sharp increase only after pain threshold was reached (Timmermann et al., 2001) but has also been found to encode non-painful stimuli and show little change during painful stimuli (Frot et al., 2007). There is debate in the literature about the exact location of SII and insula and whether it is possible to dissociate activity from the two areas (Frot et al., 2007). SII is thought to be involved in detecting and avoiding harmful stimuli and directing attention towards it (Timmermann et al., 2001) as activation in SII has been found to increase with attention (Mima et al., 1998, Nakamura et al., 2002).

1.6.3 Anterior Cingulate Cortex

The ACC is a projection target for the medial nociceptive system (see Section 1.3) (Buchel et al., 2002). Their nociceptive neurons have large receptive fields and show some coding of intensity (Buchel et al., 2002). The ACC is part of the limbic system and has a role in the affective and emotional side of pain, in fact activity in the ACC can be seen when witnessing other people's pain, in the absence of any noxious stimulus being delivered (Benuzzi et al., 2008). In a study by Rainville et al (1997) using positron emission tomography (PET), participants were hypnotised and instructed to find a stimulus either more or less unpleasant and activity in the ACC was the only area that correlated to the unpleasantness, confirming it's role in the negative affect of pain. It is thought to be involved in response selection, such as pain avoidance behaviour and integrating emotional and cognitive inputs (Treede et al., 1999). ACC is often activated in anticipation of pain (Hsieh et al., 1999) and when attending to a noxious stimulus (Sawamoto et al., 2000, Frankenstein et al., 2001). The cingulate cortex is not a homogenous area and has been found to have

functionally distinct regions (Mohr et al., 2005, Vogt, 2005), in fact the regions involved in attention are found to be more anterior and those involved in the processing of pain as more posterior (Davis et al., 1997, Buchel et al., 2002). Vogt (2005) proposed a 4 region model of the cingulate, splitting it into ACC, middle cingulate cortex, posterior cingulate cortex and retrosplenial cortex, each with slightly different roles in emotional processing. The rostral ACC has been implicated in emotional processing and it has been found to be activated during both opioid and placebo induced analgesia (Petrovic et al., 2002a).

Both the anterior and mid cingulate cortex have been found to be active during visceral and somatic stimulation (Vogt, 2005). ACC activity is commonly seen during somatic pain (Buchel et al., 2002, Coghill et al., 1999) and has been found to activate a spatially distinct region from visceral stimuli with visceral ACC activation being found more rostral than somatic ACC activation (Strigo et al., 2003).

1.6.4 Insula

The insula is often combined with SII when speaking of pain centres as the parasylvian region or parietal operculum, as anatomically, they are very close together (Kakigi et al., 2005). According to many, the most consistently activated region during somatic and visceral pain is the insula (Brooks and Tracey, 2007, Derbyshire, 2003). It receives nociceptive input from brainstem areas such as the periaqueductal grey, rostral ventromedial medulla and nucleus cuneiformis (Tracey and Mantyh, 2007) and projects to the amygdala, which is an important centre in the limbic system. The insula is part of the limbic system and is thought to be involved in affective and emotional processing of pain (Apkarian et al., 2005). The amplitudes of laser evoked potentials (LEPs) from the insula were found to increase when a stimulus became painful (Frot et al., 2007) and its activation has been found to relate to stimulus intensity (Bornhovd et al., 2002). The insula is also believed to have a role in visceral sensory and motor information (Treede et al., 1999). It integrates the

affective impulses with a reactive component i.e. the motivation to create a behaviour to avoid the pain.

The insula is not a homogenous area, different parts are involved in different aspects, for example activation in the anterior insula is seen in many pain studies (Dunckley et al., 2005, Strigo et al., 2003). Strigo et al (2003) demonstrated bilateral activation in anterior insula in response to cutaneous pain, but lower activation and only in the right anterior insula in visceral pain. This finding indicates there may be some differences in how visceral and cutaneous stimuli are processed within the insula. During direct cortical stimulation of the insula, both painful and non-painful sensations were elicited in the posterior region, it showed a somatotopic organization and there was some overlap between painful and non-painful sensation (Ostrowsky et al., 2002). Activity in the insula is reduced during distraction (Qiu et al., 2004) suggesting it is involved in attentional processing.

1.6.5 Prefrontal Cortex

PFC is involved in the cognitive-evaluative components of pain (Lorenz et al., 2003) and is found to be activated in many experimental pain studies (Dunckley et al., 2005, Wise et al., 2007, Porro et al., 2002, Peyron et al., 1999, Frankenstein et al., 2001). It is thought to be involved in planning behaviour and selective attention and vigilance to a stimulus (Derbyshire et al., 1997, Lorenz et al., 2003). The grey matter density in the dorsolateral prefrontal cortex (DLPFC) has been found to be much lower in chronic back pain patients relative to controls (Apkarian et al., 2004). This implicates PFC as a site of neurodegeneration in chronic pain, although the reason for this neurodegeneration is not yet fully understood (Tracey and Mantyh, 2007).

DLPFC is thought to have an impact on behavioural control. In a study by Dunckley et al (2007), DLPFC was found to be involved in attentional switching between tasks. It is thought that it may be able to actively manipulate the behavioural response to pain using top-down mechanisms. It may also be involved in bottom-up processing by influencing the strength of connection between the brainstem and the thalamus and

therefore decreasing pain perception (Dunckley et al., 2007, Tracey and Mantyh, 2007). Activity in the anterolateral PFC was found to increase with perceived control over pain, creating an analgesic effect (Wiech et al., 2006). The medial PFC is involved in self-focus and rumination and has been found to have a higher activation during pain studies in women than men (Straube et al., 2008).

1.7 Evoked potentials/fields

Both PET and functional magnetic resonance imaging (fMRI) have contributed greatly to pain research in discovering the key cortical areas involved in pain processing and giving detailed spatial information. The next step is to comprehend what is happening on a temporal basis in more detail. Both EEG and MEG are able to provide this information.

There are two types of response when looking at EEG and MEG data. There are evoked responses which are phase or time-locked to the stimulus and therefore when many trials are repeated and averaged together, a robust stereotypical response can be seen to a particular stimulus. For example somatosensory evoked potentials/fields (SEPs/SEFs) always have the same general morphology although the latency and amplitude may change. The other type of response is induced which means it is non-phase or time-locked to the stimulus. This is lost during averaging of trials and so another method is needed in order to investigate these changes in the frequency dynamics of the cortex. There will be more on these induced responses later in the chapter (see Section 1.8), the following section will focus on evoked responses.

1.7.1 Somatosensory evoked potentials/fields

SEPs have been used clinically for many years, in order to diagnose abnormalities and pathologies in both the peripheral and central nervous system. The stimulation technique for SEPs that is most commonly used is transcutaneous electrical stimulation applied to the median nerve at the wrist (Cruccu et al., 2008). Electrical stimulation activates mechanosensitive peripheral fibres as well as nociceptive fibres,

so SEPs resulting from this form of stimulation will be a combination of different fibre activation making it more difficult to separate out each component. LEPs selectively activate nociceptive fibres (Aδ and C) and by changing the protocol it is possible to activate either fibre group (Forss et al., 2005).

Figure 1:4 shows an example of a SEP from the contralateral SI (taken from Ploner et al 1999)

SEPs can be separated into different sections; early (<100ms for upper limb stimulation), late (200-500ms) and ultra-late (>500ms) (Treede et al., 2003). Early components of the evoked potential are thought to be due to activity in contralateral SI and bilateral SII (Garcia-Larrea et al., 2003) and are commonly seen as a negative followed by a positive peak termed N1-P1 (see Figure 1.4). Late components show a negative-positive complex also which is termed N2-P2, for a CO_2 laser stimulus on the hand N2 occurs at around 240ms and P2 at 380ms (Treede et al., 2003). These components show abnormalities in many clinical conditions, such as fibromyalgia (see Section 1.12) or neuropathic pain, or if lesions are present in different parts of the nervous system (Treede et al., 2003). Ultra-late components of the evoked response are due to the unmyelinated C fibres but are often masked by the earlier Aδ response. There are many ways of unmasking these components, for example low intensity stimulation over a larger area preferentially stimulates C fibres (Cruccu et al., 2008, Raij et al., 2004).

1.7.2 Visceral evoked potentials/fields

The evoked response to experimental visceral stimulation has been reported by many using different modalities of pain such as mechanical (Hobson et al., 2000b) and electrical (Hobson et al., 2000a, Hecht et al., 1999). A triphasic response is generally reported with P1, N1 and P2 components (see Figure 1.5) (Hobson et al., 2000a).

Figure 1:5 shows an example of a visceral evoked potential in response to a painful electrical oesophageal stimulus. This figure was taken from Hobson et al 2000a.

These are at latencies of around 88.4±11.5ms for P1, 145.6±18.2ms for N1 and 227.9±24.6ms for P2 (Hobson et al., 2005) and are thought to originate in the somatosensory cortex. The latencies of the visceral evoked response tend to be longer than for the equivalent early components of somatic stimuli, In a study by Schnitzler et al (1999), somatic stimuli of the median nerve elicited an evoked response between 22-45ms after stimulation whereas distal oesophageal stimulation elicited an evoked response with a peak at ~135ms.

1.8 Oscillations

Alongside the evoked responses found in MEG data (see Section 1.7), there are responses that are not time-locked to the stimulus and these are known as induced

responses. The brain oscillates at various different frequency bands and it is the changes in these oscillations that may be key in unravelling how the brain responds to pain. This section will describe the cells involved in creating these oscillations and then the role of each different frequency band and how they relate to pain research.

1.8.1 Cell types involved in oscillations

There are many different cell types within the human brain, all with different roles in producing neural activity. Using neuroimaging techniques such as EEG and MEG it is possible to record the summated activity of many neurons. The cells predominantly involved in creating these oscillations are thought to be pyramidal cells (Traub et al., 2003). These are much larger than most other cell types in the brain and the frequency at which they oscillate appears to be controlled by cells called interneurons (Dupret et al., 2008), which act as an inhibitory influence on pyramidal cell firing as well as their own (Fries et al., 2007). This relationship regulates the rate at which pyramidal cells fire and therefore determines the frequency of their oscillations. Different oscillations are thought to use slightly different mechanisms, for example gamma oscillations use gap junctions between interneurons in order to transmit the signal quickly across a population of cells (Traub et al., 2003, Whittington and Traub, 2003). The principal cells involved in gamma oscillations are thought to originate from fast rhythmic bursting neurons in layers II/III (Cunningham et al., 2004) whereas beta oscillations are thought to originate from layer V neurons in the somatosensory cortex (Roopun et al., 2006).

1.8.2 Theta

Theta frequency is commonly thought of as between ~3.5-7Hz (Basar et al., 1999). Theta oscillations are often seen in frontal areas of the cortex, for example in response to bimodal sensory stimulation (Basar et al., 1999). Theta has been linked to gamma oscillations and it is hypothesised that ripples of gamma oscillations can be paced at a theta frequency (Ward, 2003, Fries et al., 2007), although this topic still

needs more research. Theta has been seen to increase in forefrontal and central medial frontal cortices during the cold pressor test (CPT) (Chang et al., 2002), it has also been found to decrease in frontal areas during CPT (Chang et al., 2005, Dowman et al., 2008) showing an increase after CPT (Chang et al., 2005). Theta has been implicated in chronic pain conditions, showing higher baseline levels when compared to healthy controls in both visceral (Drewes et al., 2008) and somatic pain syndromes such as complex regional pain syndrome and neurogenic pain (Walton et al., 2010, Sarnthein and Jeanmonod, 2008).

1.8.3 Alpha

Alpha oscillations (~7-14Hz) (Basar et al., 2001) are seen during low levels of arousal and the early stages of sleep in the occipital cortex and alpha is immediately reduced when eyes are opened (Hari and Salmelin, 1997, Teplan, 2002). Alpha has been found to decrease in response to painful stimulation using both laser (Ploner et al., 2006b, Ploner et al., 2006a, Raij et al., 2004) and CPT (Chang et al., 2002, Chang et al., 2005, Dowman et al., 2008). This decrease can be seen over a variety of areas including somatosensory cortex, posterior parietal cortex, and temporal regions. A decrease in alpha has also been seen during anticipation of a painful electrical stimulus in SI (Babiloni et al., 2004, Babiloni et al., 2006) and the decrease was stronger than when anticipating non-painful stimuli. In fact the strength of alpha was found to negatively correlate with the participants pain ratings (Babiloni et al., 2006). It is thought that an active suppression of cortical activity relating to distractions is able to focus attention on a painful stimulus (Ward, 2003) and that alpha may have a role in this. The decrease in alpha during painful stimuli is stronger during attention than in distraction (Ohara et al., 2004, Ohara et al., 2006).

1.8.4 Beta

Beta frequency (~15-25Hz) oscillations are most commonly found in the motor cortex, and are thought to be the natural idling frequency in this area. Voluntary movement is

associated with a decrease in power in the beta band followed by an increase in beta after the movement has finished to a level higher than the baseline, this is known as beta rebound (Pfurtscheller and Lopes da Silva, 1999, Jurkiewicz et al., 2006). The decrease in beta is thought to allow movement and it is this decrease that is absent in Parkinson's disease patients (Mallet et al., 2008), the beta rebound may be acting to recalibrate the sensorimotor system after a movement (Baker, 2007). Beta is also seen to decrease during both tactile (Cheyne et al., 2003, Gaetz and Cheyne, 2006) and painful (Raij et al., 2004) stimuli in MI. A decrease in beta has been seen in SI (Ohara et al., 2006) and SII (Ohara et al., 2004) during attention to a painful stimulus as opposed to distraction from it. Beta has been seen to increase in fronto-temporal areas in response to CPT (Chang et al., 2002).

1.8.5 Mu

The mu rhythm is a combination of upper alpha (~10Hz) and lower beta (~20Hz) rhythms (Hari and Salmelin, 1997). A decrease in mu power has been seen during painful stimuli (Ploner et al., 2006a, Ploner et al., 2006b, Raij et al., 2004, Cheyne et al., 2003, Gaetz and Cheyne, 2006) as well as during movements (Pfurtscheller and Lopes da Silva, 1999, Hari and Salmelin, 1997, Jurkiewicz et al., 2006). It is thought that mu suppression prior to movement may act as a priming of motor areas so that they are prepared for the movement (Pfurtscheller and Lopes da Silva, 1999).

1.8.6 Gamma

It has been hypothesised that gamma oscillations (>30Hz) are involved in many higher cognitive tasks (Ward, 2003) such as attentional processing (Bauer et al., 2006, Hauck et al., 2007a) and may be important in binding theory (Engel et al., 2001). Binding theory states that in order to comprehend the world around us, we must bring all the different features of a stimulus together to form a coherent percept (Treisman, 1996). It is hypothesised that this may occur due to the different areas of the brain involved oscillating in synchrony with one another at a gamma frequency

(Engel and Singer, 2001, Engel et al., 1997), however this may be an oversimplification (Kaiser and Lutzenberger, 2003).

There are 3 types of gamma response (Tallon-Baudry and Bertrand, 1999); firstly the gamma evoked response which is time and phase-locked to the stimulus, secondly the steady-state response which is periodically modulated and thirdly the induced response which can range from ~30-100Hz. They are each involved in sensory and cognitive processing in different ways, not all of which are understood as yet. The induced response is most commonly associated with complex cognitive tasks requiring understanding and perception (Ward, 2003). Gamma oscillations are created via an interconnecting network of interneurons and pyramidal cells in the cortex (Fries et al., 2007). The interneurons provide an inhibitory influence over the pyramidal cells so that they can only fire at a certain window during the cycle. Those that receive the strongest excitation are able to fire earliest in the cycle and this may be driven by the stimulus features which they are coded to respond to. The adjustment of spike timing in the gamma cycle may therefore be a mechanism for information processing (Fries et al., 2007). It is thought therefore that gamma oscillations may be capable of encoding information about sensory stimuli.

Increases in power in the gamma frequency band have been seen in response to many different sensory stimuli such as visual in both humans (Hadjipapas et al., 2007) and primates (Logothetis et al., 2001) and auditory stimuli (Kaiser and Lutzenberger, 2003). An increase in gamma oscillations was seen over SI in pain studies in response to both electrical (De Pascalis et al., 2004, De Pascalis and Cacace, 2005, Hauck et al., 2007a, Hauck et al., 2008) and laser stimuli (Gross et al., 2007). Gamma oscillations have also been found during non-painful stimuli (Tecchio et al., 2003, Tecchio et al., 2008, Fukuda et al., 2008). Some found the gamma response to pain to be induced (Gross et al., 2007) and related to higher cognitive processing such as attention (Hauck et al., 2007a). The gamma response seen in other studies was phase-locked (De Pascalis et al., 2004, De Pascalis and Cacace, 2005) or began as phase-locked and with time became induced (Fukuda et al., 2008).

The increase in gamma oscillations after pain has generally been seen within the first 500ms; De Pascalis et al (2004) report a gamma increase (~40Hz) between 0-150ms, Gross et al (2007) find gamma oscillations (60-95Hz) between 100-300ms after the stimulus. However, Hauck et al (2007a) report both an early gamma increase (60-80Hz) between 50-250ms (pattern I in Figure 1.6), but also another later gamma increase between 400-600ms at a higher frequency band of 120-140Hz (pattern III in Figure 1.6) which is affected by the level of attention paid to the painful stimulus.

Figure 1:6 shows time-frequency representations over the somatosensory cortex in both hemispheres during painful intracutaneous stimuli. **A** shows the non-time locked or induced power and **B** shows the time-locked/evoked power, **C** shows the location of MEG sensors over the somatosensory cortex. Red colour shows an increase in power at that frequency band and blue reflects a decrease in power. The patterns labelled I and III show two different gamma oscillations in response to pain. Patterns II and IV show a beta suppression followed by a rebound and V is thought to show an increase in delta. This figure is taken from Hauck et al (2007).

Gamma oscillations in response to pain have been linked to the perception of pain; Gross et al (2007) found that even at the same stimulus intensity around pain

threshold level, if the participant rated the stimulus as painful, then the gamma response was stronger than if they rated it as non-painful. In some cases, the gamma response has been found to be an accurate predictor of the participants pain ratings (De Pascalis et al., 2004).

1.9 Sensitization

There is a phenomenon in the nervous system called sensitization in which the response to strong stimuli increases over time as the individual becomes sensitized to it, this is also known as 'wind-up' and is a temporal summation phenomenon (Clauw, 2009, Staud et al., 2007). This can happen at different levels of the nervous system; it can happen at the nociceptors on the surface as part of an inflammatory response often involving opioid receptors (Stein et al., 2009); it can also happen centrally in the dorsal horn where nociceptive neurons respond more strongly to peripheral stimuli, and this is thought to be due to excitatory amino acids and peptides being released into the dorsal horn (Willis and Westlund, 1997). Central sensitization can lead to allodynia (feeling non-painful stimuli as painful) and secondary hyperalgesia (feeling a painful stimulus as much more intense than it would normally feel in the surrounding area) (Maihofner et al., 2009), whereas peripheral sensitization leads to primary hyperalgesia (this is an increased response to pain only in the receptive field of the peripheral sensitization) (Wiech et al., 2005). NMDA receptors and glutamate play an important role in the induction of activity-dependent central sensitization (Latremoliere and Woolf, 2009). It is thought that the brainstem has an important role in maintaining central sensitization (Lee et al., 2008).

1.10 Habituation

An important issue to consider when designing an experimental pain study is the possibility of habituation over time. This results in a decreased response to the same stimulus as it is repeated many times (Greffrath et al., 2007). Some studies have looked specifically at habituation, in order to discover how much cortical responses

change with repeated stimulation. Both fMRI (Bingel et al., 2007) and EEG (Greffrath et al., 2007) have been used to investigate this and both showed that the participants pain perception decreased across the experiment as did the amplitude of the evoked response, there were also changes in the involvement of different areas of the cortex as habituation occurred. Sometimes habituation and sensitization can occur in the same experiment, for example habituation occurs across the whole experiment whereas within each stimulus block, sensitization is seen (Christmann et al., 2007). It is important to randomise stimuli in order to avoid order effects and habituation. Protocols must be considered very carefully in order to balance performing enough trials in order to get a reliable response but not so many that the response is attenuated towards the end.

1.11 Psychological Modulators of Pain

The relationship between injury and pain, once thought to be constant, is now understood to be highly complex and dependent on many things; aspects of one's personality, gender, age, cultural background, past experience and many psychological factors too. Many of these are being explored experimentally in order to better comprehend this complex balance. It is important to unravel these influences over pain as anticipation and anxiety in chronic pain patients is often a debilitating additional problem to the pain. If the psychological modulations of pain can be better understood, it could lead to strategies, therapies and possibly pharmacological intervention that would improve the quality of life for chronic pain patients (Eccleston, 2001).

1.11.1 Anticipation

The brains response to anticipating pain has been investigated by many in recent years. Babiloni et al (2006) used EEG to look at pain anticipation, using both laser and electrical noxious stimuli. They looked into the frequency dynamics in the alpha band and found a general decrease in alpha during the anticipatory period. This is

thought to be associated with a change in arousal, however there was a lack of specific spatial localisation for this study.

Cortical activity has been found in response to anticipation in SI (Porro et al., 2002, Babiloni et al., 2007, Straube et al., 2008), ACC (Sawamoto et al., 2000, Porro et al., 2002, Davis et al., 1997, Hsieh et al., 1999, Fairhurst et al., 2007), parietal operculum (SII/Insula) (Porro et al., 2002, Babiloni et al., 2007, Sawamoto et al., 2000, Fairhurst et al., 2007, Ploghaus et al., 1999, Wise et al., 2007) and PFC (Porro et al., 2002, Hsieh et al., 1999, Carlsson et al., 2000) using a variety of neuroimaging techniques. It appears from these studies that using a visual warning cue followed by a painful stimulus whether it is laser, electrical or chemical, displays activation during both anticipation and pain phases in most of the areas considered to be part of the pain neuromatrix. The studies using EEG seem to report less distinct areas of activation but have the advantage of good temporal resolution. Sawamoto et al (2000) suggest that the predictability of the noxious stimulus has an effect on the anticipatory response, when non-painful and painful stimuli are presented in a randomised order. The anticipatory response to the uncertain non-painful stimuli was heightened compared to the control of certain non-painful stimuli as the nature of the stimulus was unpredictable.

1.11.2 Anxiety

The anxiety of the participant can also have an impact on the response to pain (Ploghaus et al., 2001). Warbrick et al (2006) used electrical stimuli and only changed the instructions given by the researcher between conditions, one intended to make the participant anxious about the painful stimuli that were to be administered and one with more neutral instructions. All participants in this study were female, so it was not possible to assess the gender differences in anxiety. The subjective rating of pain intensity and unpleasantness were higher in the anxiety driven condition than the control and there were alterations in components of the event-related potential, namely a larger amplitude in the N140. In a study by Frot et al (2004) looking at sex

differences to pain, it was found that although women rated the painful stimulus as more intense, males showed higher anxiety levels in relation to the pain than women.

1.11.3 Attention

Whether a participant attends to or is distracted from the painful stimulus has a great effect on cortical activation of the pain areas. Qiu et al (2004) used laser pulses and MEG to investigate this. A mental calculation task was given to distract the participant in one condition and the participant was asked to attend to the stimulus in the other condition. In the distraction condition, all sources showed a reduction in amplitude of the evoked response, especially in SII, insula and cingulate, indicating that these areas are involved in cognitive function. Yamasaki et al (1999, , 2000) looked into attention effects on evoked fields using MEG and EEG but found it hard to find any changes in SI and SII in the distraction task, the later components (after 200ms) had reduced amplitude in areas of the limbic system but spatial localisation was not precise as only 37 channels were used for the MEG.

Most work done on attentional mechanisms until recently has focussed on evoked potentials, but Hauck et al (2007a) began to investigate the frequency dynamics using MEG and an oddball paradigm with rare and frequent intracutaneous electrical stimuli. Changes in all frequency bands were observed; delta oscillations showed an increase in power with directed attention and a higher stimulus intensity, beta showed a suppression and rebound after the painful stimulus and gamma band increased in power with directed attention. These results show great potential for unravelling the oscillatory dynamics in attention to pain but there is still a need for better spatial localisation. The analysis was only done at sensor level and results were taken from an average of all sensors across all participants. The changes in the gamma frequency were <1% increase compared to baseline. It would be advantageous to perform source space analysis and to investigate the changes in gamma oscillations within each individual.

1.11.4 Control

Control or even perceived control over pain can have a huge effect on how pain is perceived. In a clinical setting, if patients are given coping strategies to deal with their pain post surgery, they report significantly less pain than those who did not receive the same instructions (Melzack, 1996). Also if women in labour are given some control over aspects of the delivery process then there appears to be less pain and tiredness (Eccleston, 2001). This can also be investigated experimentally.

Helmchen et al (2006) used fMRI with self-administered and externally generated thermal contact stimuli. Activation in SII and insula appeared to respond independently of the mode of application whereas SI was only activated in the externally generated model and did not show increased activity in the self-administered condition. Functional segregation in the ACC has been seen in response to control experiments (Mohr et al., 2005). The posterior ACC had a linear increase in the externally generated condition but no increase in activity in the self-administered model, whereas the perigenual ACC increased in activation with self-administered stimulation and decreased in activation with the externally generated stimulus. The midcingulate cortex showed activation independent of mode of application (Mohr et al., 2005). These experiments show it is possible to investigate the influence of control in areas of the pain neuromatrix.

1.11.5 Placebo

The placebo effect is a well known phenomenon but is still not fully understood. Placebo analgesia in functional brain imaging is a fairly new area of investigation but has come up with some interesting results. Kong et al (2007) used fMRI with painful thermal stimulation to investigate placebo analgesia using a sham acupuncture needle as the placebo manipulation. Subjective pain ratings were significantly reduced in the placebo condition and significant differences in activation were seen in

bilateral rostral ACC, lateral PFC, right anterior insula, supramarginal gyrus and the left inferior parietal lobule, most of which are known to be part of the pain matrix.

Wager et al (2004) used fMRI with noxious electrical pain stimulation and a topical 'analgesic' cream as a placebo manipulation. They found a reduction in reported pain and brain activity in ACC, SII, insula and thalamus. An increase in prefrontal activity was seen in anticipation of noxious stimuli.

1.12 Chronic Pain Syndromes

Chronic pain is a very debilitating and costly problem. The main conditions included in this group are fibromyalgia and neuropathic pain, both of which are thought to involve a malfunction in central or sometimes peripheral pain processing although some of the mechanisms remain elusive. Neuropathic pain is thought to affect between 3-8% of the population (Gilron and Coderre, 2007), it is defined as "pain caused by a lesion of the nervous system" (Gilron and Coderre, 2007). It has many different causes such as diabetes or certain infectious diseases (O'Connor and Dworkin, 2009).

Fibromyalgia is characterised by widespread chronic pain (>3 months) and multiple tender points over the body (Kroenke et al., 2009), and is often accompanied by a myriad of other symptoms such as sleep disturbance, fatigue, depression and, on occasion, impaired cognitive function (Clauw, 2009). Fibromyalgia has a genetic component in that first-degree relatives are 8 times more likely to develop it compared to the general population. It is often triggered by environmental factors such as physical trauma, emotional stress or an infection (Clauw, 2009).

Visceral pain syndromes are often grouped into the category of functional gastrointestinal disorders (FGIDs) with irritable bowel syndrome and non-cardiac chest pain being two of the most common (Aziz et al., 2000a). The pathology behind these disorders is often unknown although they are thought to involve abnormal sensory processing or a hyperexcitability in the visceral pain pathways (Sarkar et al., 2001). Neuroimaging techniques are very useful in investigating these issues

(Sharma et al., 2009). The main symptom in these disorders is a heightened sensitivity to normal gut function (Aziz et al., 2000a).

1.13 Therapy and Drug Treatments for Chronic Pain syndromes

There are many options for pharmacotherapy in treating chronic pain. There are non-opioid analgesics such as non-steroidal anti-inflammatory drugs (NSAIDs) and cyclo-oxygenase-2 (COX-2) inhibitors (Kroenke et al., 2009). Many physicians have found opioid analgesics to be ineffective in the treatment of chronic pain disorders such as fibromyalgia, however tramadol which is a mu opioid agonist has been found to have a beneficial effect. It also inhibits the reuptake of serotonin and norepinephrine (Kroenke et al., 2009).

Some studies have found that serotonin and noradrenergic activity is attenuated in fibromyalgia patients (Clauw, 2009). Antidepressants are very commonly prescribed in chronic pain disorders as they have a beneficial effect on these systems. Tricyclic antidepressants are often chosen despite the problematic side effects (O'Connor and Dworkin, 2009). An alternative to these is selective serotonin reuptake inhibitors or the more recent serotonin-norepinephrine reuptake inhibitors which have proven to be effective in both managing the depression that often occurs with chronic pain and also demonstrating an analgesic effect (Nitu et al., 2003).

Non-pharmacological therapies are important in chronic pain disorders as there are many other symptoms as well as the pain to contend with and the patient’s quality of life can be hugely affected by their condition. It is valuable to have a programme of care with many different aspects to it. Both cognitive behavioural therapy and regular cardiovascular exercise have been found to be efficacious in treating fibromyalgia (Clauw, 2009). These are important factors in the patient’s lifestyle and can lead to them having a greater feeling of control over their condition which may subsequently improve other symptoms. Another alternative to these therapies is the more invasive spinal cord stimulation in which pain transmission can be inhibited by electrical

stimulation of the dorsal column of the spinal cord using an implant (Brook et al., 2009).

1.14 Summary

One of the main issues in pain research is the subjectivity of an individual's response in describing the pain they are experiencing. As discussed above, people are affected by what they are told by the experimenter or in a clinical setting by their Doctor; they are affected by their preconceptions of what their symptoms are and how they expect different treatments to work. It is very difficult to obtain a standardised unbiased response as no one is able to know how that person is feeling and what sensations they are experiencing.

A key aim of pain research would be to find an objective biomarker within the activity of the brain from which we can tell how much pain an individual is in. This would allow better understanding of their condition and also make it easier to test the efficacy of different drugs and therapies. Previous research using PET and fMRI has made it clear what areas are activated during pain perception and in part what roles each area plays, however these techniques are unable to investigate the temporal changes over the course of the pain experience in any detail. It is in the frequency dynamics of the cortex that a pattern specific to pain may be elucidated, a consistent change in a particular frequency band that indicates when a person is in pain or not. MEG is well placed to explore this exciting new area of oscillatory dynamics in pain. Gamma frequency band is known to be important in complex cognitive tasks and in binding features of a stimulus together, it has also been seen in response to experimental pain in a few studies (Hauck et al., 2007a, Gross et al., 2007). It is a possibility that changes in the gamma frequency band may give us clues as to the mechanisms of pain perception and how and why this can vary so dramatically.

The studies in this thesis aimed to explore these issues by looking at the changes in oscillatory dynamics using MEG. In the first study, anticipation and pain were

investigated using both painful and non-painful electrical median nerve stimulation and visual cues. The second study explored how oscillatory patterns change between visceral and somatic electrical sitmulation looking at both evoked and induced responses. The third study investigated a more clinically relevant pain using a version of the cold pressor test and the fourth study went back to electrical stimulation but with 4 different intensities in order to investigate whether the oscillations seen changed linearly with stimulus intensity.

2 Magnetoencephalography and Analysis methods

This chapter consists of an explanation of Magnetoencephalography, how it works and its advantages and disadvantages relative to other neuroimaging techniques. This is then followed by a description of protocols used, the acquisition of MEG data, data processing and analysis tools used for the studies in this thesis.

2.1 Magnetoencephalography

2.1.1 Basic Principles

MEG takes advantage of Maxwell's equation which states that any electrical current will produce a magnetic field flowing around it. This magnetic field is what an MEG system measures in units of Tesla (T). The electrical current is thought to be generated mainly by the pyramidal cells (or principal cells) of the cortex, which are larger than other types of cells such as glial or stellate cells (Dupret et al., 2008). Current flows along their axons and dendrites at an angle perpendicular to the sheet of grey matter in the brain. The direction of the electrical current flow is important as MEG is better at picking up currents that are tangential to the surface, radial sources may produce magnetic fields that do not protrude outside the head (Hamalainen, 1993).

MEG uses superconducting quantum interference devices (SQUIDs). These are made up of a superconducting ring with one or two Josephson junctions, these are weaker links which restrict the current flow around the ring (Hamalainen, 1993). In order to function, the SQUIDs must be supercooled, and for this reason the dewar of the MEG is filled with liquid helium. The magnetic flux, created by the magnetic fields emanating from the head, enters the superconducting ring, changing the impedance in the loop, this change in impedance can be calculated by feeding a current through it and measuring the voltage (Hamalainen, 1993). SQUIDs are highly sensitive and can record the magnetic fields created from the electrical currents firing inside the

cortex. The neuromagnetic signals are 1 in 10^9 of the earth's geomagnetic field and are generally in the range of 50-500fT (1 femtoTesla = $1x10^{-15}$ Tesla) (Hamalainen, 1993).

The MEG system is housed in a magnetically shielded room to eliminate the majority of the background magnetic noise created by fluctuations in the earth's geomagnetic field. These can be caused by lifts, moving vehicles, electrical equipment, phones etc (Singh, 1995) although there is still the potential for noise created physiologically by the heart and skeletal muscles. Complex mathematical algorithms are used to solve what is termed the inverse problem, which is how to estimate the cerebral sources of the measured distributed magnetic field (Hamalainen, 1993). It has no unique solution and there are different analysis techniques attempting to solve this problem (see Section 2.2.6) all with their advantages and disadvantages (Barnes et al., 2006, Hillebrand et al., 2005).

2.1.2 Advantages

MEG offers many advantages over other techniques and whilst it is similar in many ways to EEG, it has some beneficial differences. The spatial resolution of MEG is better than EEG as it is not influenced by the inhomogeneities in the head such as the skull and the meninges. The EEG signal is distorted by these, making source reconstruction much more challenging. Also MEG is better at picking up tangential currents than EEG. It is similar to EEG in that it has excellent temporal resolution in the order of milliseconds, which is key in investigating very quick changes in brain activity. MEG and EEG allow us to investigate the frequency dynamics of the cortex, in other words how the frequency of brain waves changes over time due to a certain task or at resting state. This information gives us key insights into how the brain interprets the information it receives.

MEG is non-invasive and this in turn means that it is easier to obtain participants for research studies and it is possible to repeat experiments on the same participant a number of times without any negative consequences. Another advantage of MEG

compared to PET and fMRI is that it is a direct measure of neuronal activity, it is created by the electrical currents flowing due to neurons firing in synchrony. This means it is a more reliable account of brain activity and is less likely to be affected by other confounding variables.

2.1.3 Limitations

MEG is inferior to fMRI in terms of spatial resolution, although it uses magnetic resonance imaging (MRI) structural anatomical scans to coregister with the data, in order to see where the activity originates (Singh, 1995) (see Section 2.2.4). MEG spatial resolution is limited by the source reconstruction methods used and to what accuracy they can measure activity. These methods are developing and improving all the time in order to obtain the most reliable source reconstruction possible. The fact that the magnetic field strength decreases with distance from the detection coils means that it is very difficult to look at any deep structures using MEG and it can only reliably pick up sources from the cerebral cortex (Hamalainen, 1993, Hillebrand and Barnes, 2002).

As a magnetic field is created around an electrical current, depending on which way the current is facing it is sometimes difficult to pick up the resulting magnetic fields outside the head, and therefore MEG is unable to pick up truly radial sources effectively, which was thought to include most gyri (Hamalainen, 1993). However Hillebrand and Barnes (2002) showed that it is only a small portion at the crest of gyri that MEG is unable to detect and that the majority of cortical signals can be picked up using MEG.

In order to obtain a satisfactory signal to noise ratio (SNR) it is necessary to repeat trials a large number of times in MEG experiments. This may be problematic in that the participant's vigilance will not remain constant throughout the experiment and may therefore induce differences across trials. Another consideration is the need for the participant's head to be very still throughout a MEG experiment in order to provide accurate source reconstruction, both these issues are also relevant to other

neuroimaging techniques. In order to deal with the latter issue, new MEG scanners have been developed that constantly monitor head movement and allow the participant to move within the dewar, this is particularly advantageous with children who find it difficult to sit still for long periods of time.

2.2 MEG acquisition and Analysis Methods

2.2.1 Protocols

2.2.1.1 Psychophysics

A common problem when diagnosing patients complaining of acute or chronic pain, is that the physician must rely solely on the patients description of the pain. Pain is a difficult sensation to describe, it is very emotive and can manifest itself in many different ways.

Many people have created both qualitative and quantitative questionnaires in order to standardise pain responses, helping physicians to categorise patients more easily and potentially diagnose them better. In pain research, there are a few key questionnaires or scales commonly used to establish the amount and type of pain an individual is in. Often they are asked to scale the intensity or unpleasantness of their pain on a numerical scale (0-100) or perhaps to mark on a line where one end is ‘no pain whatsoever’ and the other end is ‘worst pain imaginable’, this is called a visual analogue scale (VAS). These scales are useful, however one person’s idea of worst pain imaginable may differ from the next. For example, if one has had a serious sports injury and the other has never broken a bone, they will both have different concepts of the worst possible pain. However, a study was performed investigating this technique and it found that as long as the pain anchors of worst pain imaginable were sufficiently extreme then these scales were a robust measure of pain (Dannecker et al., 2007). As an alternative to a simple numerical scale, there is a Likert scale (Cruccu et al., 2004) which still works on a 0-10 basis but each number is

linked to a written explanation of what sensation this number represents, for example '5=moderately painful'.

Another commonly-used method for attempting to make qualitative information about pain quantifiable is the McGill pain questionnaire (Melzack, 2005). This involves a list of different descriptive words often used for pain, these are split into sensory and affective categories. The questionnaire requires the individual to rate whether they felt that the painful sensation is described by any of these and they are given the options of not at all/mild/moderate/severe. These are then given a numerical value so different painful sensations and individuals can be compared.

A major issue with experimental pain research is in the instructions and explanations given by the experimenter. This can affect how the individual responds dramatically, for example, Warbrick et al (2006) reported that when given different instructions about a forthcoming painful stimulus, individual's anxiety was very different depending on what words were used.

There are various different questionnaires designed to quantitate an individual's personality traits or more specifically anxiety. The Spielberger state and trait questionnaire can be used to create an anxiety score for participants and they can then be ranked according to their anxiety (Spielberger, 1983).

All these questionnaires can still be very subjective as different individuals interpret what is asked of them differently. There is a need in pain research for objective measures of pain that are not affected by the subjectivity of an individual's response.

2.2.1.2 Types of Experimental Pain

There are many ways of producing pain experimentally, and all have advantages and disadvantages logistically and in producing a clear and reproducible evoked response. Depending on what aspect of pain is being investigated, it is important to consider all of these factors before beginning experiments.

2.2.1.2.1 Thermal

Laser is commonly used in pain experiments as it selectively activates nociceptive fibres (Qiu et al., 2004). Aδ and C nociceptive fibres can be differentiated with laser stimulation by varying the surface area and the intensity as C fibres have a higher density and lower activation threshold than Aδ fibres (Forss et al., 2005, Raij et al., 2004). It is easy to vary the intensity using laser to give a non-painful warm stimulus ramping up to pain tolerance level and it can be precisely controlled. Laser is also good for eliciting evoked responses (Lorenz and Garcia-Larrea, 2003). However a disadvantage with laser stimulation is that in order not to damage the skin, or for habituation or nociceptor sensitization to occur, the area of stimulation has to be constantly moved (Legrain et al., 2002). Another alternative is a Contact Heat Evoked Potential Stimulator (CHEPS) which involves a thermode placed on the skin which heats up to noxious temperatures (Adjamian et al., 2009). This is very easy to control although issues have been found when using this technique in a MEG system due to stimulus artefact.

The cold pressor test is a classic form of experimental tonic pain, whilst being very painful it is also very affective and tends to induce more emotion than other modalities mentioned (Fulbright et al., 2001) and is biologically closer to chronic pain syndromes (Chen et al., 1989). Typically participants place a limb into ice cold water around 1^{o}C for up to five minutes or to the participants tolerable limit (Backonja et al., 1991). This can be a disadvantage as some people have a low tolerance, leaving the experimenter with insufficient data.

2.2.1.2.2 Mechanical

Mechanical stimuli have been used in some pain studies in order to give a more biologically relevant stimulus. Examples of mechanical stimuli commonly used are a nail pressor in which a probe is forced down onto the nail bed until painful, or a balloon distension in the oesophagus or rectum. Most of the pain we experience on an everyday basis will be mechanical (e.g. stubbing a toe) and these stimuli will

activate not only nociceptors but also mechanoreceptors on the surface. Mechanical stimuli are used in both somatic (Arendt-Nielsen et al., 1999) and visceral (Hobson et al., 2000b) studies as a robust and controlled way of creating pain.

2.2.1.2.3 Chemical

Chemical stimuli such as injection of capsaicin (Mohr et al., 2008), ascorbic acid (Porro et al., 2002) or ethanol (Hsieh et al., 1999) are very effective in generating strong burning pain which lasts for a number of minutes, but often has the disadvantage of involving an injection which participants may find distressing. The alternative is to use topical creams but this is less commonly reported. Chemical stimuli are very good for visceral pain studies as they provide a similar sensation to naturally occurring visceral pain such as acid reflux.

2.2.1.2.4 Electrical

Electrical stimulation is simple to use and effective at generating different intensities of pain and non-noxious sensory stimuli similar to laser stimulation (Hobday et al., 2000, Hobson et al., 2005). Frequency, intensity and duration can be altered easily to provide different stimulations, however there is a restricted range for each of these factors. A practical problem with electrical stimulation in electrophysiological techniques is that a stimulus artefact may be recorded. However, as this is consistent between trials, it can normally be excluded using analysis techniques. Another disadvantage is that electrical stimulation produces a sensation not normally encountered (Babiloni et al., 2007), and it is not part of our evolutionary experience in comparison to thermal or mechanical stimuli which our bodies and minds are used to dealing with. It may therefore be less biologically relevant and more difficult to generalise its effects to clinical populations, although this problem is applicable to most types of experimental pain.

Electrical stimulation was chosen for the majority of studies in this thesis due to the fact that it is easy to control, is compatible with the MEG system and easily produces both sensory and painful stimuli.

2.2.1.1 Thresholding

In order to ascertain the appropriate stimulus intensity for each individual, thresholding was performed to find their sensory and pain threshold for that particular stimulus. For both somatic and visceral electrical stimuli, the electrodes were put in place and then thresholding could begin. Using a stimulator, the intensity was lowered to 0mA and gradually increased whilst triggering a pulse to fire at ~1Hz. For the protocols involving a train of electrical pulses, the experimental protocol was used for thresholding to get an accurate portrayal of the intensity that would be felt during the experiment (2s train with a rest period between each train). The participant was instructed to notify the experimenter when a sensation was first noticed. At this stage, the intensity would be lowered and increased 3 times in order to ascertain an accurate reading of sensory threshold. The intensity was then increased again and the participant was instructed to notify the experimenter when the sensation became painful to them, again when this point was reached, the intensity was decreased and increased 3 times in order to ensure an accurate reading.

The same was then done for pain tolerance, the participants were instructed to comment when the intensity of the pain was as high as they could tolerate. The stimulus was never given at this level experimentally, but it gave a range within which the painful stimuli would be applied. For the protocols in Studies 1 and 2, there was only one painful and one sensory stimulus, the sensory stimulus was taken as 50% between sensory and pain threshold and for the painful stimulus, it was taken as 50% between pain threshold and pain tolerance. For Study 4 in which there were 4 intensities; low and high sensation were 25 and 75% between sensory and pain threshold respectively and low and high pain were 25 and 75% between pain threshold and pain tolerance respectively.

2.2.1.2 Presentation, Triggers, Markers

In order to include markers in the data clarifying when certain events took place, Presentation software was used. In Study 1, markers were put in place for all the different visual cues (rest, anticipation/pain, recovery) and markers were put in for each electrical pulse. Triggers came from the Computer with Presentation software to the monitor cueing the visual stimuli and the electrical stimulator to respond. These triggers were then transmitted to the MEG computer and included in the recorded data for analysis. For studies 2 and 4 only a trigger for the electrical stimulus was necessary from Presentation. For study 3, triggers were added to the data manually using a button press to indicate when each event began and ended (baseline, warm start, warm end, cold start, cold end), also a button press was used each time the participant gave a Likert scale rating and these were annotated for later analysis.

2.2.2 Acquisition

The magnetic flux resulting from electrical current flow in the cortex was recorded by SQUIDs held inside a liquid-helium filled dewar. The system used in Aston University was a 275 channel CTF MEG system (CTF Systems Inc, Vancouver, Canada). Data was recorded at a sampling rate of 600Hz for Studies 1 and 3. For studies 2 and 4, 1200Hz was used as the sampling rate in order to look at frequencies up to 150Hz. The highest frequency it is possible to study with MEG is a quarter of the sampling rate (Hamalainen, 1993). The MEG scanner was housed in a magnetically shielded room, the wall of which comprised an aluminium shell lined with a high permeability alloy called mu metal. This cut down on the environmental noise due to electrical equipment, fluctuations in the earth's geomagnetic field etc, as the magnetic fields recorded from the cortex are tiny compared to the background noise in the environment. The length of each trial was programmed into the protocol, as was the number of trials. Study 1 consisted of 30 20s trials in each block, Study 2 had 60 5s trials in each block, Study 3 was 16 60s trials and Study 4 had 60 5s trials for each block containing the 2s train of pulses whereas for the 5s train, each trial was 10s and

there were 30 trials. Head localization was monitored continuously, and if the participant moved more than 5mm from the original position then the data was not used as this could lead to problems localising activity. Triggers were programmed into the protocol if it was necessary to have markers in the dataset indicating different events (see section 2.2.1.4).

2.2.3 Data Filtering, Artefact screening

Once the MEG data had been recorded, it was then viewed using the software DataEditor (CTF Systems Inc, Vancouver, Canada). The data was scanned for artefacts due to eye blinks, electromyography (EMG), also known as muscle artefacts, and also artefacts from any electronic equipment being used to deliver the stimuli. Trials or channels that contained large artefacts were removed from the dataset. Pre-processing was then performed on the data; this involved activating 3rd gradient noise reduction which is able to remove environmental noise from the data that is picked up by reference coils. The DC offset was removed (based on the pre-trigger period). Any noise created by the power line at 50Hz was removed using a notch filter with a width of around 0.6Hz. A high and low-pass filter was added to the data from 1-100Hz although this varied in some cases if it was necessary to focus on a particular frequency band.

2.2.3.1 Independent Component Analysis (ICA)

In Study 2, a stimulus artefact was present in the visceral data of some participants from the oesophageal electrical catheter. ICA is able to separate out components of the data that have a consistently similar pattern and are repeated a number of times throughout the data. It also shows a topographic map of each components origin. It is then possible to see which components are artefactual, i.e. originating in the eyes for eye blinks or towards the throat for the oesophageal catheter, these can then be removed from the data (Hyvarinen et al., 2010). This was performed on the visceral data for a number of participants in Study 2 and improved the evoked response data greatly.

2.2.4 Coregistration

In order to map the MEG data on to anatomical areas of an individual's brain, it was necessary to perform coregistration. At the beginning of each MEG experiment, 3 electromagnetic head coils were placed at the nasion, left and right preauricular points of the participant. A 3-dimensional digitizer (Polhemus isotrak system, Kaiser Aerospace Inc, Colchester, Vermont, USA) was used to digitize the surface of the participants head in relation to a reference point. The 3 coils were then plugged into a head box in the MEG system which records the position of these coils using a position sensing device and therefore is able to record the location of the participant's head inside the dewar during the recording (Singh, 1995). This information was then mapped on top of (coregistered with) the participants previously obtained anatomical MRI (Singh et al., 1997). This surface matching involves minimizing the squared Elucidean distance between the polhemus surface and the MRI surface, the algorithm is repeated 20 times in order to get the most accurate fit and is accurate to within 5mm (Adjamian et al., 2004). Therefore once source reconstruction analysis has been performed, it is possible to see in which anatomical areas of the cortex the changes in frequency power are located.

2.2.5 Event related fields (ERFs)

The raw MEG data was averaged over all trials and then channels were grouped according to location in the dewar in order to see the evoked response to the stimulus at the sensor level (see Figure 2.1). This was informative in terms of time windows and areas to investigate. Using synthetic aperture magnetometry (SAM) (see Section 2.2.6), coordinates were found that were peaks of activity in the cortex. Using these coordinates, 'virtual electrodes' (VEs) could be created. This reconstructs the data focusing on the activity arising from that precise source. It is then possible to load this information into a temporal display to see the evoked response at that location (see Figure 2.1).

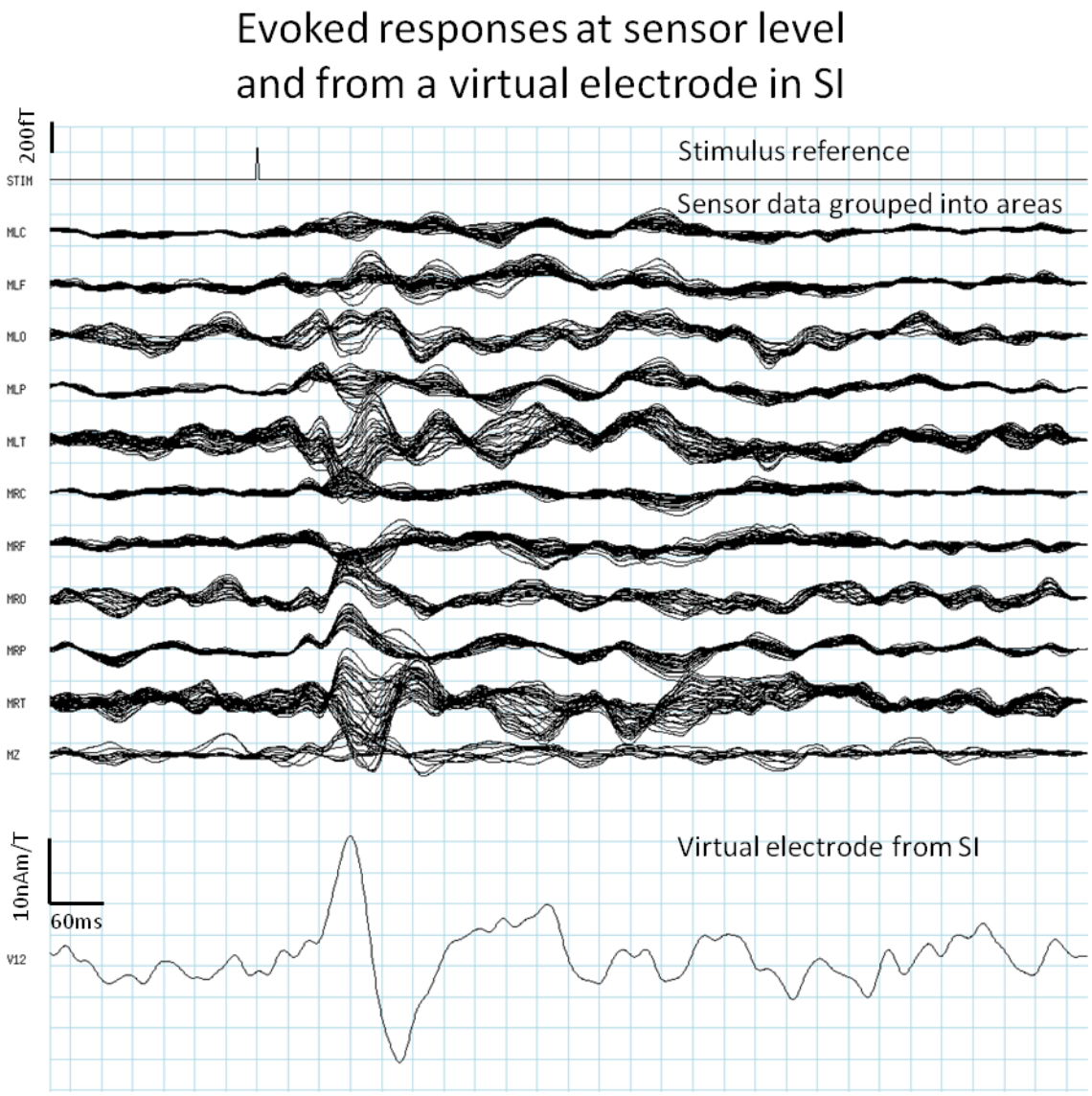


Figure 2:1 shows an example of sensor level data grouped into channel areas (eg MRT= middle right temporal) and then below from a virtual electrode taken from a SAM peak in SI. The onset of the electrical stimulus used to create an evoked response can be seen on the top line.

2.2.6 Synthetic Aperture Magnetometry (SAM)

Once MEG data is acquired, it is then necessary to use the sensor level data to infer the location of the source of the magnetic fields. This is termed the inverse problem (Singh, 1995). There is no unique solution to the inverse problem, however if

additional information is added, a good estimation can be calculated (Barnes et al., 2006).

SAM is a non-linear adaptive beamforming analysis technique. An optimal spatial filter is constructed for every voxel of the brain using certain parameters such as time windows and frequency band (Vrba and Robinson, 2001). The MEG data is then passed through this spatial filter to create a narrow beamformer which has the same millisecond resolution as the original data (Barnes and Hillebrand, 2003). A beamformer focuses on a specific spatial location and attenuates signals from all other areas (Cheyne et al., 2008). SAM makes a comparison between two states at each voxel; an active period of the data and a passive period (e.g. pain vs baseline) in the frequency bands selected. A t-test is done in order to determine whether there are any significant differences in power between active and passive states at each voxel.

SAM is excellent at eliminating sources of noise, for example if there is an artefact from an electrical stimulus then SAM is able to recognise that it is outside the dewar and has the same effect on all the channels and therefore eliminates this component from the data. However, a limitation of SAM is that it treats any highly coherent sources (i.e. sources that oscillate completely in phase with one another) as originating from a single source. This can be problematic if there is bilateral activation of an area in the brain that is highly coherent, SAM will find a single peak in the dominant hemisphere. It is also restrictive in that this form of SAM requires an active and passive state, comparing the two in order to find activity in the active phase, this assumes that there is little variation in the passive period which is often untrue.

2.2.6.1 Group SAM

Group SAM enables one to see whether there are any trends of activity, key areas or frequency bands that are involved in the task in question across a group of participants. In order to perform Group SAM, each individual's activity must be mapped onto a template brain. This is because each individual's brain anatomy is

different to the next and in order to compare them, they must be moulded onto the same template (Singh et al., 2003). This is done using statistical parametric mapping (SPM) software (SPM, UCL, London); each individual's MRI anatomical image is resliced to the same orientation and position of the SAM functional volume. This can then be spatially normalised to a standard MNI template brain, this is done at each frequency band, for each participant. Once all the SAM images have been normalised, it is possible to create group averages (Singh et al., 2002), these images can then be displayed on a template brain using MRI3DX (see Section 2.2.8).

There are two types of statistical analysis performed by Group SAM; simple effects and random effects (Singh et al., 2003). With simple effects, the magnitude of response at each voxel can be pooled across all participants and the T values can become probabilities against the null hypothesis. This is a sensitive form of analysis, especially if using small numbers of participants, however there is the issue that the group image may be dominated by one individual's response (Singh et al., 2003). The random effects model uses both intra and inter-individual variance and performs a t-test on the data, this makes responses seen in this model more reliable but often requires a larger sample size (Singh et al., 2003). If particular areas show changes in frequency power that are consistent across the group then these will be evident in the group image.

2.2.6.2 Statistical Non-Parametric Mapping (SnPM)

Group SAM merely creates a grand group average of the data across participants. In order to find out if there are any statistically significant changes in frequency band power in specific areas at the group level, SnPM analysis is used (Nichols and Holmes, 2002). This performs non-parametric repeated measures statistical tests on the group data, it also corrects for multiple comparisons.

2.2.7 Time-frequency Spectrograms

From the coordinates created from peaks in SAM analysis, virtual electrodes (VEs) can be created. The MEG data is then analysed focussing on this coordinate in the cortex and then changes in power across frequency bands can be seen across a trial.

2.2.7.1 Averaged spectrograms

In each MEG dataset, a number of trials are recorded and then averaged in order to be able to provide a robust response to a stimulus. Having decided on a coordinate from a particular area of the cortex (from a SAM peak), a spectrogram of each trial is created showing how the power of different frequency bands changes across the trial, then the spectrograms of each trial are averaged together to create an averaged spectrogram for that stimulus. It is possible to see both how the frequency bands relate to each other and how a particular frequency band changes across the course of the trial. A disadvantage of the wavelet analysis used to create spectrograms is that the same wavelet width or sampling rate is used for all frequencies and at higher frequencies (>100Hz) temporal resolution is lost.

2.2.7.2 Bootstrap spectrograms

As a more robust, nonparametric measure of changes in power in certain frequency bands compared to averaged spectrograms, a bootstrap spectrogram can be calculated. This compares an active period to a baseline period. A bootstrap resamples the data by taking all the time points across active and passive periods and creating two new random populations from this combination. It repeats this resampling 500 times and then calculates whether the original data shows a significant difference between the active and passive periods as compared with the 500 random populations (Graimann et al., 2002). The bootstrap gives percentage change in frequency power.

2.2.7.3 Group Spectrograms

Group Spectrograms allow us to see if changes in frequency band power are consistent across a group of participants. An averaged spectrogram must be created for each individual first, using the same time windows and frequency range for each participant; these can then be averaged together.

2.2.7.4 Envelopes

Depending on the protocol used, sometimes it is not possible to average across many trials. For example, the cold pressor test (CPT) is only one trial with the participant's hand in ice cold water. If this is the case, then envelope analysis can be used to look at temporal changes in frequency bands of interest across a particular time interval. The data is read in and weighted to a particular location (VE) in the cortex that is specified by the covariance matrix within a weights file previously created. The data is band pass filtered to a particular frequency band over the selected time interval. The root mean square (RMS) of the power of each sample is then calculated which makes every value positive, allowing the visualisation of comparative power change in the frequency band across the time interval. A graph is produced of time versus frequency power and patterns can be easily seen.

2.2.8 MRI3DX

MRI3DX is software written by Krish Singh in order to visualise and manipulate functional data on anatomical scans or a template brain (http://www.cubric.cf.ac.uk/Documentation/mri3dX/). It is used in individual and Group SAM in order to indicate areas showing an increase or decrease in power in particular frequency bands, in order to look for general trends and also in order to display the data in a clear and comprehensible way for publications.

3 Study 1
Investigating cortical oscillations during both anticipation and perception of a noxious electrical stimulus using Magnetoencephalography

3.1 Abstract:

Pain is a multi-dimensional experience comprising both perceptive and affective components. Anticipation is a preparatory response that involves arousal, attention, anxiety, conditioning and cognitive appraisal. The aim of the present study was to investigate the oscillatory changes in key areas of the pain neuromatrix in anticipation of and during both non-painful and painful electrical stimuli using MEG.

Using visual cues, the participants were guided to anticipate a train of electrical pulses at both painful and non-painful levels on the right median nerve at the wrist. The painful and non-painful stimuli were separated into two separate blocks. Each trial in a block contained 5s rest phase, 10s anticipation phase (during which the train of electrical pulses would be given, the onset of this was jittered) and 5s recovery phase. Each block had 30 trials and each phase of the trial was signalled by a different coloured shape.

During the anticipatory period in the pain block, a statistically significant decrease in gamma frequency power (30-80Hz) ($p<0.05$) was seen in ipsilateral SI across the group. A decrease in gamma power was also seen in contralateral SI but it did not reach significance at the group level ($p=0.1$). During the pain period, a statistically significant increase in power was seen in the gamma band in contralateral SI ($p<0.05$).

This increase in gamma oscillations during painful electrical stimulation was seen in 66% of participants. The time-frequency spectrograms showed a decrease in the bandwidth of this pain-related gamma response during the course of the stimulation (60-80Hz to 45-60Hz). In 66% of participants that showed gamma oscillations during

pain, the increase in gamma oscillations was still seen in the non-painful block although it was not as strong.

A trend was seen with a decrease in both the alpha and beta bands in SI during both painful and non-painful stimuli in Group SAM although neither reached significance. Evoked responses were seen in key areas of the neuromatrix (SI, SII, ACC and Insula) in the majority of participants. 22% of participants showed an increase in gamma band in SII in response to painful electrical stimuli as well as in SI.

This study suggests that change in the gamma frequency range may be an important component in pain and sensory perception and attentional processing and that even when anticipating pain there are cortical changes in areas associated with pain processing in preparation for the stimulus.

3.2 Introduction:

Pain research is of utmost importance as many people are affected by chronic pain syndromes, as well as the general population being exposed to pain regularly in their daily life, due to injuries or as a symptom of another health problem. In order to differentially diagnose between pain disorders, it is important to understand the mechanisms behind the pain. For example, hypersensitivity is caused by changes in peripheral receptor function or central pathways leading to decreased pain thresholds, such as occurs in complex regional pain syndrome (de Mos et al., 2009). Whereas hypervigilance is when the physiological pain response is normal, but the subjective reaction is larger than expected for that intensity (Hobson et al., 2006). These two conditions would therefore necessitate different treatments.

Pain is multi-dimensional in that there are components of pain perception related to sensory discrimination such as stimulus location, and components related to the affective side of pain such as anxiety (Melzack and Casey, 1968). It is important to understand the affective aspects as these psychological issues can often become a debilitating additional problem, and can have an impact on the sufferer's everyday life (Eccleston, 2001). These issues can be investigated experimentally.

Anticipation is a key component of pain processing. It is a preparatory response and has been found to recruit areas of the pain matrix before any pain has been given (Porro et al., 2002), as highlighted in Chapter 1: Section 1.11.1). Understanding how the brain reacts during anticipation may be key in unravelling the more affective and emotional components of pain (Porro et al., 2002) and how we can influence these psychologically in our endeavour to change pain perception. The brain's response to anticipating pain has been investigated by many in recent years as can be seen in Table 3:1.

Author and Year of publication	Stimulus	Warning cue	Imaging Technique	Areas found active during anticipation
Ploghaus (1999)	Painful and non-painful thermal stimulation (heat)	2 coloured lights signalling which stimulus to expect	fMRI	Medial frontal lobe, insula, cerebellum, ACC
Hsieh, J. C., S. (1999)	Injection of ethanol in arm	Injection of saline	PET	ACC, ventromedial PFC, PAG
Carlsson, K., P. (2000)	Tickling sensory stimulation on bottom of right foot	Visual cue – red square Varied stimulus onset time	fMRI	Contralateral SI, bilateral inferior parietal, SII, right ACC, right prefrontal cortex
Sawamoto, N., M. (2000)	Laser stimulation to dorsum of right hand, non-painful and painful	Stimulus given at fixed time interval after start of each trial	fMRI	ACC, parietal operculum, posterior insula
Porro, C. A.(2002)	Ascorbic acid subcutaneous injection into dorsum of foot	Cleaning foot with wipe	PET	Foot area of SI, rostral anterior cingulate, medial prefrontal cortex, anterior insula, anteroventral cingulate
Wager, T. D., J. K. (2004)	Electrical stimulation at painful and non-painful intensities + placebo	Visual cue – red or blue	fMRI	Increase in PFC during anticipation with placebo
Warbrick, T., D. (2006)	Electrical stimulation to finger	Primed for anxiety or neutral condition with research instructions	EEG	No source analysis performed – EEG sensors mentioned (Cz)
Babiloni, C., A. (2006)	Painful laser stimulation	3 visual stimuli	High res EEG	Frontal regions, parietal regions (change in alpha)
Fairhurst, M. (2007)	Painful thermal stimulation to dorsum of hand	Visual cue (variable ISI)	fMRI	Right PAG, nucleus cuneiformis, ventral tegmental area, entorhinal cortex
Babiloni, C., A. (2007)	Laser and electrical stimulation, non-painful and painful	3 visual cues	High res EEG	Right posterior parietal, bilateral medial premotor, left SI
Wise, R. G. (2007)	Thermal stimulation (+midazolam/saline)	Learned association with coloured light for non-painful and painful	fMRI	ACC, contralateral anterior insular cortex, ipsilateral SII/posterior insula
Straube, T., S. (2008)	4 different intensities of subcutaneous electrical stimulation to finger		fMRI	Medial PFC

Table 3:1 shows a summary of recent neuroimaging studies on pain and anticipation and the cortical areas that were activated in each. ISI = inter-stimulus interval, fMRI = functional magnetic resonance imaging, PET = positron emission tomography, EEG = electroencephalography, ACC = anterior cingulate cortex, PFC = prefrontal cortex, PAG = periaqueductal grey, SI = primary somatosensory cortex, SII = secondary somatsensory cortex.

The studies in Table 3:1 demonstrate that using a visual warning cue followed by a painful stimulus, whether it is laser, electrical or chemical, reveals activation during both anticipation and pain phases in most areas considered to be part of the pain neuromatrix (SI, SII, ACC, insula) as well as other areas involved in higher cognitive function (PFC).

Over the years, much has been discovered about the oscillatory patterns in the brain, from *in vitro* electrophysiology work (Traub et al., 2003) and from animal work (Ray et al., 2008). As we progress up to the human, we can begin to relate the changes in oscillatory patterns to different functions, such as those involved in a painful experience, and unravel to some extent how the brain integrates all the sensory information it receives. This can be done using intracortical electrodes (Fukuda et al., 2008) but also with non-invasive techniques such as EEG (Babiloni et al., 2006) and MEG (Cheyne et al., 2008).

The studies in Table 3.1 using EEG reported less distinct areas of activation, but had the advantage of good temporal resolution. This superior temporal resolution enables the changes in frequency dynamics during pain and anticipation to be investigated, although there are few studies focusing on this area at present. Babiloni et al (2006, , 2007) used EEG to look at pain anticipation, to both laser and electrical noxious stimuli. They investigated the frequency dynamics of alpha (6-12Hz) and found a general decrease in this bandwidth during the anticipation period, at electrodes in frontal, central and parietal regions. It was suggested that this could be associated with a change in arousal, however there was a lack of specific spatial information about which cortical areas were involved in these changes as only sensor data was analysed.

The predictability of a painful stimulus has been found to have an effect on the anticipatory response, as observed by Sawamoto et al (2000). When non-painful and painful stimuli were presented in a randomised order, the anticipatory response in the ACC and parietal operculum to uncertain non-painful stimuli was heightened compared to the control of certain non-painful stimuli, due to the unpredictable nature of the stimulus.

The anxiety of the participant has been found to affect the anticipatory response to pain. Warbrick et al (2006) used electrical stimuli and changed only the instructions given between conditions. One intended to make the participant anxious about the

painful stimuli that they were to be given and one gave more neutral instructions. The subjective rating of pain intensity and unpleasantness were higher in the anxiety driven condition than the control and the amplitude of the N140 component of the evoked response was increased in the anxiety condition compared to the neutral condition.

It is known that pain perception and it's cortical activation can be modulated using distraction (Ohara et al., 2004). Qiu et al (2004) used laser pulses and MEG to investigate this. A mental calculation task was given to distract the participant in one condition and the participant was asked to attend to the painful stimulus in the other condition. In the distraction condition, the RMS of components of the ultra-late laser evoked field (LEF) (1M and 2M) was reduced compared to the control condition. These components were found to be from dipoles in SI, SII, insula and cingulate cortex.

Yamasaki et al (1999, , 2000) also looked into attention effects on evoked fields using MEG and EEG. They were unable to find any changes in the earlier components of the evoked response during the distraction task compared to the control condition. The only component that was affected in the MEG data was the later N140-P230 peak to peak amplitude which was reduced in the distraction condition. Later components of the EEG evoked response at latencies of 240ms and 340ms were reduced in amplitude during the distraction task. It was thought that these were generated by multiple sources including areas of the limbic system.

Many quite simple experiments have been carried out on human participants investigating areas involved during somatic experimental pain. Ploner et al (2000, , 2001, , 2002, , 2004, , 2006a, , 2007) performed a number of experiments using MEG and laser noxious and non-noxious stimuli, to investigate the involvement of different areas of the pain neuromatrix. They found activity in contralateral SI and almost simultaneously bilateral SII activation in response to their laser stimuli. The timing of SII activation in these studies would suggest that there is direct input from the

thalamic nuclei to SII rather than information travelling via SI as has been previously suggested (Frot and Mauguiere, 1999). The role of SI and SII has also been explained further in that SI activity appears to be linearly related to stimulus intensity, whereas SII becomes more active when the stimulus is painful (Buchel et al., 2002, Timmermann et al., 2001, Maihofner and Kaltenhauser, 2009, Frot et al., 2007, Bornhovd et al., 2002, Coghill et al., 1999).

Hauck et al (2007a, , 2007b, , 2008) investigated cortical responses to pain using MEG. In one study, attention was altered and in another the cue to pain time delay was varied, in order to vary the participant's expectations. Most work done on attentional mechanisms until then had looked at evoked potentials, but Hauck (2007a) began to investigate the frequency dynamics using MEG. An oddball paradigm was used with rare and frequent intracutaneous electrical stimulation. Changes in all frequency domains were observed. Delta oscillations showed an increase in power with directed attention and higher stimulus intensity, beta showed a suppression and rebound after the painful stimulus and gamma band increased in power with directed attention.

These results show great potential for unravelling the oscillatory dynamics in attention to pain but there is still a need for more specific spatial localisation. Hauck et al (2007) used two 31 channel dewars placed over the SII cortices. Time-frequency representations were created by averaging across 31 sensors for each participant and across 20 participants. Averaging over both sensors and participants means that some of the detail of the data may have been lost. For example, the exact spatial location of each frequency band is unclear and some of these changes may originate from areas outside the somatosensory cortex. Also the changes in spectral power that they were reporting were very small, the gamma oscillations that increased with directed attention were an increase of <1% from baseline.

Hauck et al (2007b) also investigated the effects of varying the cue-to-pain time delay. They found that a longer interval led to a higher pain intensity rating due to

greater expectation levels. Activity in the midcingulate cortex was found to increase with an increasing delay. These results indicate that anticipation, expectation and attention are all capable of varying the subjective responses given by subjects and also modulate the oscillatory dynamics in areas of the pain neuromatrix.

Another study that investigated the frequency dynamics of pain was by Gross et al (2007). An increase in gamma oscillations was seen in this study in response to painful laser stimulation. Behavioural data indicated that around pain threshold, if the participant rated the stimulus as painful then there was a stronger increase in gamma oscillations than at the same intensity when they rated it as non-painful. From this they hypothesised that gamma oscillations have an important role in pain perception.

3.3 Experimental Rationale

The patterns of cortical oscillations in response to somatosensory stimuli are still not completely transparent. Further research needs to be done in order to create more robust, reproducible evidence on how these oscillations vary with different physiological and psychological modulators and which cortical areas are involved.

MEG data is rich with spatial and temporal information. It allows us to investigate the role of different areas of the pain neuromatrix and the possible roles of particular frequency bands in pain processing. The aims of this study are therefore to explore the oscillatory dynamics during anticipation and perception of both painful and sensory stimuli and to see if it would be feasible to modulate these both psychologically and with different modalities of pain using different paradigms.

According to the literature, SI, SII, ACC and insula have all been activated in response to anticipation and pain. This study aims to explore the changes in different frequency bands within these areas in order to further understand their role in pain perception. In particular, gamma frequency has been observed during pain in SI and this study aims to further elucidate how gamma oscillations are modulated during

both anticipation and pain in SI and whether it is present in other areas of the pain neuromatrix.

Median nerve stimulation was chosen as the stimulus as it is known to produce strong evoked responses and creates a clear, strong sensation which can be localised to the hand area of the SI cortex (Schnitzler et al., 1999, Frot and Mauguiere, 1999, Chen and Herrmann, 2001, Fukuda et al., 2008). A train of electrical pulses was used in order to provide a longer, more tonic-like stimulus as it was thought that this was more likely to drive an anticipatory response than just a single, brief pulse.

In order to confirm whether any oscillatory patterns were due to the perception of the electrical stimulation and not as a result of the sensation caused by the thumb twitch elicited by the electrical stimulation, the protocol was repeated with digital stimulation instead of median nerve stimulation in Study 1 part B. The frequency of the electrical pulses was also changed from 10Hz to 7Hz to investigate what effect this had on the oscillatory dynamics. 10Hz was originally chosen as the frequency for the train of electrical pulses due to pilot testing which found this to be the most effective at creating the illusion of a constant stimulus and creating significant pain in the participant.

3.4 Materials and Methods:

3.4.1 Participants:

12 healthy participants (7 male; age range 23-45years) took part in this study. All were free of any neurological or pain disorders and none were taking medication at the time of the study. Anatomical Magnetic Resonance Images (MRI) were taken for each of these individuals and were made available for analysis. 3 participants could not be used for the final analysis due to problems with coregistration. Informed consent was obtained from all participants and the local ethics committee approved the experimental protocol.

3.4.2 Stimulus:

Electrical pulses were delivered via a constant current stimulator (Model: Digitimer Ltd, Welwyn Garden City, DS7A). Two electrodes were placed on the right wrist of each participant over the median nerve. The duration of each electrical pulse was 200μs. A train of electrical pulses was delivered at a frequency of 10Hz for a period of 2s as the stimulus.

Thresholds were obtained by administering pulses at 1Hz. The current (range from 0mA to 100mA) was started below sensory threshold and was increased incrementally at a rate of ~1mA/s. The participant was instructed to notify the experimenter when a sensation was first felt, when the sensation became painful and when the participant was unwilling to experience a higher intensity due to the strength of the pain. Four measurements were taken; sensory threshold, first appearance of thumb twitch, pain threshold and pain tolerance. Once each level was reached, the current was then increased and decreased around that intensity three times with feedback from the participant in order to ensure an accurate threshold. A sample of the 10Hz stimulation was given to each participant before each block to ensure that the stimulus was at the correct level.

3.4.3 Experimental Procedure:

Two 10 minute blocks were administered in the experiments. One block involved a sensory stimulus (50% between sensory threshold and pain threshold) and one block had a painful stimulus (50% between pain threshold and pain tolerance) (see Chapter 2: Section 2.2.1.1).

The stimulator administered the electrical stimuli at predetermined times indicated by Presentation software (Neurobehavioral Systems Inc, California, USA). This software was used to write a code for the protocol, detailing triggers to be sent to a monitor displaying visual cues, to the electrical stimulator and to the MEG acquisition computer. This allowed precise timing of each event and triggers were sent to the

MEG computer, indicating when each event happened in order to coordinate the event with changes in brain activity during analysis.

The participant was warned to anticipate the electrical stimulus using visual cues displayed on a monitor (Sony Trinitron Multiscan G520 21”). This was placed outside the shielded room and could be seen by the participant through a small window, the monitor was ~1m away from the participant’s head. A green square represented a rest period (5s duration), a red square represented the anticipation/pain phase (10s duration) and a green circle represented the post-pain phase (5s duration) (Figure 3:1).

During the anticipation/pain phase, the onset of the electrical train was randomised. The train could appear, at 1 second intervals, between 1s after the red square appeared to 8s after it appeared. Its onset was therefore less predictable to the participant and the delay was randomized throughout the study. This variability in stimulus onset was intended to increase the participant’s anticipation and anxiety.

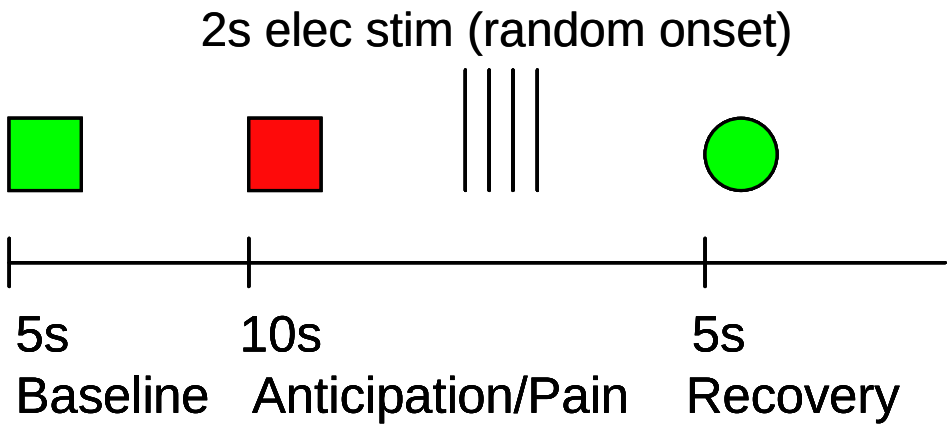


Figure 3:1: Schematic diagram of protocol showing visual cues and timings of a single trial

There were 2 blocks (sensory and pain) of 10 minutes; each block had 30 trials of which 20 trials had an anticipation period of 5s or more. Those with an anticipation period of less than 5s were not used for analysis of anticipation, leaving 20 trials. All

30 trials were used to investigate the pain period. The participant was given an example of the electrical train before beginning each block. The order in which the participants received the blocks was randomized throughout the study.

Participants filled in a Spielberger State anxiety questionnaire (Spielberger, 1983) after thresholding and before the first block, then again after the study in addition to a McGill pain questionnaire (Melzack, 1975).

3.4.4 MEG recordings:

Participants were seated in a magnetically shielded room and viewed the visual cues, presented on a computer monitor, through a window in the room. Neural activity was recorded using a 275-channel CTF MEG system (CTF Systems Inc, Vancouver, Canada) at a sampling rate of 600Hz. 30 trials were recorded, each 20s in duration. Head localisation was continuously monitored throughout each recording to ensure the participant had not moved more than 5mm from their original position. Pre-processing was completed using 3rd gradient noise reduction and removing the DC offset based on the whole trial (see Chapter 2: Section 2.2.2 and 2.2.3 for more details). The 50Hz power line was taken out with a width of 0.5Hz. The trials were scanned for movement or EMG artefacts and if necessary a trial was removed. An average of all the trials for each participant was scanned for blink artefacts but none were consistent across trials and it was not necessary to remove them.

3.4.5 Coregistration:

A 3-dimensional digitizer (Polhemus isotrak system, Kaiser Aerospace Inc, Colchester, Vermont, USA) was used to digitize the surface of the participants head and this information was then coregistered with the participants previously obtained anatomical MRI which gives accuracy to within 5mm (Singh, 1995, Adjamian et al., 2004) (see Chapter 2: Section 2.2.4 for details).

3.4.6 Data Analysis:

3.4.6.1 Synthetic Aperture Magnetometry (SAM):

SAM is a beamformer which enables changes in power in certain frequency bands over the cortex to be observed between active and passive states, this technique is described in more detail in Chapter 2: Section 2.2.6. SAM comparisons were made by comparing 5s of the anticipatory phase (active) to 5s of the baseline phase (passive) and comparing 2s of the pain phase (active) to 2s of the baseline phase (passive). These comparisons were performed in both the pain and sensory blocks. The frequency bands used for SAM comparison were 3-7Hz (Theta), 7-14Hz (Alpha), 15-25Hz (Beta) and 30-80Hz (Gamma).

Group SAM (Singh et al., 2003) was performed on this data in order to find out if there were any changes in frequency band power that were consistent across the entire group. Each participant's activity at each frequency band was mapped onto a template brain, the participants' activity was then averaged together in order to provide a group image (see Chapter 2: Section 2.2.6.1 for details). This was done for both anticipation and pain comparisons, for both pain and sensory stimuli and for each frequency band. SnPM (Nichols and Holmes, 2002) was performed on this data in order to explore the statistical significance of changes in power across the group in each frequency band (see Chapter 2: Section 2.2.6.2 for details).

3.4.6.2 Time-Frequency Analysis (Spectrograms):

Using the Group Data, key regions of interest (ROIs) were identified (SI, SII, ACC, Insula). This information was then used to refer back to the individual SAM data. SAM peaks in the individual that were spatially coincident with ROIs from the Group data and had a pseudo t value of ≥1 were used for further analysis. The coordinates in these ROIs formed VEs (see Chapter 2: Section 2.2.5) (Barnes and Hillebrand, 2003) which were used to create time-frequency representations or spectrograms (see

Chapter 2: Section 2.2.7). These demonstrated how the oscillatory dynamics varied across a trial at a particular location.

Average time-frequency spectrograms were created comprising baseline, anticipation, pain and recovery periods with a frequency range of 1-100Hz. In addition, further spectrograms were produced for just the 2s stimulation period from 1-150Hz to investigate higher frequency gamma oscillations. Bootstrap spectrograms provided a more robust indication of significance of changes in oscillatory power (see Chapter 2: Section 2.2.7.2 for details). These were created comparing 2s of baseline with the 2s pain period between 1-100Hz.

3.5 Methods for Study 1 part B

3.5.1 Participants

3 healthy participants (2 male; age range 22-31years) took part in this study. All were free of any neurological or pain disorders and none were taking medication at the time of the study. Anatomical Magnetic Resonance Images (MRI) were taken for each of these individuals and were made available for the analysis. Informed consent was obtained from all participants and the local ethics committee approved the experimental protocol.

3.5.2 Stimulus

Electrical pulses were delivered via a constant current stimulator (Model: Digitimer Ltd, Welwyn Garden City, DS7A). Two electrodes were placed on the right index finger of each participant. The duration of each electrical pulse was 200μs with a frequency of 7Hz for a period of 2s as the stimulus.

For further details on methods see section 3.4 as the rest of the method for this study was identical to study 1 apart from that already mentioned.

3.6 Results:

3.6.1 Behavioural Data:

No significant difference was found between the Speilberger anxiety scores before and after the pain run ($t_{(5)}$=1.06, p=0.32). The McGill scores were calculated and split into sensory and affective descriptive words (Melzack, 1975). Only 13% of the total score was made up of affective words and only 33% of participants used affective descriptive words to describe the pain. As can be seen from Figure 3.2, the most commonly used words to describe the electrical pain were 'shooting', 'stabbing' and 'sharp'.

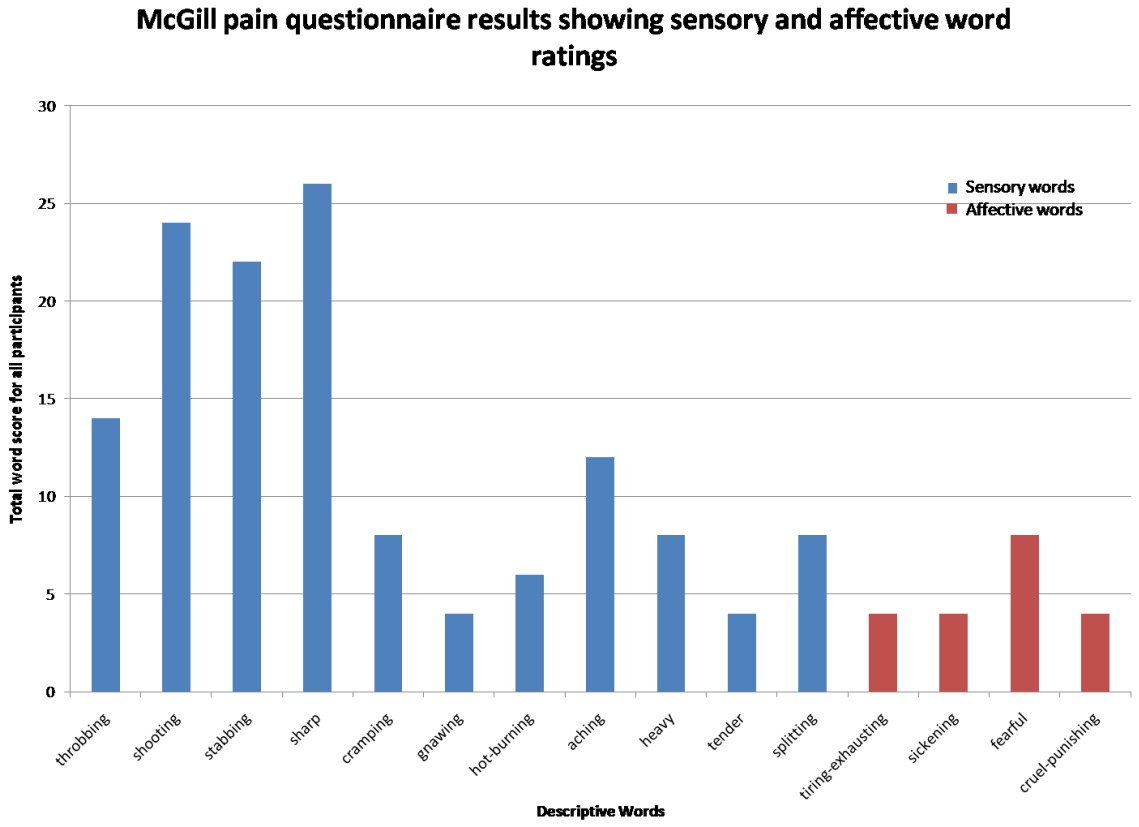


Figure 3:2 illustrates the ratings given in the McGill pain questionnaire in response to painful electrical stimulation. In blue are sensory descriptive words and in red are affective descriptive words. The y axis shows the total score of each word for all participants.

3.6.2 Pain thresholds:

Each individual participant's sensory (ST) and pain thresholds (PT) were determined and stimulation levels calculated from these at the beginning of the study. Table 3.2 shows the sensory and pain thresholds for all participants including a multiplier (PT/ST) in order to give some indication of the range between the two values across participants. The range for ST was 1.4-3.4mA and for PT was 7.4-15mA.

Pain and sensory thresholds for each participant			
Participant	ST (mA)	PT (mA)	Multiplier
P1	3.2	10.0	3.2
P2	1.6	3.9	2.4
P3	3.0	10.0	3.3
P4	1.4	7.4	5.3
P5	2.0	8.2	4.1
P6	2.3	7.2	3.1
P7	3.4	15.0	4.4
P8	3.0	10.0	3.3
P9	2.8	13.0	4.6

Table 3:2 shows each participant's sensory and pain thresholds and the multiplier.

3.6.3 SAM activation:

SAM peaks were found in key areas of the pain neuromatrix (SI, SII, ACC, Insula) during both the anticipatory period and the pain period, although there was some variability between individuals (see Table 3.3).

SAM peaks found in each ROI during each SAM comparison for all participants								
	SI		SII		ACC		Insula	
Participant	sens	pain	sens	pain	sens	pain	sens	pain
P1	A + S	A + S	A + S	A + S	S	A + S	S	-
P2	A + S	A + S	A + S	A + S	A + S	-	A + S	A + S
P3	A + S	A + S	A + S	-	A + S	A	A	S
P4	A + S	A + S	S	A	A + S	S	A + S	-
P5	A + S	A + S	S	-	S	A	-	A + S
P6	S	A + S	-	A + S	A + S	A + S	S	A + S
P7	A + S	A + S	S	A + S	A + S	A + S	A	A + S
P8	A + S	A + S	-	-	A + S	A + S	S	A + S
P9	A + S	A + S	-	S	A + S	A + S	A	A + S

Table 3:3 demonstrates whether or not each participant showed a peak of SAM activity with a pseudo t≥1 in each of the key areas of the pain neuromatrix during both the sensory and pain runs. A = peak during anticipation vs baseline, S = peak during stimulus vs baseline, - = no peaks ≥1.

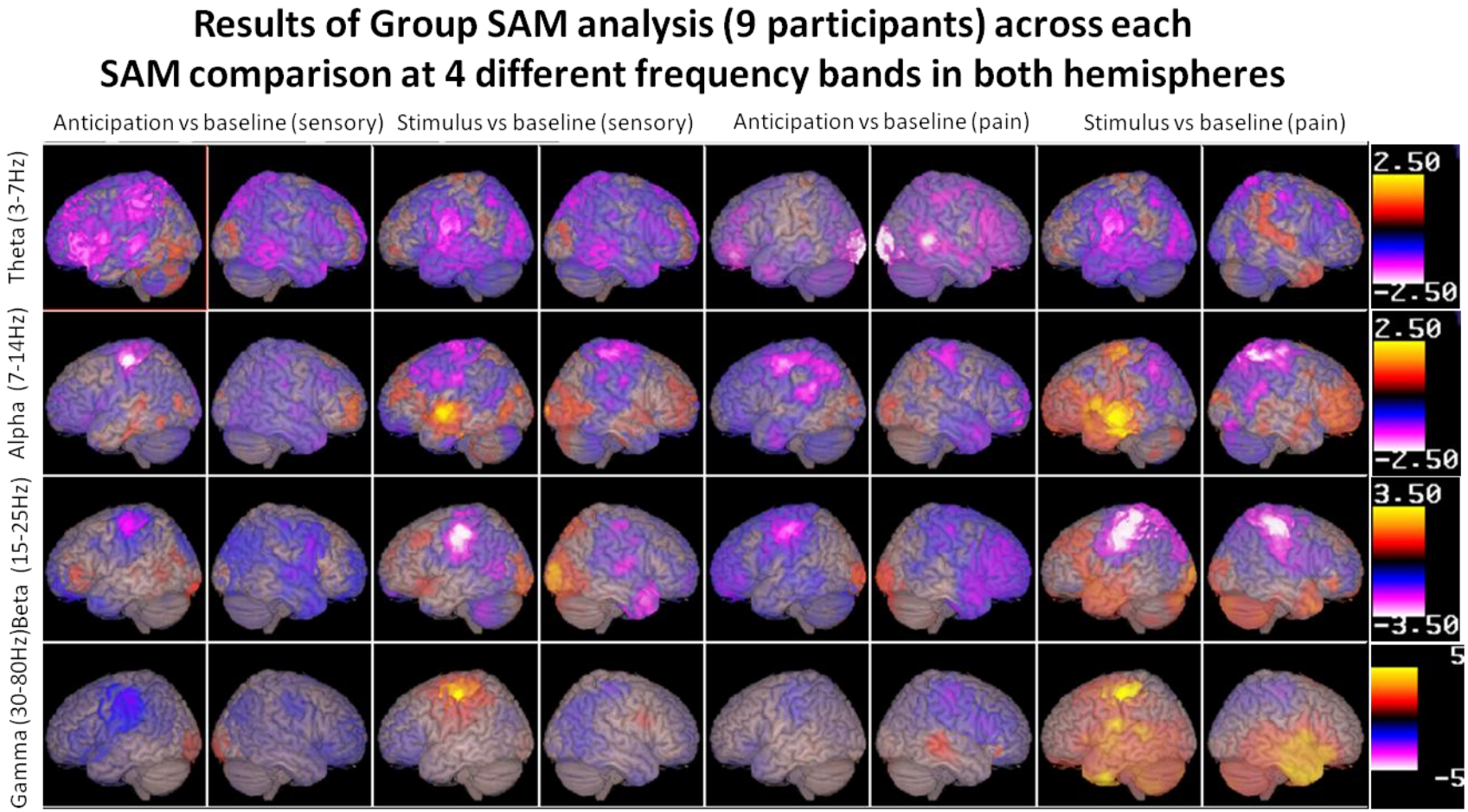


Figure 3:3 illustrates Group SAM data of all 9 participants. The rows indicate different frequency bands and each pair of columns represent different SAM comparisons showing both anticipation vs baseline and stimulus vs baseline during both the sensory and pain blocks. Purple/Pink colours indicate a decrease in power and Orange/Yellow indicate an increase in power. A surface rendering function was used to bring the interior activity to the surface.

3.6.4 Group Data:

Each participant's activity was normalized to a template brain and they were then analysed at group level using Group SAM and SnPM, the results of which can be seen in Figures 3.3-3.7.

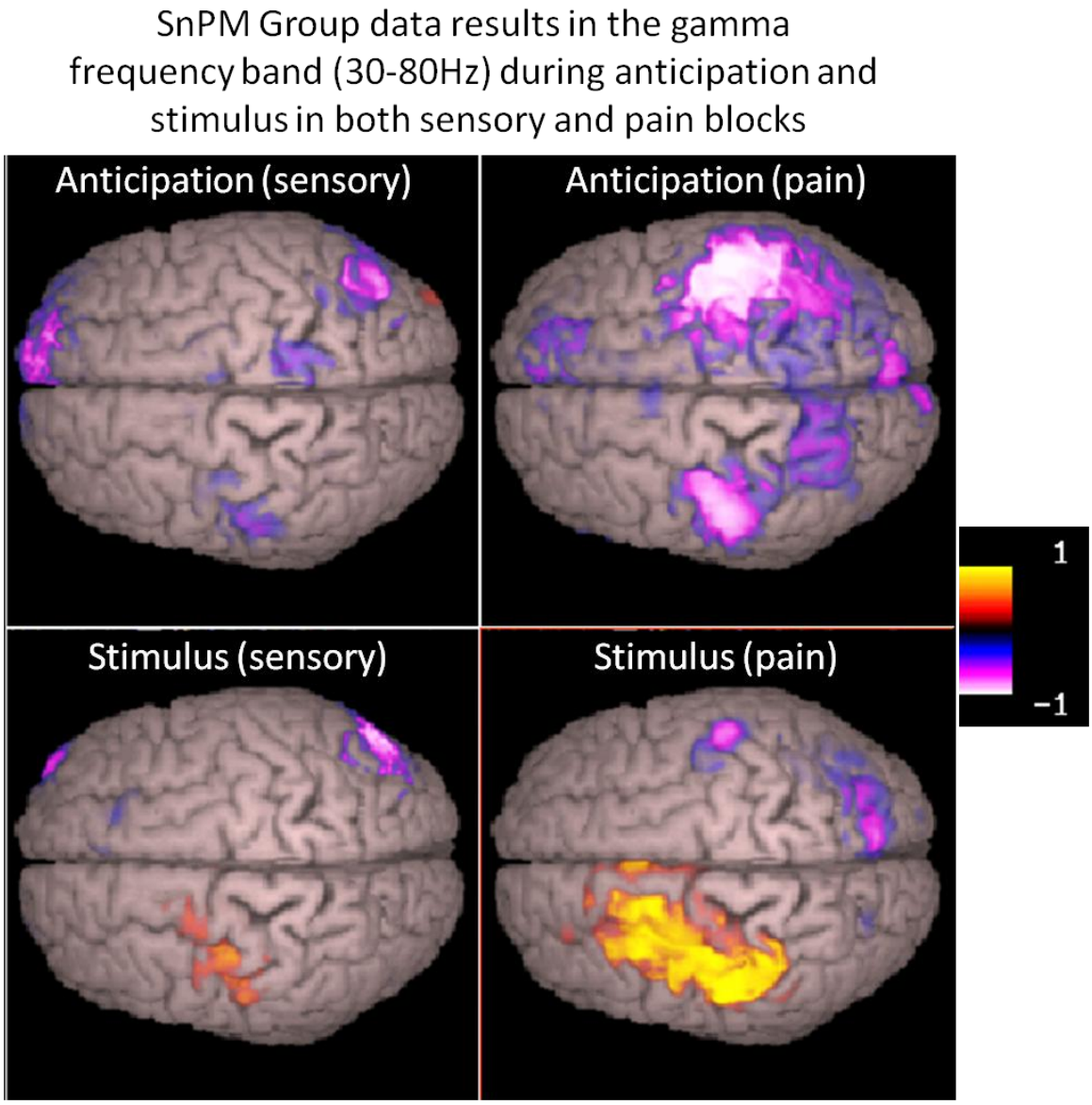


Figure 3:4 demonstrates results of SnPM analysis performed on group data in the gamma frequency band (30-80Hz). The top row shows the SAM comparison of anticipation to baseline and the bottom row indicates the stimulus period compared to baseline. The left hand column is during the sensory run and the right hand column is during the pain run. Red and orange indicate an increase in power in the gamma band and purple and white indicate a decrease in power in the gamma band. The scale demonstrates the confidence interval so anything above 0.95 or below -0.95 is a statistically significant change across the group.

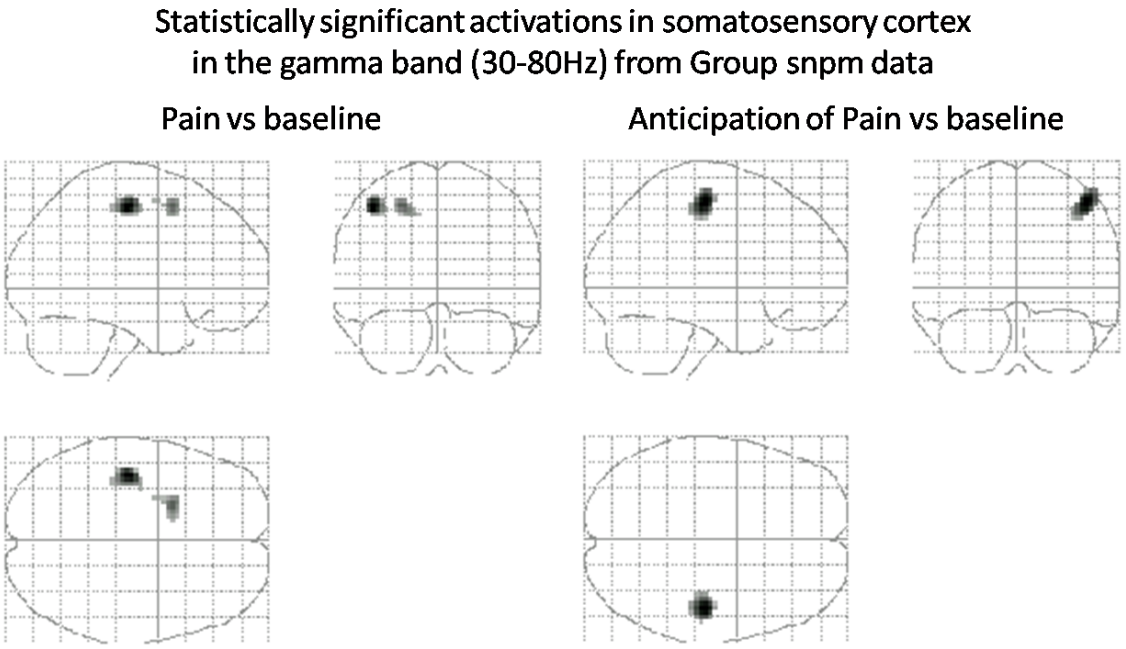


Figure 3:5 shows statistically significant data (i.e.p<0.05) found from SnPM analysis in the gamma band (30-80Hz). There was a significant decrease in power in the gamma band in an area corresponding to ipsilateral (right) SI during anticipation of pain which was not significant during anticipation of sensation (right-hand side). There was also a significant increase in power in the gamma band in an area corresponding to contralateral SI during the painful stimulus (left-hand side) which did not reach significance during the sensory stimulus.

From the SnPM data (Figures 3.4 and 3.5) (Nichols and Holmes, 2002), it is possible to see that during the anticipatory period in the pain block, a statistically significant decrease in power in the gamma band (30-80Hz, $p<0.05$) was observed in ipsilateral (right) SI. It appeared that gamma power decreased in contralateral (left) SI however this was not found to reach significance at the group level ($p=0.1$). During the pain period, a statistically significant increase in power was seen in the gamma band in contralateral SI ($p<0.05$). This significant anticipatory desynchronization and pain synchronization was not observed during the sensory block.

During anticipation in the sensory block, SnPM found statistically significant decreases in power in the theta range (3-7Hz) in frontal areas and the alpha range (7-14Hz) in the ipsilateral SI ($p<0.05$) (see Figure 3.6). In the gamma band, during anticipation, a statistically significant decrease in power was seen in an area corresponding to the anterior cingulate cortex (see Figure 3.6). During anticipation of

the sensory stimulus, a statistically significant decrease in power in the theta band was seen in the occipital cortex ($p<0.05$) (see Figure 3.7). During the sensory stimulus there was a significant decrease in power in the gamma band in the posterior parietal cortex ($p<0.05$) (see Figure 3.7).

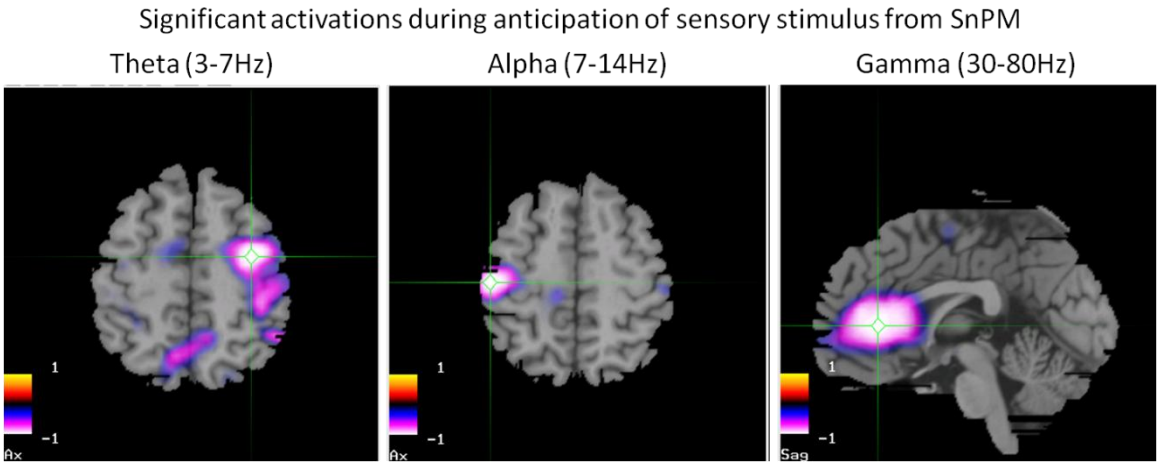


Figure 3:6 shows the significant activations during anticipation of the sensory stimulus in theta, alpha and gamma from Group SnPM results. Any activity that is >0.95 or <-0.95 is statistically significant.

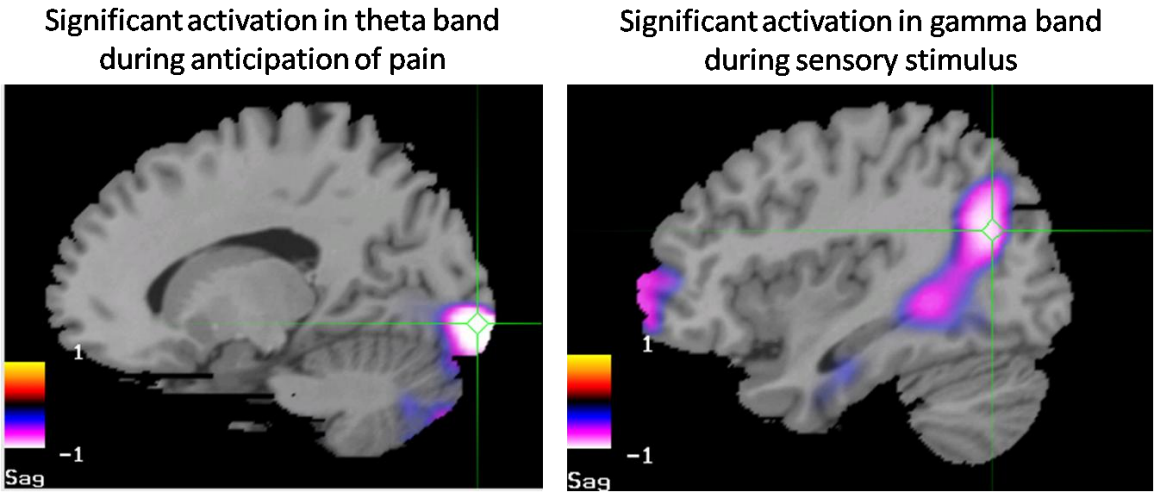


Figure 3:7 shows significant activation in the theta band during anticipation of pain corresponding to the occipital cortex and significant activation in the gamma band during the sensory stimulus in the posterior parietal cortex from Group SnPM results.

3.6.5 Evoked Fields:

With an averaged dataset, the VEs from SAM coordinates were used to look at the profile of the evoked response across a trial from key ROIs (see Figures 3.8 and 3.9). In the SI of 78% of participants, the amplitude increased in pain compared to sensation, an example of this is shown in Figure 3.8. After the first stimulus the amplitude of the positive peak (~70ms) decreased and then plateaued (see Figures 3.9 and 3.10), this was seen across all participants. There was only 100ms between each pulse so the later aspects of the evoked response may have been cut off, however it was possible to see the earlier components.

Figure 3:8 shows the difference in amplitude of the evoked response in sensation and pain in contralateral SI of a representative individual (P4).

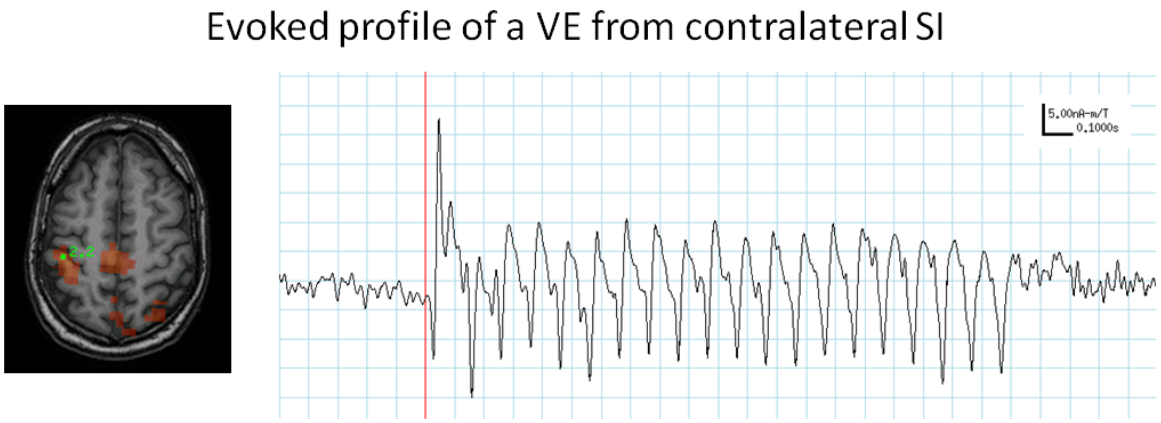


Figure 3:9: shows the evoked response from a VE in contralateral SI in a representative individual (P1) (coordinate shown on the left). The red line indicates the onset of the first stimulus in the train (each subsequent stimulus is a box later). It is possible to see the evoked response to every electrical pulse, even though there is only 100ms between each stimulus. The first response has a larger amplitude of the positive peak (~70ms component) than the rest, whereas the earlier negative peak (~20ms component) remains reasonably consistent compared to baseline.

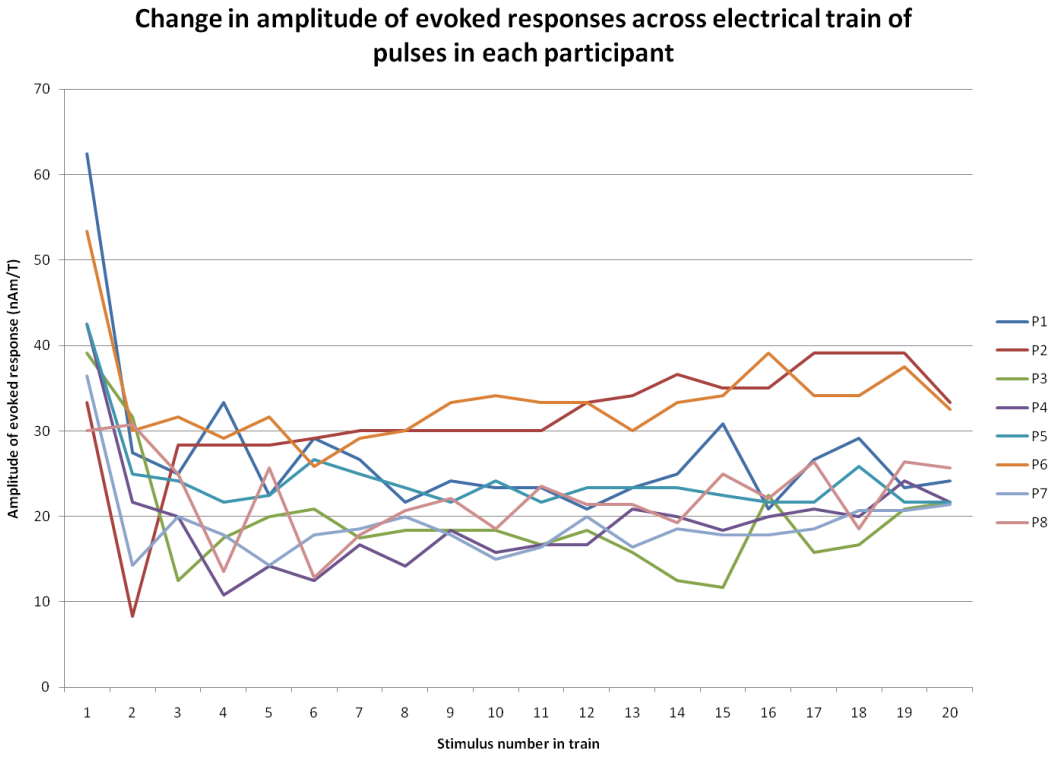


Figure 3:10 illustrates how the amplitude of the 70ms positive evoked response peak varied across the train of electrical pulses during the pain run. Each line represents one participant. A similar pattern was seen during the sensory block although the initial decrease was not as sharp.

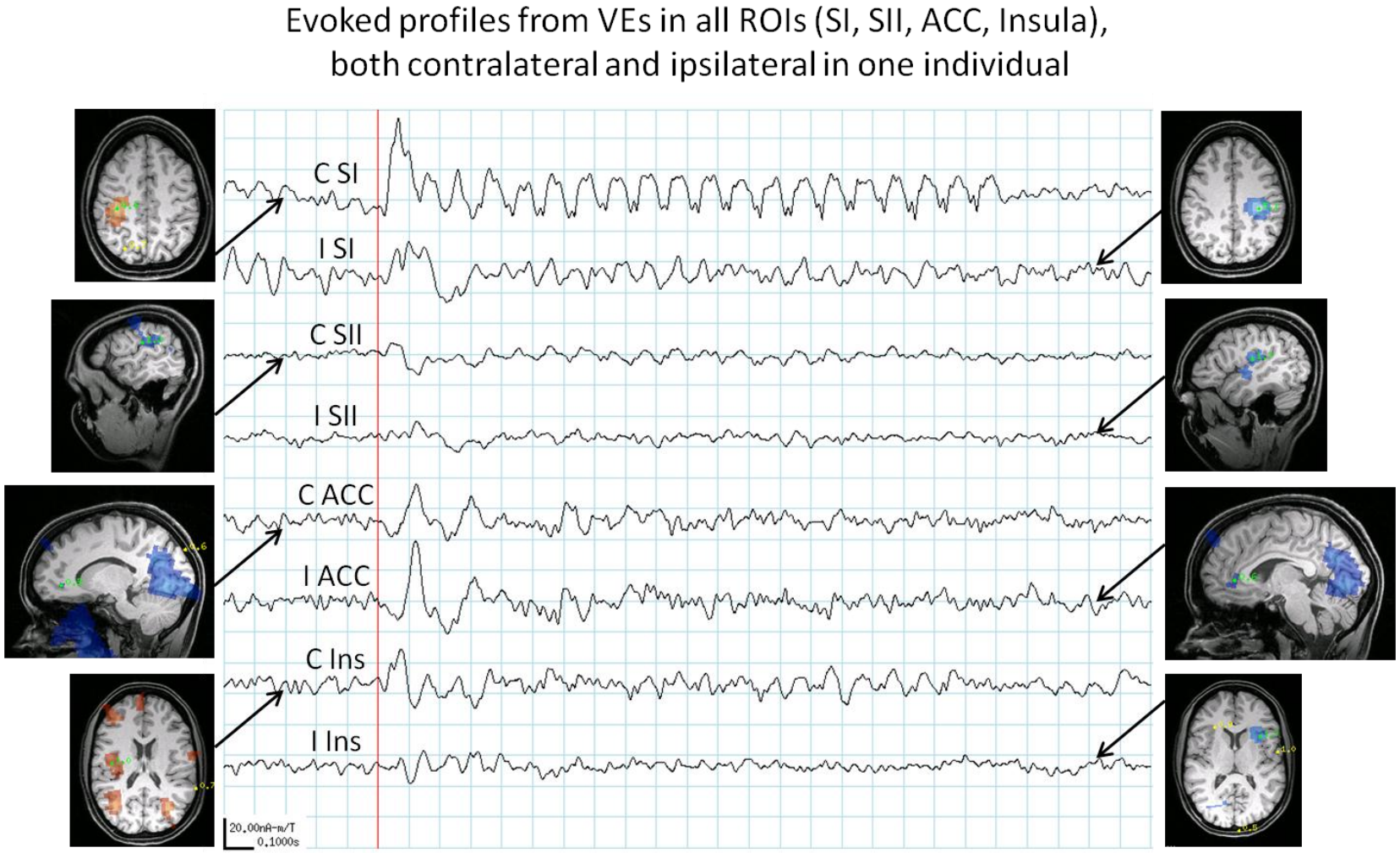


Figure 3:11 shows the evoked response profiles from an averaged dataset of all 30 trials, to the train of electrical pulses in different areas of the pain neuromatrix (contralateral and ipsilateral SI, SII, ACC and Insula) in a representative individual (P2). The exact locations of the coordinates used for the VEs can be seen in the MRI images next to each profile. It is also possible to see whether the coordinate was from an increase or a decrease in power (blue shows a decrease and orange shows an increase). The coordinates used for the evoked profiles in the MRI images are green.

The evoked response seen in both contralateral and ipsilateral SI was clear in response to each stimulus, whereas with the other areas of the pain matrix (SII, ACC, Insula), only the evoked response to the first electrical pulse stood out, although small evoked responses could be seen in the rest of the train (see Figure 3.11).

3.6.6 Time-Frequency Spectrograms:

From the peaks found in SnPM, it was necessary to reference back to the SAM peaks in those areas for each individual. The coordinates from these SAM peaks were then used to create time-frequency spectrograms.

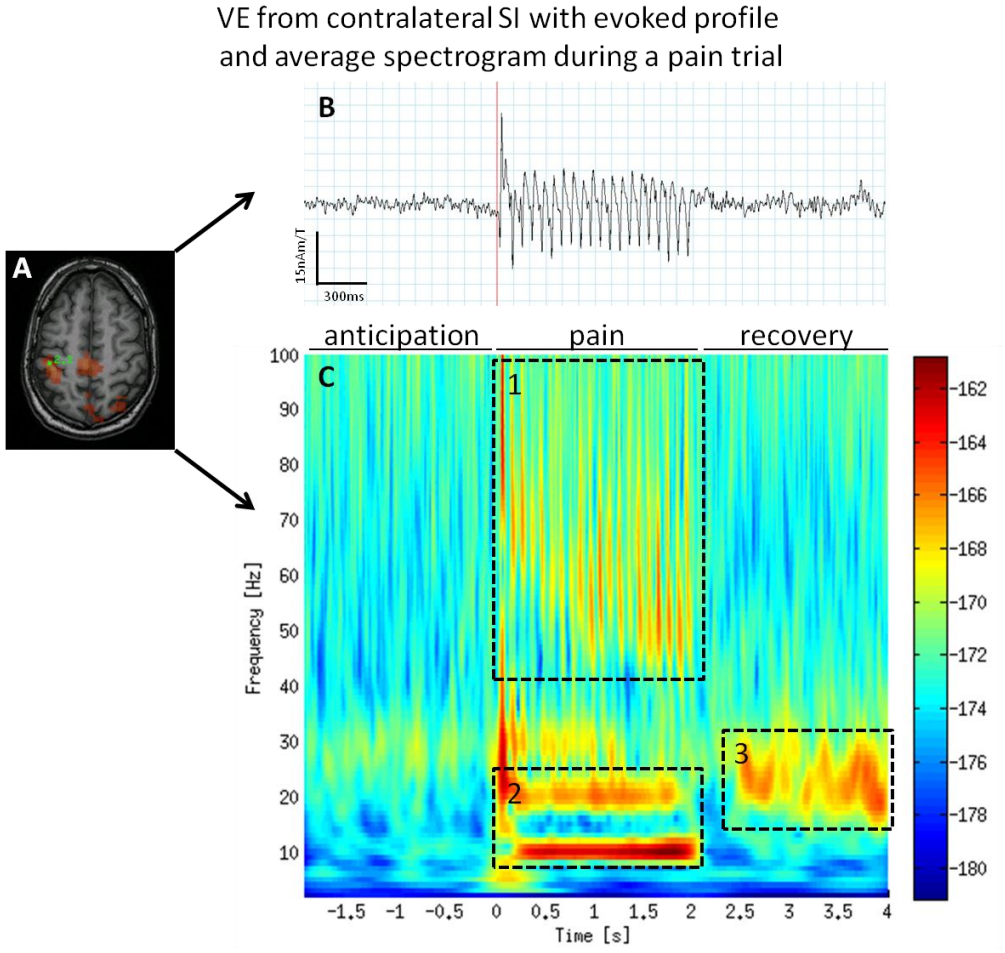


Figure 3:12 shows a virtual electrode coordinate from contralateral SI found from a peak of SAM activity (A) during the pain block in a representative individual (P1) which is then used to create a profile of the evoked field (B) and a time-frequency spectrogram (C) at that location. There is a clear increase in gamma activity (30-80Hz) during the painful electrical stimuli (Box 2). In the spectrogram (C), the x axis represents time in seconds, the y axis represents frequency and the colour scale represents power of each frequency band, red indicating high power and blue indicating low power.

During the pain run, the spectrograms of contralateral SI showed a strong beta power between 20-30Hz in the anticipation period. There was an increase in power at 10Hz in 89% of participants during pain and 66% of participants showed an increase in both 10 and 20Hz during pain (Box 2) although the 20Hz pattern appeared to stop just before the stimulus offset whereas the 10Hz rhythm stayed consistent throughout the train of pulses. There also seemed to be a slight decrease in the high beta band in the majority of participants as can be seen around 25-35Hz (see Figures 3.12 and 3.14).

In spectrograms of ipsilateral SI during the pain run, there was a high beta power between 20-30Hz during anticipation which disappeared during the pain response and then reappeared as a beta rebound after the pain stimulus (see Figure 3.13).

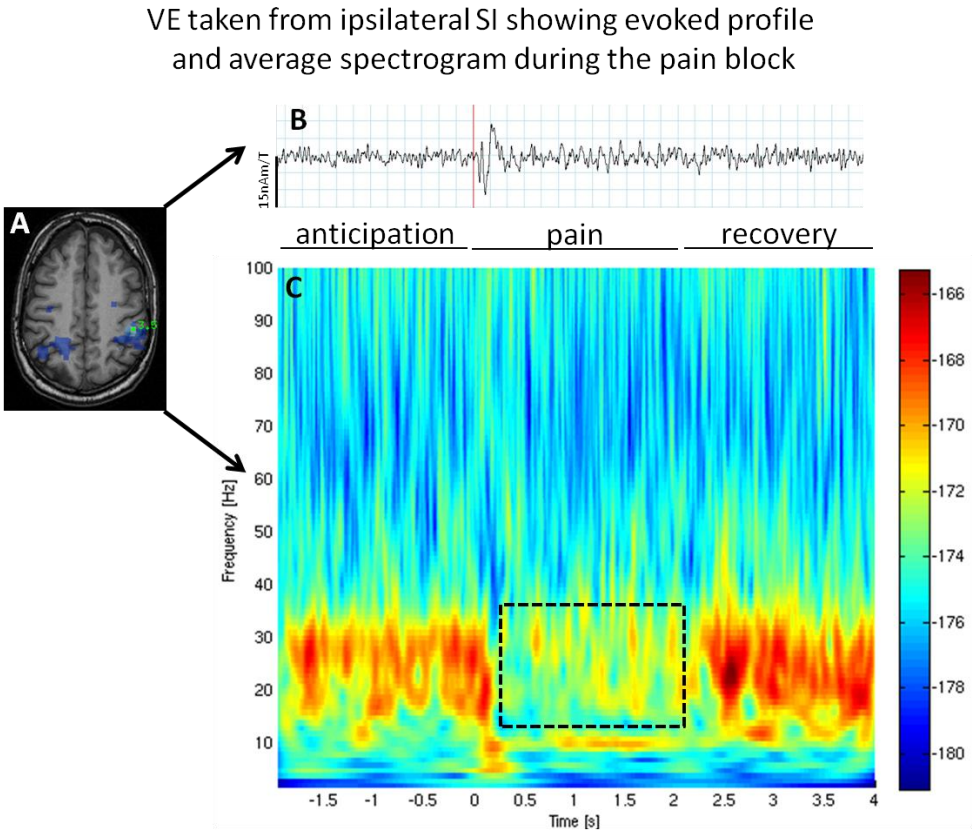
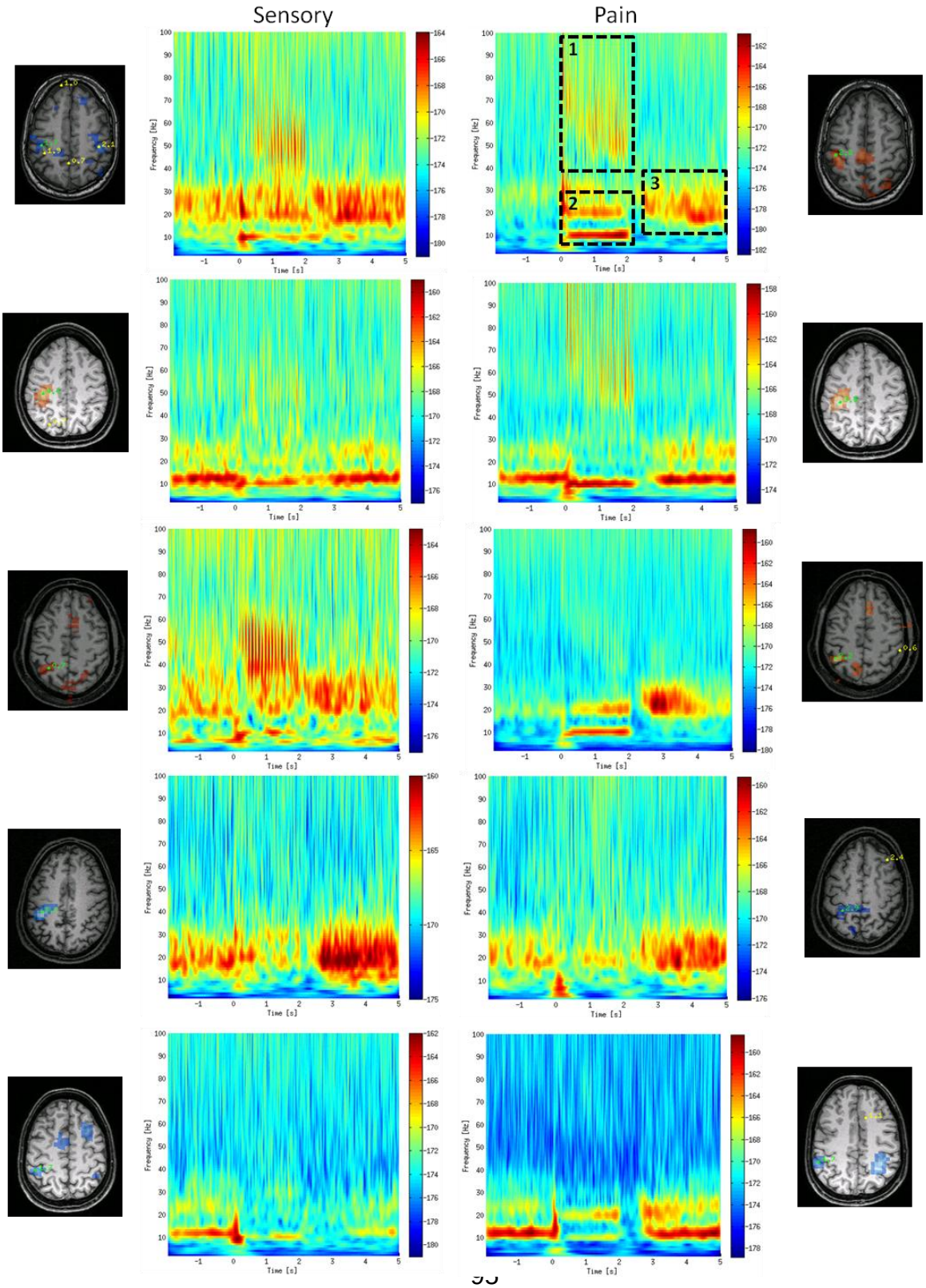


Figure 3:13 shows an average spectrogram from the ipsilateral SI of a representative individual (P1) during the pain block. A shows the location of the VE in SI, B shows the evoked response profile and C shows an average spectrogram. The dotted box highlights the decrease in beta frequency seen during pain.

As demonstrated in Figure 3.12 and Figure 3.14 in the right hand column (pain run), an increase in power was seen in the gamma band (30-80Hz) in 66% of participants (Box 1). Figure 3.15 shows a bootstrap spectrogram from individual P1 which compared the stimulation period to baseline and the scale shows percentage increase in power compared to baseline. The percentage increase in response to sensation and pain are next to each other to allow for comparison between the two. The frequency range of this gamma change decreased over time, commencing between ~60-80Hz at stimulus onset and ending between ~45-60Hz at stimulus offset, this can be seen clearly in Figure 3.15. After the pain stimulus, there was a beta rebound seen between 20-30Hz in 78% of participants (Figure 3.12 Box 3).

During the sensory run, some of the features of the pain run were still present but a lot weaker (see Figure 3.14 and 3.15). 44% of participants still showed an increase in gamma oscillations during the sensory stimulus although it was not as strong and at a lower frequency (~40-70Hz).

VEs from contralateral SI and their average spectrograms during sensory and pain blocks in all participants



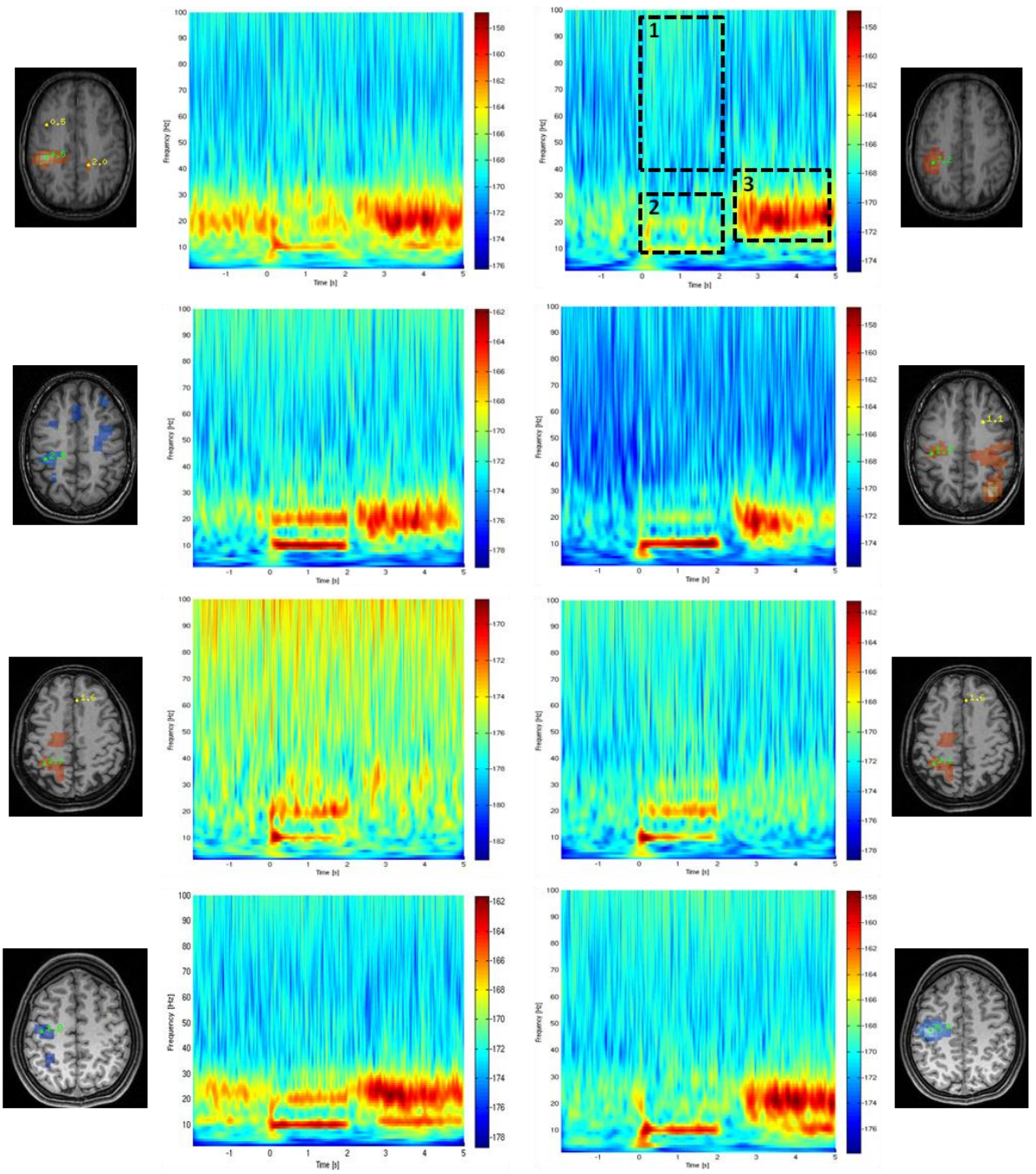


Figure 3:14 shows all 9 participants that took part in the study (each one occupying a row). The left hand column shows the MRI coordinate in contralateral SI of the SAM peak during the sensory run. The averaged spectrogram from this location can be seen next to it. The right hand side is the same but during the painful run. In the MRI images, the coordinates used for the spectrograms are shown in green. The x axis is time in seconds and the y axis is frequency in Hz. Across the x axis, -2 to 0s is anticipation, 0-2s is the stimulation period and 2-5s is the recovery period. It is possible to see across the group the variance in the frequency dynamics, particularly the increase in gamma oscillations during pain. The top right spectrogram indicates key areas to look at. Box 1 covers the gamma increase during stimulation. Box 2 shows the increase at 10 and 20Hz during stimulation and Box 3 shows the beta rebound during the recovery period.

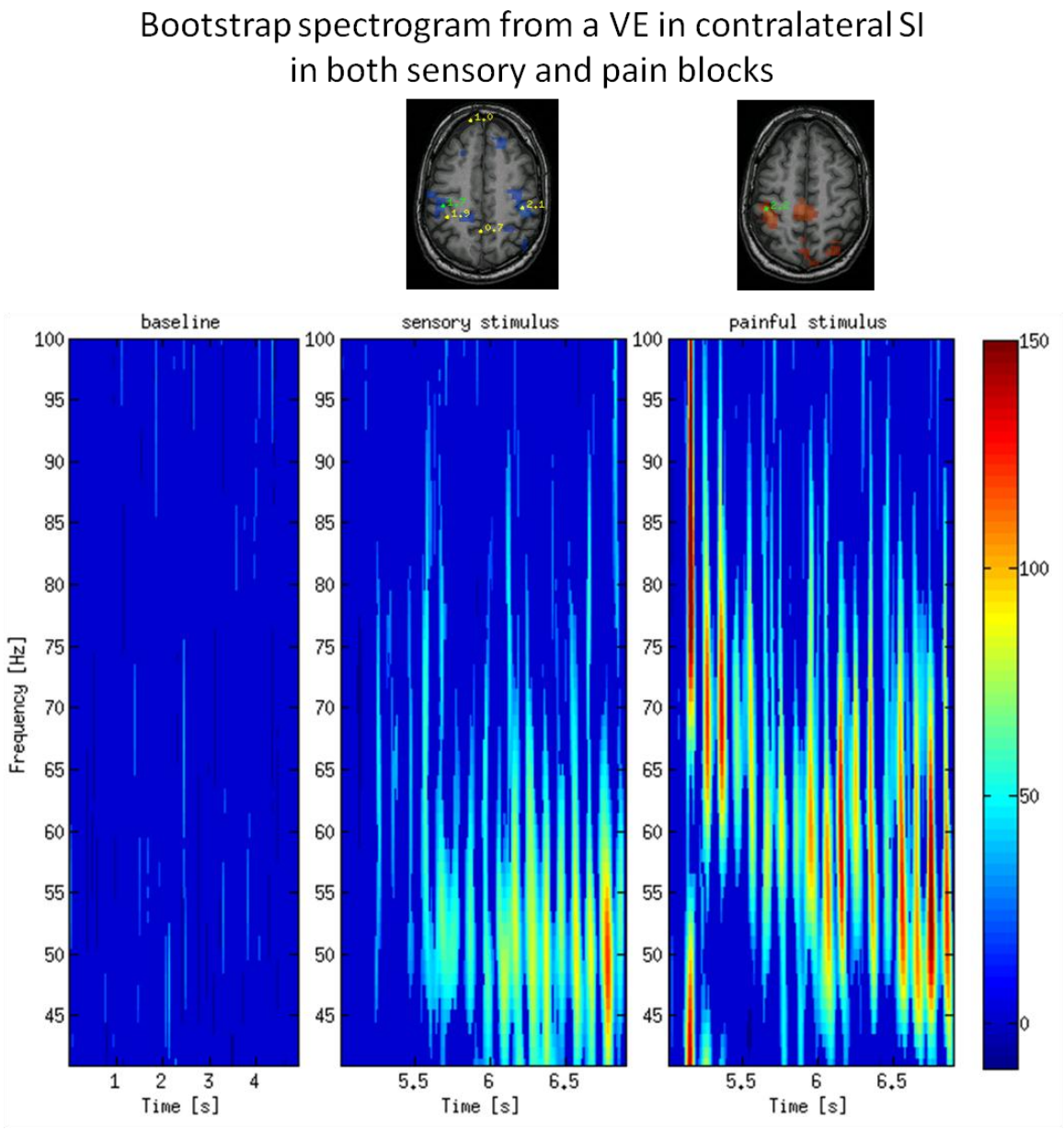


Figure 3:15 shows two bootstrap spectrograms comparing the stimulus period to a baseline period (figure on left). Again the x axis shows time in seconds and the y axis is frequency as in the average spectrograms, however the colour bar in bootstrap spectrograms demonstrates percentage change in power relative to the baseline period, red indicating an increase of up to 150%. Above each spectrogram can be seen the MRI coordinate of the SAM peak from which the spectrogram was generated (peak coordinate in green) in the contralateral SI of a representative individual (P1). The middle figure is during the sensory stimulus and the right is during the painful stimulus. An increase in gamma power can be seen during both sensation and pain, however during pain, the percentage increase in gamma oscillations is stronger and at a higher frequency bandwidth.

In order to determine whether pain threshold had an effect on the presence of gamma oscillations, the pain thresholds of those that did and did not show a gamma response

were compared in a T-test, however there was no significant difference in pain thresholds between groups ($t_{(8)}$=0.79, p=0.45).

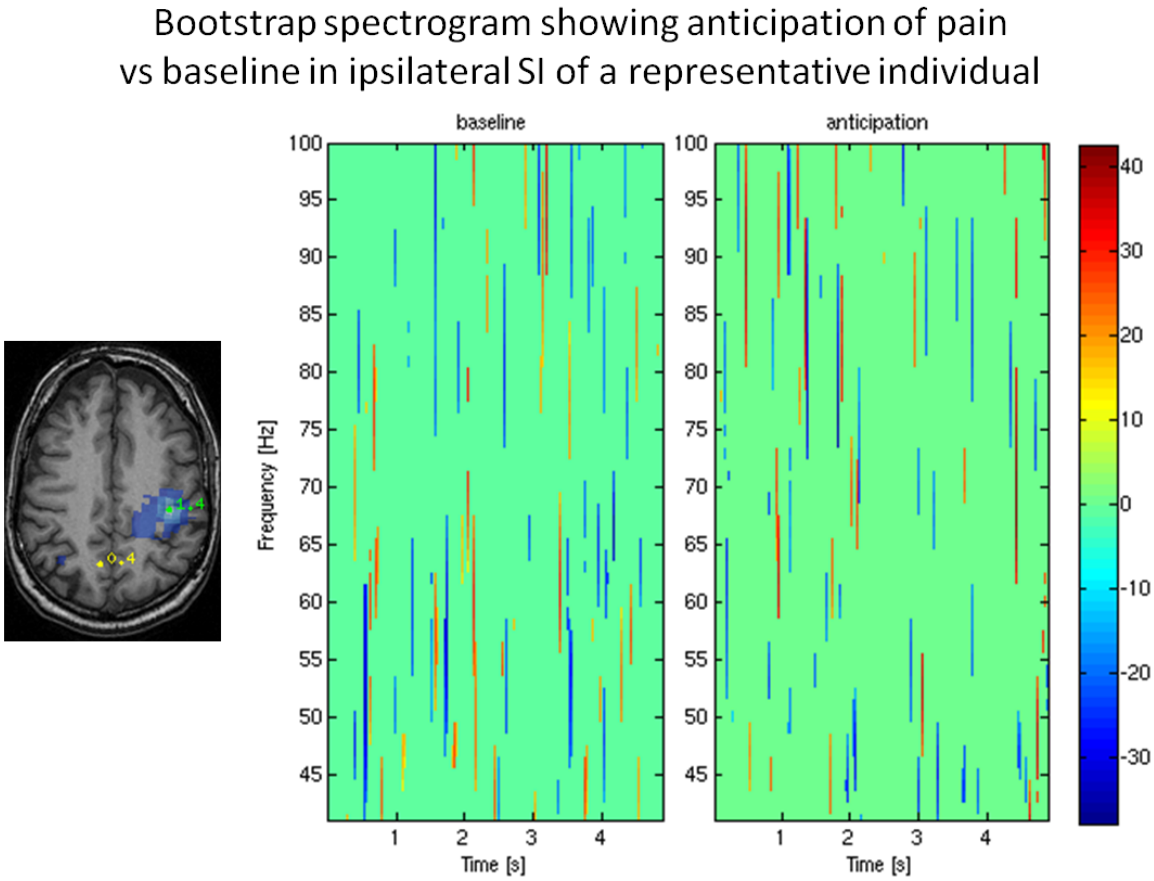


Figure 3:16 shows a bootstrap spectrogram of the gamma range from a VE in ipsilateral SI comparing anticipation of pain to baseline in a representative individual (P1). Although a statistically significant decrease was seen in ipsilateral SI during anticipation of pain from group analysis, it was not possible to see any decrease in the gamma band in individual spectrograms.

In Figure 3.16, the MRI shows a SAM peak in ipsilateral SI which demonstrates a decrease in gamma frequency (30-80Hz) during anticipation of pain. This decrease in gamma oscillations was found to be statistically significant at the group level but when looking at individual bootstrap spectrograms there is no obvious decrease from baseline to anticipation in any participants.

Figure 3.17 shows an average spectrogram of the gamma band from 40-150Hz across the 2 seconds of pain stimulation. An increase in gamma oscillations in

response to each electrical stimulus in the train was evident, it also showed how the frequency of the gamma oscillations changed across the train, beginning at 70-120Hz and ending between 50-80Hz. The strength of the first gamma increase was stronger than the subsequent responses. There appeared to be no higher frequency gamma response as has been suggested by other researchers. This downward shift in frequency has also been seen in *in vitro* preparations (Bracci et al., 1999) after tetanic stimulation from gamma to beta frequency (see Figure 3.18).

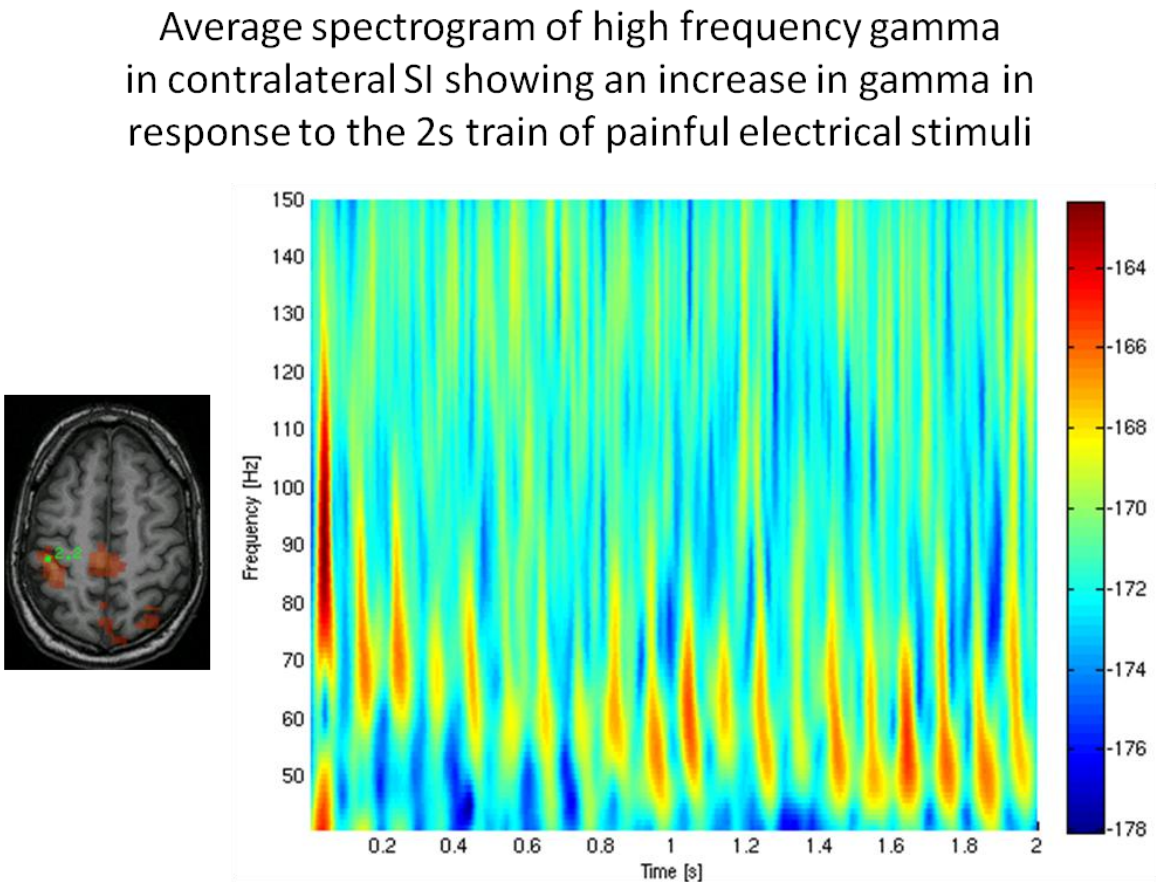


Figure 3:17 shows a average spectrogram from the contralateral SI of a representative individual (P1) showing the high gamma frequency band (40-150Hz) during 2s of painful electrical stimulation.

Figure 3:18 shows the shift from gamma to beta frequency oscillations in rat hippocampal slices *in vitro* after tetanic stimulation. This figure is taken from Bracci et al 1999.

Coordinates taken from SII were used to create evoked profiles and averaged spectrograms. During pain, 33% of participants showed bilateral SII activity, 33% of participants showed only ipsilateral SII activity and 33% of participants had no clear peaks in SII. In 50% of the participants who showed SII activity, a clear evoked response was evident (Figure 3.19, Box 1). 22% of participants showed an increase in gamma power in contralateral SII during pain although it was not as strong as that seen in SI. Figure 3.19 shows an example from one of the individuals that showed a gamma increase (Box 2). There was an increase in power at the onset of the train between 5-10Hz (Box 4) which coincided with the evoked response (Box 1). A decrease in beta power can also be seen (Box 3) followed by a rebound in the recovery period.

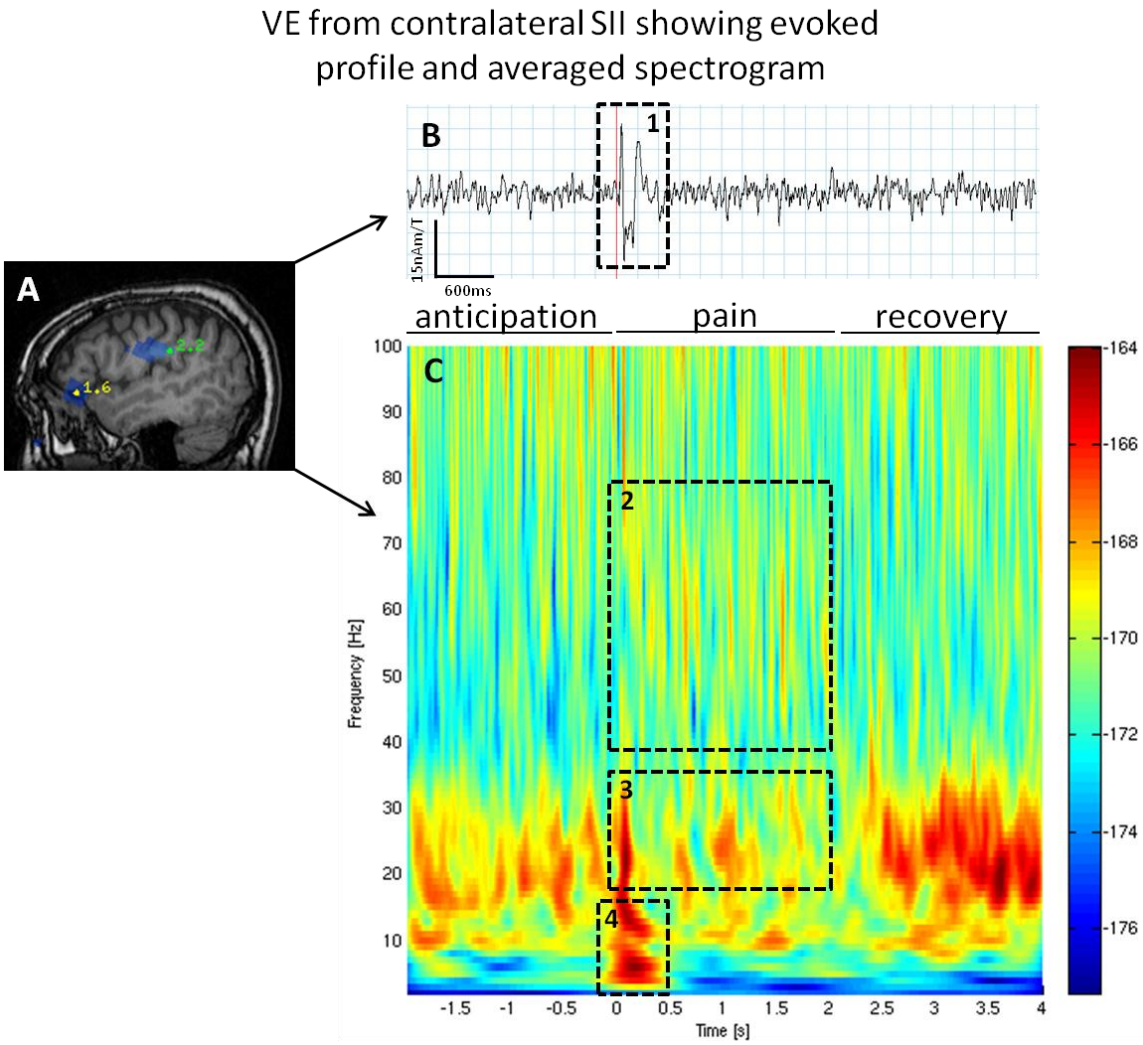


Figure 3:19 shows an averaged spectrogram (C) and the evoked response profile (B) from a virtual electrode in contralateral SII (A) in one participant (P1). Box 1 shows the evoked response profile to the first pulse of the train. Box 2 shows an increase in gamma frequency during the painful stimulus. Box 3 highlights a decrease in beta power during pain and Box 4 shows an increase in 5-10Hz at the onset of the stimulus coinciding with the evoked response.

All 9 participants had SAM peaks in the ACC, although not all in every SAM comparison (see Table 3.3). Spectrograms of ACC showed a continuous beta activity (20-30Hz) although it was less well defined than in SI. 78% of participants showed an increase in power around 5-10Hz which coincided with the evoked response to the first stimulus (see Figure 3.20). 22% of participants showed continuous gamma activity throughout the trial from 50-70Hz.

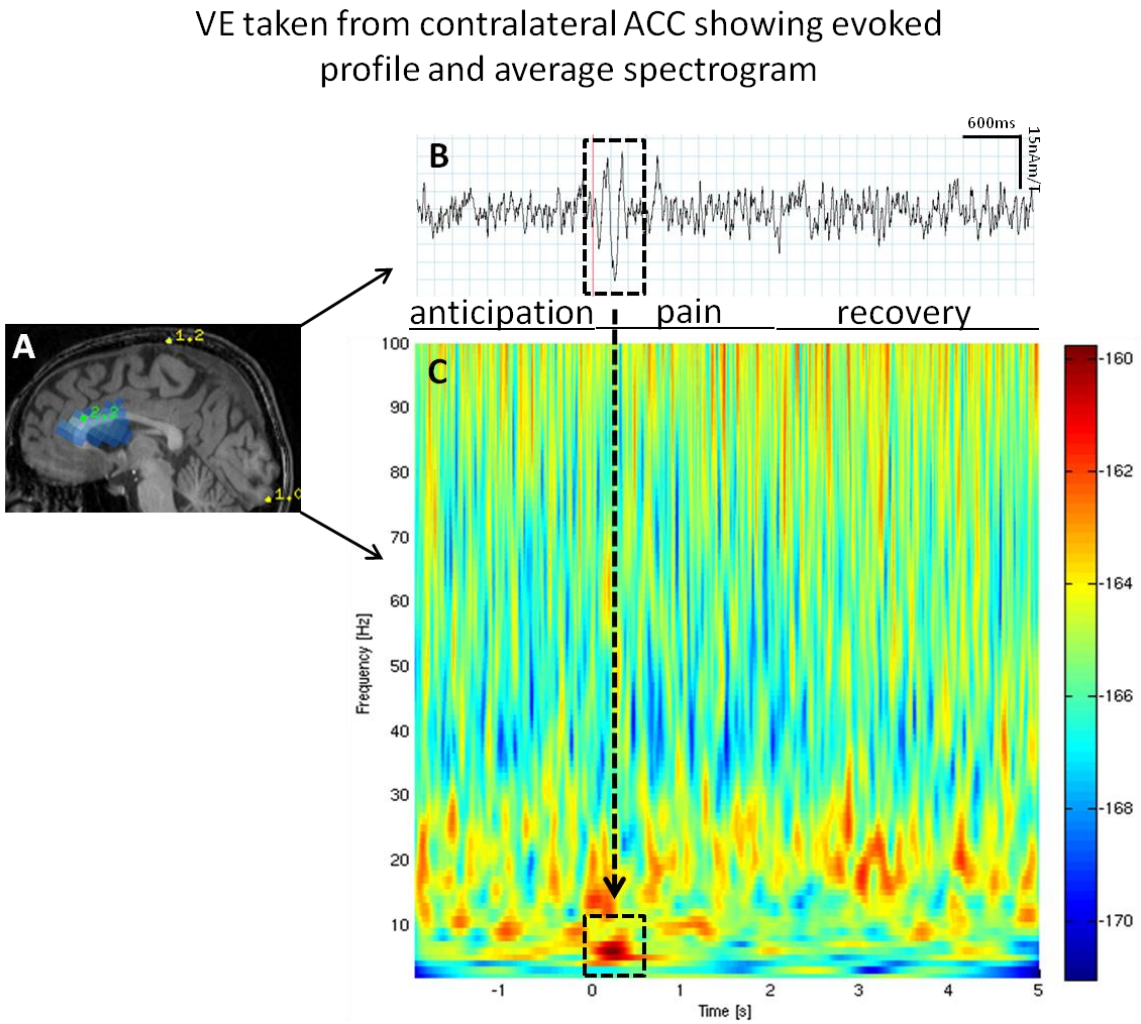


Figure 3:20 shows a virtual electrode (A) taken from the left ACC of a representative participant (P4). B shows the evoked field profile and C shows a time-frequency spectrogram from that location. There is a clear evoked response to the stimulus onset at 0s (red line in B) however there is very little change in the frequency dynamics in left ACC.

All 9 participants showed SAM peaks in the insula, although not consistently across all SAM comparisons (see Table 3.3). Coordinates from the Insula also showed some changes in spectral patterns (see Figures 3.21 and 3.22). 22% of participants showed a slight increase in gamma oscillations during pain (see Figure 3.22) but the rest showed no obvious changes in the gamma band (see Figure 3.21). There was an increase in power between 5-10Hz at the beginning of the train which can be linked to components of the first evoked response.

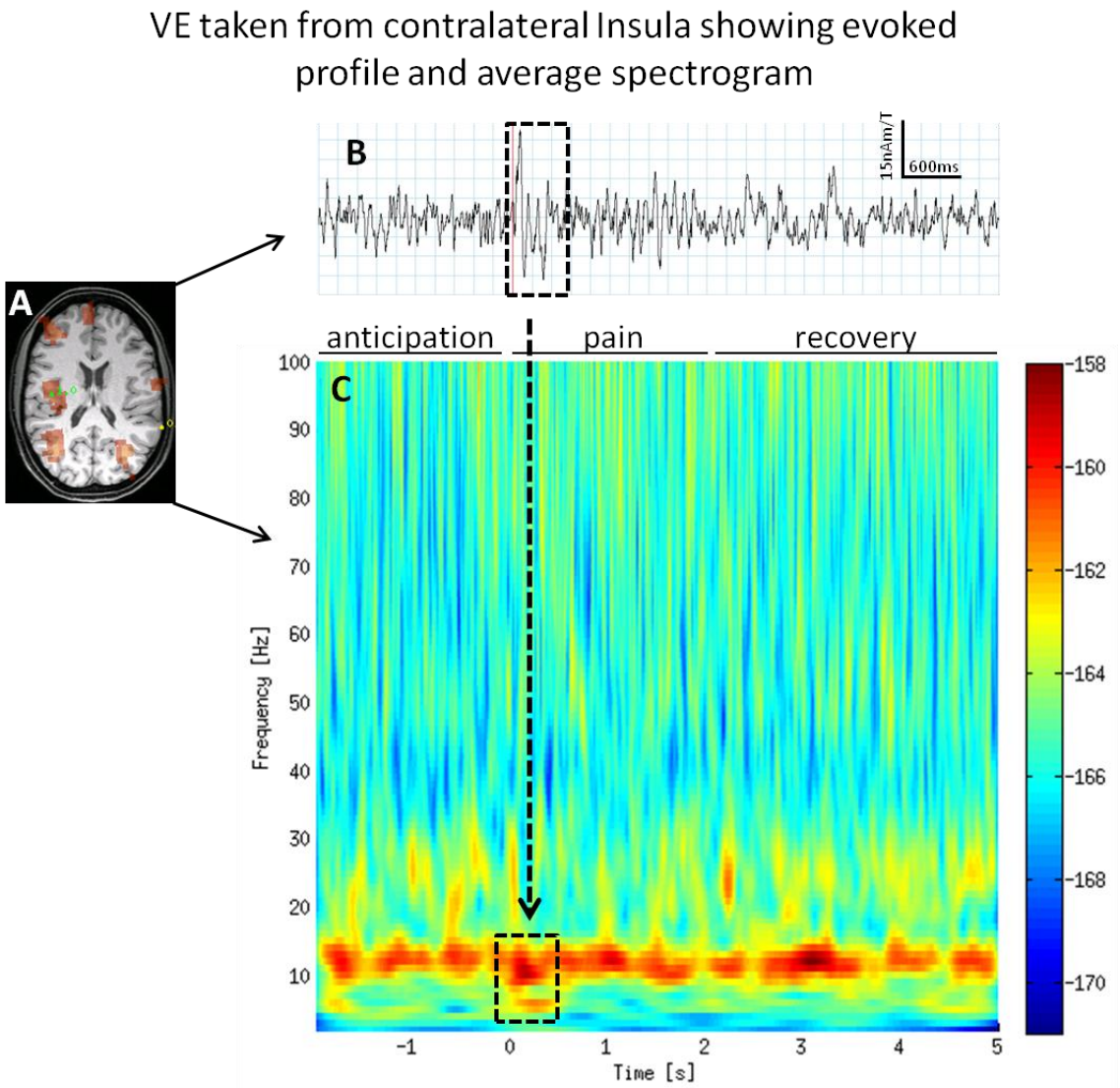


Figure 3:21 shows a virtual electrode (A) from the left insula of one participant (P2). B shows the evoked field profile and C shows a time-frequency spectrogram from this location. There is a clear evoked response to stimulus onset as seen in the highlighted box in B which coincides with an increase between 5-10Hz in the averaged spectrogram, however there is very little change in the frequency dynamics seen in the insula in this participant.

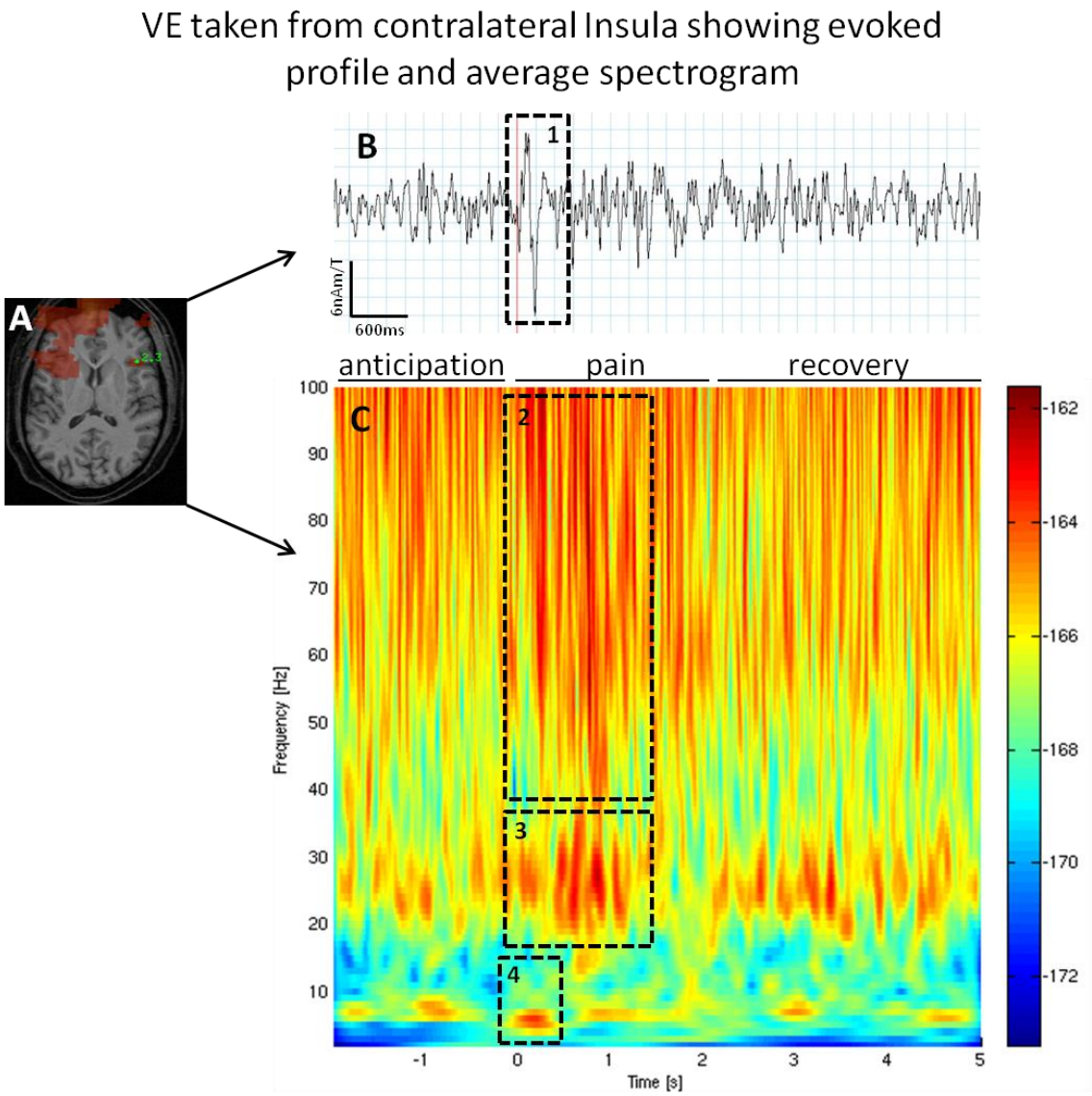


Figure 3:22 shows a virtual electrode (A) from the right insula of one participant (P3). It shows the evoked field profile (B) and a time-frequency spectrogram (C) from this location. A slight increase in gamma (Box 2) and beta (Box 3) bands can be seen after the stimulus onset at 0s as well as a strong evoked response to the first stimulus in the train (Box 1) coincident with an increase in power at stimulus onset at around 5Hz (Box 4).

3.6.7 Results for Study 1 part B

The protocol to Part B changed the stimulus to digital rather than median nerve stimulation and 7Hz train of pulses replaced 10Hz trains. VEs were found from SAM peaks in each individual in SI and these were used to create spectrograms. In the average spectrograms from SI of all 3 participants, there was no 10 and 20Hz

component seen during painful stimulation. A decrease in beta frequency was seen during the stimulus which rebounded afterwards (see Figure 3.23: Box 2). It was possible to see gamma oscillations in the SI of one of the participants (see Figure 3.23: Box 1).

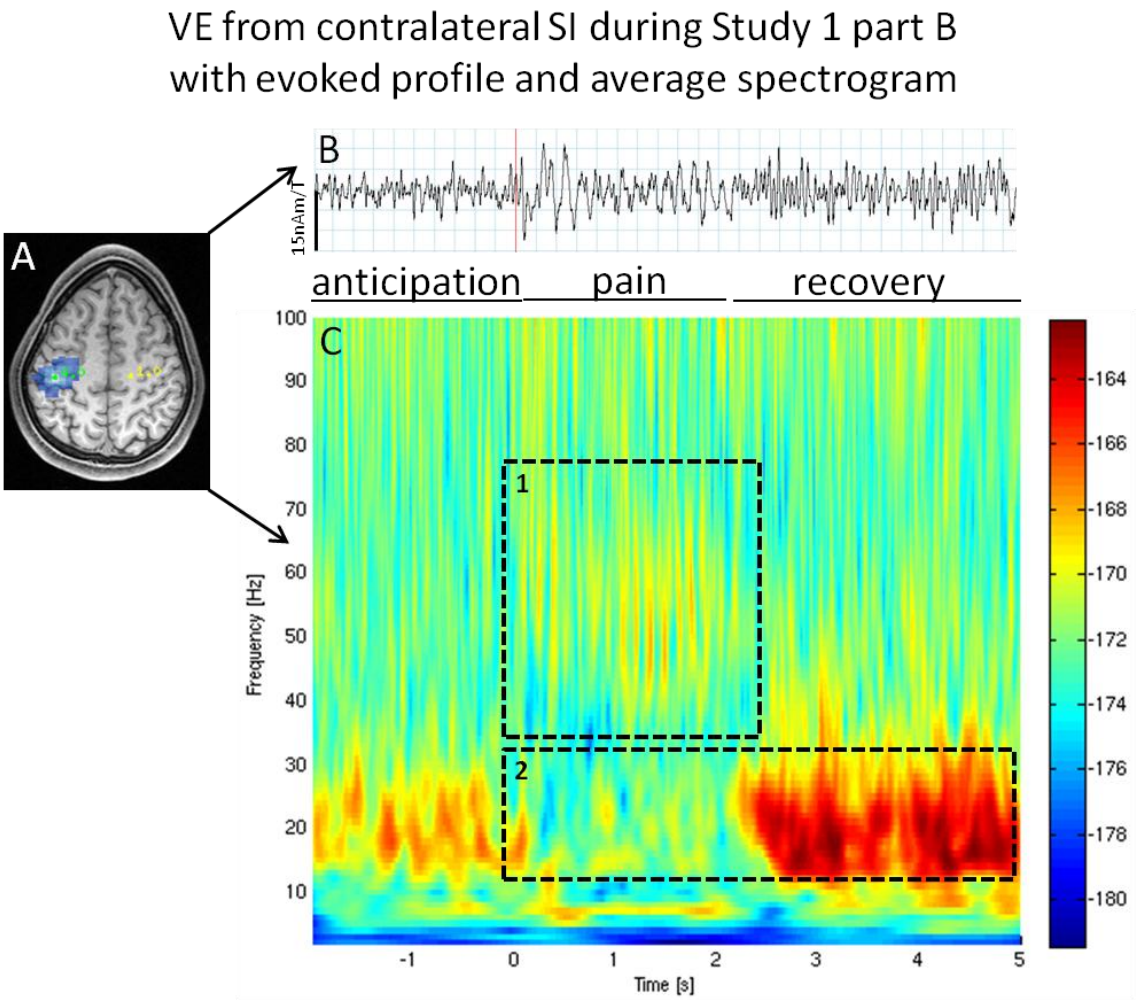


Figure 3:23 shows an average spectrogram from a VE in the contralateral SI of a participant (S2). It is possible to see an increase in the gamma band (Box 1) and a decrease in the beta band during the stimulus period followed by a rebound during the recovery period (Box 2).

3.7 Discussion:

3.7.1 Summary of key findings

Peaks of cortical activity were found in key areas of the pain matrix (SI, SII, ACC, Insula), during both anticipation and pain, in the majority of participants (see Table 3.3). This demonstrates that the pain matrix was active even without a painful stimulus during anticipation of pain. Sensory-discriminative areas such as SI and SII were activated by expectation of the upcoming pain as well as the more affective areas such as ACC and insula (Melzack and Casey, 1968).

Time-frequency spectrograms demonstrated interesting oscillatory changes in SI. The alpha band was seen to decrease during both anticipation of pain, pain and sensory stimuli compared to baseline (see Figures 3.3 and 3.6). An increase was seen at 10Hz and 20Hz (Figure 3.12) but over the whole width of the beta band (15-25Hz), a decrease could be seen compared to baseline levels (Figure 3.3), this was then seen to rebound to a level higher than baseline during the recovery period (Figures 3.12 and 3.14).

One of the key findings in this study was the change seen in the gamma band during both anticipation and pain. A significant decrease in gamma power was seen during anticipation in the ipsilateral SI followed by a significant increase in gamma power during pain in the contralateral SI at the group level using SnPM analysis.

The profile of the gamma increase during pain was of interest as the bandwidth appeared to decrease along the train perhaps due to habituation or a cellular mechanism also seen in *in vitro* preparations, this will be discussed further in Section 3.7.2.2.1 (Traub et al., 1999).

The results of group SnPM analysis also demonstrated significant activity in other areas of the cortex, such as ACC and occipital cortex. SAM peaks were found in SI,

SII, ACC and Insula in the majority of participants (see Table 3.3) and many showed clear evoked responses in all these areas (see Figure 3.11).

This study shows that there are interesting changes in the oscillatory dynamics during both anticipation and pain in SI, especially in the gamma frequency range which may have an important role in pain and sensory processing. Each of these findings will be discussed in further detail below.

3.7.2 Activity in SI

3.7.2.1 Oscillatory dynamics during anticipation

3.7.2.1.1 Gamma (30-80Hz)

A decrease in the gamma band was seen during anticipation of the painful stimulus in the ipsilateral SI, this was found to be statistically significant at the group level using SnPM (see Figure 3.5). To date, this is the first time that a decrease in gamma oscillations has been reported during anticipation of a painful stimulus.

Changes in gamma power during pain have previously been linked to attentional factors by Hauck et al (2007a) who found that during attention to pain, the gamma response was stronger than during distraction (see Chapter 1: Figure 1.6). The decrease in gamma oscillations seen during anticipation in this study could potentially agree with this suggestion that gamma oscillations are involved in attention and arousal during anticipation of a forthcoming stimulus. The fact that it is a decrease in power may indicate some top-down inhibitory feedback mechanism that is attempting to control the oncoming pain.

When looking at each individual's spectrogram, the decrease in gamma oscillations in anticipation compared to baseline was not evident (see Figure 3.16). This is likely to be due to the fact that the decrease in each individual was small but it was consistent across the group. 33% of participants had a SAM peak (≥1) showing a decrease in gamma power in ipsilateral SI during anticipation of pain.

3.7.2.1.2 Alpha (7-14Hz)

During this study, a decrease in alpha band in anticipation of pain was seen over SI cortex in individuals although this did not reach statistical significance at the group level (see Figure 3.3). A decrease in alpha band during anticipation of experimental pain has been reported previously, which is thought to be due to an increase in arousal and attention during expectancy of pain (Babiloni et al., 2006).

Alpha has also been found to increase in areas of sensory cortex other than the type that is being administered, for example if an auditory stimulus is expected then an increase in alpha is seen in visual cortex which is thought to suppress information from distracters and allow focus on the stimulus being administered (Ward, 2003).

3.7.2.1.3 Beta (15-25Hz)

A trend for beta power (15-25Hz) to decrease could be seen in the group data in SI (Figure 3.3) during anticipation of both sensory and painful stimulation, however this did not reach significance at the group level. A decrease in beta band has been seen during pain and attention to pain in previous studies (Ohara et al., 2006, Raij et al., 2004), however these did not look at the anticipatory response. A decrease in beta has also been seen during preparation for a movement (Jurkiewicz et al., 2006) and is thought to be required to initiate a movement. The decrease seen during anticipation in this study may demonstrate a preparation for the sensory or painful stimulus and perhaps facilitating a movement away from the stimulus. Although a jitter was included so that the exact timing of the pain was unpredictable to the participant in order to diminish a preparatory response.

3.7.2.2 Oscillatory dynamics in SI during electrical stimulation

3.7.2.2.1 Gamma (30-80Hz)

Gamma oscillations showed an increase in power in the contralateral SI during painful electrical stimulation in the average spectrograms of 66% of participants (see Figures

3.12 and 3.14). This finding is consistent with other studies which see an increase in gamma oscillations in response to painful intracutaneous electrical (Hauck et al., 2007a) and laser (Gross et al., 2007) stimulation. This increase in gamma oscillations was found to be significant at the group level using SnPM analysis (see Figure 3.5).

The gamma oscillations during sensory stimulation showed a trend to increase (see Figure 3.4) however this did not reach significance at the group level. An increase in gamma oscillations was still seen in 44% of participants during sensory stimulation (see Figure 3.14). This could have a number of explanations. It is possible that the participant's pain threshold altered between blocks meaning that what was once sensory may have become painful. However, in order to counteract this, an example of the train was given before each recording to ensure the stimulus level was appropriate and if not it was altered accordingly. Another explanation is that all participants who showed gamma oscillations during the sensory stimulus had received the pain block first and therefore may have sensitized to the stimulus (Staud et al., 2007).

The final explanation could be that gamma oscillations relate to stimulus intensity rather than pain and is activated during sensation regardless of whether the stimulus is noxious. Figure 3.15 supports this theory as a gamma increase can clearly be seen during the sensory block, however the increase is not as strong as during pain and it is at a lower bandwidth (60-80Hz during pain, 45-60Hz during sensation). Activation seen in SI has been found to correlate with stimulus intensity previously (Bornhovd et al., 2002, Timmermann et al., 2001) although these studies did not mention oscillatory dynamics. It is possible that SI's involvement in encoding stimulus intensity is managed through gamma oscillations.

It has been suggested that gamma oscillations may be involved in binding information from different areas of cortex together (Treisman, 1996, Engel and Singer, 2001) and may be able to encode information about sensory stimuli within its oscillations (Fries et al., 2007). It is possible that the difference in frequency or strength of gamma

oscillations between the pain and sensory stimuli reflect some encoding of the stimulus intensity.

As an alternative explanation for the gamma seen in this study, it could be that it was related to attention rather than pain specifically. Pain is of very high behavioural importance and demands a high level of attention in order for one to react appropriately to the cause of the pain. This could explain why changes in gamma oscillations were seen during the anticipatory period. Gamma oscillations during pain has been linked to attention previously by Hauck et al (2007a), in which a late (400-600ms), high frequency (120-140Hz) gamma oscillations gave a stronger increase when attention was focused on the painful stimulus as opposed to during distraction (see Chapter 1: Figure 1.6). Spectrograms from this study up to 150Hz did not reveal these later, higher gamma oscillations (see Figure 3.17). The difference in gamma oscillations seen in this study and in Hauck et al (2007) may be explained by the fact that during the study by Hauck et al, participants were required to actively attend to the stimuli whereas they were passively attended in this study.

With the small sample size of 9 participants, no firm conclusion can be made about whether the gamma band has some relevance specifically to pain processing or a more general response to stimulus intensity. More research needs to be done investigating the profile of this gamma response during different intensities of stimulus and different types of pain in order to elucidate its role in somatosensory processing.

In those participants that showed an increase in gamma oscillations to pain, it was possible to see a gamma increase in response to each stimulus in the electrical train (see Figure 3.17). The most interesting phenomenon was that the gamma response to each pulse changed across the train in that the frequency bandwidth decreased. Figure 3.17 demonstrates that during the pain block, the gamma increase started between 65-100Hz and at the end of the train it was between 45-75Hz. This pattern was seen during median nerve stimulation in another study by Fukuda et al (2008) using intracortical electrodes. High frequency gamma oscillations (100-250Hz) were

seen in SI from ~13ms after median nerve stimulation which then slowed to around 100Hz and spread over a larger area of cortex. This was also seen in an experimental pain study using CPT, with the oscillation beginning at 80Hz, 26ms after the stimulus and reducing to 10Hz after 160ms (Chen and Herrmann, 2001). This gamma to beta shift has also been seen in response to novel auditory stimuli in human EEG (Haenschel et al., 2000).

A shift from gamma down towards beta frequency is a common phenomenon seen with *in vitro* preparations of rat hippocampal slices in response to tetanic stimulation (Whittington et al., 1997, Traub et al., 1999, Bracci et al., 1999). The interneurons in the cortex fire at gamma frequency and, as a result of their inhibitory effect on the pyramidal cells, entrain the population to oscillate at gamma frequency; this is the signal recorded in MEG (Murakami and Okada, 2006). If stimulated tetanically *in vitro*, there is an increase in the excitatory influence of the pyramidal cells due to an increase in the amplitude of excitatory post-synaptic potentials (EPSPs) and afterhyperpolarizations (AHPs). Pyramidal cells naturally fire at a lower frequency (low-beta) than interneurons and as a consequence of their increased influence, either directly or indirectly, the field oscillation is slowed to beta frequency (see Figure 3.18).

33% of participants showed no apparent gamma oscillations during pain. The reason for the absence of gamma oscillations in these individuals is still uncertain. However, the pain thresholds of these individuals were the lowest in the group and those that had the highest pain thresholds showed strong, clear gamma responses. A t-test was performed in order to determine whether the pain thresholds were significantly different between those that were gamma responders and non gamma responders but it was not found to be significant ($p=0.45$). This may be due to the limitation of having a small sample size as there may not be enough statistical power with so few participants.

3.7.2.2.2 Alpha (7-14Hz)

During both pain and sensory stimuli, a decrease in alpha was seen over SI in the group image, although it was found to be stronger and more widespread during pain (see Figure 3.3). Alpha is known to relate to levels of arousal and is shown to decrease during pain as the individual is more aroused in a painful situation (Babiloni et al., 2006). Gamma oscillations have been found to increase during attention (Hauck et al., 2007a) and alpha to decrease (Babiloni et al., 2006), it has been hypothesised that a decrease in alpha power may facilitate the increase in gamma oscillations leading to binding of stimulus features (Ward, 2003). Both a decrease in alpha and an increase in gamma were seen in this study but it cannot be stated whether one had any influence on the other. Due to the small sample size, it would be unwise to rely on statistics from a correlation between alpha and gamma in this study.

3.7.2.2.3 Beta (15-25Hz) and Mu (10 and 20Hz)

During baseline/anticipation periods, a strong power can be seen between 20-30Hz in SI in most participants (see Figures 3.12, 3.14), this seems to disappear during the stimulation period although it is obscured by the strong increase seen at 10Hz and 20Hz. An oscillatory rhythm made up of 10 and 20Hz components is commonly referred to as a mu rhythm (Hari and Salmelin, 1997). A decrease in both beta and mu rhythms has been seen during tactile stimulation previously (Cheyne et al., 2003, Gaetz and Cheyne, 2006). The increase in 10 and 20Hz in this experiment has not been previously reported. Study 1 Part B used a 7Hz stimulus in order to establish whether the 10Hz and 20Hz rhythms were due to the cortex being driven at this frequency by the 10Hz electrical stimuli. No increase was seen at 10Hz and 20Hz during the 7Hz electrical train. Therefore it can be assumed that these rhythms were an artefact of the stimulus rather than a physiological phenomenon (see Section 3.7.4.3). During the recovery period, in the majority of participants, a large increase in the beta band (20-30Hz) can be seen. This is known as ‘beta rebound’ and is a

common phenomenon during movement and also sensory stimulation (Pfurtscheller and Lopes da Silva, 1999, Cheyne et al., 2008, Cheyne et al., 2003).

3.7.2.2.4 Evoked response profile

Figure 3.8 shows the first evoked response to both painful and non-painful sensations, the first component of the evoked response could be seen at around 20ms corresponding to the 20ms component that is well documented in the literature, this was then followed by the next component at ~70ms, these latencies are consistent with 20ms and 70ms components seen in the literature (Kakigi et al., 2000, Della Penna et al., 2004). Across the train of painful electrical pulses, the amplitude of the 70ms component of the evoked response decreased dramatically from the first pulse to the second and then began to plateau out (see Figure 3.9, 3.10) whereas the amplitude of the 20ms component appeared to remain relatively stable (see Figure 3.9). This may indicate a habituation to the stimulus, either at a peripheral level in the receptors or at a central level. A similar phenomenon has been seen previously (Huttunen, 2010), in that a component of the evoked response at a latency of 35ms was seen to decrease in amplitude with repeated median nerve electrical stimulation. This was linked to the augmenting response seen in *in vitro* preparations which indicates that this decrease in amplitude is due to a decrease in the inhibitory post synaptic potentials (IPSPs).

3.7.3 Activity in other areas of the cortex

Other areas of the cortex, in addition to SI, commonly found to be activated during pain are SII, ACC and Insula (Peyron et al., 2000). In this study, SAM peaks were found in these areas although not in all participants (see Table 3.3). This fits with fMRI literature which finds reproducible activity in these areas (Dunckley et al., 2005, Wise et al., 2007, Straube et al., 2008). Peaks were found in SI in 100% of participants and all showed a clear evoked response to pain, 67% of participants had peaks in SII and out of these only 50% showed clear evoked responses. 89% of participants showed peaks in ACC and 75% of these showed clear evoked

responses, whereas peaks in the insula were found in 78% of participants of which 71% showed clear evoked responses.

Generally, the oscillatory dynamics in SII, ACC and insula appeared not to vary dramatically between anticipation or stimulus and baseline, however there were still some key changes. There was no significant activation in SII found at the group level but SAM peaks in individuals were found in SII cortex (pseudo t ≥1). Peaks were found in 33% of participants during anticipation of sensation and 44% of participants during anticipation of pain. During pain, 56% of participants showed peaks in SII and during the sensory stimulus, 66% of participants showed peaks in SII. During the pain run, 33% of participants showed bilateral SII activity, 33% of participants showed only ipsilateral SII activity and 33% of participants had no clear peaks in SII. In the literature, the SII is commonly activated bilaterally (Coghill et al., 1999, Timmermann et al., 2001), in this study it was seen in only a third of participants. It is possible that this is due to the way SAM analysis is performed. It treats highly coherent bilateral sources as originating from the same point and finds a peak in the dominant location, it is possible that bilateral activity did occur in SII but that SAM only showed it as coming from one hemisphere.

In the SII of two participants, an increase in gamma oscillations could be seen during painful stimulation similar to SI (see Figure 3.19). SI and SII are both known to be involved in the sensory-discriminative processing of somatosensory stimuli (Timmermann et al., 2001) so perhaps gamma oscillations are encoding some information about the stimulus in these two areas. It has been hypothesised that the gamma frequency is involved in binding information from different areas of the cortex into a coherent percept (Engel et al., 2001). The gamma oscillations found in both SI and SII could support this hypothesis and infer a functional connection between these two areas with the neurons in both areas firing in synchrony at gamma frequency. A decrease in beta can also be seen in SII in Figure 3.19 but this was only present in 1 participant. The majority of other participants showed a strong 20-30Hz component

throughout the trial but it did not vary during the stimulus. In one participant an increase at 10 and 20Hz could be seen similar to that seen in SI.

SnPM found a variety of areas of the cortex to be significantly activated at the group level. During the anticipation of the sensory stimulus there was a significant decrease in theta activity in frontal cortex and a significant decrease in alpha in the contralateral SI. Changes in frontal theta have been seen in both experimental pain studies (Chang et al., 2002, Chang et al., 2005) and clinical pain studies (Sarnthein and Jeanmonod, 2008) and it is hypothesised that theta could be involved in the pathology of chronic pain syndromes (Drewes et al., 2008). It is not clear why a significant decrease was seen in theta during anticipation of sensation but not anticipation of pain. A decrease in alpha is thought to be associated with an increase in arousal (Teplan, 2002), in this situation, as the participant was anticipating a stimulus, a decrease in alpha was seen during anticipation of the painful stimulus in some individuals but it did not reach significance at the group level and was less focal than during anticipation of sensation (see Figure 3.3).

There was a significant decrease in gamma oscillations seen in the ACC during anticipation of the sensory stimulus but not anticipation of pain. Activity in the ACC has been seen in anticipation of pain previously in both fMRI and PET studies (Hsieh et al., 1999, Sawamoto et al., 2000), and in anticipation of both sensory and painful stimuli (Yaguez et al., 2005). Peyron et al (1999) suggested that rather than being involved in intensity encoding of stimuli, the ACC forms part of the attentional network. There have not been many MEG/EEG studies investigating anticipation of pain, and those that have mostly used sensor space analysis as opposed to source space analysis (Babiloni et al., 2006, Warbrick et al., 2006).

Gamma power was seen to decrease in both the ipsilateral SI and ACC during anticipation of the stimulus. This could suggest a functional link between the two areas during anticipation, in order to bind different aspects of the experience together as suggested by Engel et al (2001). It is not clear why this change in gamma power

was apparent during anticipation of the sensory stimulus but not the pain stimulus. It may be that changes in gamma oscillations in the ACC during anticipation of pain were more widespread and that there was not enough overlap between participants for significance to occur at the group level. A clear evoked response could be seen in 78% of participants in the ACC, there were few induced changes apparent in the spectrograms (see Figure 3.20) except for in 22% of participants, a continuous gamma activity was seen throughout the trial from 50-70Hz, this gamma response did not appear to vary between the baseline and stimulation period. It is not clear what this gamma response relates to although it is possible that it reflects a constant state of anxiety and attentional arousal throughout the trials (Frankenstein et al., 2001).

There was a significant decrease in gamma oscillations in the posterior parietal cortex during the sensory stimulus. The posterior parietal cortex has been activated in many previous studies in response to pain (Peyron et al., 1999, Forss et al., 2005, Nakata et al., 2008), however in this study it was significantly activated at group level during sensation but not during pain. This may be due to the activity during pain being more widespread and variable across individuals and therefore not reaching significance level in group analysis.

3.7.4 Methodological issues:

3.7.4.1 Participants

Participants were experienced MEG study volunteers, this may have biased the results as they are likely to have had a lower level of anxiety compared to naïve participants and may have experienced electrical stimulation before. This may mean that the participants were not a truly representative sample of the general population. The fact that participants were experienced in the MEG meant that there was less movement and less likely to be any artefacts in the data as they were very compliant. There were 9 participants in this study; this is an acceptable number for a neuroimaging study and 66% showed an increase in gamma oscillations. In order to

apply these changes to the general population, this increase in gamma oscillations will need to be reproduced in more participants.

3.7.4.2 Psychophysics

Another issue of contention is the psychophysics of thresholding. The instructions given by the researcher can alter the participant's interpretation of what each threshold should be, and the instruction can be interpreted differently by individuals. It is a common issue in experimental pain studies and there are various different options used to try and keep the participants understanding of pain thresholds as consistent as possible. The researcher used descriptive words to explain what the sensory threshold, pain threshold and pain tolerance levels should be. For future studies in this thesis, the language used by the researcher will be kept consistent between participants in order to limit the variability. Also a Likert scale will be used to rate the stimulus intensity after each block (Cruccu et al., 2004), to ensure the stimulus was at the correct intensity. The Likert scale is a scale from 0-10 with verbal explanations of the sensation at each number, the participants can then explain their sensation in terms of numbers.

Another issue during thresholding was that in this study a single electrical pulse was used to calculate the thresholds rather than the train that the participant would experience during the experiment. An example of the train was given to the participant before each block to ensure it was at an appropriate level, however the next studies will use the actual experimental stimulus for thresholding purposes to obtain a more accurate threshold.

3.7.4.3 Stimulus

Electrical pulses were used to deliver the pain and sensory stimuli. Electrical stimulation has the disadvantage of being less biologically relevant (Babiloni et al., 2007) than other forms of pain such as CPT or mechanical stimuli as it is not experienced regularly in daily life. Also it is not able to selectively stimulate

nociceptors as laser is able to and therefore activates a combination of sensory and nociceptive-specific fibres. It is likely that electrical stimulation activates Aβ (sensory) and Aδ (nociceptive) fibres. C fibres are also nociceptive specific but are more commonly associated with longer lasting, dull, aching types of pain (Forss et al., 2005). The results of the McGill questionnaires (see Figure 3.2) demonstrate that the sensation felt by the participants was much closer to first pain, mediated by Aδ fibres. It cannot be specified whether the changes in oscillatory dynamics were due to sensory or nociceptive fibres, however during pain experienced in everyday life, both sensory and nociceptive fibres are activated together. It is also a possibility that the contact between the electrodes and the skin changed across the experiment altering the conductance and therefore the strength of the stimulus, although the strength of the stimulus was checked before proceeding with each block.

During the stimulation period, an increase in power around 10Hz and 20Hz could be seen in a number of participants in SI (89% of participants during pain, 56% of participants during sensation). This could be a genuine physiological increase in these frequency bands referred to as the mu rhythm (Hari and Salmelin, 1997), however the electrical pulses were administered at 10Hz and this could in turn be driving the cortex to oscillate at this frequency and at a harmonic of 20Hz. In order to uncover why there was an increase at 10 and 20Hz, Study1b was performed using a lower frequency stimulus (7Hz). During 7Hz trains of electrical stimulation, no increase in 10 and 20Hz was seen. This indicates that in Study 1, the 10 and 20Hz component was likely to be due to the stimulus being administered at 10Hz and therefore is not part of the physiological response to pain.

Median nerve stimulation has been used in many somatosensory and pain studies previously (Schnitzler et al., 1999, Frot and Mauguiere, 1999, Chen and Herrmann, 2001). It produces strong evoked responses and creates a clear, strong sensation which can be localised to the hand area of the SI cortex (Fukuda et al., 2008). A disadvantage of median nerve stimulation is that it activates both sensory and motor fibres (shown by a thumb twitch during stimulation). The oscillatory dynamics seen

during stimulation in the first study could be due to sensation of the electrical stimulation or as a result of the sensation triggered by the thumb twitch created by the activation of motor fibres.

In order to answer this question, Study 1b used digital stimulation which only contains sensory fibres in order to ascertain whether these oscillatory dynamics can be attributed to sensory processing of the electrical stimulus. An increase in gamma oscillations was seen during 7Hz painful digital electrical stimulation in 33% of participants in Study 1b, as was a decrease in beta in 66% of participants during stimulation, followed by a rebound. This would suggest that the oscillatory dynamics seen in Study 1 were not due to the sensation of the thumb twitch but are part of the processing of the electrical stimulus.

3.8 Conclusion

Gamma oscillations were found in 66% of participants during pain in this study and in 44% of participants during sensation, this would suggest that gamma oscillations are not specific to pain per se but may encode some other aspect of the stimulus such as stimulus intensity. The frequency of the gamma oscillations was found to decrease across the train of stimuli, and this may be due to habituation. A significant decrease in gamma oscillations was seen during anticipation of pain, this may reflect attentional processing of painful stimuli. Gamma oscillations were not present in all participants; this may simply be due to individual differences or may be related to specific aspects of an individual's personality and how they respond to pain. It is still not clear exactly what role gamma oscillations play in somatosensory processing; whether it applies to different types of stimuli and whether it relates to intensity or the painful nature of a stimulus. The next studies in this thesis will attempt to answer some of these questions.

4 Study 2
Investigating the temporal patterns of cortical activity in response to visceral and somatic electrical painful stimulation using Magnetoencephalography

4.1 Abstract:

Different types of pain give different sensory and emotive responses depending on the quality, severity and nature of the pain. Experimental visceral pain has been described as more unpleasant and emotionally distressing than somatic for the same intensity of stimulus (Strigo et al., 2002). It is poorly localised and is often referred to somatic structures (Aziz et al., 2000b). Visceral and somatic sensations have different somatotopic organisation in the somatosensory cortex (Strigo et al., 2003, Strigo et al., 2005) and are thought to involve the emotive areas of the brain in different ways (Derbyshire, 2003).

The aim of this study therefore was to explore the different contributions of areas of the pain neuromatrix involved in both sensory-discriminative and emotional aspects of pain and to understand the complex changes in oscillatory dynamics during pain and how these differ according to whether the stimulus is visceral or somatic.

MEG recordings were made during electrical stimulation of the right index finger and the distal oesophagus using skin electrodes and a naso-oesophageal electrical catheter respectively. Both modalities were administered at a painful and non-painful level, these were carried out in separate blocks. Each electrical pulse lasted 200μs and the stimulus was administered at a rate of 0.2Hz.

In the somatic data, an increase in gamma frequency oscillations was observed during pain in SI in 64% of participants at a frequency band of 65-95Hz. This gamma pattern was not seen in the visceral data. Evoked responses were seen in SI during somatic pain but the gamma response was not coincident with these, it was later at a latency of ~100-250ms. There was a decrease in beta (15-30Hz) in SI after the

stimulus which then rebounded after a few hundred milliseconds, this was seen in both visceral and somatic datasets although was less reproducible in the visceral data. Evoked responses could be seen in all areas of the pain neuromatrix however there was little induced activity seen in SII, ACC and Insula.

This study shows us that the gamma response seen in Study 1 is reproducible with one brief stimulus as well as a train. It also demonstrates that the gamma response seen in this study is not simply a part of the evoked response but may be partly induced and therefore involved in higher order cognitive processing. It is also apparent that the gamma oscillations seen in response to somatic stimuli were not observed in visceral stimulation. These results suggest that somatic and visceral pain may be processed differently in the cortex, specifically SI.

4.2 Introduction:

Different types of pain give different sensory and emotive responses depending on the quality, severity and nature of the pain. Experimental visceral pain has been described as more unpleasant and emotionally distressing than somatic for the same intensity of stimulus (Strigo et al., 2002). It is poorly localised and is often referred to somatic structures (Aziz et al., 2000b). This referral is thought to be due to the fact that visceral afferents and somatic afferents converge on the same spinal neurones (Aziz et al., 2000a).

Visceral pain tends to engage very different physiological responses and behaviours to somatic pain such as hypotension, quiescence and a loss of interest in the environment (Strigo et al., 2003). Somatic pain can often engage the fight or flight response and increase blood pressure in order to prepare the body to withdraw from a painful stimulus (Strigo et al., 2003).

Experimental visceral pain is often found to be more unpleasant and emotionally distressing than somatic pain even at the same stimulus intensity (Strigo et al., 2002). From this, one could hypothesise that it will activate areas of the brain involved in the

more affective side of pain, such as ACC and insula, although both somatic and visceral pain have been seen to activate these regions (Derbyshire, 2003).

Somatic pain is known to be transmitted by two types of peripheral fibres; Aδ and C fibres. Aδ fibres are myelinated and therefore have a high conduction velocity (~5-30m/s) (Forss et al., 2005), these lead to a sharp, immediate, pricking pain called first pain. C fibres are unmyelinated and therefore slower to conduct impulses (~0.5-2m/s) (Forss et al., 2005), these lead to a later duller, aching pain called second pain (Willis and Westlund, 1997, Ploner et al., 2002). These fibres are frequently activated together, although during experimental pain paradigms it is possible to selectively activate Aδ or C fibres depending on the experimental stimulus, surface area and intensity (Raij et al., 2004).

The distal oesophagus is most often used to administer visceral pain in an experimental setting (Hobson et al., 2005). The distal oesophagus is very different physiologically to the proximal oesophagus in terms of muscle and innervations (Aziz et al., 2000b). The proximal oesophagus (top one-third) has striated muscle whereas the distal oesophagus (bottom two-thirds) has smooth muscle. The vagal afferents from the distal portion of the oesophagus are mainly unmyelinated whereas those from the proximal oesophagus are mainly myelinated (Aδ fibres). The proximal oesophagus has more spinal innervations than the distal, for these reasons many refer to the proximal oesophagus as being somatic rather than visceral (Aziz et al., 2000b).

Both the vagus and the spinal nerve innervate the viscera (Fitzgerald et al., 2007). Vagal afferents respond to fairly low-threshold stimuli but saturate before strong pain levels are reached (Sharma et al., 2009). Spinal afferents are thought to be mostly nociceptive and whilst they still respond differently to different intensities, it is at a much higher threshold than vagal fibres (Hobson et al., 2000a).

The evoked response to experimental visceral stimulation has been reported by many using different modalities of pain such as mechanical (Hobson et al., 2000b) and

electrical (Hecht et al., 1999). In the distal oesophagus, a triphasic response is generally reported with P1, N1 and P2 components (Hobson et al., 2000a). These are at latencies of around 88.4±11.5ms for P1, 145.6±18.2ms for N1 and 227.9±24.6ms for P2 (Hobson et al., 2005). The latencies of the visceral evoked response tend to be longer than for somatic stimuli. Latencies for somatic evoked responses in the upper limb are commonly seen as early as 20ms (Kakigi et al., 2000, Della Penna et al., 2004). The somatic evoked response also shows a triphasic shape (Ploner et al., 2000). The amplitude of evoked responses is often found to be linearly related to stimulus intensity in somatosensory cortices (Hobson et al., 2000a).

There are many conflicting results found across neuroimaging studies with regards to the exact involvement of different areas of the cortex in visceral and somatic sensation. Firstly, looking at the primary and secondary somatosensory cortices (SI and SII), some studies suggest that when comparing visceral and cutaneous pain, there is a marked difference in the involvement of these two areas. During somatic pain studies, contralateral SI activation and bilateral SII activation are commonly seen (Timmermann et al., 2001, Ploner et al., 2002). Activation in SI during somatic pain has been seen to increase exponentially with increasing stimulus intensity (Bornhovd et al., 2002, Della Penna et al., 2004) whereas it has been hypothesised that SII responds in an S-shaped function, encoding whether a stimulus is noxious or not (Timmermann et al., 2001).

Aziz et al (2000a) suggest that visceral sensation primarily activates SII whereas the activity in SI is more vague, this is supported by a number of other researchers (Schnitzler et al., 1999, Strigo et al., 2003). Schnitzler claims that the lack of SI representation could be an explanation of the poor localization of visceral pain as it is suggested that SII is not somatotopically organised to the same degree as SI (Schnitzler et al., 1999) although some disagree with this (Strigo et al., 2005). Hobson et al (2005) found parallel activation of SI and SII in response to distal oesophageal electrical stimulation whereas Hecht et al (1999) found SI was activated before SII following oesophageal electrical stimulation. There is still much debate about the

exact involvement of SI in visceral pain but the activation of SII is seen in the majority of studies.

Activation in both the anterior and mid cingulate cortex has been found during both visceral and somatic stimulation and these areas are thought to be important in the affective response to pain. ACC activation has been found to increase linearly with stimulus intensity (Coen et al., 2007, Buchel et al., 2002), although this is not found in all studies (Bornhovd et al., 2002). ACC activity is commonly seen during somatic pain (Buchel et al., 2002, Coghill et al., 1999) and has been found to activate a spatially distinct region from visceral stimuli (Strigo et al., 2003). Vogt (2005) explored the involvement of different subregions of the cingulate cortex in pain and stated that visceral responses were commonly found in pregenual and subgenual ACC.

Derbyshire (2003) reviewed visceral research and found that the ACC was one of the areas activated in the majority of studies. The ACC or perigenual cingulate cortex is thought to be involved in visceromotor control and in regulating the emotional responses to external stimuli such as pain. The mid cingulate is more involved in attention, selecting an appropriate response to stimuli and preparing the motor system for the chosen action (Aziz et al., 2000b).

According to many, the most consistently activated region during somatic and visceral pain is the insula (Brooks and Tracey, 2007, Derbyshire, 2003). The insula is thought to have a very important role in pain processing and is involved in integrating sensory, motor and affective information and making decisions on which behaviours to make as a result (Brooks and Tracey, 2007, Aziz et al., 2000a). The insula is anatomically very close to SII and their activation is sometimes hard to separate. Frot (2007) believes that this is important as SII and insula process somatosensory stimuli differently and the insula is more multi-modal receiving a greater range of sensory input.

There have been many papers written on visceral pain with regards to evoked responses, however there seems to be few visceral papers investigating the

oscillatory dynamics using EEG or MEG. Drewes et al (2008) found that patients with chronic pancreatitis had a much higher level of theta activity in response to oesophageal electrical stimulation at pain threshold compared to healthy controls. Furlong et al (2004) demonstrated a decrease in the beta band over the pre and postcentral gyrus during water infusion, tongue thrusts and the initiation of swallowing. These studies demonstrate that oscillatory dynamics may have relevance in functions of the gut and may also relate to clinical conditions. Understanding the oscillatory dynamics during visceral pain may be of great importance in understanding how visceral stimuli are processed and how they differ from somatic processing.

There have been many changes reported in oscillatory dynamics during somatic pain. Alpha power has been seen to decrease during expectancy of pain (Babiloni et al., 2006). Beta has been seen to decrease immediately after a sensory stimulus and then rebound to a higher level than baseline (Pfurtscheller and Lopes da Silva, 1999, Cheyne et al., 2003). It has also been seen to decrease during painful stimuli (Ohara et al., 2004, Ohara et al., 2006, Raij et al., 2004). Abnormal levels of theta are often seen in chronic pain patients, for example neurogenic pain (Sarnthein and Jeanmonod, 2008) and an increase in gamma oscillations has been found in response to experimental somatic pain, both in the literature (Hauck et al., 2007a, Gross et al., 2007) and in Study 1. It is possible that changes in the frequency dynamics are important in the processing of pain but this is not yet fully understood.

The importance of research into experimentally induced visceral and somatic pain is due to the large incidence of functional gastrointestinal disorders (FGID) and chronic pain syndromes in the population as well as the more acute pain experienced by the entire population on occasion. Functional dyspepsia is thought to affect around 20% of the population and irritable bowel syndrome affects around 10% (Talley, 1998). Neuropathic pain affects around 3% of the population and fibromyalgia affects approximately 1% and is much more common in women than men (Kroenke et al., 2009).

In order to fully understand the cortical processing of visceral and somatic pain and their similarities and differences, more research needs to be done on their precise spatial localization, evoked responses and how the patterns of oscillations change across the two modalities in response to pain. These studies have the potential to give us information that can then be used on patients in order to understand their conditions better and to establish the efficacy of any new treatments available.

4.3 Experimental Rationale

Activity in key areas of the pain neuromatrix has been seen during both visceral and somatic pain although the involvement of some of these areas is still under debate. This study will allow us to directly compare cortical activations and evoked and induced changes to both visceral and somatic pain. The fact that visceral pain is often found to be more unpleasant than somatic pain experimentally may mean that there is a difference in how the affective areas are involved in processing each type and how these compare and contrast with the involvement of sensory-discriminative areas.

In Study 1, an increase in gamma oscillations was seen after electrical stimulation which was much stronger during pain than when the stimulus was non-noxious. Using a different modality of pain such as visceral, will enable us to see if this change in gamma oscillations can be applied to a variety of stimuli, or whether it is specific to somatic electrical stimulation. This study aims to discover whether the increase in gamma power seen during painful somatic stimulation can be reproduced with distal oesophageal stimulation.

4.4 Materials and Methods:

4.4.1 Participants:

12 healthy participants (6 female; age range= 21-36 years) took part in this study. All were free of any neurological or pain disorders and none were taking medication at

the time of the study. Anatomical Magnetic Resonance Images (MRIs) were acquired for each of these individuals and were made available for the analysis. Informed written consent was obtained from all participants and the local ethics committee approved the experimental protocol.

4.4.2 Stimulus

Electrical pulses were delivered via a constant current stimulator (Model: Digitimer Ltd, Welwyn Garden City, DS7A). The duration of each electrical pulse was 200μs with a frequency of 0.2Hz. There were 60 stimulations altogether.

4.4.2.1 Somatic stimulations

For the somatic stimulations, the skin was rubbed with an alcohol wipe in order to ensure good contact, then two disk electrodes were placed on the inside of the right index finger of each subject, towards the tip, approximately 1cm apart.

4.4.2.2 Visceral stimulations

For the visceral stimuli, the participants were intubated with a commercially manufactured naso-oesophageal tube (Gaeltec, Dunvegan, UK) with a pair of platinum bipolar ring electrodes sited 5cm from the tip of the intraluminal catheter. The catheter was constructed from nylon tubing covered with stainless steel braid and sheathed in silicone rubber. The electrodes were connected to a constant-current, high-voltage stimulator (Model DS7, Digitimer, Welwyn Garden City, UK).

Participants were intubated with the tube nasally, a water based lubricant jelly was used to ease the intubation. Using the centimetre markings on the catheter, placement in the oesophagus could be accurately measured based on insertion distance from the nose and knowledge of average oesophageal length in adults (Li et al., 1994). Catheters were placed 35cm *ab nares* in order to stimulate the distal oesophagus. A maximum of 2 further attempts at oesophageal intubation were undertaken if the first one was not successful, with a period of at least 5 minutes

between each. Participants were informed at the beginning of the experiment that they could withdraw consent at any point and oesophageal intubation would be discontinued.

4.4.2.3 Thresholding

The current (ranging from 0mA to 100mA) was started below sensory threshold and gradually increased during thresholding. Thresholds were obtained by administering pulses at 1Hz and increasing the current incrementally at a rate of ~1mA/s. At the point a threshold was reached, the current was increased and decreased three times in order to ensure an accurate threshold. Three measurements were taken; sensory threshold, pain threshold and pain tolerance. Clear instructions were given to the participants of what sensation should be felt at each threshold. The value for the non-painful block was taken as 50% between sensory threshold and pain threshold (although this was checked before recording to ensure it was still felt but not painful). The value for the pain block was taken as 50% between the pain threshold and pain tolerance, again this was checked before recording. Further details on how thresholding was performed can be found in Chapter 2, section 2.2.1.1.

4.4.3 Experimental procedure:

4 datasets were collected for each participant; visceral sensory, visceral pain, somatic sensory, somatic pain. The stimulation blocks comprised 200μs electrical pulses at 0.2 Hz for 60 trials, these lasted 5 minutes. To eliminate the possibility of order effects, the order in which visceral and somatic stimuli were given was randomised as well as whether sensory or pain blocks were administered first. Participants filled in a McGill pain questionnaire after each block of electrical stimulation and also rated the intensity and unpleasantness of the stimulus on a 0-10 VAS where 0= no pain and 10 = worst pain imaginable.

4.4.4 MEG recordings:

Participants were seated in a magnetically shielded room. Neural activity was recorded using a 275-channel CTF MEG system (CTF Systems Inc, Vancouver, Canada) at a sampling rate of 1200Hz (see Chapter 2: Section 2.2.2 for details). 60 trials were recorded, each 5s in duration, with 2.5s pre-stimulus and 2.5 post-stimulus. Pre-processing was completed using 3rd gradient noise reduction and removing the DC offset based on the whole trial (see Chapter 2: Section 2.2.3 for details). The 50Hz power line was taken out with a width of 0.6Hz. The trials were scanned for blink artefacts. One participant had large blink artefacts due to an MRI earlier the same day, this participant was discarded from any further analysis, and therefore data will only be shown from the remaining 11 participants. For the other participants, no artefacts were considered to be consistent across trials and it was not necessary to remove them.

There was a strong stimulus artefact seen during visceral blocks due to the close physical proximity of the catheter to the MEG channels. In order to view the raw data, independent component analysis (ICA) was performed on these datasets. ICA is able to separate out components of the data that have a consistently similar pattern and are repeated a number of times throughout the data, those that originate from sources outside the head can then be removed (see Figure 4.1 for an example) (Hyvarinen et al., 2010) (see Chapter 2: Section 2.2.3.1 for details).

Figure 3:18 shows the shift from gamma to beta frequency oscillations in rat hippocampal slices *in vitro* after tetanic stimulation. This figure is taken from Bracci et al 1999.

Coordinates taken from SII were used to create evoked profiles and averaged spectrograms. During pain, 33% of participants showed bilateral SII activity, 33% of participants showed only ipsilateral SII activity and 33% of participants had no clear peaks in SII. In 50% of the participants who showed SII activity, a clear evoked response was evident (Figure 3.19, Box 1). 22% of participants showed an increase in gamma power in contralateral SII during pain although it was not as strong as that seen in SI. Figure 3.19 shows an example from one of the individuals that showed a gamma increase (Box 2). There was an increase in power at the onset of the train between 5-10Hz (Box 4) which coincided with the evoked response (Box 1). A decrease in beta power can also be seen (Box 3) followed by a rebound in the recovery period.

stimulus artefact during this period. The frequency bands used were 3-7Hz (Theta), 7-14Hz (Alpha), 15-30Hz (Beta), 30-100Hz (Gamma) and 60-100Hz (higher gamma).

4.4.6.2 Time-Frequency Analysis (Spectrograms):

SAM peaks in the individual that were spatially coincident with ROIs from the Group data (SI, SII, ACC, Insula) and had a pseudo t value of ≥1 were used for further analysis. The coordinates in these ROIs formed VEs (see Chapter 2: section 2.2.5 for details) (Barnes and Hillebrand, 2003) which were used to create time-frequency representations or spectrograms (see Chapter 2: Section 2.2.7 for details). These spectrograms covered 1.5s before the stimulus and 1.5s after and the frequency range was 1-100Hz. After looking at these results, bootstrap spectrograms were produced using 500ms before and after stimulus and a frequency range of 60-100Hz. Also average spectrograms were created from 0-80Hz to investigate the patterns in the lower frequency bands.

4.5 Results:

4.5.1 Behavioural Data:

The participants' ratings for intensity and unpleasantness differed significantly for the visceral pain block ($t_{(10)}$=-2.21, p=0.04) with unpleasantness ratings higher than intensity, whereas they did not differ for the somatic pain block ($t_{(10)}$=0.81, p=0.44). The visceral pain was rated as more unpleasant compared to intensity than the somatic pain (see Figure 4.2). The rating of stimulus intensity was higher in the somatic pain block than the visceral pain block, although this did not reach significance ($t_{(10)}$=1.71, p=0.11). There was no significant difference in the McGill descriptive word pain ratings between somatic pain and visceral pain although there are some differences as shown in Figure 4.3. The majority of the words used to describe the stimuli were sensory, few used affective words. The score for sensory words appeared to be generally higher for the somatic pain stimulus than for visceral.

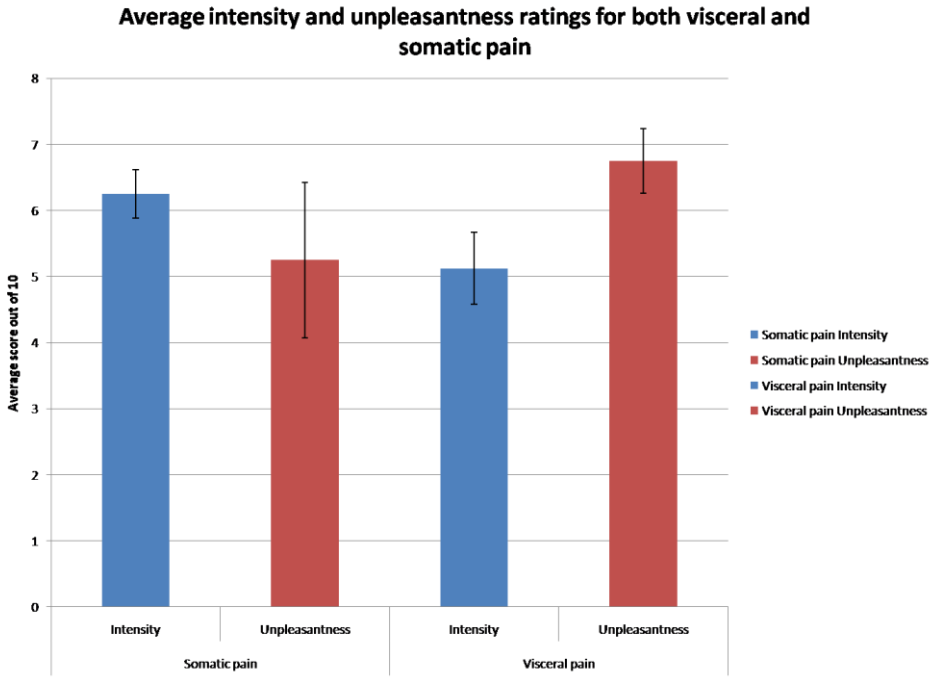


Figure 4:2 shows the average ratings of intensity and unpleasantness for both somatic and visceral pain, the error bars show the standard error for each rating.

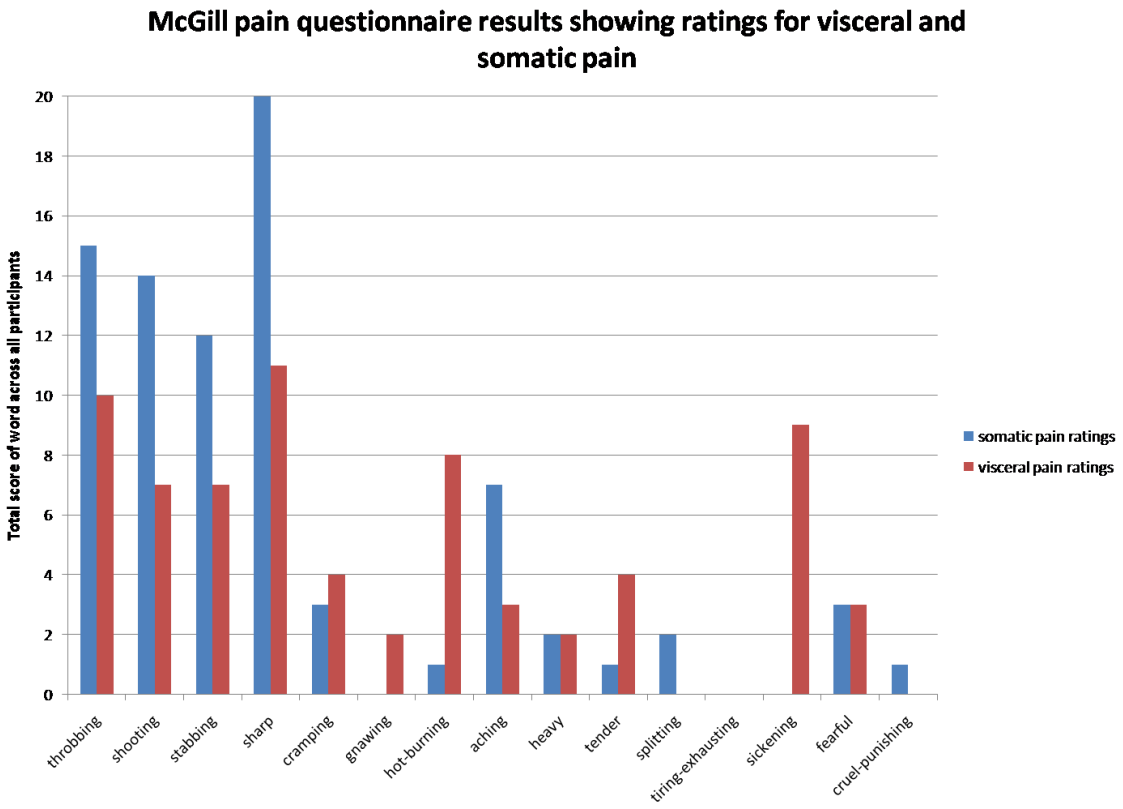


Figure 4:3 shows the results of the McGill pain questionnaire for somatic and visceral pain showing which descriptive words were used to describe both modalities of pain.

4.5.2 Pain thresholds:

Each participant's sensory (ST) and pain (PT) thresholds were determined and stimulation levels calculated at the beginning of the visceral and somatic blocks. Table 4.1 shows the sensory and pain thresholds for both somatic and visceral stimuli in all participants. The multiplier (PT/ST) indicates the range between the two values. The sensory threshold occurs at a much lower intensity in somatic stimulation compared to visceral. The multiplier ranges from 2.3-55 for somatic and 1.6-3.6 for visceral.

Pain and sensory thresholds for somatic and visceral stimuli in all participants						
Participant	**Somatic ST (mA)**	**Somatic PT (mA)**	**Multiplier**	**Visceral ST (mA)**	**Visceral PT (mA)**	**Multiplier**
VS001	1	6	6.0	21	51	2.4
VS003	1.5	7.3	4.9	12	36	3.0
VS004	3.3	17	5.2	30	49	1.6
VS005	1.1	2.5	2.3	11	40	3.6
VS006	1	55	55.0	5.3	19	3.6
VS007	2	6	3.0	6.5	23	3.5
VS008	1.5	15	10.0	24	81	3.4
VS009	2.5	10	4.0	45	90	2.0
VS010	17	60	3.5	17	48	2.8
VS011	1.8	16	8.9	36	82	2.3
VS012	1.8	7.3	4.1	17	61	3.6

Table 4:1 shows the sensory and pain thresholds of each participant for both somatic and visceral stimulation. It also shows the multiplier as an indication of how the range between sensory and pain threshold varied between modalities and participants.

4.5.3 SAM activation:

From SAM comparisons, peaks were found in key areas of the pain matrix although the number and strength of these peaks varied across individuals. Table 4.2 shows which areas showed SAM peaks with a pseudo t ≥1 during somatic and visceral pain in all participants. Activity was found consistently in SI during somatic and visceral pain whereas activity in the other areas (SII, ACC, insula) was less consistent.

SAM peaks of all participants in key areas of the pain neuromatrix during visceral and somatic sensation and pain

	SI				SII				ACC				Insula			
	Somatic		Visceral		Somatic		Visceral		Somatic		Visceral		Somatic		Visceral	
	S	P	S	P	S	P	S	P	S	P	S	P	S	P	S	P
VS001	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	Y	N	N	Y
VS003	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	Y	N	N	Y	Y
VS004	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	N	N	Y	Y	Y
VS005	Y	Y	Y	Y	N	N	Y	Y	N	N	Y	Y	Y	Y	Y	N
VS006	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	Y	Y	N
VS007	Y	Y	Y	Y	N	Y	Y	Y	N	Y	N	N	Y	N	N	N
VS008	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	Y	Y	N	Y
VS009	Y	Y	Y	Y	Y	Y	Y	N	N	Y	Y	Y	Y	Y	N	N
VS010	Y	Y	Y	Y	N	Y	Y	N	Y	N	N	N	N	Y	Y	Y
VS011	Y	Y	Y	Y	Y	Y	N	Y	Y	N	N	N	Y	Y	Y	N
VS012	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	Y	N	Y	Y
Total	11	11	11	11	8	10	8	8	3	6	3	5	7	7	7	6

Table 4:2 shows which areas of the cortex demonstrated SAM peaks greater than a pseudo t of 1 in each participant. The last row shows the total number of participants that showed activity in that area during that stimulus, this is out of a total of 11 participants.

Group SAM was performed on this data, the results of which can be seen in Figure 4.4. A focal decrease was seen in the beta band over the somatosensory cortex during somatic sensation and pain. There was a strong increase in gamma band during somatic pain and to a lesser degree somatic sensation which was not apparent during visceral stimuli. More widespread changes were seen in theta and alpha frequency bands.

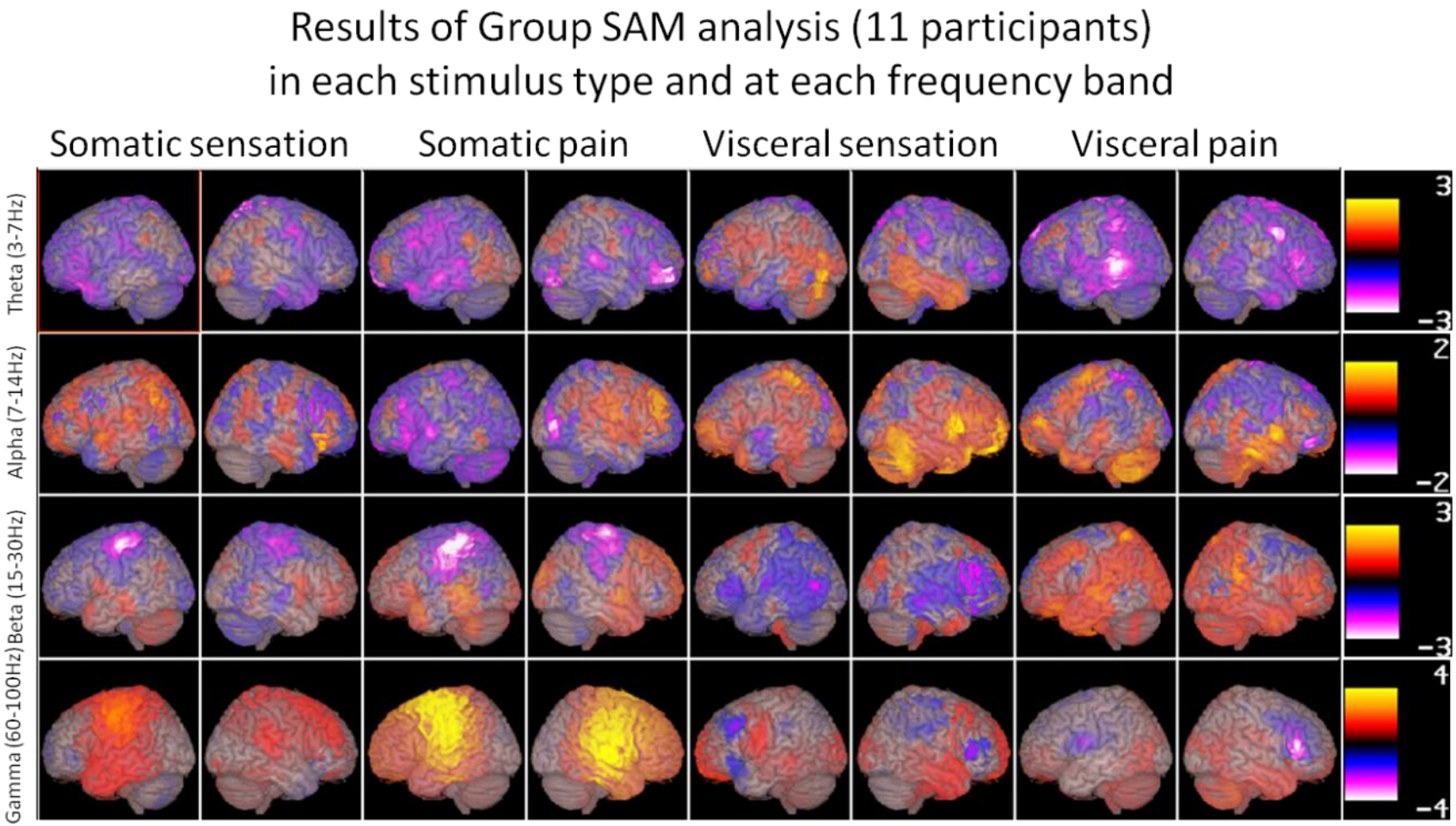


Figure 4:4 shows Group SAM data of all 11 participants. Each row is a frequency bands and each pair of columns is a type of stimulus, both sensory and painful and somatic and visceral. The activity from the interior of the brain was brought closer to the surface in order to make it clearer in this figure using a surface render function. Increases in power are shown by red/yellow colours and a decrease in power is shown by purple/white colours. A decrease in power can be seen in beta during somatic stimulation (both sensory and pain) over the somatosensory cortices. Also a strong, more widespread increase can be seen in the gamma band during somatic stimulation (especially pain).

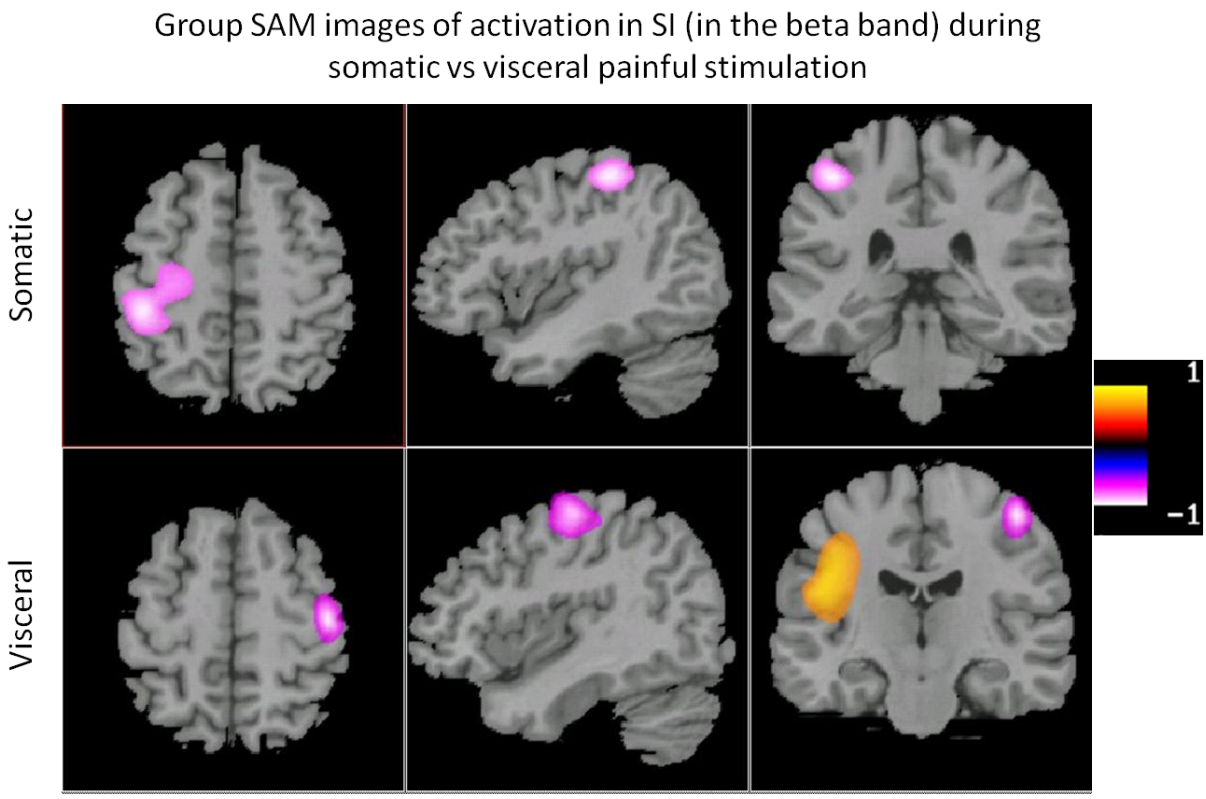


Figure 4:5 shows the spatial localization of the decrease in power in SI in the beta band during somatic pain vs visceral pain.

The spatial localization of activity in SI during somatic and visceral pain can be seen in Figure 4.5. The beta band was chosen as peaks in SI were seen consistently in all participants in this frequency band. Somatic pain activated the left SI contralateral to the stimulus in an area corresponding to the hand region. During visceral pain the right SI was activated at the group level in a region lateral to the hand area.

SnPM was performed on the pain datasets in the study for both visceral and somatic stimuli. A statistically significant decrease was seen in beta power (15-30Hz) during somatic pain in left and right SI/precentral gyrus (Figure 4.6). A significant decrease in beta was also seen during visceral pain in the right SI (Figure 4.7). There was a statistically significant increase in gamma oscillations during somatic pain over left primary and secondary somatosensory cortices which was not found during somatic sensation or visceral stimuli (Figure 4.8). There were no statistically significant changes found in theta or alpha bands from SnPM in somatic or visceral stimuli.

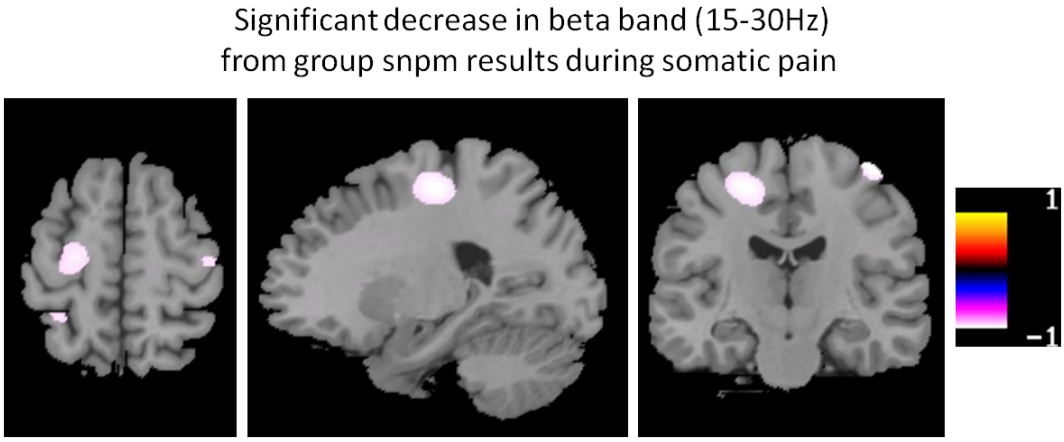


Figure 4:6 shows the activity over the somatosensory cortex and precentral gyrus that was shown to be significant across the group in the beta band (15-30Hz) during somatic pain.

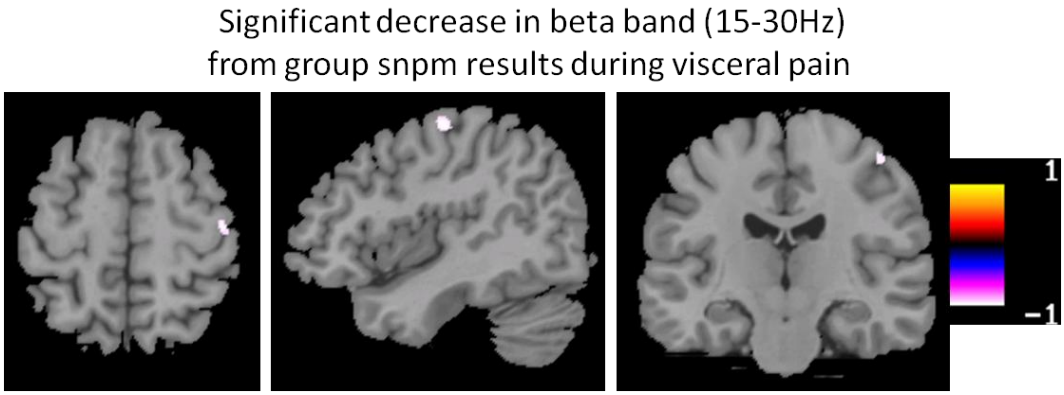


Figure 4:7 shows the activity over the right somatosensory cortex in the beta band (15-30Hz) during visceral pain.

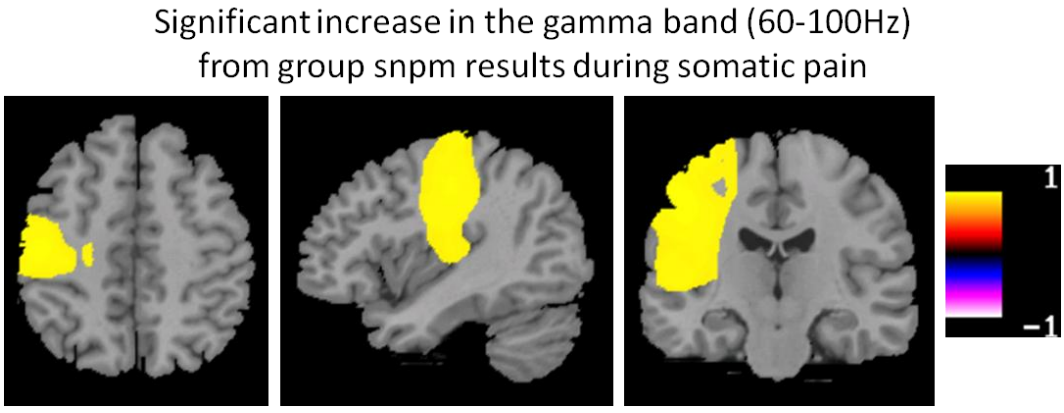


Figure 4:8 shows the statistically significant increase over SI (and SII) in the gamma range (60-100Hz) during somatic pain.

4.5.4 Evoked fields:

Averaged datasets were created around the electrical stimulus. Weights files were created of the VEs found from SAM peaks (pseudo t ≥ 1) in key ROIs in the pain matrix (SI, SII, ACC, Insula). These were loaded into an averaged dataset to see the evoked response from that location more clearly as illustrated in Figures 4.9 and 4.10. A strong evoked response can be seen in SI in both somatic and visceral stimulation, this is discussed in more detail in section 4.5.5.2. Other areas of the neuromatrix also showed clear evoked responses although these had a smaller amplitude (Figures 4.9 and 4.10). Average latencies of the peak of evoked responses in key areas of the pain matrix are shown in Table 4.3.

Average latencies of largest peak-to-peak amplitudes of evoked responses in key areas of the pain matrix during both somatic and visceral pain				
	Somatic Pain (ms)	Standard deviation	Visceral Pain (ms)	Standard deviation
SI	25	6	79	27
SII	76	24	73	30
ACC	146	46	142	50
Insula	119	33	130	39

Table 4:3 shows the average latencies of the largest first peak of the evoked response comparing visceral and somatic pain in key areas of the pain matrix. There is a considerable difference between the latencies of somatic and visceral pain in SI, however in the other areas, the latencies for somatic and visceral are similar.

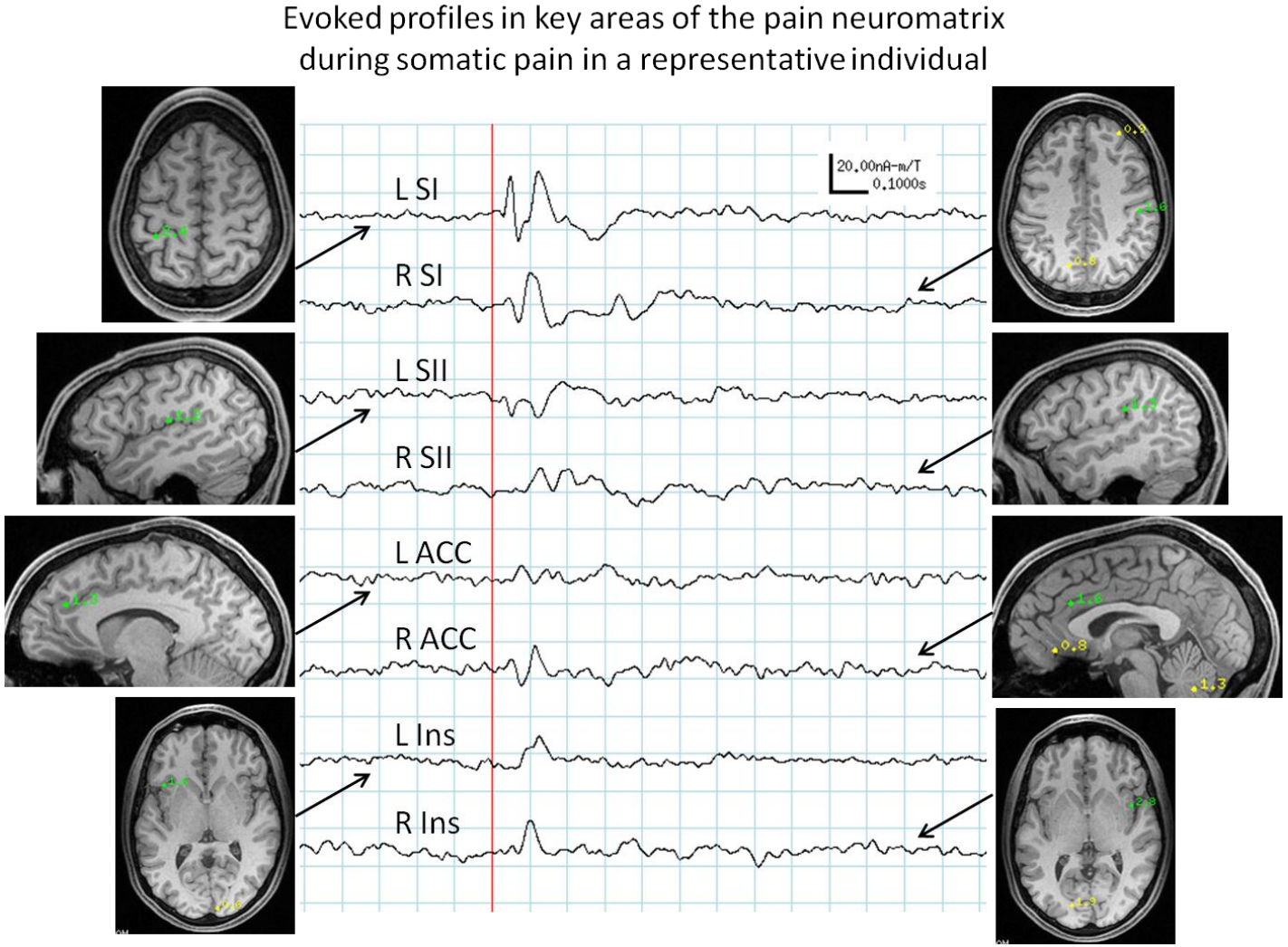


Figure 4:9 shows profiles of the evoked response in the somatic pain block from VEs in one representative individual (VS004) in various areas of the pain neuromatrix. VEs 1 and 2 are left and right SI, VEs 3 and 4 are left and right SII, VEs 5 and 6 are left and right ACC and VEs 7 and 8 are left and right insula. The location of each VE is shown in the MRI to the side of the evoked response.

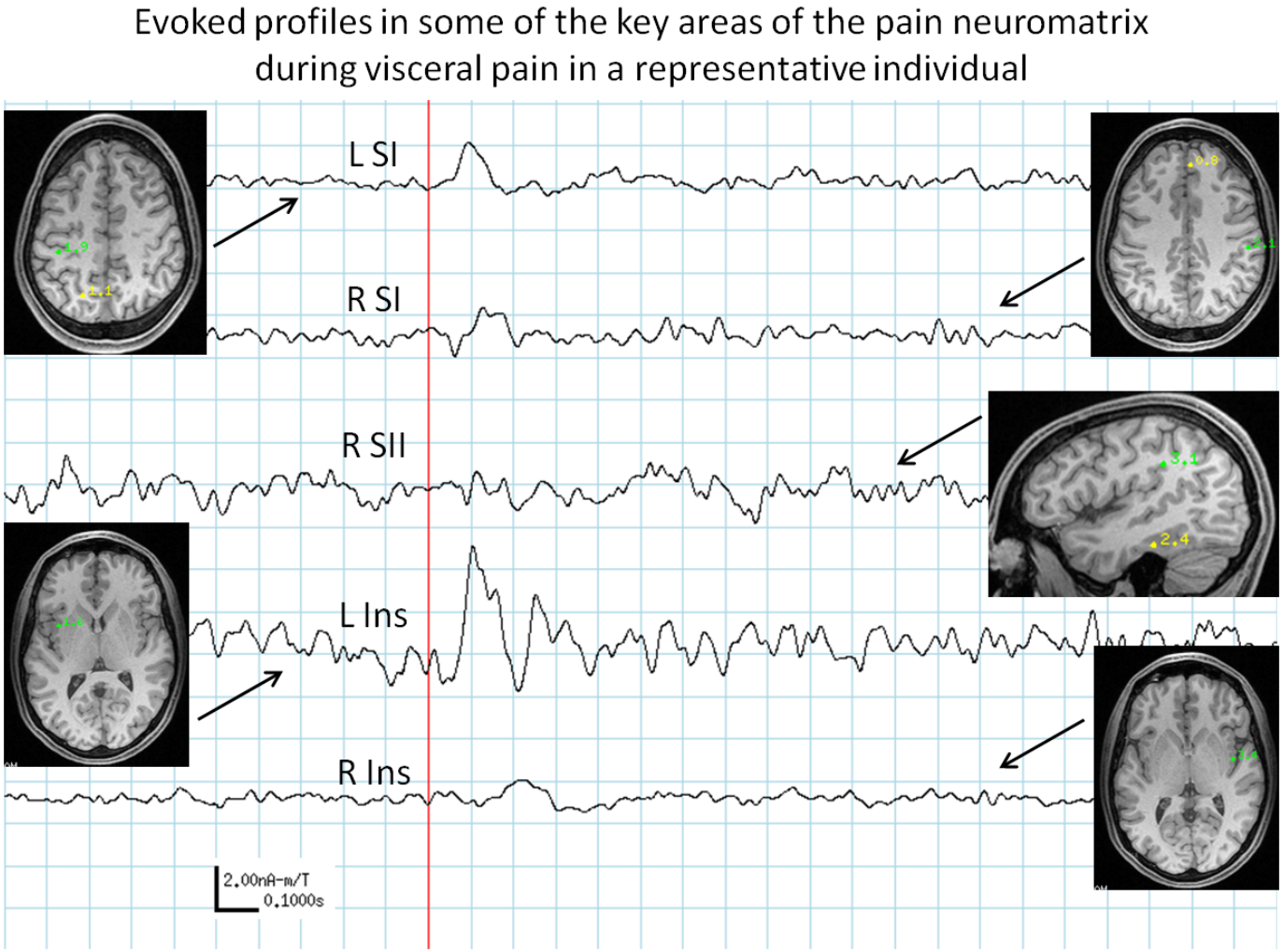


Figure 4:10 shows the profiles of the evoked responses during the visceral pain block from VEs in one representative individual (VS004) in some areas of the pain neuromatrix. VEs 1 and 2 are left and right SI, VE3 is right SII and VEs 4 and 5 are left and right insula. The location of each VE is shown in the MRI to the side of the evoked response. There were no peaks of SAM activity in the ACC of this individual, however an evoked response from the ACC during visceral pain can be seen in Figure 4.21 in another individual.

4.5.5 Spectrograms:

4.5.5.1 Somatic Data

In the somatic pain data, an increase in power in the gamma range was evident in 64% of participants in SI from ~100-250ms between ~65-95Hz in average spectrograms. This was not seen in any participants during somatic non-painful sensation.

In order to further investigate this pattern, bootstrap spectrograms were performed on data from these participants, focusing on the time period and frequency range in question, using 500ms before and after the stimulus and 60-100Hz bandwidth (see Figures 4.11 and 4.12).

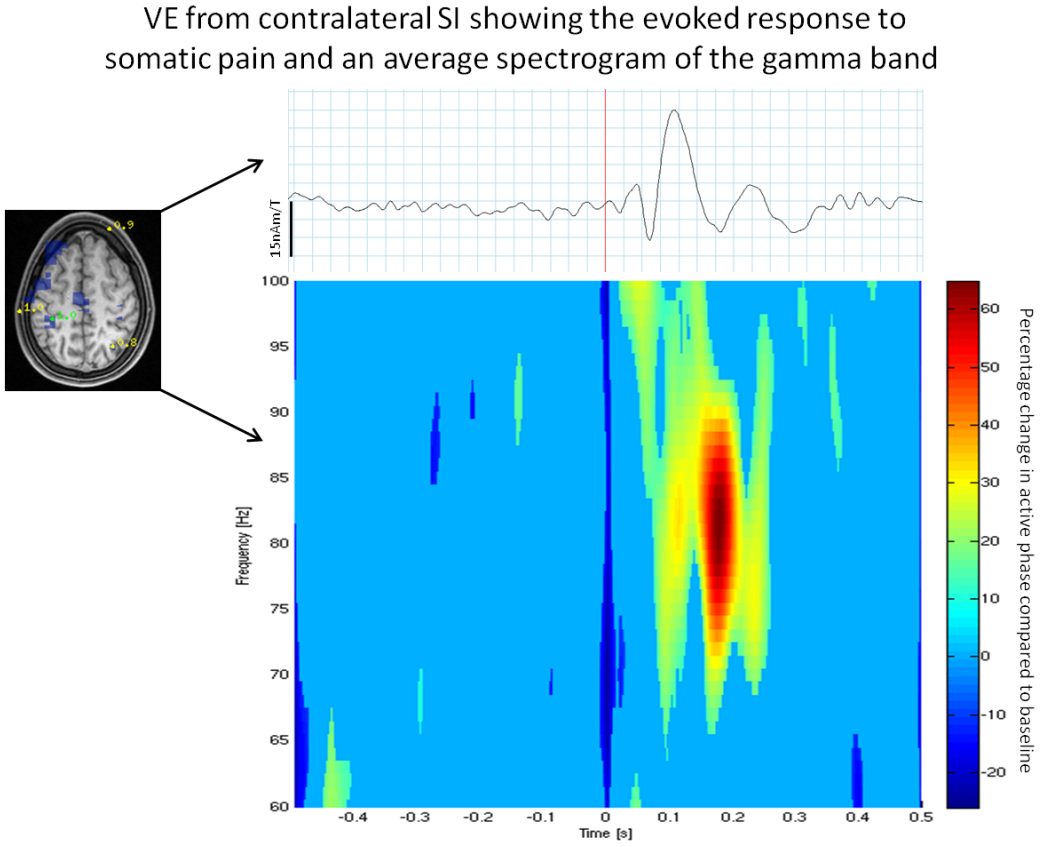
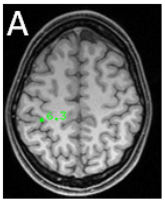
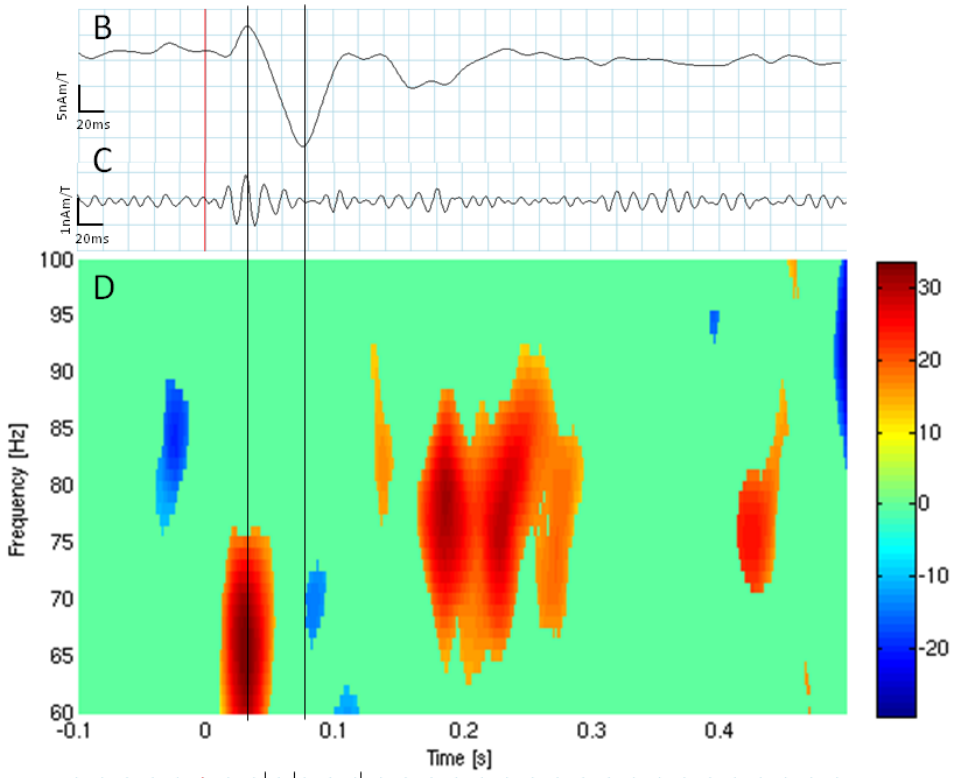


Figure 4:11 shows the evoked response and a bootstrap spectrogram from the left SI (see MRI on left) of a representative individual (VS008) during the somatic pain block. The time of electrical stimulation is at 0s.

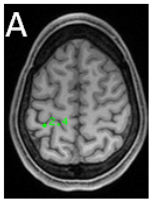
All participants that showed a gamma increase in response to somatic pain from virtual electrodes in contralateral SI showing the evoked response filtered from 1-50Hz, 60-100Hz and bootstrap spectrograms

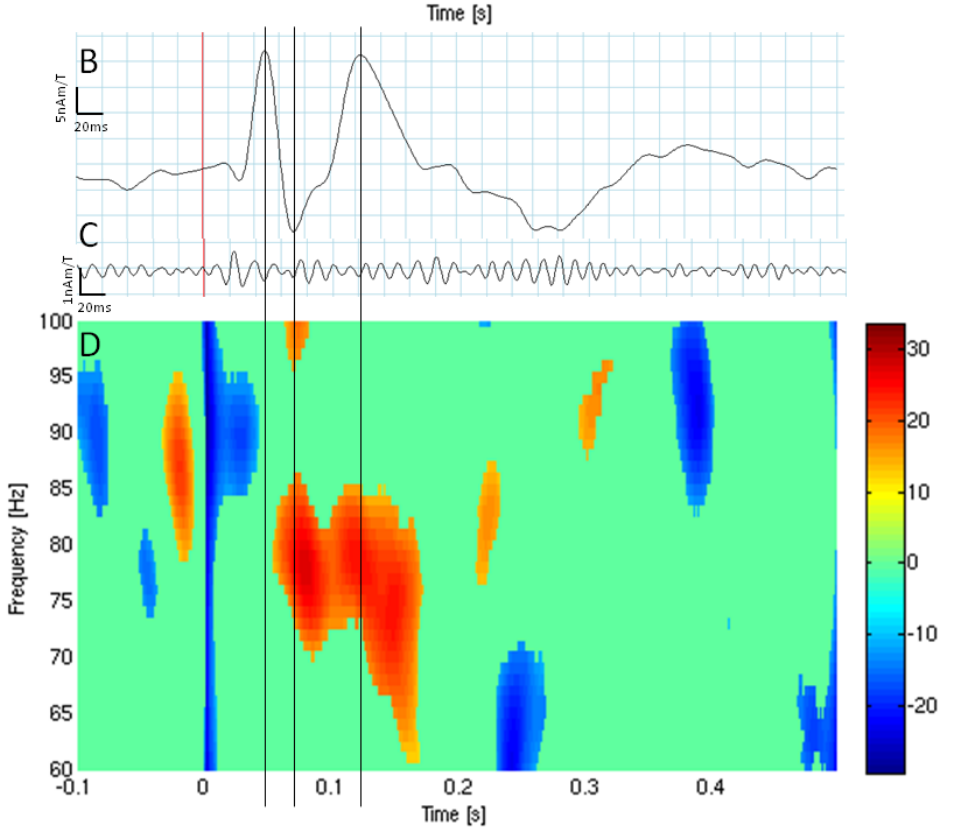
VS003



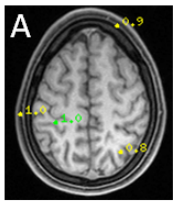


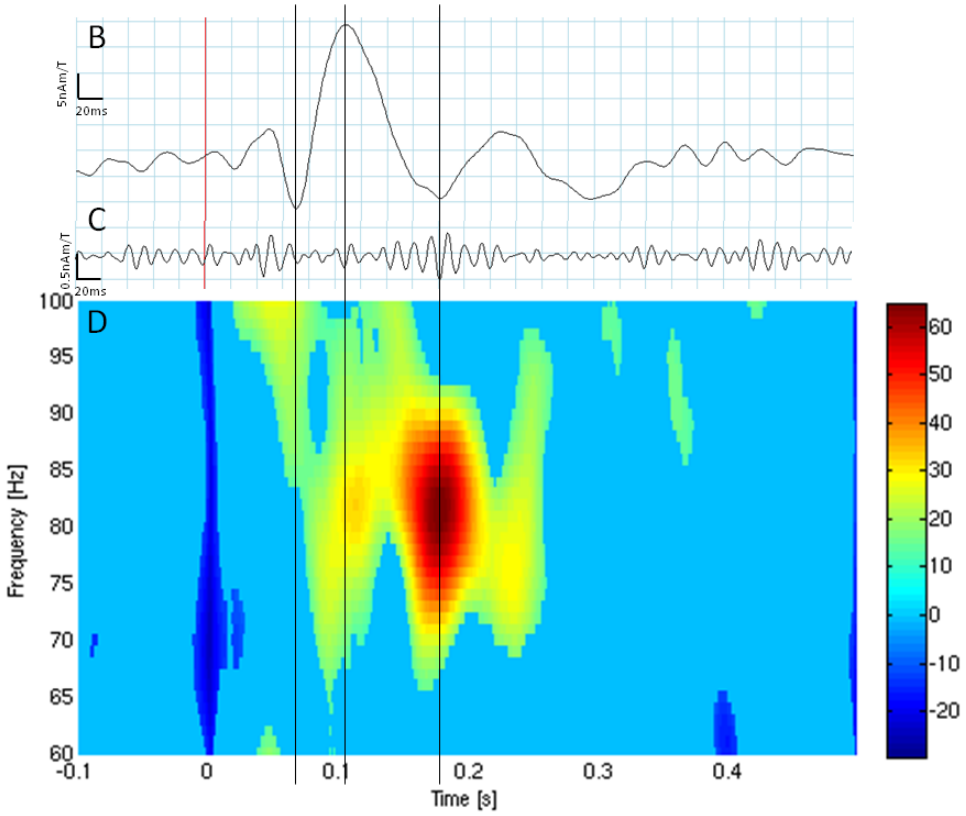
VS004



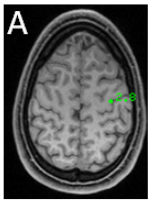


VS008


A


B
5nAm/T
20ms
C
0.5nAm/T
20ms
D
Frequency [Hz]
Time [s]

VS009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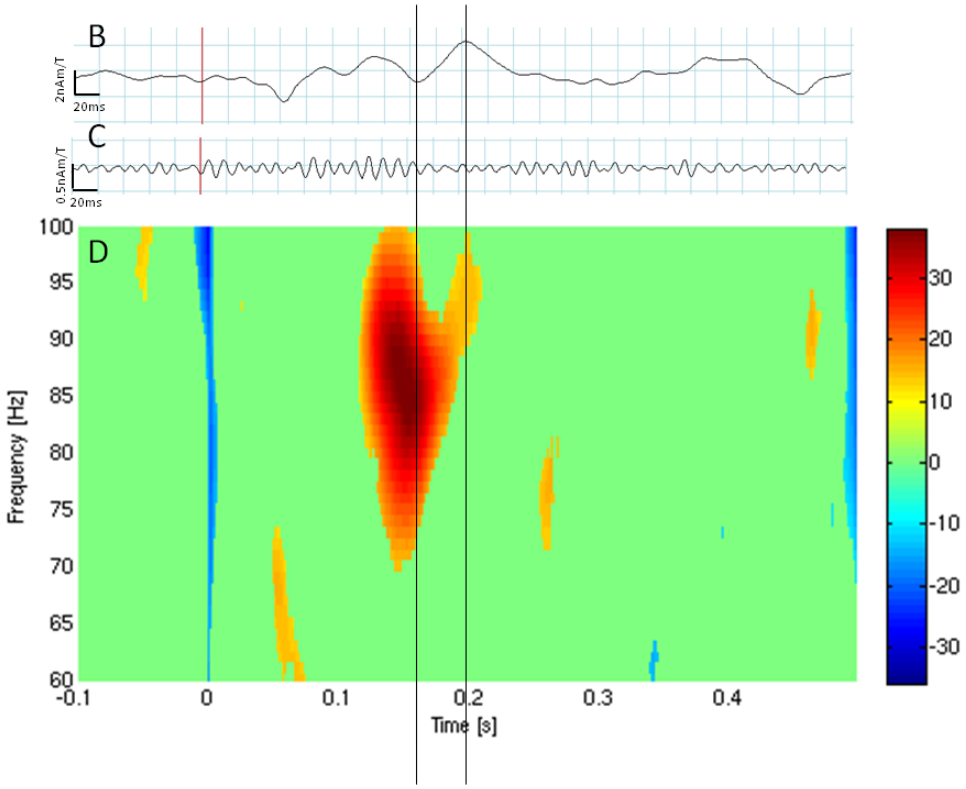
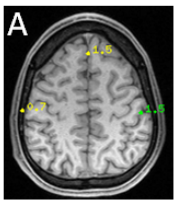
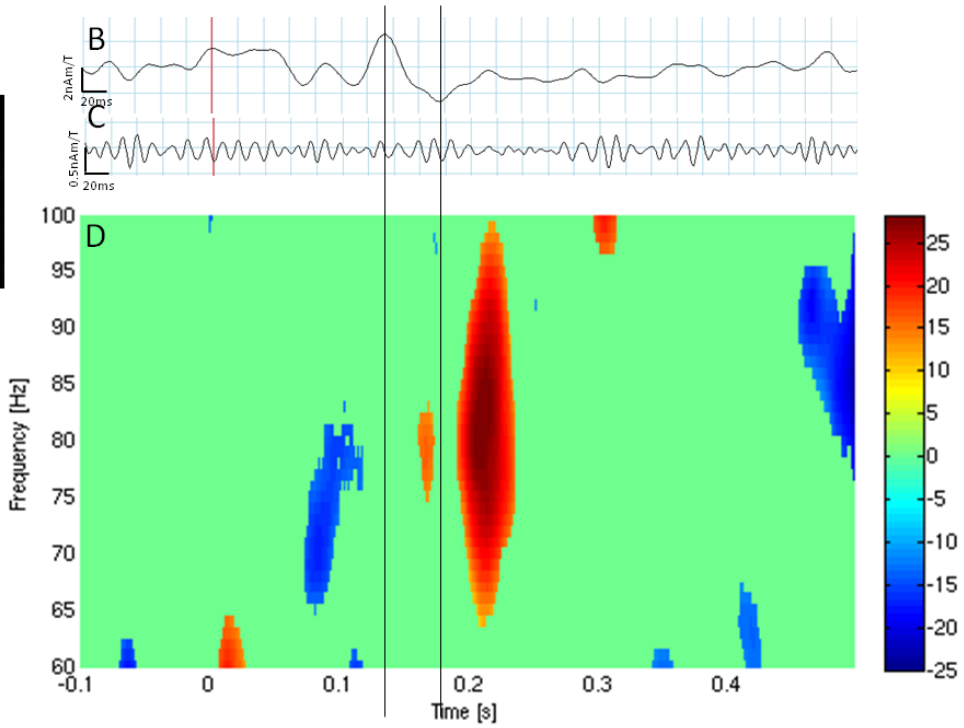
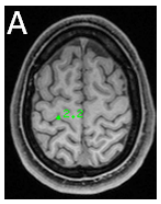

B
2nAm/T
20ms
C
0.5nAm/T
20ms
D
Frequency [Hz]
Time [s]

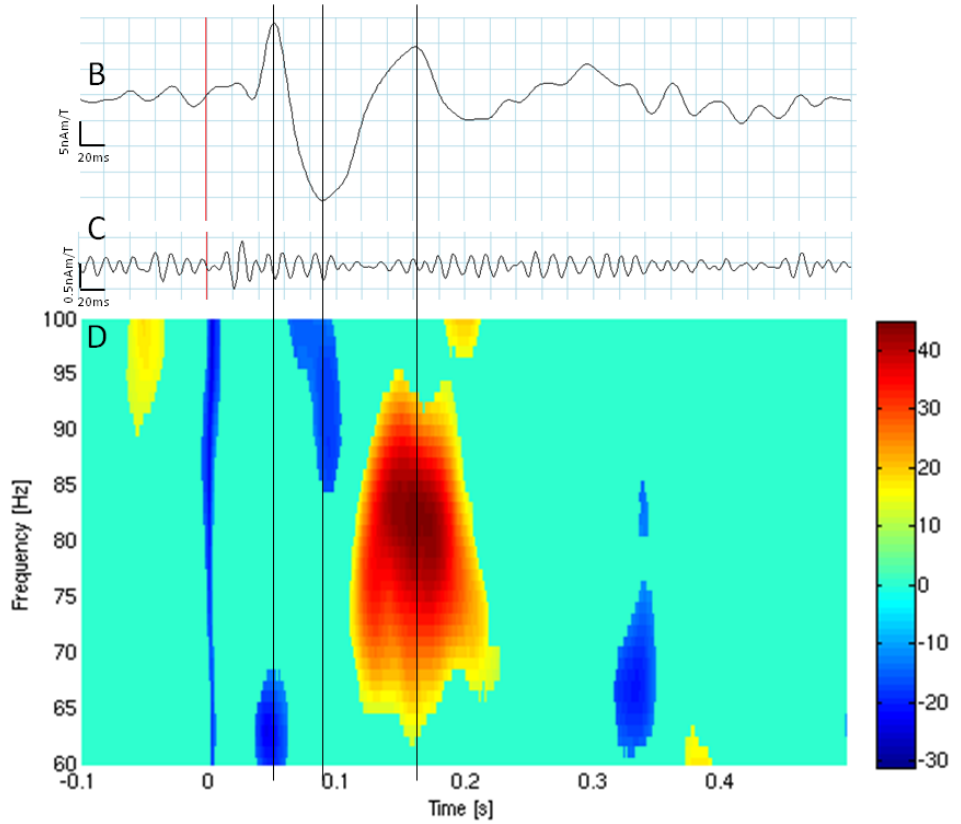
VS010


A


B
2nAm/T
20ms
C
0.5nAm/T
20ms
D
Frequency [Hz]
100
95
90
85
80
75
70
65
60
-0.1
0
0.1
0.2
0.3
0.4
Time [s]
25
20
15
10
5
0
-5
-10
-15
-20
-25

VS011


A


B
5nAm/T
20ms
C
0.5nAm/T
20ms
D
Frequency [Hz]
100
95
90
85
80
75
70
65
60
-0.1
0
0.1
0.2
0.3
0.4
Time [s]
40
30
20
10
0
-10
-20
-30

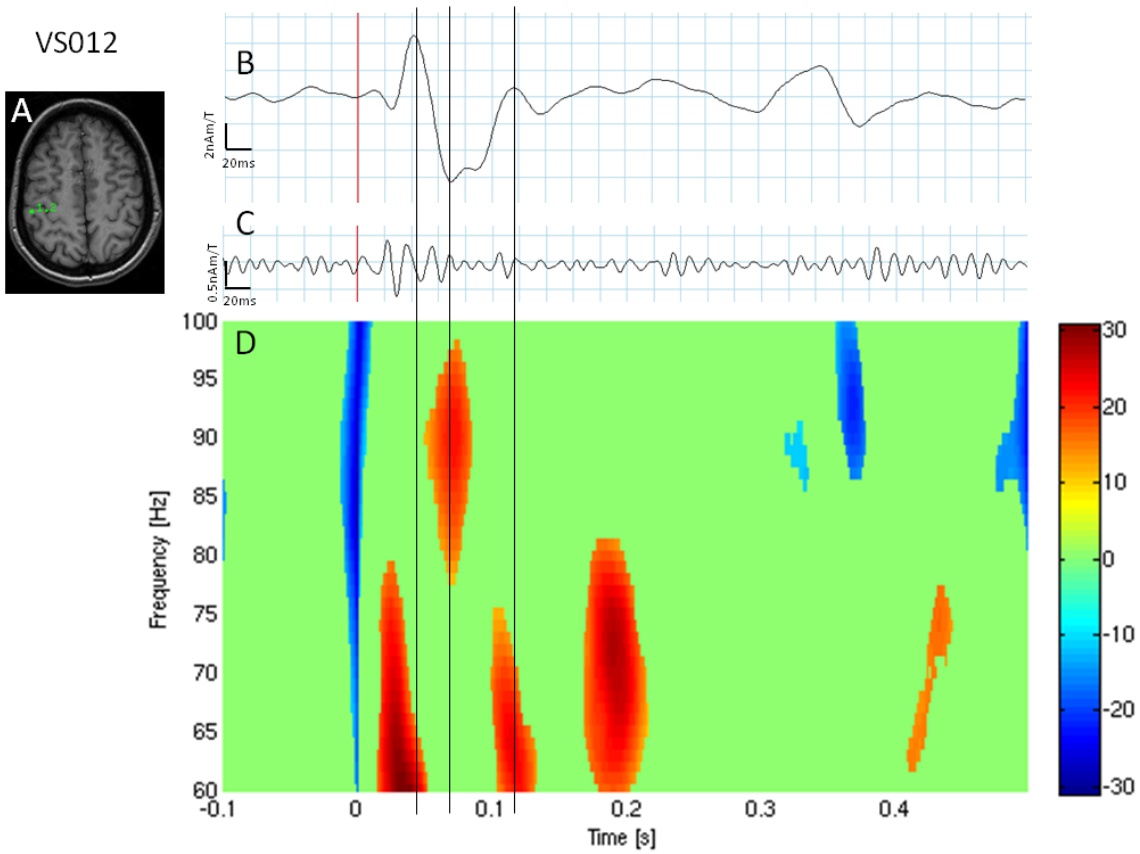
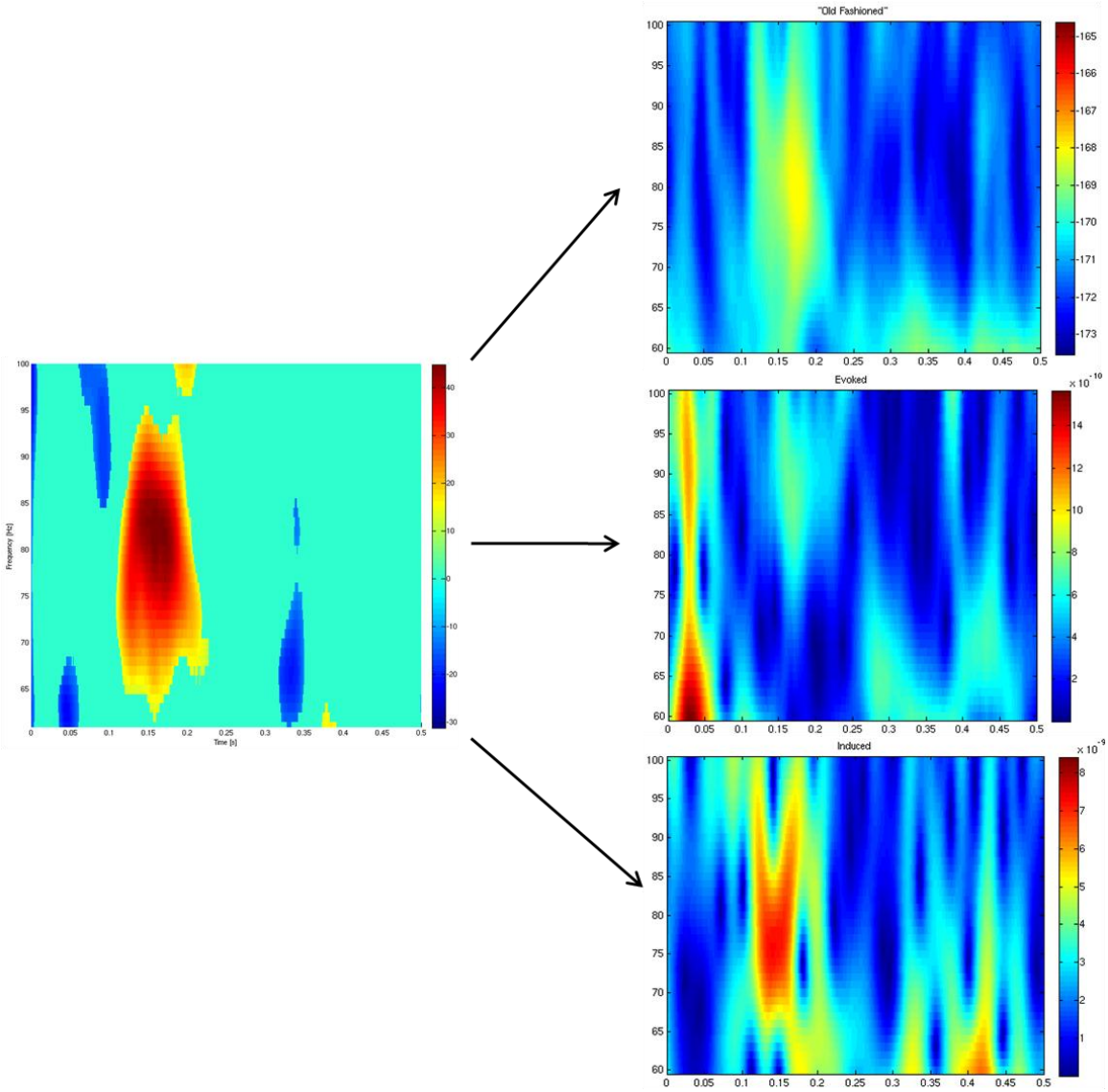


Figure 4:12 shows the evoked fields filtered from 1-50Hz (B) and filtered from 60-100Hz (C). Bootstrap spectrograms (D) show the increase in gamma oscillations found in 7 of the 11 participants. The location of the VEs are shown in the MRIs (A) (coordinate in green), all are in either left or right SI.

Figure 4.12 shows all participants who demonstrated an increase in gamma oscillations in response to somatic pain. The evoked response profile can be seen filtered between 1-50Hz and filtered between 60-100Hz. Lines have been drawn down from the key peaks in the evoked response and it is clear that this does not always correspond to the timing of the gamma burst. A t-test was performed on the pain thresholds of those that showed gamma oscillations against those that did not and the result was not significant ($t_{(10)}$=0.18, p=0.86), however there was a disadvantage in the small sample size.

Gamma increase to somatic pain showing bootstrap spectrogram and average spectrogram split into evoked and induced components



4:13 shows a bootstrap spectrogram of the gamma increase in VS011 on the left and on the right is the average spectrogram in original form (top), and then split into the evoked data (middle) and the induced data (bottom).

Figure 4.13 demonstrates the combination of evoked and induced components to the gamma response in one individual. It clearly shows that there is a strong induced component to the gamma increase. Across the group this was variable and it generally appeared to be a combination of both evoked and induced components.

Average spectrograms from 0-90Hz from SI demonstrate changes in the lower frequency bands during somatic pain (Figure 4.14). All participants showed a 20-30Hz strong beta band component during baseline which decreases immediately after the stimulus between 0-500ms (see Figure 4.14). In 64% of participants this beta then rebounded after ~500ms or so to a higher level than it was seen in the baseline between 0.5-1.5s after the stimulus.

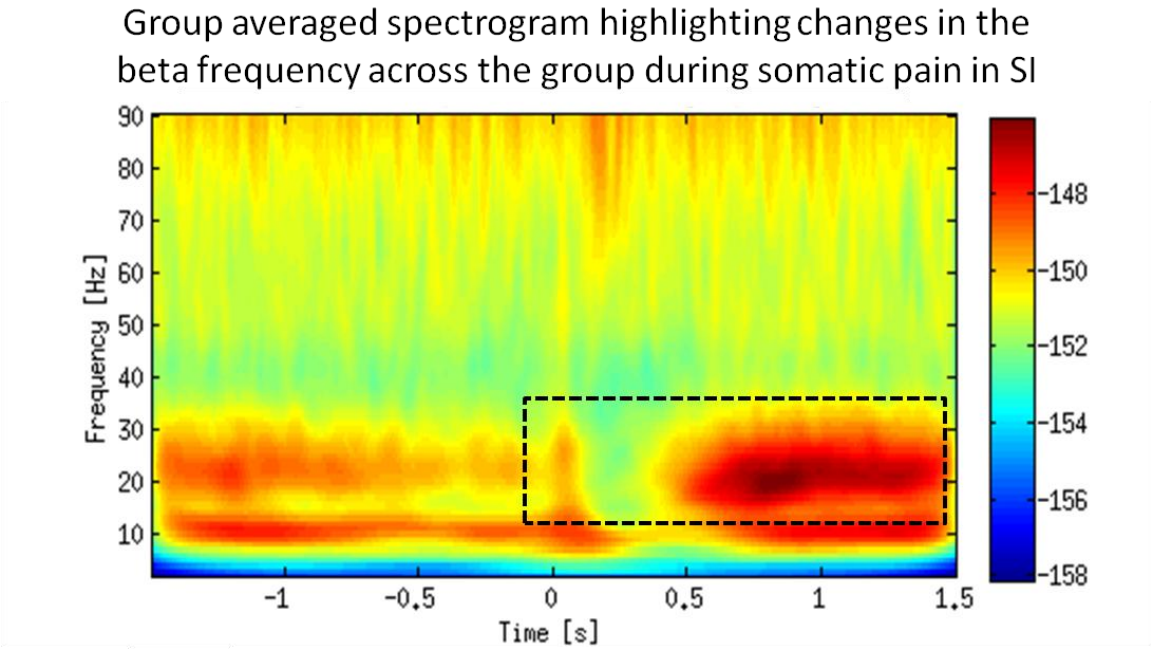


Figure 4:14 shows an averaged spectrogram across all participants in the somatic pain block in SI. The decrease in beta power after the stimulus is followed by a strong rebound and is consistent across the group (highlighted in dotted box). An increase around 5-15Hz can be seen around the stimulus followed by a decrease around 10Hz from ~250-550ms which then returns to baseline levels.

45% of participants showed a consistent 10Hz activity (alpha) during baseline, in 27% of participants it disappeared immediately after the stimulus and then rebounded later. In 73% of participants in SI, an increase in power was evident in the averaged spectrograms between 5-15Hz which coincided with the evoked response which can be seen in the group spectrogram in Figure 4.14.

In other areas of the pain neuromatrix (SII, ACC, insula), it was possible to see an increase around 10Hz which was coincident with the evoked response, but induced

changes in the frequency dynamics were less evident in response to somatic stimulation compared to SI (see Figures 4.15-4.17).

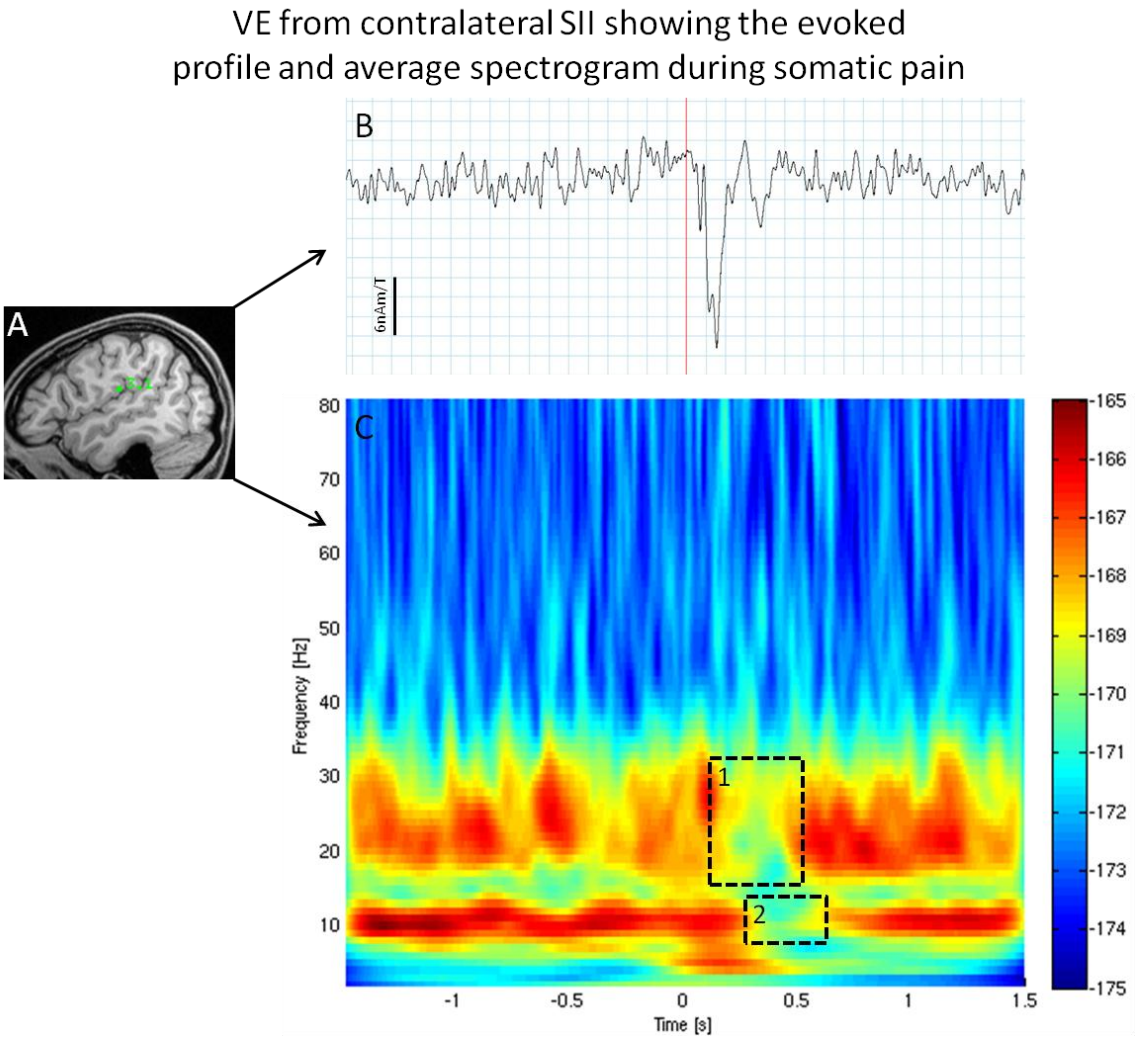


Figure 4:15 shows the evoked profile (B) and an average spectrogram (C) from a VE (A) in left SII during somatic pain in a representative individual (VS003).

In Figure 4.15, a VE in contralateral SII cortex showed a decrease in both alpha (Box 2) and beta frequency (Box 1) bands which returned to baseline levels very quickly. An increase around 5Hz could also be seen immediately after the stimulus which coincided with the evoked response seen above.

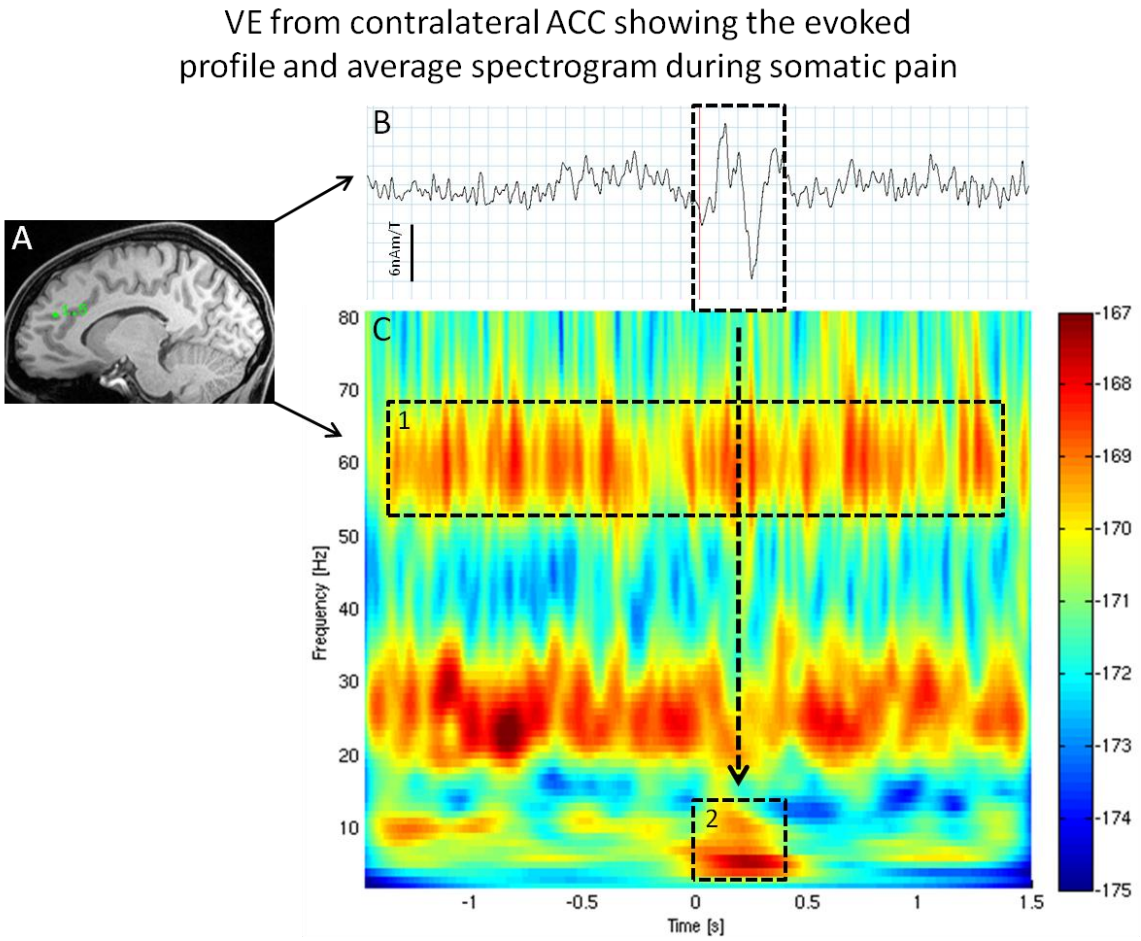


Figure 4:16 shows the evoked profile (B) and an average spectrogram (C) from a VE (A) in left ACC during somatic pain in a representative individual (VS003). This was only seen in 2 participants.

Figure 4.16 demonstrates an interesting phenomenon in the ACC. A constant high power in the gamma frequency band can be seen (Box 1), this was evident in 2 participants. A small decrease in beta band can be seen although it is quite variable in the baseline also. A strong increase can be seen around 5Hz which coincides with the evoked response (Box 2).

There were few changes found in the spectrograms of the Insula, as can be seen in Figure 4.17, although an increase at around 10Hz is apparent following the stimulus which corresponds to the evoked response (shown above it).

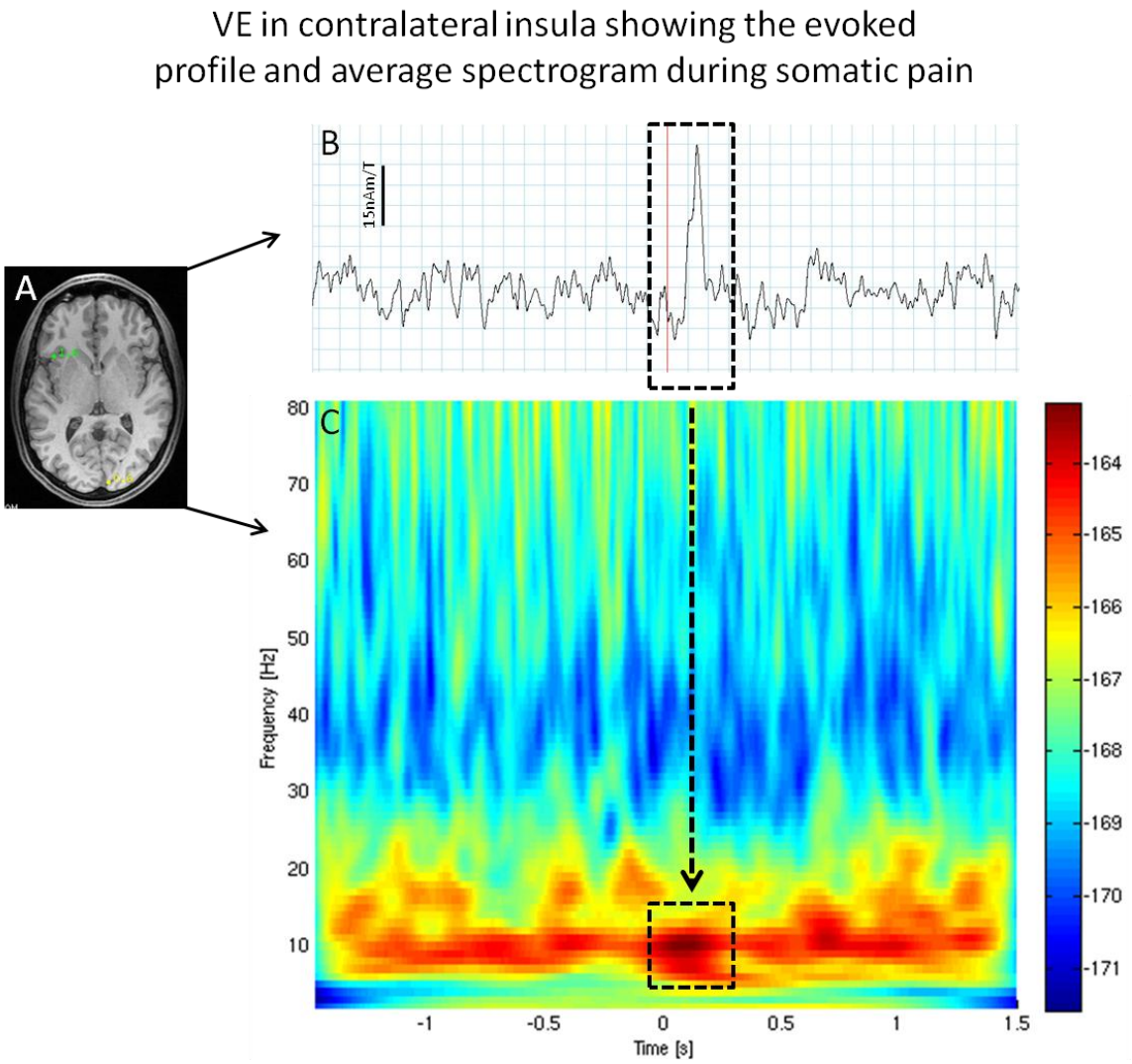


Figure 4:17 shows the evoked profile (B) and an average spectrogram (C) from a VE in left insula (A) during somatic pain in a representative individual (VS004).

4.5.5.2 Visceral Data

During visceral stimulation in SI, no gamma response was evident in average or bootstrap spectrograms in any participants despite a clear evoked response still being present (see Figure 4.18). A decrease in alpha (~10Hz) and beta (~20-30Hz) was seen in SI in 45% of participants which returned to normal between ~500-600ms (see Figure 4.19). A delay was seen between the peak of the evoked response during somatic pain and visceral pain across the group, 25±6ms and 79±27ms respectively (see Table 4.3).

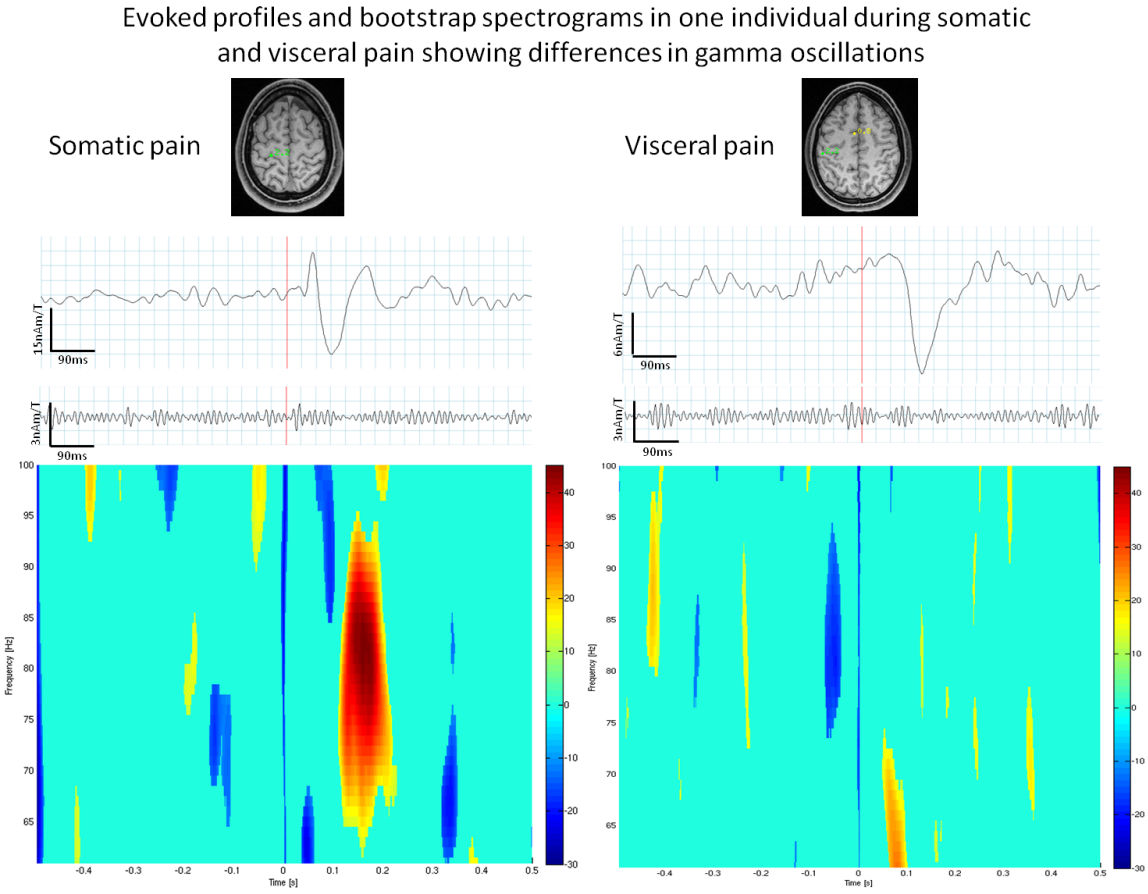


Figure 4:18 shows the evoked response profile and bootstrap spectrogram during somatic pain and visceral pain in one representative individual (VS011). A strong increase in gamma oscillations can be seen following the somatic pain, however this is not present in the visceral pain even though an evoked response is still evident.

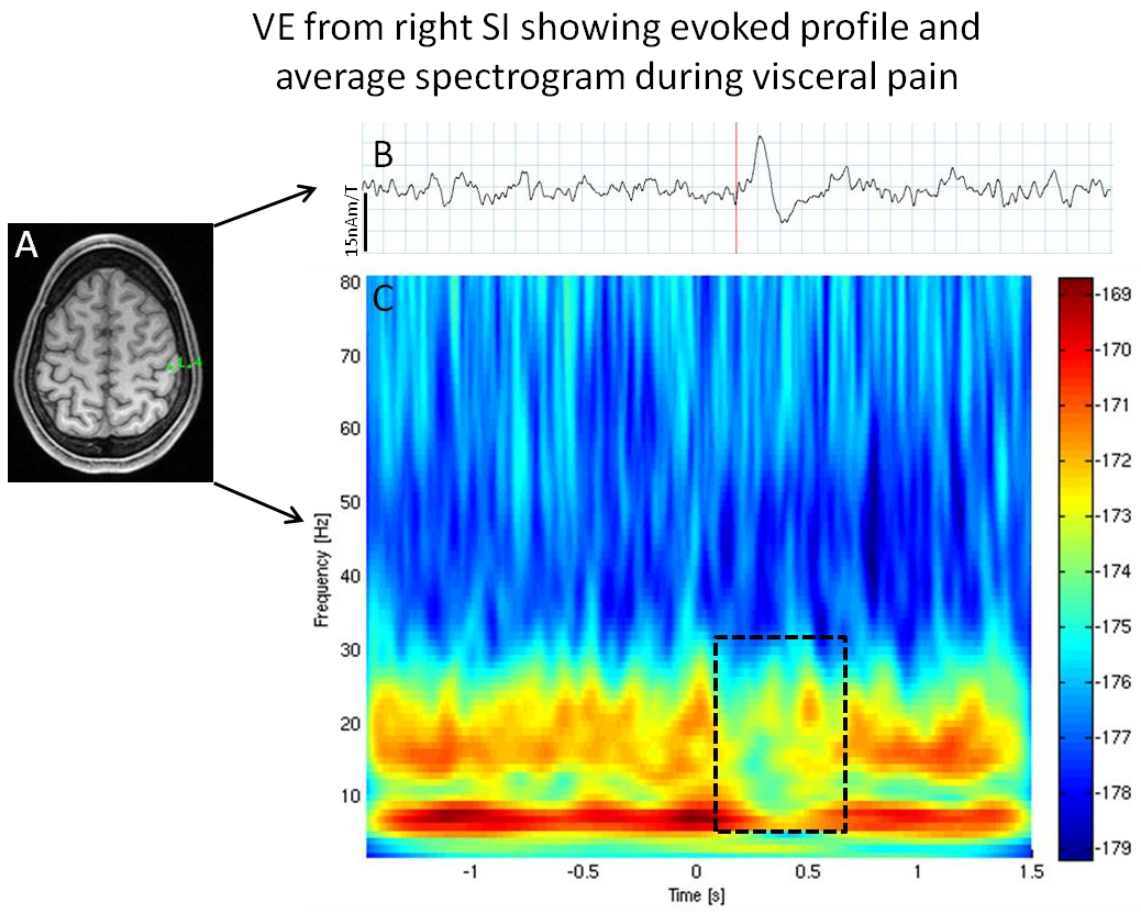


Figure 4:19 shows the evoked profile and an average spectrogram of the right SI during visceral pain in one representative individual (VS004). A decrease in both alpha and beta is apparent immediately after the stimulus (seen in dotted box).

Few induced changes were apparent in other areas of the neuromatrix during visceral stimulation, however clear evoked responses could be seen (see Figures 4.20-4.22). In SII, an increase at around 5-10Hz was seen which coincided with the peak of the evoked response (see Figure 4.20).

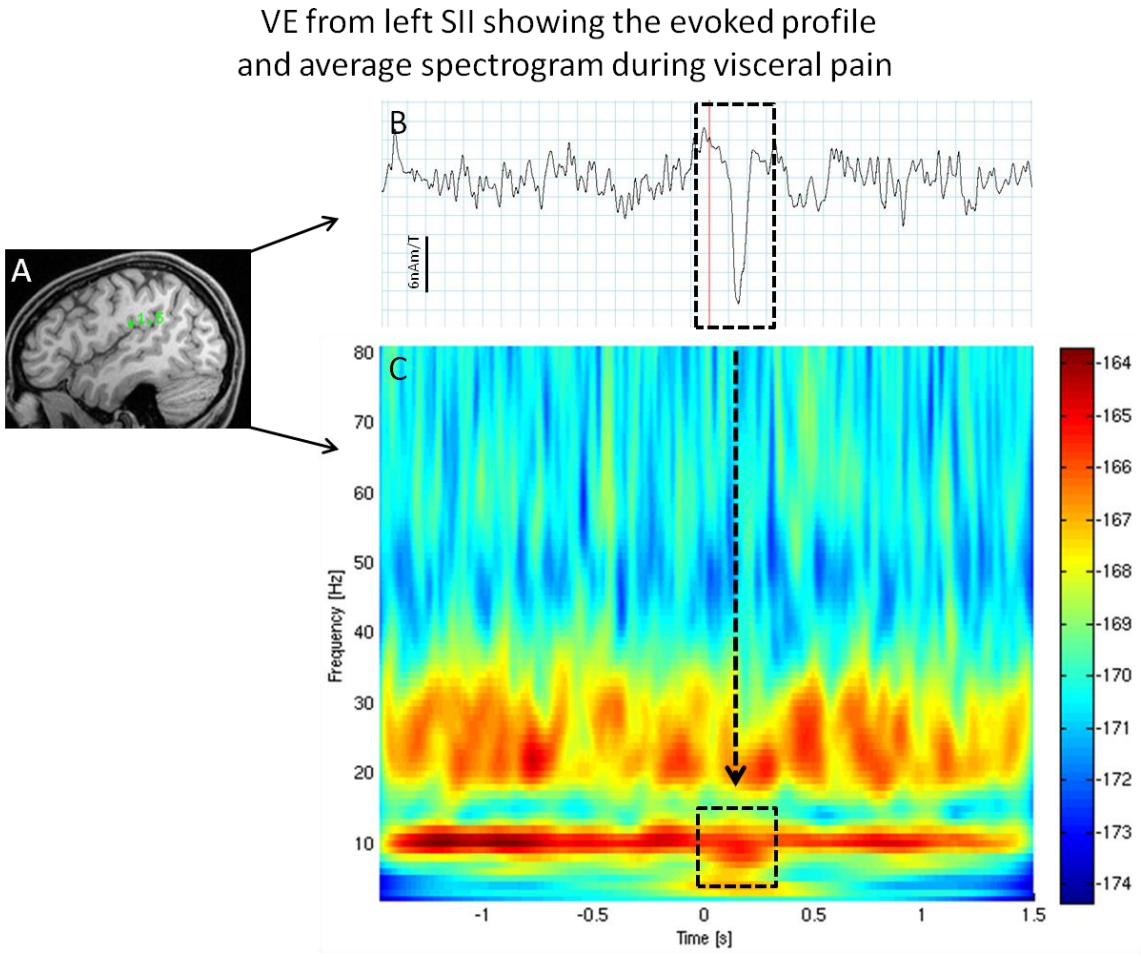


Figure 4:20 shows the evoked profile and an average spectrogram from a VE in left SII during visceral pain in a representative individual (VS003).

Similar to the somatic data, a consistently high gamma power was seen in the ACC as well as a slight decrease in the beta frequency and an increase in 5-10Hz (corresponding to the evoked response) (see Figure 4.21).

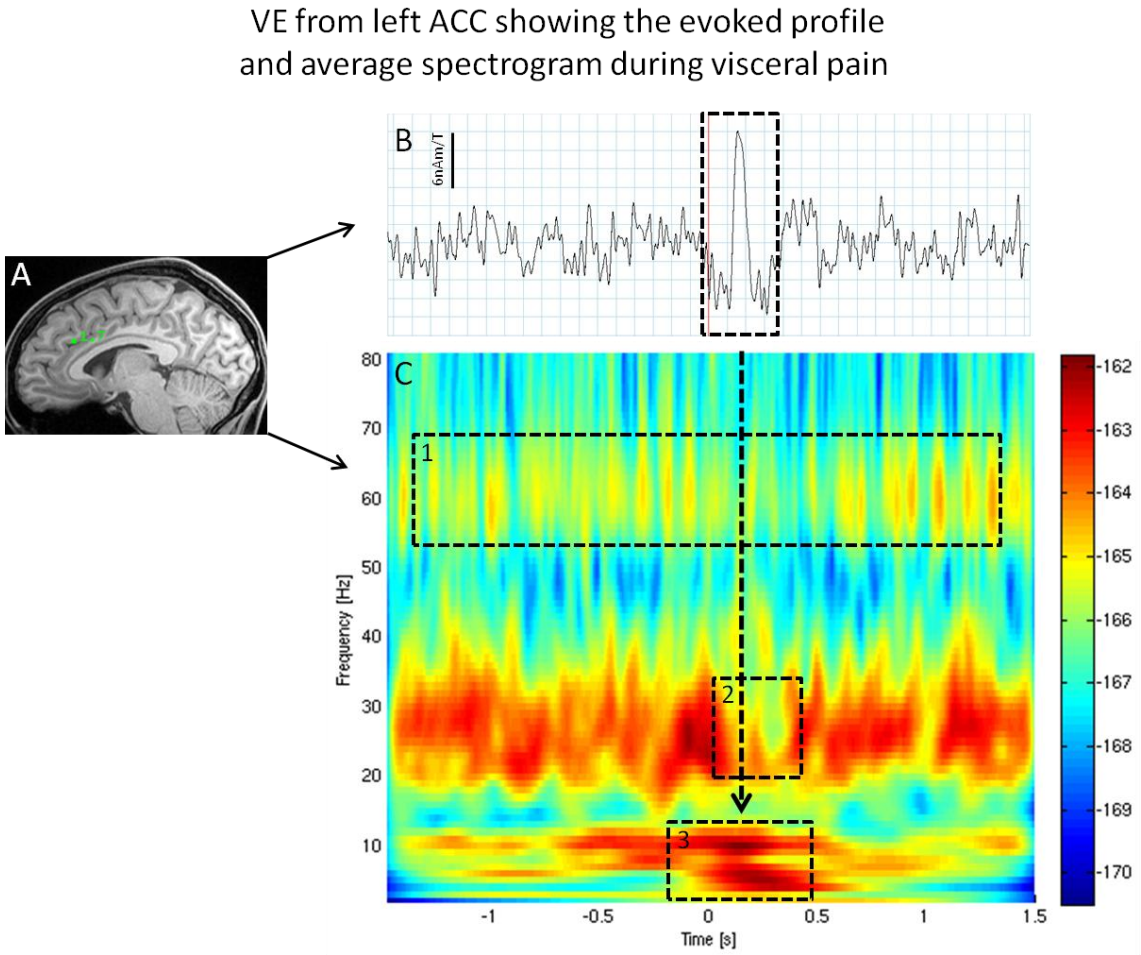


Figure 4:21 shows the evoked profile and an average spectrogram from a VE in left ACC during visceral pain in a representative individual (VS003).

In the Insula, no changes were evident in the oscillatory dynamics except for an increase around 5-10Hz which coincided with the evoked response (see Figure 4.22).

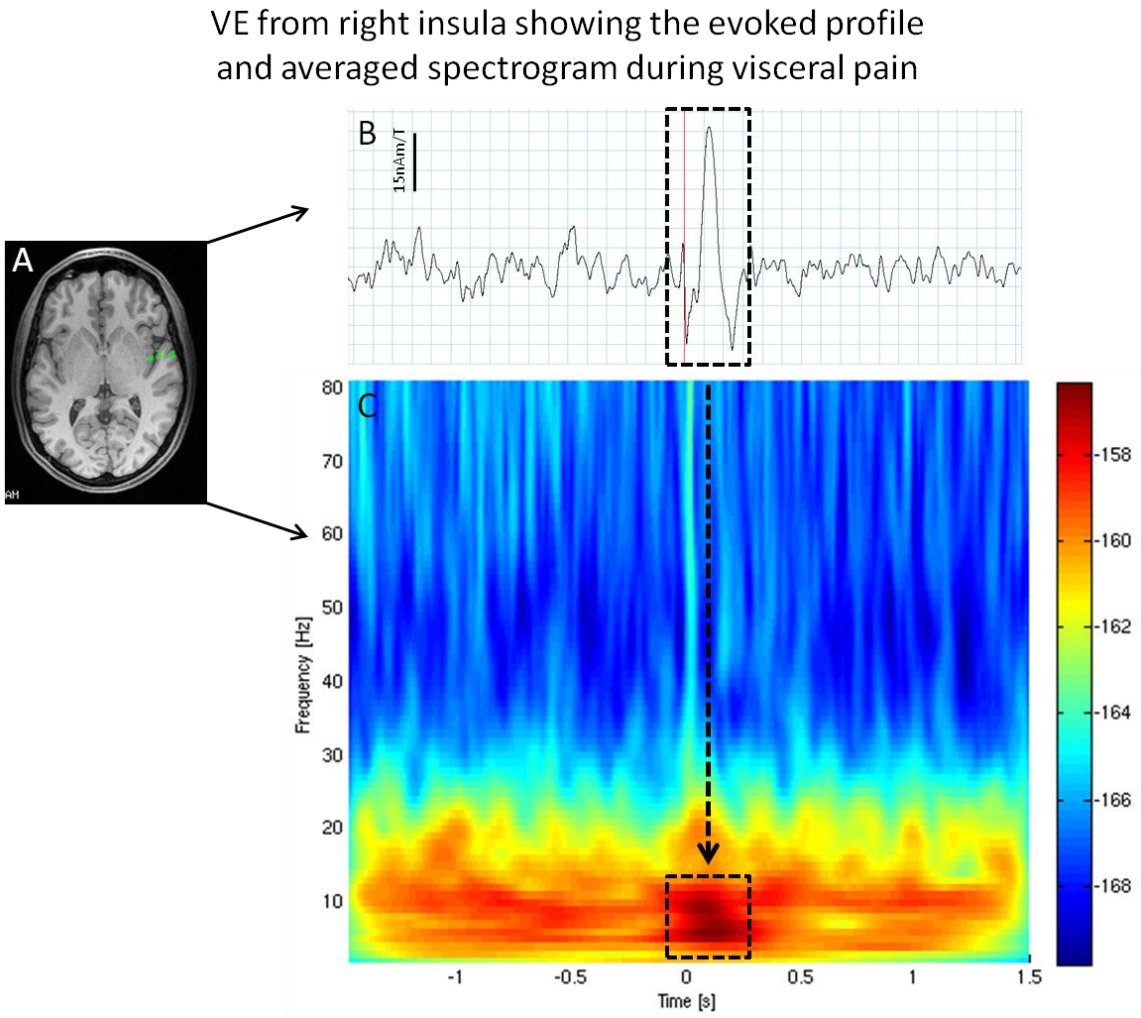


Figure 4:22 shows the evoked profile and an average spectrogram from a VE in right insula during visceral pain in a representative individual (VS004).

4.6 Discussion:

4.6.1 Summary of key findings

Activity was found in key areas of the pain matrix during both somatic and visceral stimulation. In somatic pain, 64% of participants showed an increase in gamma oscillations ~100-250ms after the stimulus between 60-95Hz (Figure 4.12). This was not seen following visceral stimulation. The timing of the gamma increase did not coincide with the main components of the evoked response in SI during somatic pain, it was found to have an induced component to it implying it may have a role in higher order cognitive processing (Tallon-Baudry and Bertrand, 1999).

SAM activations were found in SI during visceral stimulation despite debate over its involvement in the literature (Aziz et al., 2000a). Clear evoked responses were seen in SI in response to both somatic and visceral stimulation. A decrease in both alpha and beta bands was seen in SI and SII in somatic pain, however few induced changes were apparent in ACC and Insula. Visceral stimulation was rated as more unpleasant than somatic stimulation (Figure 4.2), however no obvious difference was apparent in activations of the emotive areas of the pain matrix (ACC, Insula).

4.6.2 Primary Somatosensory Cortex

4.6.2.1 Spatial localization of visceral vs somatic

All participants showed peaks from SAM analysis in SI in both visceral and somatic stimuli (see Table 4.2). The role of SI in visceral processing has been debated in the literature. Differences seen may be due to whether the proximal or distal oesophagus is stimulated as the proximal oesophagus contains striated muscle which is more likely to have SI representation whereas the distal oesophagus is smooth muscle and is therefore under autonomic control and less likely to be represented in SI (Aziz et al., 2000b). In a study by Schnitzler et al (1999) using MEG, there was no activation seen in SI following distal oesophageal electrical stimulation and Aziz et al (2000b)

found that distal oesophageal stimulation bilaterally activated an area at the junction of SI and SII. However, others have found clear activity in SI during oesophageal stimulation in a variety of neuroimaging techniques. Coen et al (2007) found bilateral activation of SI during painful distal oesophageal balloon distension using fMRI. Hobson et al (2005) found clear evoked responses from the abdominal and trunk regions of SI during painful electrical oesophageal stimulation using MEG. Peaks in SI during visceral stimulation in this study were seen in both hemispheres and slightly lateral to the hand area, although only the right SI showed significance at group level. During somatic stimulation, activity was in the left SI in an area corresponding to the hand region (see Figure 4.5).

4.6.2.2 Evoked profile

A clear evoked response could be seen in the SI of all participants during somatic pain and 91% of participants in visceral pain. The shape of the evoked responses showed some variance across participants but most commonly had a triphasic morphology (Ploner et al., 2000, Hobson et al., 2000a) with the latency of the first component at ~25±6ms during somatic pain and ~79±27ms for visceral pain. The latency of the first component for somatic pain is comparable with the 20ms component common in the literature (Kakigi et al., 2000, Della Penna et al., 2004). The latency of the first response in visceral is comparable with data from Hobson et al (2005) who found first SI response at 88.4±11.5ms. This delay in latency for visceral pain could be due to the activation of different fibres or a different population of neurons or perhaps due to it being an indirect pathway as referred pain to a somatic area.

There was a consistent difference across the group in the amplitude of the evoked response in that it was larger for somatic than visceral pain, this can be seen in Figure 4.18 from the different scales used. Both the digit and the oesophagus contain Aδ and C nociceptive fibres, but from the latencies it is most likely that the electrical stimulation in this study activated Aδ fibres.

4.6.2.3 Changes in gamma band

In Study 1, an increase in the gamma band was seen in left SI in response to a train of electrical stimuli on the right median nerve. In this study, a single pulse was given to the right index finger and an increase in gamma oscillations in SI was seen in 64% of participants during somatic pain (45% in left SI and 18% in right SI) (Figure 4.12) while a clear evoked response was seen in all participants. No change in gamma oscillations was apparent during somatic non-painful stimulation.

It is important to define whether the increase in gamma oscillations seen during somatic pain was a transient synchronization caused by the evoked response or whether it was a signal of something more complex occurring in SI. As can be seen in Figure 4.12, the gamma response shown in the bootstrap spectrograms (D) occurred between 100-250ms in all participants whereas the key components of the evoked response (B) were earlier than this (~70ms). The evoked response filtered to gamma frequency can be seen in part C of these figures. It is clear that the evoked gamma response does not coincide with the increase in gamma oscillations seen in the bootstrap spectrogram.

Only 64% of participants showed an increase in gamma oscillations whereas all showed a clear evoked response in SI, also a clear evoked response was seen in the SI during visceral pain, however there was no increase in gamma oscillations observed. It is possible that the stimulus during visceral pain was not strong enough as 45% of participants reached the maximum threshold for the electrical stimulator before reaching their pain tolerance. Also pain thresholds (in mA) for oesophageal stimulation were much higher than for somatic so it is possible that the same strength of pain was not elicited in the visceral pain blocks. It may be that the oesophageal electrical stimulation was not driving the cortex to the same level of neural synchrony as strongly as during somatic pain and therefore did not show any change in gamma oscillations. These results suggest that the gamma oscillations seen in this study do

not form a simple relationship with the evoked response but have a more complex role in somatosensory processing.

Hauck et al (2007a) and Gross et al (2007) both demonstrated a similar increase in induced gamma oscillations to this study in response to experimental pain in SI. They related it to attention to pain and pain perception respectively, however they provided no sensory comparison to investigate whether gamma oscillations were also present in non-painful stimuli. The results of this study suggest that gamma oscillations do not appear to respond specifically to painful stimuli as it was not seen during visceral pain, it may be encoding different information about the stimulus. Gamma oscillations have been found to have a role in other sensory modalities. Hadjipapas et al (2007), found that within the temporal frequency characteristics of gamma oscillations, information about the spatial frequency of visual stimuli was encoded. It is possible that rather than simply being a biomarker for pain, gamma oscillations encode important information about particular aspects of the stimulus within its frequency characteristics. Further studies need to vary different aspects of the stimulus such as intensity or whether the pain stimulus is tonic or phasic to investigate how the gamma oscillations are affected.

The latency of the gamma response in this study (~100-250ms) suggests that it may form part of the induced response involved in higher order cognitive processing such as attention or emotion, the induced component of the gamma response can be seen in Figure 4.13. Gamma oscillations have been linked to attention previously by Hauck et al (2007a) who have seen 2 gamma responses, both induced. One of the gamma responses was similar to that seen in this study in terms of both frequency band (60-80Hz) and timing (50-250ms). They also saw later, higher-frequency gamma oscillations (400-600ms, 120-140Hz) that they found strengthened with increased attention to the pain. They stated that both gamma responses were induced, not evoked. A paradigm involving both attention to, and distraction from, a somatic painful electrical stimulus would reveal if the gamma oscillations seen in this study involved attentional factors. Changes in oscillatory dynamics in response to attention to a

painful stimulus have been reported previously by Ohara et al (2004, , 2006) who showed that a decrease in both alpha and beta bands was seen in SI during attention as compared with distraction from a painful laser stimulus.

36% of participants did not show an increase in gamma oscillations in response to somatic pain, even though their evoked response profile was similar to those that did. In Study 1, gamma oscillations were only seen in 50% of participants. The reason some individuals do or don't show gamma oscillations is yet to be understood. In Study 1, it was hypothesised that it may be linked to an individual's pain thresholds however the results of t-tests comparing pain thresholds in gamma responders and non-gamma responders in both studies were not significant. This suggests it is unlikely that the presence of gamma oscillations relates to an individual's pain threshold.

4.6.2.4 Changes in beta band

Beta oscillations were found to decrease after somatic stimulation (~200-500ms) in the SI of all participants. In 64% of participants, around 500-600ms, a rebound was observed back to a level higher than the baseline (Figure 4.14). This was also seen in visceral stimulation but was less consistent across the group (45% of participants) (Figure 4.19). Decreases in beta power in SI were found to be significant during both visceral and somatic pain using SnPM analysis (Figure 4.6, 4.7), during somatic pain the left SI was significantly activated and during visceral pain, the right SI. This fits with the literature in that a decrease and rebound has been seen in the mu rhythm (10 and 20Hz) over sensorimotor cortices in response to the offset of a movement (Hari and Salmelin, 1997, Pfurtscheller and Lopes da Silva, 1999) and in response to painful stimulation (Ohara et al., 2004, Raij et al., 2004, Ploner et al., 2006b, Hauck et al., 2007a). However, in some studies looking at tonic cold pain, there is an increase seen in beta power over fronto-temporal areas of the cortex (Chang et al., 2002).

4.6.2.5 Alpha and Theta

During somatic pain, 45% of participants showed a constant 10Hz activity throughout the baseline in SI, in 27% of participants this decreased immediately after the painful stimulus and then returned to normal (see Figure 4.14). During visceral pain, 45% of participants showed a decrease in alpha (~10Hz) in SI which returned to baseline soon after (~600ms) (see Figure 4.19). This matches the literature that states that a decrease in the mu rhythm (10 and 20Hz) is seen in response to painful stimulation (Ploner et al., 2006a). There were no significant changes in alpha or theta bands found at the group level using SnPM analysis in either somatic or visceral stimulation. Changes in theta band have been seen during CPT, often in frontal areas (Chang et al., 2002, Chang et al., 2005, Dowman et al., 2008), however changes in theta during electrical pain are not commonly reported.

4.6.3 Other areas of the cortex

Many of the studies published in the literature on the affective areas involved in pain (ACC, Insula) use fMRI or PET. This shows that these regions are commonly activated during pain, however they lack temporal detail. EEG and MEG studies have investigated evoked responses in these areas (Hecht et al., 1999, Ploner et al., 2002, Garcia-Larrea et al., 2003, Hobson et al., 2005, Forss et al., 2005, Inui et al., 2006, Christmann et al., 2007) but none of these mention how the oscillatory dynamics vary during pain in these areas of the cortex. It is thought that the ACC and Insula have an important role in the affective processing of pain (Vogt, 2005, Rainville, 2002) (see Figures 4.16-4.17, 4.21-4.22).

As can be seen in Table 4.2, SAM peaks were consistently found in SI (100% across all blocks and all participants) however, SII was less consistent (77%) and ACC and Insula even less (39% and 61% respectively). The signal originating from the ACC is generally radial in orientation which may mean that it is harder to pick up in MEG than in other imaging techniques (Garcia-Larrea et al., 2003), although Hillebrand and Barnes (2002) have indicated that this may not be such a problem as first thought. It

was hypothesised that visceral painful stimulation would involve the affective areas of the pain matrix more heavily, however this was not apparent. Both types of pain elicited SAM peaks in SII, ACC and insula and the majority of participants showed evoked responses in these regions. However, there was little difference seen in cortical activity between somatic and visceral pain.

The McGill scores in Figure 4.3 showed that few participants used affective words to describe the stimuli. Visceral pain was rated as more unpleasant than somatic pain (see Figure 4.2). The process of being intubated may have made the participants anxious, however once they had experienced the stimuli they were to receive during MEG recordings, they may have become more relaxed. They had been informed about what the stimulus would be and that there were no side effects or consequences of the electrical stimuli. The stimulus in this study was phasic, tonic stimuli such as cold pain might be more likely to drive affective aspects and coping strategies during pain whereas phasic stimuli may not be strong enough or last long enough to create this type of response.

4.6.3.1 SII

91% of participants showed activation in SII during somatic pain; of these, 60% showed bilateral SII activation and 40% showed activation only in right SII. In visceral pain, 73% of participants showed activation in SII, only 24% of which were found to be bilateral, 38% only showing activation in right SII and 38% only in left SII. All participants who had SAM peaks in SII showed clear evoked responses in somatic pain and 75% of participants who had activity in SII in visceral pain showed clear evoked responses.

80% of participants during somatic pain and 38% of participants during visceral pain showed a decrease in both alpha and beta bands after the pain (~200-600ms) and subsequently returned to baseline levels or higher (>600ms) (see Figures 4.15 and 4.20). During experimental pain studies bilateral activation of SII is commonly found in both somatic and visceral pain (Schnitzler et al., 1999, Ploner et al., 1999,

Timmermann et al., 2001). In this study, 55% of participants showed bilateral SII activation during somatic pain whereas only 18% showed bilateral SII activation during visceral pain. It is possible that bilateral activation in SII was not seen due to the limitations in SAM analysis. SAM treats any highly coherent sources as originating from a single location, this enables it to eliminate sources of environmental noise but also may mean that activity is seen in only the dominant hemisphere.

The average latency of the 1st peak of the evoked response in SII was ~76±24ms and ~73±30ms for somatic pain and visceral pain respectively. There was no apparent difference in evoked response latency between somatic and visceral pain in SII unlike SI. Frot et al (1999) found SEPs in SII with peaks at N70 and P90 in response to electrical stimulation of the median nerve at the wrist, these were ~40ms later than the evoked responses seen in SI. During digital electrical stimulation in this study, the latency of the first peak of the evoked response in SI was 25±6ms whereas in SII it was 76±24ms. This agrees with data by Frot et al (1999) that the SII evoked response is later than SI.

4.6.3.2 ACC

SAM peaks were found in the ACC in 55% of participants during somatic pain and 45% of participants during visceral pain (see Table 4.2). During both somatic and visceral innocuous stimuli, only 27% of participants showed activity in the ACC (see Table 4.2). All participants that showed ACC activation during somatic pain had clear evoked responses (see Figure 4.16) whereas only 60% of participants that showed ACC activation during visceral pain showed clear evoked responses (see Figure 4.21). Evoked responses seen in ACC were biphasic with a peak around 146±46ms in somatic pain and 142±50ms in visceral pain. The standard deviation shows that there was inter-individual variability in the latencies however there did not appear to be a difference between somatic and visceral latencies. Evoked responses in the ACC were found using MEG during distal oesophageal electrical stimulation in a study by Hobson et al (2005), the latencies were 104.7±15ms in the perigenual

cingulate, 95.6±11ms in the mid-cingulate and 106.5±22ms in the posterior cingulate. The latencies found in this study were slightly delayed compared to the latencies found by Hobson et al (2005), however there was a large variability in the latencies of the responses in this study.

Evoked potentials/fields from the anterior cingulate have been investigated in a number of studies, although sometimes with conflicting results. It is thought that cortical activity from the ACC is likely to be radial in direction, suggesting that it is harder to pick up with MEG than EEG (Garcia-Larrea et al., 2003, Christmann et al., 2007). This could be the reason that only 39% of participants showed ACC activation during this study, however Hillebrand and Barnes (2002) indicate that this should not be as great a problem as previously stated. Previous EEG studies have found activation in the anterior mid cingulate corresponding to Brodmann Area 24 (BA24) in response to painful electrical stimulation of the thumb (Christmann et al., 2007) and in fMRI using non-painful oeospheal balloon distension (Aziz et al., 2000b). Activation in ACC has also been found using MEG and painful laser stimuli previously (Ploner et al., 2002). Ploner et al (2002) found the first peak in ACC at 188ms and a later peak at 782ms and Christmann et al (2007) saw activation in the ACC at 200ms using EEG. A review by Garcia-Larrea et al (2003) claims that evoked responses to pain in ACC are commonly found later than this at around 325-350ms and are biphasic. The latencies of the evoked responses in this study correspond more closely to the work of Ploner (2002) and Christmann (2007).

Different regions of the cingulate cortex are believed to be involved in different processes (Vogt, 2005) such as pain processing in the pregenual ACC and visceral integration in the subgenual ACC. Aziz et al (2000b) found that both proximal and distal oesophageal balloon distension activated the anterior midcingulate cortex (BA24) whereas only distal activated the rostral perigenual cingulate cortex corresponding to Broadmann area 32. Anterior mid cingulate is most commonly reported but laser evoked potentials (LEPs) have also been reported in more posterior parts of the ACC (Garcia-Larrea et al., 2003). There is no consistent

difference apparent in the spatial location of ACC activity between somatic and visceral stimuliin this study, there is a large amount of variance between individuals in the spatial location of peaks in the cingulate cortex.

An interesting phenomenon is the high gamma power that was seen throughout the trial in both somatic and visceral pain in the ACC (see Figures 4.16, 4.21), this gamma response did not appear to vary in response to the stimulus. It was between ~50-70Hz and was seen in 66% of participants that showed ACC activity in somatic pain and in 20% of participants that showed ACC activity in visceral pain. The reason for this gamma response is unclear, although it could be related to a constant state of anticipation or anxiety of being in a MEG scanner and participating in a pain experiment. Gamma oscillations in ACC has not been reported in the literature.

4.6.3.3 Insula

64% of participants showed activation in the insula during somatic pain, 29% of which showed bilateral activation. During visceral pain, 55% of participants showed activation in the insula, 20% of which were bilateral. All participants that showed activity in the insula in somatic pain had clear evoked responses. 83% of participants that showed activity in the insula during visceral pain had clear evoked responses. The average latency of the peak of the evoked response during somatic pain in the insula was 119±33ms and for visceral pain was 130±39ms. There was no significant difference between these latencies. There were no clear changes apparent in the oscillatory dynamics from VEs in the insula in any subjects apart from an increase at around 5-15Hz which coincided with the evoked response (see Figures 4.17 and 4.22).

There is much controversy in the literature about the difference between SII and insula (Frot and Mauguiere, 2003, Frot et al., 2007). As they are located so closely anatomically, it is often difficult to separate them into different functional regions and are sometimes considered together as the parietal operculum (Sawamoto et al., 2000), or parasylvian cortex (Frot and Mauguiere, 2003). The peak of the evoked

response in SII in this study was 76±24ms and for insula was 119±33ms for somatic pain and 73±30ms for SII and 130±39ms for insula in visceral pain. This indicates that it is possible to observe a delay in latency from SII to insula distinguishing the two regions. Also there was a decrease in the mu rhythm in SII in 73% of participants during somatic pain whereas there were no apparent changes during somatic pain in oscillatory dynamics in the insula.

Frot et al (2003, , 2007) were also able to distinguish between the two regions based on a delay of ~50ms between SII and insular evoked responses. Timmermann et al (2001) found different results in that SII showed little change at sensory levels but increased in amplitude at a level above pain threshold. Frot et al (2007) stated that this finding was probably a mixed signal dominated by insular responses. Bornhovd et al (2002) combined the two areas as SII/posterior insula and saw an increase in the BOLD response with increasing pain intensity in their study, however activation in this area did not correlate with non-painful stimulus intensities.

4.6.4 Methodological issues:

The electrical catheter used for distal oesophageal stimulation caused a large stimulus artefact when stimulating in the MEG data. This made it problematic to look at the raw sensor data initially but ICA was used in order to eliminate the artefact effectively. In some participants, the artefact was localised during SAM analysis to the back of the throat, which then allowed it to be disregarded from further analysis. MEG data using the same equipment as in this study with oesophageal electrical stimulation has been published previously (Hobson et al., 2000a, Hobson et al., 2005).

It is possible that there was an anticipatory response during the rest period as the timing between each pulse was predictable, this may have masked changes in oscillations. However in the previous study, gamma was found to decrease during anticipation, so if an anticipatory response was seen it would be more likely for the gamma to come out as significant during the pain period.

4.7 Conclusion

The increase in gamma oscillations seen during somatic pain did not coincide with the main components of the evoked response. This suggests that the gamma response is not simply a transient synchrony driven by the evoked response but is more likely to contain induced components that are involved in higher order processing of somatic stimuli. Gamma oscillations were not seen during distal oesophageal pain, and it may be that the evoked response seen in SI during visceral pain is indirect activation due to referred pain to a somatic structure. Perhaps the visceral stimulus was not sufficient to drive the SI cortex to oscillate at a gamma frequency due to the temporal response properties of visceral afferents. The evoked responses seen in SI during distal oesophageal stimulation indicate that SI is involved in the processing of visceral stimuli to some degree. The timing of the gamma increase did not coincide consistently with the main components of the evoked response and aspects of the gamma response were found to be induced. These results indicate that the gamma response seen in this study is not purely part of the early evoked response and may be involved in higher order processing of pain.

5 Study 3: Investigating the cortical oscillatory responses to a cold pressor task using Magnetoencephalography

5.1 Abstract:

The cold pressor test (CPT) has been used extensively in cardiovascular and autonomic studies due to its profound effects on blood pressure and heart rate. It is also a valuable tool for assessing pain processing as it is a more biologically relevant stimulus than electrical or laser and is more akin to the chronic pain experienced by many. The aim of this study is to investigate whether the changes in frequency dynamics shown in the previous studies can be replicated during CPT, allowing a deeper understanding of the roles of these oscillations during sensory and pain processing.

During a MEG recording, participants went through a baseline period, a control period using a room temperature ice pack under the palm and then 5 minutes with a cold ice pack. The participants rated their pain on a 0-10 Likert scale throughout CPT.

From bootstrap spectrograms and envelope analysis it was possible to see a decrease in the beta band (15-30Hz) during CPT in SI in 71% of participants. In 29% of participants, beta gradually returned towards the baseline level by the end. A decrease in alpha was seen in 57% of participants in SI. A decrease in both alpha and beta bands was also seen at the onset of CPT in both SII and ACC in a smaller percentage of participants, no change in theta or gamma bands was apparent. Although SAM peaks were found in the insula, there were no obvious changes in any frequency band in this area.

The decrease that was seen in beta and alpha bands in this study matches some previous EEG studies (Chen and Rappelsberger, 1994), others have seen an increase in beta although this was over temporal regions (Chang et al., 2002). No change was apparent across CPT in theta or gamma bands. Comparing this to our

previous studies, it appears that gamma changes are not found across all modalities of pain. It may still have an important role in sensory processing but it appears to be specific to certain stimuli.

5.2 Introduction:

Experimental pain studies have led to a greater understanding of how we perceive pain and the mechanisms underlying it. There are a variety of modalities of experimental pain commonly used such as electrical, thermal and mechanical. All of these have different advantages and disadvantages. Electrical and laser stimulation give the experimenter greater control over the stimulus by enabling them to vary stimulus duration and intensity easily, however the main disadvantage, with electrical stimulation especially, is that it is not a sensation commonly experienced in everyday life. It is an unusual sensation and is transient rather than tonic as most chronic pain is, and so is less biologically relevant (Babiloni et al., 2007) than, for example, thermal pain.

The aim of most experimental pain research is to further understand and improve the conditions for those suffering from chronic pain so it is important to use clinically relevant stimuli where possible. In Studies 1 and 2, electrical stimulation was used and some interesting frequency patterns were observed, especially in the gamma range in SI (see Section 3.6 and 4.5). The aim for this study was to investigate whether these frequency patterns could be replicated using a more ecologically valid and thus clinically relevant stimulus such as CPT.

CPT generally involves immersing the hand (or sometimes foot) in ice cold water for a number of minutes. This has been used for many years in cardiovascular and autonomic studies as it has a profound effect on blood pressure and heart rate, causing them both to increase (Streff et al., 2009).

CPT is known to initiate modulatory pain mechanisms within the brain (Streff et al., 2009). There are two main modulatory mechanisms: one involves a spino-bulbo-

spinal feedback loop and is known as diffuse noxious inhibitory control (DNIC) and the other involves the periaqueductal gray and rostroventral medulla (Song et al., 2006). DNIC works by inhibiting nociceptive dorsal horn activity and therefore controlling the amount of pain that is subsequently perceived.

As in other forms of experimental pain, CPT has been found to activate all areas involved in the pain neuromatrix: contralateral SI, bilateral SII, ACC and insula as well as areas of frontal cortex using fMRI (Fulbright et al., 2001) and PET (Frankenstein et al., 2001, Petrovic et al., 2002b).

EEG has been used to investigate CPT and is able to offer a high temporal resolution compared to fMRI or PET. Generally in these studies, a decrease in the alpha frequency band was seen as well as an increase in the higher beta band (Chang et al., 2002) (see Table 5.1). Theta has been found to decrease during cold stimuli and increase post-CPT but not during warm stimuli, possibly due to the more unpleasant nature of cold water to warm and therefore a different emotional response (Chang et al., 2005). Gamma oscillations are not mentioned in the majority of past studies, although in one report it was characterised as EMG artefact with a similar temporal pattern to when the participant made a wincing facial expression (Dowman et al., 2008). Details of neuroimaging CPT studies can be seen in Table 5.1.

Paper	Stimulus	Imaging technique	Areas of cortex activated	Oscillatory changes
Backonja 1991	Hand immersion in cool and painfully cold water (5 mins)	EEG	Bilateral frontal and posterior regions	Increase in alpha and beta
Chang 2002	Immersion of left hand up to wrist in ice water (2°C) for 3 min	EEG	Frontal, posterior, bi-temporal	Delta and theta increased in frontal areas, alpha decreased in posterior, beta increased in bi-temporal regions
Chang 2005	Non-painful warm (40-43°C) and cold (12-15°C) water on left hand for 2 mins	EEG	Frontal and posterior regions	Increase in theta in contralateral frontal area, alpha decrease posteriorly with rebound after end of CPT
Chen 1989	Both arms inserted into 1°C water bath for max of 3 mins	EEG	Frontal, temporal, parietal, occipital	Increased delta and beta power during cold pain
Chen 1994	Ice cube on hand for 2 mins	EEG	Central and precentral regions	Decrease in theta and alpha in central and precentral areas, increase of high beta
Chen 1998	Right hand submerged up to wrist in non-painful (15°C) and painful (0.3°C)cold	EEG	Frontal and central regions	Decrease in theta coherence over frontal areas, increased coherence in alpha between central and frontal areas
Dowman 2008	Left hand in ice water (~4°C) for 10 mins	EEG	Temporal and posterior regions	Alpha decreased over contralateral temporal and increased posteriorly, gamma increase
Ferracuti 1994	Hand immersed in painful cold water (0°C)	EEG	Parietal and frontal regions	Alpha decrease in contralateral parietal regions, delta increased bilaterally in frontal regions
Frankenstein 2001	Cold compress on right foot during attention and verbal distraction task	fMRI	ACC (BA24 and BA32)	n/a
Fulbright 2001	Cold water to foot (started non-painful at 14-20°C and went down to painful at 3-8°C)	fMRI	Bilateral postcentral gyrus, SII, frontal lobe, left insula, left thalamus, ACC	n/a
Greenspan 2008	Water bath switched from 31°C to 5°C for 2s then back	Subdural electrodes over parasylvian cortex	SII/Insula	n/a
Petrovic 2002	Left hand immersed in painfully cold water (0°C) and nonpainful water (20°C) for 2 mins	PET	SI	n/a

Table 5:1 gives an overview of key CPT neuroimaging papers

Cold pressor pain is generally thought to be derived from activation of deep C-fibres (Chang et al., 2002) whereas focal laser and electrical stimuli activate mainly Aδ fibres. However some studies have reported that Aδ fibres are also activated during cold pain (Simone and Kajander, 1997). The differences in fibre type activation between electrical and cold pressor stimulation may lead to differences in the cortical oscillatory dynamics.

Different areas of the pain matrix have been found to be active during cold tonic pain such as SII (Greenspan et al., 2008), ACC (Casey et al., 1996, Frankenstein et al., 2001) and Insula (Craig et al., 2000) using different neuroimaging techniques such as intracortical electrodes, PET and fMRI. These studies allow the areas involved to be elucidated but tell us little about the oscillatory dynamics. EEG studies have been conducted investigating how oscillatory dynamics change but mostly at sensor level without using any source analysis (Backonja et al., 1991, Ferracuti et al., 1994, Chen et al., 1998, Chang et al., 2005).

Using new analysis methods (Sekihara et al., 2002), it is possible to provide more detailed spatial information as well as temporal information with neuroimaging techniques such as EEG and MEG. As CPT is thought to be more biologically relevant than electrical stimulation, it is important to investigate how all frequency bands change during CPT, and to understand more about their role in pain perception (Chang et al., 2005, Gross et al., 2007).

SI is known to be an important component of the pain neuromatrix (Timmermann et al., 2001). In Studies 1 and 2, activation was found in both contralateral and ipsilateral SI. An increase in power in the gamma band was seen in the contralateral SI during somatic electrical stimulation but not during visceral stimulation. It is not yet understood why gamma oscillations were only seen during somatic electrical stimulation, using a different type of somatic stimulus may help us understand these differences.

5.3 Experimental Rationale

This study aimed to investigate the oscillatory dynamics during a more tonic, clinically relevant somatic pain; cold pain using an ice pack (a variation of CPT). These oscillatory dynamics could then be compared to those seen during electrical stimuli to see if there were any clear similarities or differences that might further define their role in somatosensory or pain processing. Also, in the CPT literature, the focus has

been on changes in frequency power at the sensor level (Chen and Rappelsberger, 1994), this study will apply source analysis to the data in order to see how the frequency dynamics change at particular locations in the cortex thought to be involved in pain processing.

5.4 Methods:

5.4.1 Participants:

12 healthy participants (5 male; age range= 23-42 years) took part in this study. All were free of any neurological or pain disorders and none were taking medication at the time of the study. Anatomical coregistration with MRIs were taken for 7 of these participants for MEG analysis (3 males; age range=23-35yrs). Informed consent was obtained from all participants and the local ethics committee approved the experimental protocol.

5.4.2 Stimulus:

The stimulus used was an ice pack measuring approximately 15cmx15cmx3cm which was placed under the hand (palm down) on a flat surface and an identical pack, at room temperature, was placed over the dorsum of the hand in order to apply a constant pressure to the hand ensuring maximum skin contact with the ice pack. For the control period a room temperature ice pack was used underneath the palm as well as another on top.

5.4.3 Experimental Procedure:

Three minutes of baseline were recorded at the start in which the participant remained relaxed and still (with the hand in the correct position prior to commencement). This was followed by three minutes in which a room temperature ice pack was placed under the participants hand by a researcher to avoid as much muscle tension by the participant as possible. Following this the room temperature ice

pack was removed and replaced by a frozen ice pack by a researcher (see Figure 5.1).

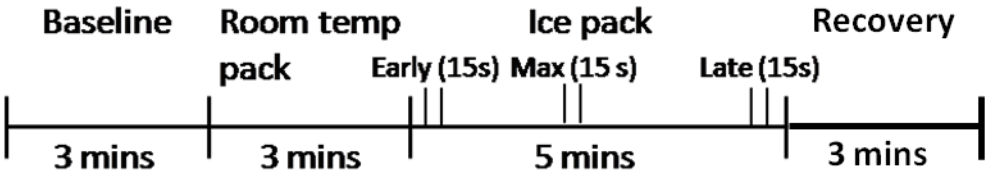


Figure 5:1 shows an illustration of the protocol with baseline, room temp pack, ice pack phases and recovery phase and within the ice pack phase: early, maximum and late which were 15s periods used for analysis.

Participants were instructed to indicate verbally on a Likert scale (Cruccu et al., 2004) what sensation/pain they felt as soon as the ice pack was placed under their hand and then every time they felt it changed to a different score throughout the 5 minutes. The Likery scale is a 0-10 scale which has words indicating different intensities of sensation and pain (0=no change, 1=slight cool, 2=cool, 3=cold, no pain, 4=slight pain, 5=mild pain, 6=moderate pain, 7=moderate-strong pain, 8=strong pain, 9=severe pain, 10=unbearable pain). A marker was manually added to the MEG recording for every verbal report and the Likert scale level recorded.

The ice pack remained under the hand for a maximum of five minutes although the participants had been told that they could ask for the pack to be removed at anytime should the pain become intolerable. All participants managed to continue for the full five minutes. After the ice pack had been removed, a three minute recovery period was recorded. The recording lasted a total of 16 minutes altogether.

5.4.4 MEG recordings:

Participants were seated in a magnetically shielded room. Neural activity was recorded using a 275-channel CTF MEG system (CTF Systems Inc, Vancouver, Canada) at a sampling rate of 600Hz in 16 epochs of 60 seconds. Preprocessing was completed using 3rd gradient noise reduction and removing the DC offset based on the whole trial (see Chapter 2: Section 2.2.3 for details). The 50Hz power line was

taken out with a width of 0.6Hz. The trials were scanned for blink artifacts but none were considered to cluster consistently across trials and so were not removed.

5.4.5 Coregistration:

A 3-dimensional digitizer (Polhemus isotrak system, Kaiser Aerospace Inc, Colchester, Vermont, USA) was used to digitize the surface of the participants head and this information was then coregistered with 7 of the participants previously obtained anatomical MRI which gives accuracy within 5mm (Singh et al., 1997) (see Chapter 2: Section 2.2.4 for details).

5.4.6 Data Analysis:

5.4.6.1 Synthetic Aperture Magnetometry (SAM):

As the dataset was made up of only one trial, it was necessary to create a number of smaller trials with markers in order for the data to be averaged and SAM analysis to be performed (see Chapter 2: Section 2.2.6 for details). 15s were found towards the end of the 3 minutes of the room temperature ice pack that were without artefact which were to be used as the baseline period. Three 15s periods during the cold ice pack were used for analysis; one as early on during the cold ice pack period as possible without any movement artefacts (Early), one immediately after the highest Likert rating for that individual (Max), and one just prior to the ice pack being removed (Late) (see Figure 5.1). Thirty markers at 0.5s intervals were placed across each of these 15s periods.

Using SAM analysis, the baseline period was compared to early, maximum and late periods of the cold pressor. The frequency bands used were 3-7Hz (Theta), 7-14Hz (Alpha), 15-30Hz (Beta) and 30-100Hz (Gamma).

5.4.6.2 Time-Frequency Analysis (Spectrograms):

Peaks were found in each individual's SAM comparisons (pseudo t≥1) in SI, SII, ACC and insula. VEs from those coordinates were used to create Spectrograms (see Chapter 2: Section 2.2.7). Bootstrap spectrograms were created (see Chapter 2: Section 2.2.7.2) comparing 30x0.5s trials of baseline with 30x0.5s trials of early, middle and late cold pressor period using the 1-100Hz bandwidth window.

Envelopes were used to give more detailed temporal information (see Chapter 2: Section 2.2.7.4). They created a profile of the change in theta, alpha, beta and gamma frequency bands and how they varied across the duration of the experiment, especially during the 5 mins of CPT. An envelope demonstrates how a particular frequency band changes across a period of time. The data was read in and weighted to a particular location (VE) in the cortex that was specified by the covariance matrix within a weights file previously created. The data was band pass filtered to a particular frequency band over the selected time interval. The RMS of the power of each sample was then calculated which made every value positive, this allowed the visualisation of comparative power change in the frequency band across the time interval.

5.5 Results:

5.5.1 Pain thresholds:

All participants tolerated the cold pressor stimulus for the entire 5 minutes. The Likert ratings during the cold ice pack varied substantially between individuals, some went up to a maximum score of 9/10 whereas others only reached 4/10. It took 67% of participants less than 1 minute to reach their highest Likert score (range=18-224s), the pain increased rapidly during this time but from then on it tended to plateau out and remained at a constant level or even decreased slightly for the remainder of the recording (see Figure 5.2).

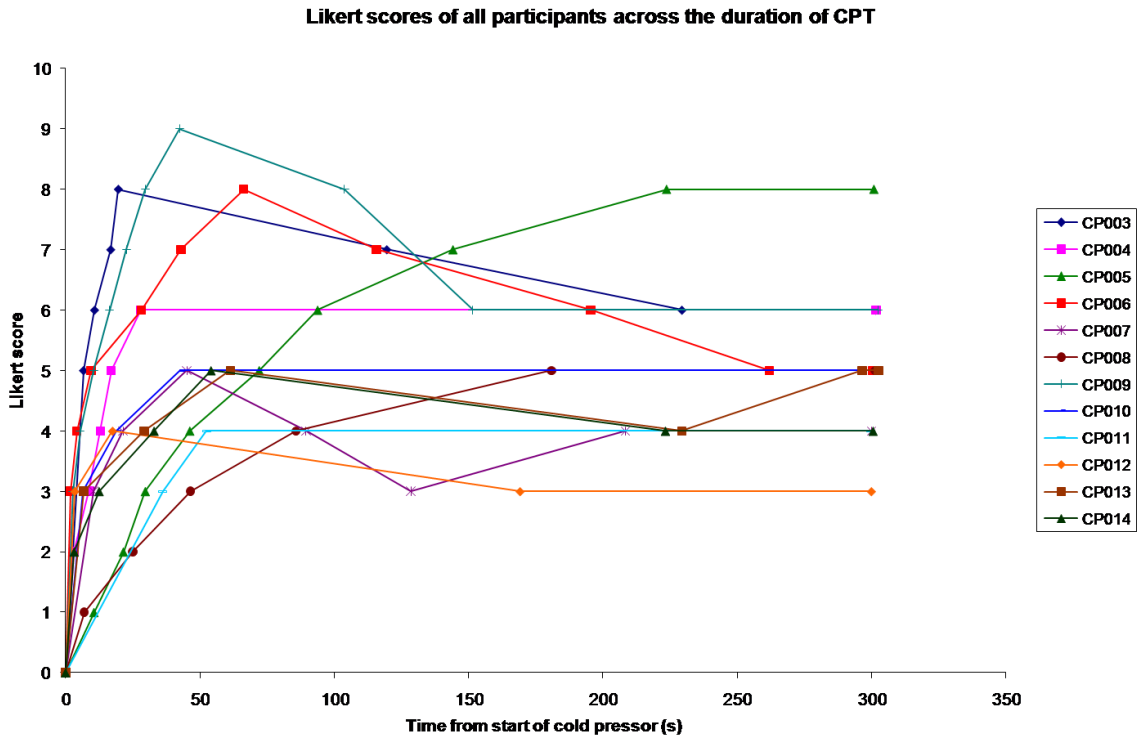


Figure 5:2 shows how the Likert scores changed across the duration of CPT in all participants.

5.5.2 SAM activation:

SAM peaks were found in key areas of the pain matrix (SI, SII, ACC, Insula) in all participants across different stages of CPT (Early, Max, Late). Group SAM data can be seen in Figure 5.3.

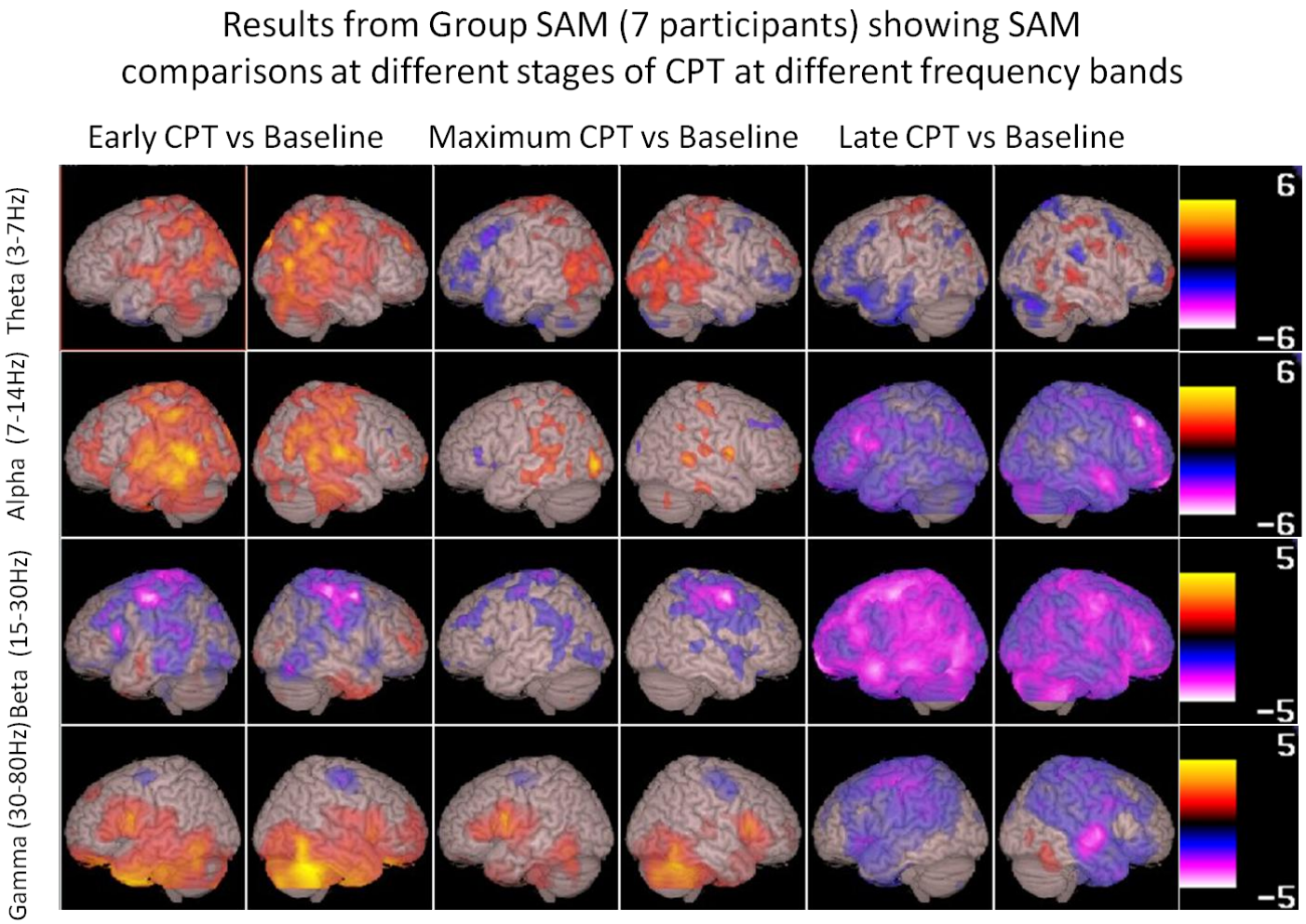


Figure 5:3 shows the Group SAM data at all frequency bands and during 3 different SAM comparisons; early stage of CPT, max Likert score and late stage of CPT. Decreases in power can be seen in purple/white and increases in yellow/orange. In order to view activity from the interior of the brain, it was brought to the surface using a surface rendering function. Widespread increases can be seen in both theta and alpha during the early stage, this changes to a decrease in alpha power in the later stage. A focal decrease can be seen in beta over the somatosensory cortex at all stages.

A decrease over the somatosensory cortex was apparent in the beta band at the early and maximum stages of CPT. Group SAM during this study was not as informative as in previous studies, it was necessary to return to the individual's SAM peaks in order to inform further analysis. No significant peaks were found from SnPM data in all frequency bands (3-7Hz, 7-14Hz, 15-30Hz, 30-80Hz).

5.5.3 Spectrograms:

In 71% of participants, when comparing all stages of CPT to baseline using bootstrap spectrograms and envelope analysis, there was a decrease in the 15-30Hz band and in 57% of participants a decrease around 10Hz (Figure 5.4, 5.7) in SI. In 29% of participants this beta power appeared to return to baseline levels towards the end of CPT (see Figure 5.7). A decrease could also be seen in alpha power in the bootstrap spectrograms in 57% of participants.

There was no apparent change in gamma or theta in SI across the duration of CPT from bootstrap spectrograms (see Figure 5.4), envelope analysis was done to explore this further.

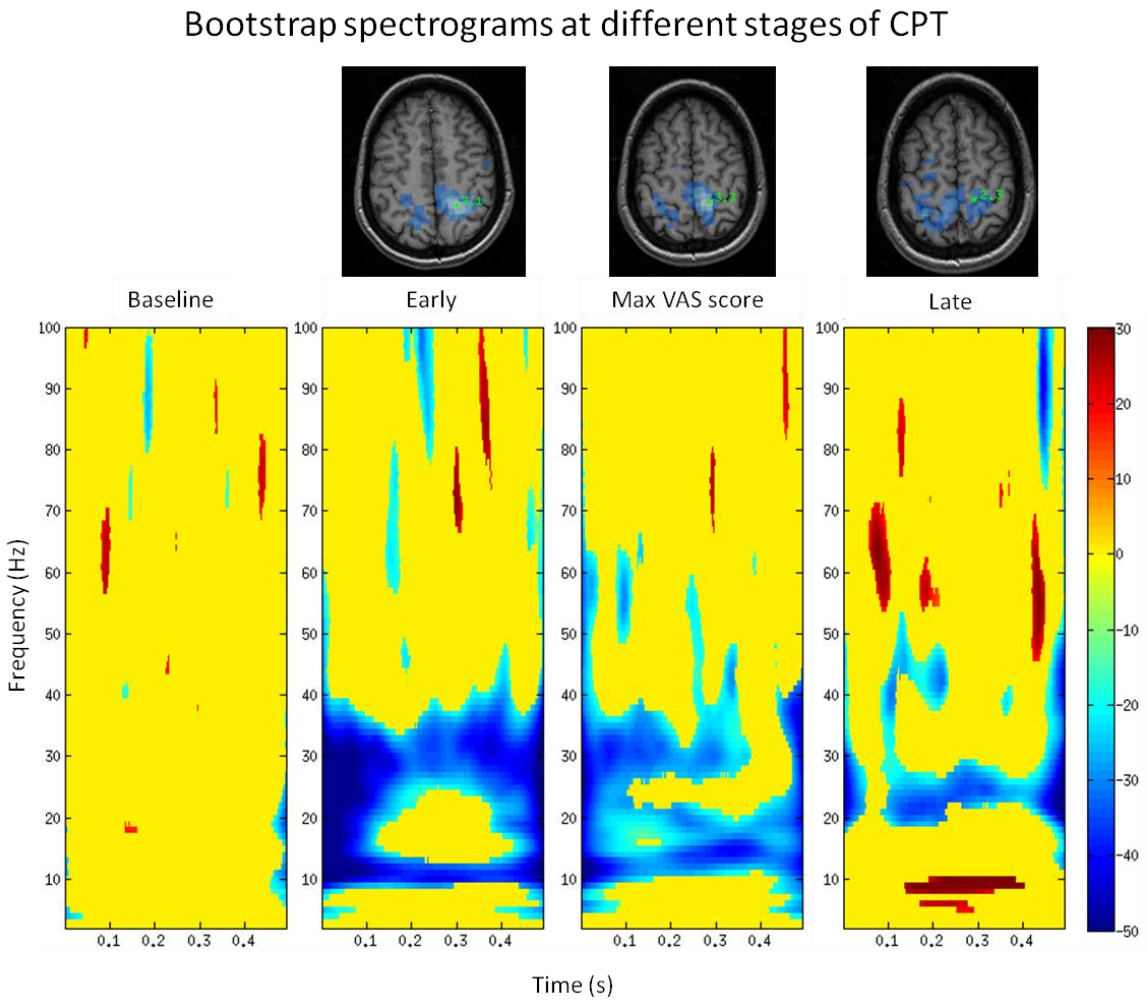


Figure 5:4 shows bootstrap spectrograms taken from VEs in SI in one representative individual (CP010). The first figure shows baseline activity, the other 3 show different stages of the CPT; Early, Maximum Likert score and Late. Each are averaged over 30 trials of 500ms. The scale on the right is percentage change compared to baseline, a decrease in power is shown as blue whereas an increase is seen as red. A decrease can be seen between 10-40Hz covering alpha and beta ranges during the early stage of CPT. This decrease seems to return towards baseline levels as CPT continues.

5.5.4 Envelope analysis

Peaks were chosen for VEs from SAM analysis that were in SI, SII, ACC and Insula and had a pseudo t value of ≥1. Four different frequency bands were investigated; theta (3-7Hz), alpha (7-14Hz), beta (15-30Hz) and gamma (30-100Hz). An envelope analysis was used in order to investigate how each frequency band varied across 2

minutes of the room temperature pack and the ice pack (5 mins) in key areas of the pain matrix.

5.5.4.1 Primary Somatosensory Cortex

Figure 5.5 shows the changes in theta band across the room temperature pack and cold ice pack in SI in all participants that showed SAM peaks in this region. There appears to be no clear change in theta between room temperature and CPT, it also appears not to change across the 5 minute duration of CPT.

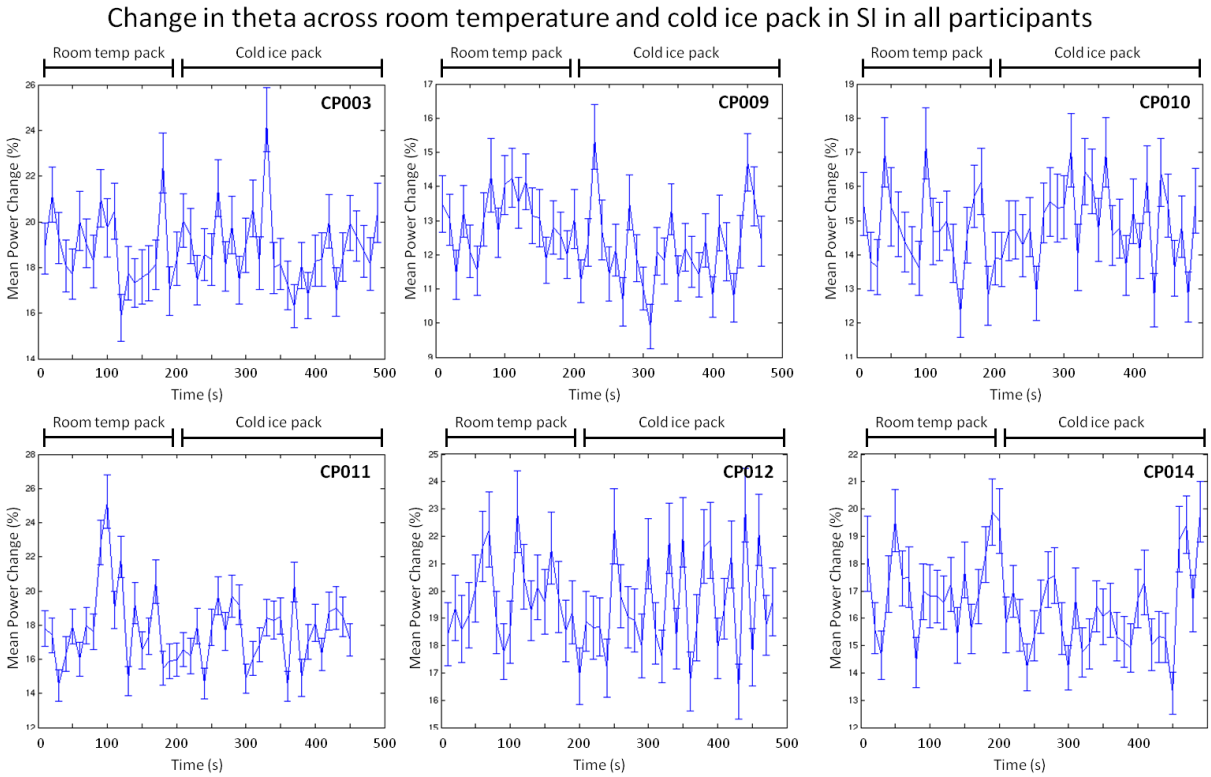


Figure 5:5 is of envelope analysis in the theta band from VEs in SI showing how theta changes across 3 minutes of the room temperature pack followed by 5 minutes of the ice pack. Each figure represents one participant.

At the onset of CPT in this study in SI, alpha power appeared to increase during the room temperature pack and then was seen to decrease at the onset of CPT in 57% of participants (CP003, CP010, CP011, CP012) (see Figure 5.6).

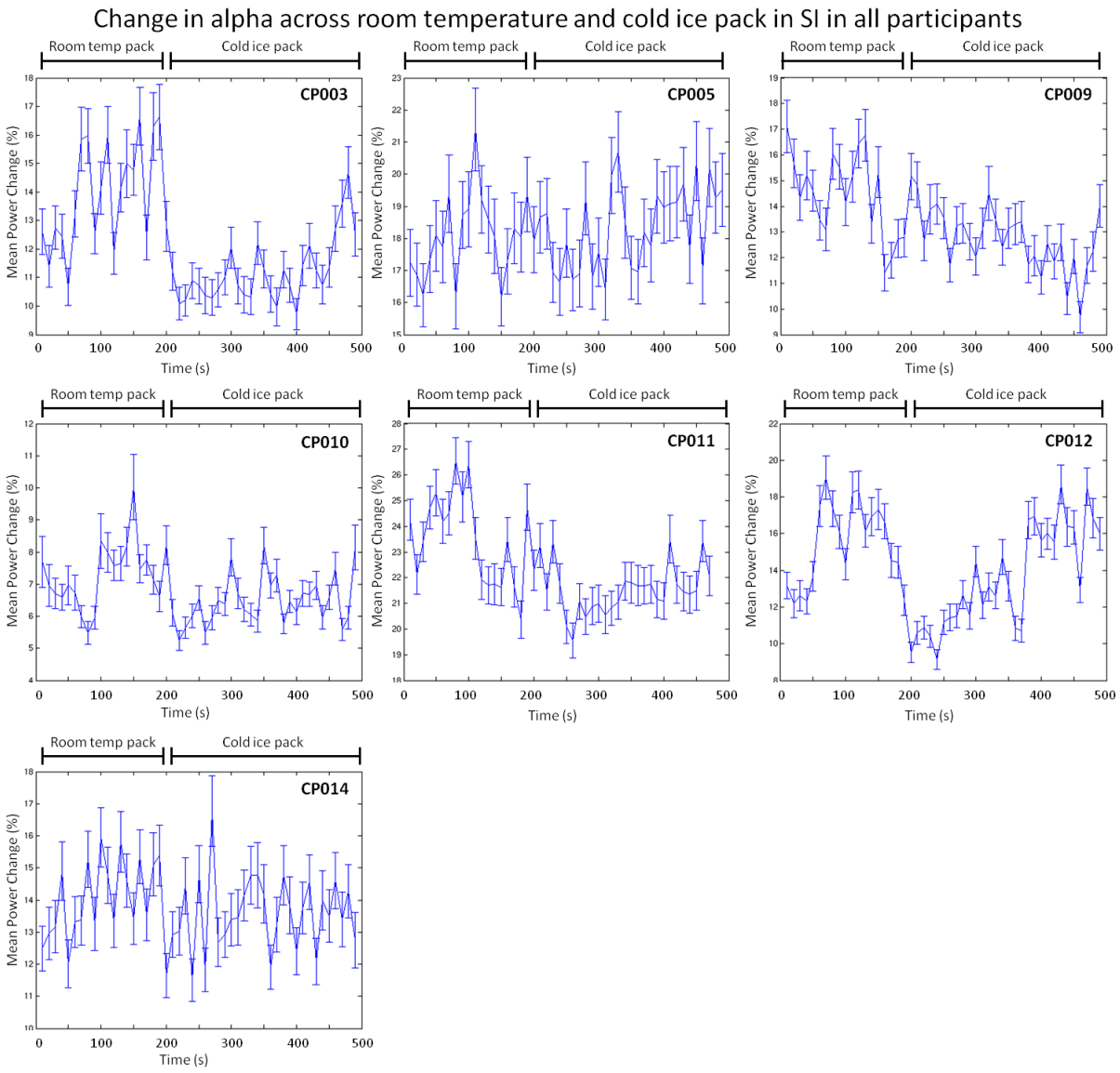


Figure 5:6 shows envelope analysis on the alpha band from VEs in SI showing how this frequency bands power changes across 3 minutes of the room temperature pack followed by 5 minutes of the ice pack.

Figure 5.7 shows how beta changed across the room temperature pack and CPT. A sharp decrease in beta power was seen after the ice pack was placed under the hand in 71% of participants (CP003, CP009, CP010, CP011, CP012). In 29% of participants, this then gradually increased back to baseline levels (CP003. CP012).

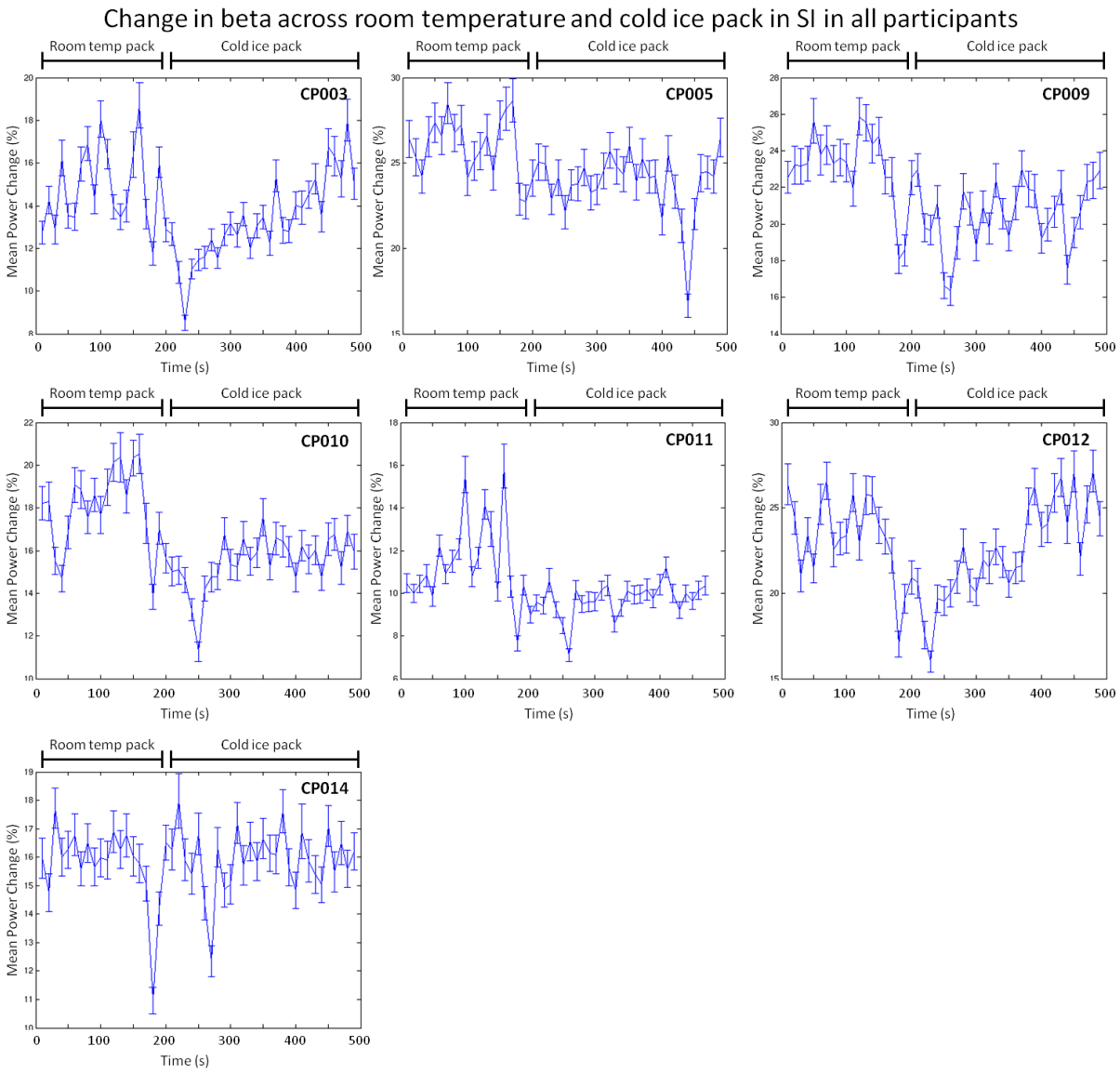


Figure 5:7 shows the change in beta (15-30Hz) in SI across 3 minutes of room temperature pack followed by 5 minutes of ice pack in all participants. 5 participants show a decrease in beta at the onset of CPT in 2 participants the beta activity appears to return to baseline levels at the end of CPT.

Envelope analysis in the gamma frequency band (30-100Hz) (shown in Figure 5.8) illustrates the profile of gamma oscillations across CPT across different participants. Little change can be seen in the gamma oscillations across the room temperature pack and cold ice pack. The increase seen in CP010 may be due to a movement artefact when the ice packs were switched.

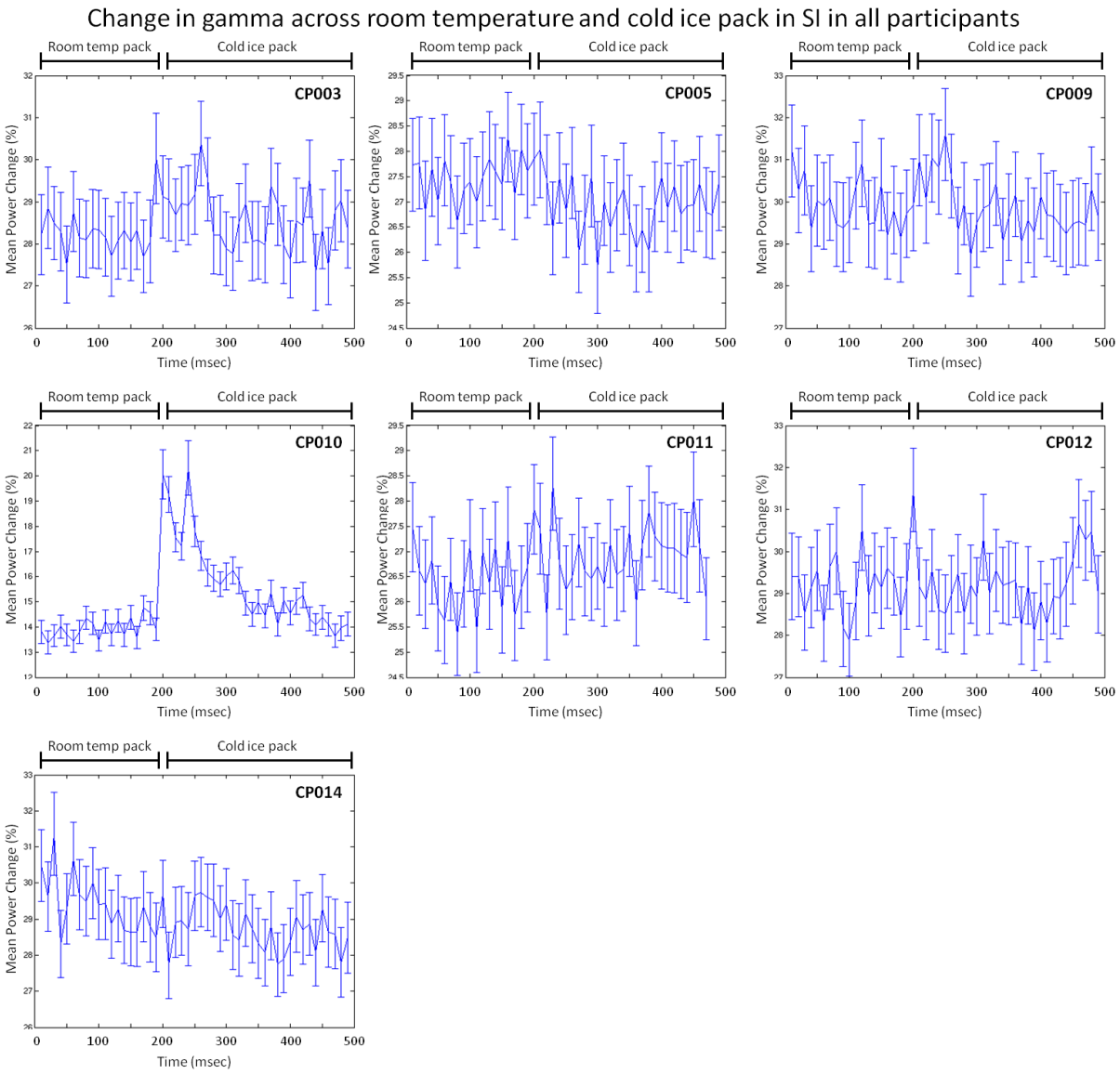


Figure 5:8 shows an envelope which shows the change in the gamma frequency band in SI across 3 minutes of room temperature pack and 5 minutes of ice pack.

5.5.4.1.1 Secondary Somatosensory Cortex

There was no obvious change in either theta or gamma frequency bands in SII across the recording. Changes in alpha and beta bands are shown below (Figures 5.9, 5.10). In Figure 5.9, alpha appears to increase during the room temperature pack in 43% of participants (CP003, CP010, CP011), and then decrease at the onset of CPT.

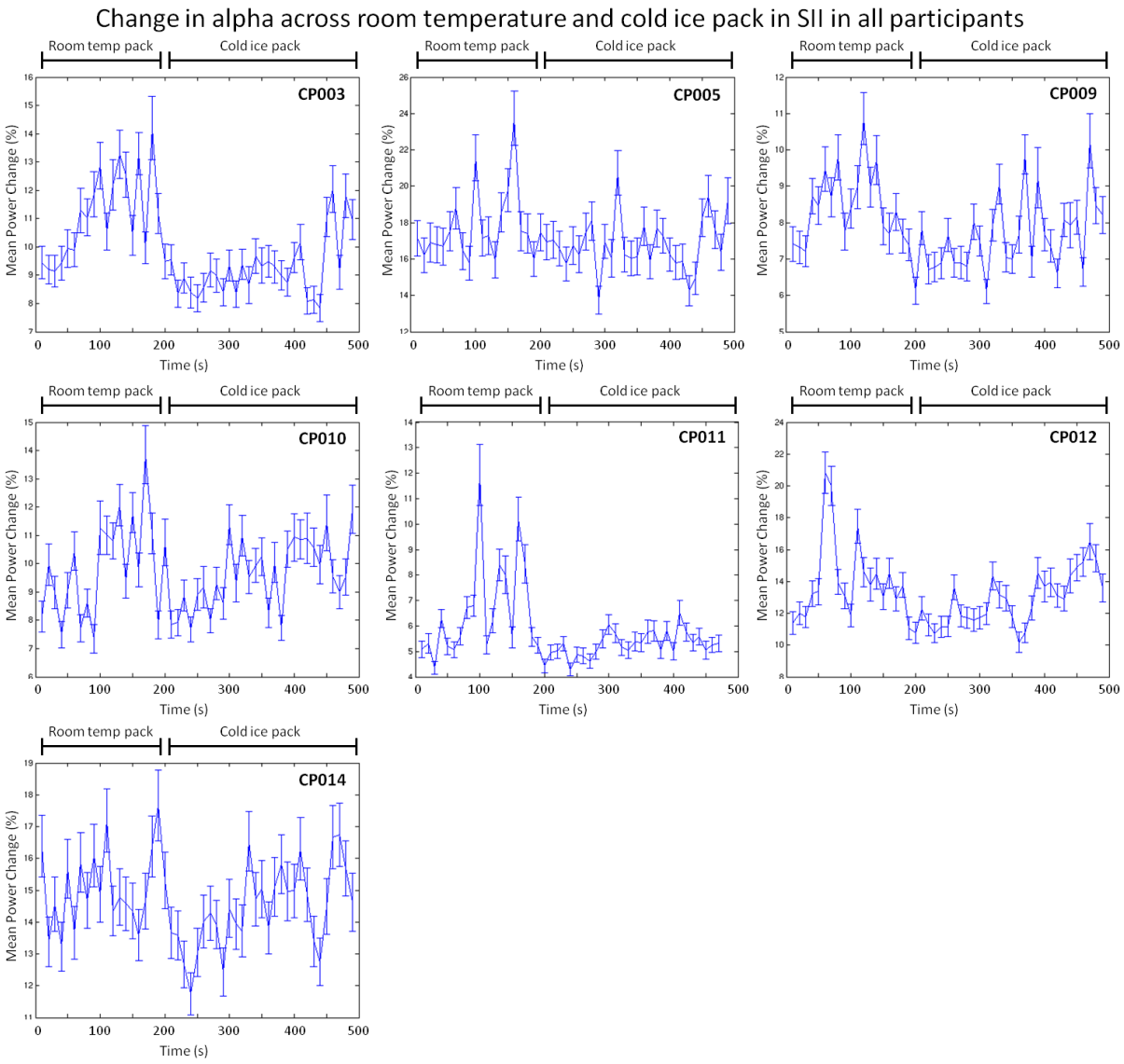


Figure 5:9 shows changes in alpha across the room temperature pack and the cold ice pack in SII across all participants.

In Figure 5.10, the changes in beta frequency band in SII can be seen across all participants. 29% of participants show a decrease in beta at the onset of CPT (CP003, CP011) similar to that seen in alpha.

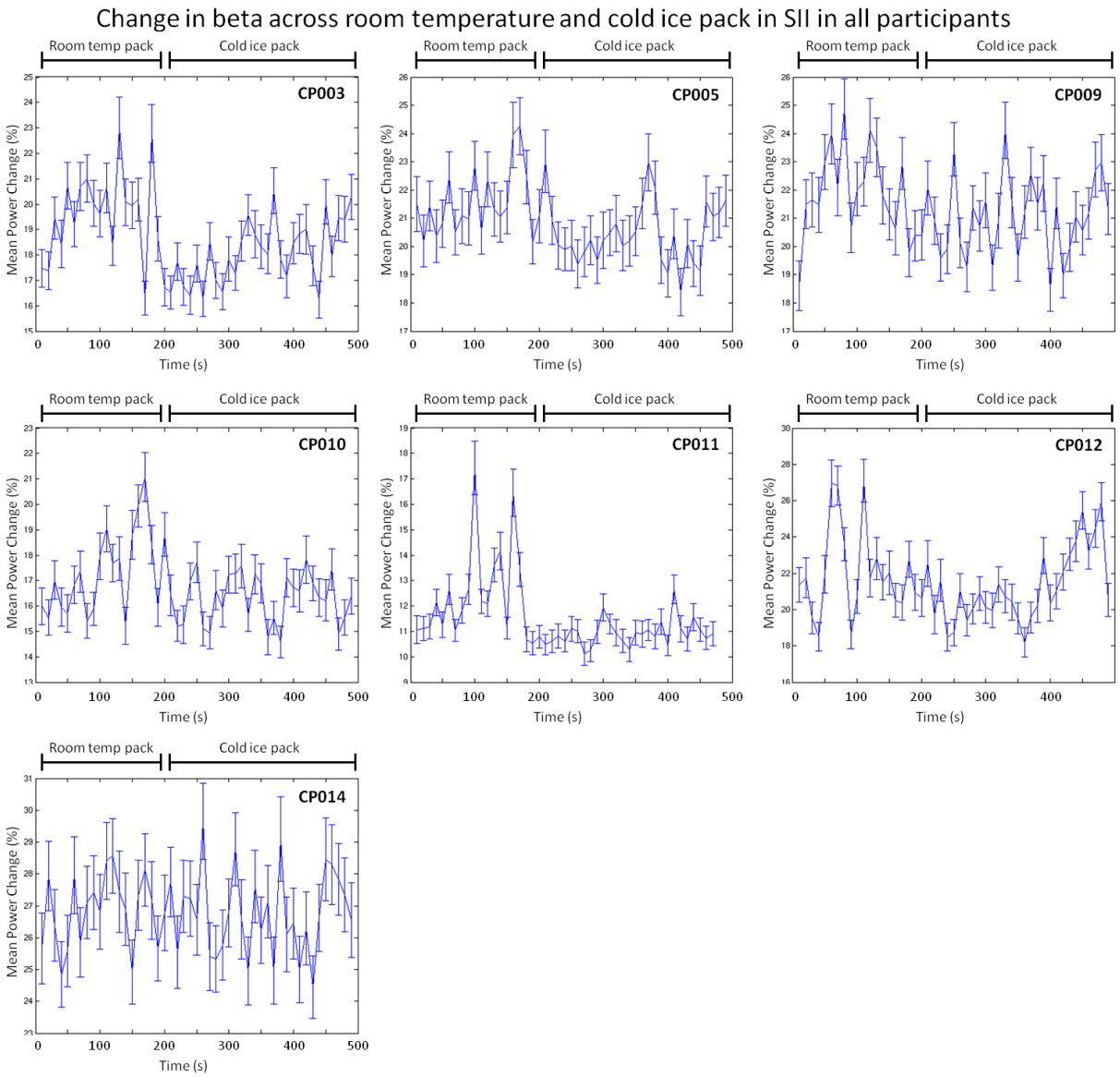


Figure 5:10 shows change in beta in all participants in SII across both room temperature and cold ice pack in all participants

5.5.4.1.2 Anterior Cingulate Cortex

No changes were apparent in theta or gamma bands across both room temperature and cold ice pack. However, 1 or 2 participants showed changes in alpha and beta frequency ranges at the onset of CPT as can be seen in Figure 5.11, 5.12. In Figure 5.11, 29% of participants (CP003, CP010) show high alpha during the room temperature pack which then decreases at the onset of the cold ice pack.

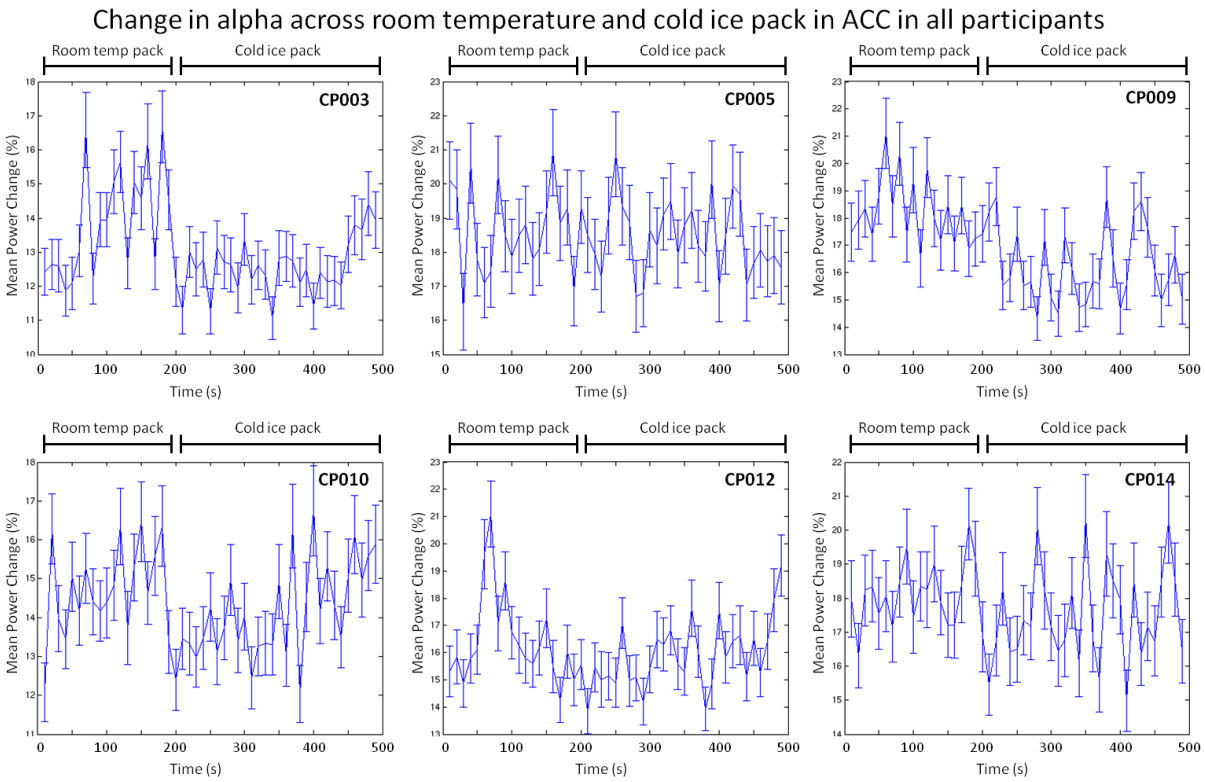


Figure 5:11 shows the changes in alpha across room temperature and cold ice pack from VEs in the ACC in all participants.

In Figure 5.12, 29% of participants (CP003, CP010) showed a decrease in beta at the onset of the cold ice pack.

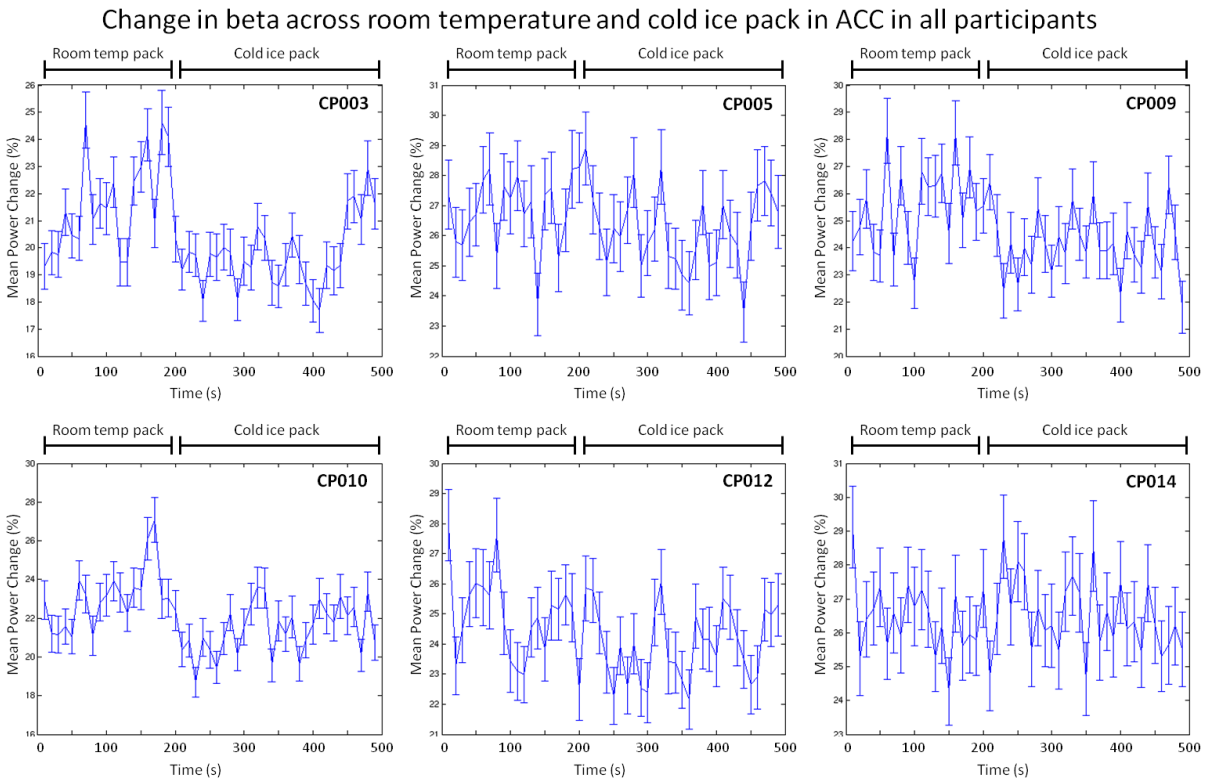


Figure 5:12 shows changes in beta across both room temperature and cold ice pack in VEs from the ACC in all participants

No changes were apparent in any participants at any frequency band from VEs in the insula.

5.6 Discussion:

5.6.1 Summary of key findings:

CPT is a very different quality of pain to electrical stimulus induced pain; it is thermal, thus recruiting different receptors and afferent nerve fibres, it is a more sustained, tonic stimulus, and is more ecologically valid and similar in quality to clinical pain (Chen et al., 1989). SAM peaks were found in key areas of the pain matrix; SI, SII, ACC and Insula. Changes were seen in alpha and beta bands during the recording. An increase in alpha was seen during the room temperature pack followed by a sharp decrease at the onset of the cold ice pack in 57% of participants in SI, 43% of participants in SII and 29% of participants in ACC. A decrease was also seen in beta

at the onset of the cold ice pack in SI, SII and ACC, 71% of participants in SI, 43% of participants in SII and 14% in ACC. In 29% of participants in SI, the beta appeared to return to baseline levels by the end of the 5 minutes of the cold ice pack.

5.6.1.1 Primary Somatosensory Cortex

5.6.1.1.1 Theta

There was no apparent change in theta in SI during CPT compared to baseline in this study (see Figure 5.5). The lack of change in theta was surprising considering previous literature on theta during CPT. Theta has been found to decrease during CPT in a number of studies using sensor space EEG recordings, especially in frontal areas (Chen et al., 1998, Chang et al., 2002, Chang et al., 2005) and central areas (Chen and Rappelsberger, 1994). However, some have seen an increase in theta in frontal areas during CPT (Ferracuti et al., 1994). It is hypothesised that low-frequency oscillations are a response to the stress created by CPT and may be involved in negative emotions (Chang et al., 2002). There may have been more global changes in theta across the cortex in this study which were not clear from the source space analysis used. There is also the possibility that changes in theta could not be found due to the fact that the recording was one long trial rather than many repeated trials.

5.6.1.1.2 Alpha

From the envelope analysis, an increase could be seen in alpha in SI during the room temperature pack period which then decreased when the ice pack was placed under the hand. This pattern was present in 57% of participants (see Figure 5.6). The increase seen during the room temperature pack may have been due to a relaxation as it required the participant to sit still and quiet in a darkened room. It is possible that the increase seen in alpha during the room temperature ice pack was due to a decrease in attention and arousal (Pfurtscheller and Lopes da Silva, 1999). The majority of participants were non-naïve and had participated in many MEG experiments previously and would therefore feel very relaxed during this time. The

decrease when the ice pack was placed under the hand is likely to indicate a higher level of arousal and attention to the painful stimulus. A similar decrease has been seen in response to CPT previously in the vicinity of the central gyrus (Chen and Rappelsberger, 1994, Dowman et al., 2008) and in posterior regions (Chang et al., 2002). A more diffuse decrease in alpha has been seen over the cortex in response to CPT (Ferracuti et al., 1994) which has been linked to a generic increase in arousal of the individual (Pfurtscheller and Lopes da Silva, 1999).

5.6.1.1.3 Beta

CPT induced a decrease in the beta band clearly in the SI of 71% of participants (see Figure 5.7), which in 29% of participants (CP003, CP012) appeared to gradually return to baseline across the 5 minutes (see Figure 5.7). Beta has been reported to change phasically in response to movement and following somatosensory stimuli in the somatosensory and motor cortex (Raij et al., 2004, Ohara et al., 2006) which is consistent with findings in this study. Beta has been found to increase during CPT; in Chang et al (2002), an increase was seen over peripheral bi-temporal regions which they interpreted as a hyperarousal due to the tonic pain. In Chen et al (1994) an increase was seen at a higher beta band (24.5-31.5Hz) over temporal regions and away from the central gyrus, in the same study a decrease in lower beta bands was seen (13-18Hz and 18.5-24Hz) in the contralateral side above the central gyrus.

The beta power in SI in this study appeared to gradually return to normal towards the end of CPT in 29% of participants (CP003, CP012), this change in beta oscillations could have been a reflection of the activation of inhibitory feedback mechanisms triggered by CPT (Streff et al., 2009) diminishing the pain felt, this was reflected in their Likert scales (see Figure 5.2), however other participants Likert ratings decreased towards the end of CPT but did not show this return to baseline in the beta band. It could also be due to peripheral receptors adapting to the stimulus (Stein et al., 2009).

5.6.1.1.4 Gamma

During CPT in this study, no change in the gamma frequency band in SI was apparent across the room temperature pack or the cold ice pack (see Figures 5.4, 5.8). This was in contrast to the gamma oscillations seen in Studies 1 and 2 in response to somatic electrical pain.

The lack of change in gamma oscillations during CPT leads us to believe that it cannot be generalised to different types of somatic pain. In Study 2, an increase in gamma oscillations were seen in response to somatic electrical pain but not visceral electrical pain. The role of gamma oscillations in somatosensory processing needs to be reassessed, it may be involved in another sensory-discriminative aspect of processing such as stimulus intensity rather than whether a stimulus is noxious. In Study 1, gamma oscillations were seen during both pain and sensation in some participants although the strength of the gamma oscillation was stronger during pain. SI activation has been found to correlate with stimulus intensity previously (Bornhovd et al., 2002), perhaps gamma oscillations are able to encode the intensity of somatic stimuli. Gamma has been found to encode information about the spatial frequency of visual stimuli within the temporal characteristic of its oscillations (Hadjipapas et al., 2007). This implies that it is quite plausible that aspects of the gamma oscillations such as frequency or intensity may encode features of sensory stimuli.

It is a possibility that a high phasic synchrony is necessary, together with a high intensity stimulus in order to measure gamma oscillations above baseline and that that is why no changes in gamma oscillations were evident in this study. Due to the nature of the stimulus, only one trial was collected and therefore changes in oscillatory dynamics may not have been obvious above the noise intrinsic in the MEG data.

A possibility for why CPT did not initiate changes in the gamma band in this study is that it is known to trigger inhibitory feedback mechanisms in the nervous system (DNIC) (Song et al., 2006)(see Section 5.2). This is thought to reduce the pain experienced by inhibiting the nociceptive dorsal horn activity (Streff et al., 2009).

These inhibitory mechanisms may diminish the pain felt during CPT, this in turn may affect the oscillatory dynamics of the cortex. Gamma oscillations have previously been linked to some form of top down inhibition of pain perceived by De Pascalis et al (2004). In this study during focused analgesia induced by hypnosis, gamma oscillations were found to be significantly reduced (De Pascalis et al., 2004). This may suggest that gamma oscillations are affected by or have a role in the amount of pain perceived due to central inhibitory controls.

5.6.1.2 Other areas of the pain matrix

This study was able to use the source analysis technique of SAM to investigate oscillatory dynamics in specific areas of cortex that were activated. Changes in both alpha and beta band were seen in SII and ACC (see Figures 5.9-5.12) in a small number of participants. 43% of participants showed a change in alpha in SII and 29% in ACC. 29% of participants showed a change in beta in SII and 14% in ACC. A decrease in alpha was seen at the onset of the cold ice pack in SI, SII and ACC (Figures 5.6, 5.9, 5.11). This widespread decrease across different areas of the cortex was likely to be associated with an increase in arousal levels across the cortex (Hari and Salmelin, 1997) due to the painful nature of the cold ice pack.

SII is believed to have a role in the sensory-discriminative aspects of pain processing (Melzack and Casey, 1968) and activity in this area has been found to increase with increasing stimulus intensity (Timmermann et al., 2001). The decrease seen in alpha and beta in this study in response to the cold ice pack indicate that this area is involved in processing pain and the decrease in alpha can be associated with an increase in arousal and attention, SII activity has previously been found to increase with increasing attention to pain (Mima et al., 1998, Nakamura et al., 2002).

The changes seen in alpha and beta in the ACC in 29% of participants showed that this area was activated and changes in oscillatory dynamics occurred in response to the painful cold stimulus. The ACC is believed to be involved in the emotional processing of pain (Vogt, 2005) and as the cold stimulus provides a tonic, sustained,

ecologically valid pain stimulus it is not surprising that affective areas such as the ACC are involved in its processing. It is not clear why only 29% of participants showed changes in the ACC. There is no apparent difference in the pain scores during CPT of these participants compared to the rest of the group.

SAM peaks were found in the insula in 86% of participants, however little change could be observed in the oscillatory dynamics during CPT. The insula has been activated during pain in previous studies (Brooks and Tracey, 2007) and is thought to be involved in integrating emotional aspects of pain with motivational impulses such as moving away from the source of pain (Peyron et al., 2002). Craig et al (2000) found activation in the anterior insula using PET that correlated with thermal intensity using a thermode on the back of the hand. Activation in the insula was also seen in another study using a CHEPS system (Roberts et al., 2008). It is possible that due to there being only one trial in the analysis as opposed to 30 to 60 trials as in the previous studies in this thesis, some of the detail of the brain activity may have been lost in that the stimulus was more prolonged and not as time locked as electrical stimulation. However, the lack of change in oscillatory dynamics in the insula is consistent with the findings of the previous studies in this thesis. In Studies 1 and 2, a clear evoked response could be seen in the insula of most participants but there was very little change in the oscillatory dynamics, the nature of the cold ice pack stimulus meant that it was not appropriate to look at evoked responses in this study but there was little change seen in oscillatory dynamics across CPT.

5.6.2 Methodological Issues:

SAM analysis is used for MEG data and works on the basis of averaging over a number of trials as was done in the previous studies. The issue with this study is that it was one long trial for each participant. In order to localise activity using SAM, smaller trials within different periods of CPT were created and then averaged. 15s from the warm ice pack period, 15s from the first part of CPT, 15s after the highest Likert score and 15s before the end of CPT were used in analysis. Within these 15s

periods, markers were placed every 0.5s (30 altogether) which were then used in the SAM analysis comparing each period of CPT to the warm baseline period. This assumes that there were no changes across these 15s period and the oscillatory dynamics were constant during that time which is not necessarily true. Peaks in the pain matrix were still found using this method of analysis and spectrograms and envelopes were created from these peaks. In order to understand the change in frequency bands across the whole profile, envelope analysis was performed on this data.

CPT normally involves immersing the hand in ice cold water, however this was found to be impractical within the MEG system. The alternative used in this study consisted of a cold ice pack placed under the hand which was found to provide a similar strong pain. This may have led to some differences compared to other studies in the literature as it was not as intense a pain as when using ice cold water and it was not over the whole surface of the hand, only the palm. This type of stimulus often causes the participant to tense the muscles of that arm and also possibly the neck. This muscle tension could lead to problems of EMG within the MEG data. In some participants this could be seen in the SAM analysis as peaks were located in the neck region. Dowman et al (2008) claim that the increase in gamma oscillations in the cortex seen in many studies is merely due to EMG artefacts. In their study, a CPT test was administered and, at a separate time, the participants were asked to contort their faces in order to create EMG artefacts. They saw an increase in gamma oscillations in CPT but found that they were similar to the increase seen during facial wincing and therefore they concluded that gamma oscillations were merely due to artefact from EMG. During this study it was found that although some EMG may have been present in the data, it localised to a source outside the brain (the neck) and no gamma oscillations were seen in the cortex. This was also seen in another study by this lab (Furlong et al., 2004), in which swallowing created a SAM peak that localised to the tongue and this could then be separated from data localising to the cortex. Therefore

gamma oscillations are not seen when there is EMG artefact and in Studies 1 and 2, gamma oscillations are seen when there is no EMG artefact present.

5.7 Conclusion

Peaks were found in key areas of the pain matrix during the cold ice pack (SI, SII, ACC, Insula) confirming their involvement in the processing of a tonic, sustained, thermal stimulus such as CPT. A decrease was seen in both alpha and beta frequency bands at the onset of the cold ice pack application. The change in alpha most likely reflects an increase in arousal due to the high behavioural importance of the painful stimulus. The decrease in beta may be associated with the impulse to remove the hand from the cause of the pain as a decrease in beta power is known to occur prior to movement (Pfurtscheller and Lopes da Silva, 1999). No change in gamma oscillations were apparent in any of the areas investigated. It may be that, in order to induce gamma oscillations, the stimulus must be strongly synchronous to drive the cortex to respond above the background activity. It is possible that due to the tonic nature of the stimulus that gamma oscillations were not apparent above the noise in the MEG data. Another possibility is that gamma oscillations are more specific to electrical stimulation or stimuli of that nature that strongly activate Aδ fibres, whereas CPT is likely to activate a combination of Aδ and C fibres as the pain felt during the cold ice pack is more similar to the C fibre mediated second pain (Forss et al., 2005). CPT is known to activate inhibitory feedback mechanisms in the brain, it is possible that these mechanisms may have had an impact on gamma oscillations.

6 Study 4: Investigating the gamma profile in SI during electrical stimulation at varying intensities using Magnetoencephalography

6.1 Abstract:

Gamma oscillations are thought to have a key role in binding different features of sensory stimuli together to create a coherent percept. They have been seen in response to many different sensory modalities; visual, auditory and somatosensory. They may be able to encode information within their firing and therefore may distinguish between different types of stimuli such as noxious or non-noxious.

In Studies 1 and 2, an increase in gamma oscillations were seen in SI in response to somatic electrical pain, and in Study 1 they could also be seen to a lesser degree during a non-painful somatic electrical stimulation in some participants. It is not clear from the previous studies whether the increase in gamma oscillations seen during electrical stimuli is linked to whether the stimulus is noxious or not or whether it is purely related to the intensity of the electrical stimulus. The aim of this study was to create a stimulus response curve to characterise the relationship between stimulus intensity and gamma oscillations in more depth.

Four different intensities of electrical stimuli were administered to the right index finger of all participants (high pain, low pain, high sensation, low sensation). Each different intensity was run in a separate block in trials of 5s comprising a 2s train of electrical pulses with 3s rest between each train. A McGill questionnaire was completed after each block. Another block was run at moderate pain intensity but with a longer train of pulses (5s) to investigate the ongoing profile of the gamma response.

An increase in gamma power was seen in SI in 50% of participants. In those that showed a gamma response, the strength of the gamma increase was found to be related to the intensity of the stimulus and there was no obvious difference when the

stimulus changed from non-noxious to noxious. After further analysis, it was found that the gamma oscillations seen in this experiment were predominantly evoked although some induced gamma oscillations were seen at the onset and offset of the train. After the large gamma increase in response to the first pulse in the longer train (5s), the gamma profile appeared to plateau out quite rapidly at a lower frequency range and a smaller bandwidth than the first.

These results indicate that the evoked gamma response found in SI may encode the stimulus intensity but does not seem to relate to the noxious nature of the stimulus. It is possible that the induced components of the gamma response that were seen in 17% of participants ~500ms after the train had ended may be involved in higher order tasks such as attentional processing.

6.2 Introduction:

Pain is of very high behavioural importance and therefore necessitates attention and focus, whereas it is easier to be distracted from merely sensory stimuli as they represent no threat to the individual. It would therefore be reasonable to expect that there were different patterns of brain activity when a sensory stimulus is given and when a painful stimulus is given.

Nociceptive-specific brain oscillations would be invaluable as biomarkers in order to investigate clinical pain patients and the efficacy of new therapies and pharmaceuticals. Gamma oscillations are a possible candidate (Gross et al., 2007) although we have to understand a lot more about the different factors affecting gamma oscillations before we can make any firm conclusions about what they are encoding.

The human brain naturally oscillates at various frequencies. Different frequency bands have been linked to different brain states. For example, alpha (~7-14Hz) is known to be involved in arousal (Babiloni et al., 2006) as it is seen during sleep and is

negatively correlated to arousal. Beta (~15-30Hz) has been found to be involved in movement (Raij et al., 2004, Pfurtscheller and Lopes da Silva, 1999, Baker, 2007).

Many theories have been created in order to explain how distinct areas of the brain combine their information to create a whole percept. A strong contender is 'binding theory' (Treisman, 1996) which suggests that in order to form a whole conscious perception of an event, neurons involved will fire in synchrony with precision within the millisecond range (Engel and Singer, 2001) and it is hypothesised that this may be in the gamma range (Tallon-Baudry and Bertrand, 1999).

There are three types of gamma response (Tallon-Baudry and Bertrand, 1999); firstly the gamma evoked response which is time and phase-locked to the stimulus, secondly the steady-state response which is periodically modulated and thirdly the induced response which can range from 30-100Hz. They are each involved in sensory and cognitive processing in different ways, not all of which are understood as yet. The induced response is most commonly associated with complex cognitive tasks requiring understanding and perception (Ward, 2003).

Gamma oscillations are seen in response to many different sensory stimuli as well as during cognitive tasks (Kaiser and Lutzenberger, 2003, Melloni et al., 2007). Binding theory has been tested thoroughly using visual tasks, for example, using illusory triangles, real triangles and no triangle stimuli (Tallon-Baudry and Bertrand, 1999). In their experiment there were two successive bursts of gamma oscillations; the first at around 100ms which was evoked but did not vary according to whether a triangle was perceived or not and the second burst which was induced and was strongest only when the stimulus was perceived as a coherent shape.

In a study by Hadjipapas et al (2007), it was found that the temporal characteristics of the gamma oscillations encoded information about the spatial frequency of visual stimuli. With reference to auditory processing, in a study by Kaiser et al (2003), an oddball paradigm was used and when the participant was instructed to attend to rare, deviant sounds, an increase in gamma oscillations was induced when these deviant

sounds were presented. These studies suggest that temporal characteristics within the gamma oscillations are able to encode specific information about features of sensory stimuli.

It is apparent that gamma oscillations can be involved in both bottom-up processing (passively digesting incoming sensory input) and top-down functions (involving active processing of stimuli) (Kaiser and Lutzenberger, 2003). Using a deafferented patient (Patino et al., 2008), a study was conducted using a task that involved compensating for a force applied to the finger which was either changing or static and the participant had to keep the forces equal using visual cues. This task involves the integration of visual, sensory and motor information however the patient had strong sensory impairment. In controls, high gamma coherence was found during the dynamic task whereas in the patient, no gamma coherence was found. This suggests that gamma coherence is involved in integrating visual and proprioceptive information and involves both ascending and descending pathways.

Gamma oscillations have been found to be involved in more complex cognitive tasks using attention, learning and memory (Ward, 2003). Theta frequency oscillations are thought to interact with gamma oscillations during memory tasks. Theta has been linked to encoding and retrieval of memory and gamma is prevalent during successful recollection (Ward, 2003). Induced gamma oscillations were found during an attentional selection and memory task (Bauer et al., 2006) and were strongest during focused attention.

There has also been research into somatosensory stimuli and gamma oscillations in the somatosensory cortices (SI and SII), often using experimentally induced pain or innocuous sensation such as laser (Gross et al., 2007) or electrical stimuli (Tecchio et al., 2008). De Pascalis et al (2004) found that phase-ordered gamma oscillations were reduced during focused analgesia induced by hypnosis. This would suggest that gamma oscillations are involved in top down control of pain perception. Gross et al (2007) found that when administering laser stimuli of the same intensity, if the

participant perceived the stimulus to be painful then the induced gamma response seen in MEG would be higher than if they did not, from this they concluded that gamma oscillations were involved in the subjective perception of pain.

Gamma oscillations have been recorded using various different techniques, such as EEG and MEG but also using intra-cranial electrode recordings in participants being evaluated for epilepsy surgery. This data is vital as it is less susceptible to noise and artefact issues than EEG and MEG. Fukuda et al (2008) found that following non-painful median nerve stimulation, a strong gamma response could be seen from intracortical electrodes. The gamma response began as phase-locked (evoked) but became non-phase-locked (induced) with time. The frequency bandwidth was initially 100-250Hz but gradually slowed to around 100Hz.

Hauck et al (2007a) found that during an oddball paradigm using intracutaneous electrical stimuli on the finger that an increase in gamma oscillations was seen after the stimulus from the somatosensory cortices. They saw two different types of gamma response; one earlier response between 60-80Hz from 50-250ms and a later component between 120-140Hz from 400-600ms, both of which were found to be induced. The later component was affected by attentional selection (i.e. whether they were actively attending to the rare stimuli). These task effects were stronger at the higher intensity stimulus.

An important question in the literature concerning the gamma response is whether it is evoked (phase-locked) or induced (non-phase-locked). Gross et al (2007) and Hauck et al (2007) believe the gamma oscillations found in their studies were induced whereas Fukuda et al (2008) saw them as being phase-locked to start with but becoming non-phase-locked with time.

6.3 Experimental Rationale

The aim of this study was to further investigate the profile of gamma activity during painful electrical stimulation in SI. In the previous studies, gamma oscillations have

been found in response to somatic electrical stimuli at both noxious and non-noxious levels. The gamma response appeared to be far stronger during pain than sensory stimuli however it was unclear whether this related to its painful nature or merely to the strength of intensity of the stimulus. In order to answer this key question, a stimulus response study must be performed using a number of different intensities at both noxious and non-noxious levels.

It is also important to determine whether the gamma oscillations found in these studies is evoked or induced as this may have an effect on the role it has in pain perception and how it relates to other literature. An interesting aspect of the gamma profile was the decrease in frequency and bandwidth across the electrical train in Study 1. It was not obvious whether it would plateau out or would continue to decrease and what this was due to. Therefore part B of this study used a longer train (5s instead of 2s) in order to develop a deeper understanding of the continuing profile of the gamma response and the possibility of sensitization or habituation mechanisms involved.

6.4 Methods:

6.4.1 Participants:

12 healthy participants (4 male; age range 24-43 years) took part in this study. All were free of any neurological or pain disorders and none were taking medication at the time of the study. Anatomical MRIs were taken for each of these individuals and were made available for analysis. Informed consent was obtained from all participants and the local ethics committee approved the experimental protocol.

6.4.2 Stimulus:

Electrical pulses were delivered via a constant current stimulator (Model: Digitimer Ltd, Welwyn Garden City, DS7A). Two electrodes were placed on the right index finger of each subject on the inner surface. Each stimulus was a train of pulses lasting

for 2 seconds. The duration of each electrical pulse was 200μs and the train was at a frequency of 7Hz. The current (ranging from 0mA to 100mA) was started below sensory threshold and gradually increased during thresholding.

Thresholds were obtained by administering trains of electrical pulses at 7Hz and increasing the current incrementally at a rate of ~0.5mA/s. Current was then increased and decreased three times in order to ensure an accurate threshold. Three measurements were taken; sensory threshold, pain threshold and pain tolerance. Four intensities were subsequently used as stimuli: low sensory (25% between sensory threshold and pain threshold), high sensory (75% between sensory threshold and pain threshold), low pain (25% between pain threshold and pain tolerance) and high pain (75% between pain threshold and pain tolerance) (see Chapter 2: Section 2.2.1.1 for more details).

6.4.3 Experimental Procedure:

Each stimulus intensity was run in a separate block. These blocks lasted 5 minutes and consisted of 60 trials of 5 seconds. Each trial consisted of 1s pre-stimulus time, 2s of electrical pulses at 7 Hz and then 2s recovery. No visual cues were given and the participant was instructed to keep their eyes open and try to focus on a central point. Each participant was instructed to fill out a McGill Pain Questionnaire after each run.

6.4.4 MEG recordings:

Participants were seated in a magnetically shielded room. Neural activity was recorded using a 275-channel CTF MEG system (CTF Systems Inc, Vancouver, Canada) at a sampling rate of 1200Hz, 60 trials were recorded, each 5s in duration. Preprocessing was completed using 3rd gradient noise reduction and removing the DC offset based on the whole trial (see Chapter 2: Section 2.2.3 for details). The 50Hz power line was taken out with a width of 0.6Hz. An average of all the trials for

each participant was scanned for blink artefacts but none were consistent across trials and it was not necessary to remove them.

6.4.5 Coregistration:

A 3-dimensional digitizer (Polhemus isotrak system, Kaiser Aerospace Inc, Colchester, Vermont, USA) was used to digitize the surface of the participants head and this information was then coregistered with the participants previously obtained anatomical MRI which gives accuracy within 5mm (Singh, 1995) (see Chapter 2: Section 2.2.4 for more detail).

6.4.6 Data Analysis:

6.4.6.1 SAM analysis:

SAM comparisons were made comparing 2s of the stimulation phase (active) to 2s of the rest phase (passive) in the four different intensity blocks using the frequency bands 3-7Hz (Theta), 7-14Hz (Alpha), 15-30Hz (Beta) and 30-100Hz (Gamma). See Chapter 2: Section 2.2.6 for an extended explanation of SAM analysis.

Group SAM (Singh et al., 2003) and SnPM (Nichols and Holmes, 2002) were used on this data in order to find out if there were any consistent changes in frequency bands across the entire group.

6.4.6.2 Time-frequency Spectrograms:

Average spectrograms were created using coordinates from peaks found in each participant's SAM comparisons within SI, with a pseudo t value of ≥1 (see Chapter 2: Section 2.2.7 for details). These spectrograms covered 1s before the stimulus, the 2s train of pulses and 1s of recovery, 4s in total. The frequency range was 1-100Hz. Spectrograms were also created from 50-200Hz in order to look for high frequency gamma oscillations but none were apparent. After looking at these results, bootstrap

spectrograms were produced focusing on the gamma response using 1s of rest phase and the 2s stimulus period and with a frequency range of 20-80Hz.

In order to investigate whether the gamma response seen was evoked or induced, 3 spectrograms were created; one original (containing evoked and induced activity), one with only the time-locked or evoked activity and one with only induced activity. To create an original spectrogram, a time-frequency representation for each of the 60 trials was created showing how the power changed across all frequency bands, and then the spectrograms for all 60 trials were averaged together. In order to create a purely evoked spectrogram, the 60 trials were first averaged together and then a spectrogram was created from this. This means that any data that was not time-locked across trials was removed. In order to create the induced spectrogram, the evoked spectrograms were subtracted from each individual spectrogram of the original data set, and the induced was what remained.

6.5 Materials and Methods (Study 4 part B):

6.5.1 Experimental Procedure:

The same participants were used for this study with the same index finger electrodes. In this study, the differences were in the stimulus intensity which was 50% between pain threshold and pain tolerance and the stimulation period carried on for 5 seconds as opposed to 2s. This was then followed by a rest period of 5 seconds and there were 30 trials collected instead of 60. In all other respects the methods remained the same for both studies.

6.6 Results:

6.6.1 Behavioural data:

The McGill scores for high pain and low pain were found to be significantly different ($p=0.0006$) from each other in terms of sensation, as were low pain and high

sensation (p=0.004), indicating that the different runs were clearly felt as different strengths of intensity. Few participants used the affective words to describe the stimuli indicating that the stimuli were not very emotive or upsetting. The most commonly used words to describe the sensation were 'throbbing', 'shooting', 'stabbing' and 'sharp' (see Figure 6.1).

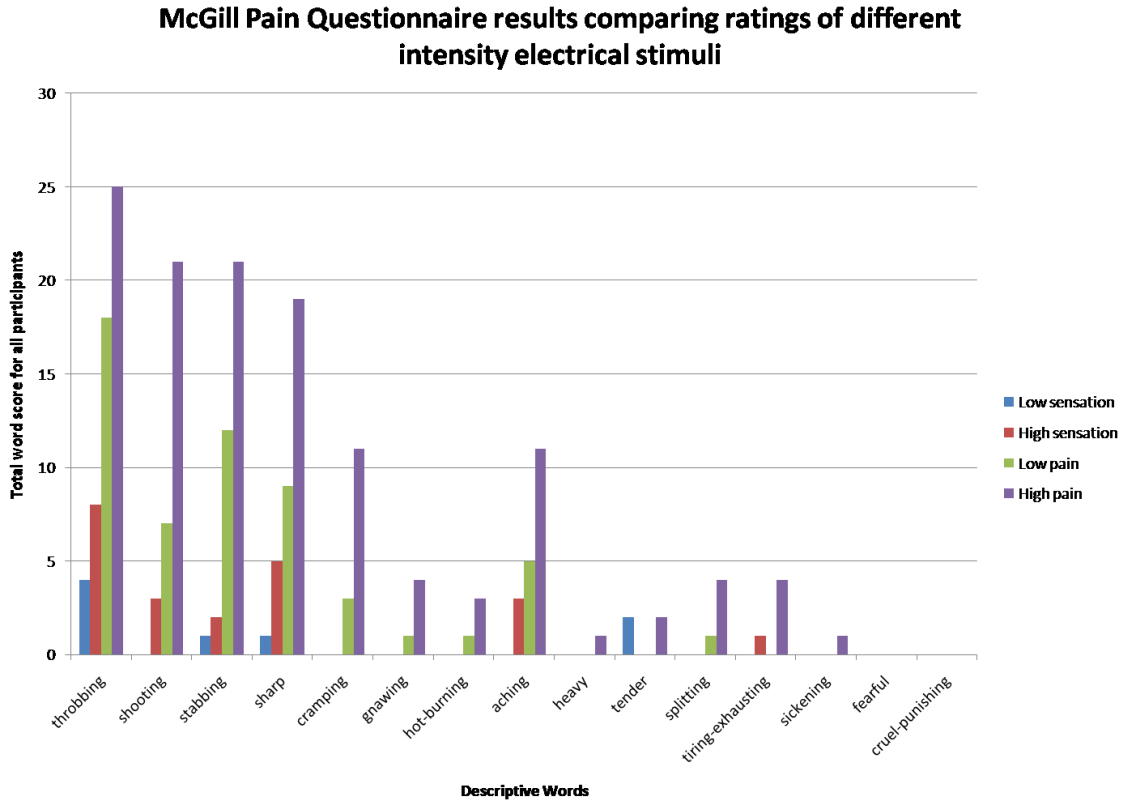


Figure 6:1 shows the results of the McGill pain questionnaire as a total of all participants' scores, to each descriptive word, at each different intensity. It is clear how the score increased with intensity and that very few affective words were used to describe the sensation.

6.6.2 Pain thresholds:

Each individual participant's sensory and pain thresholds were determined and stimulation levels calculated at the beginning of the experiment. These can be seen in Table 6.1. The multiplier gives an indication of the range between the two thresholds across the group, this ranged from 2.4 to 6.2.

Pain and sensory thresholds of all participants			
Participant	ST (mA)	PT (mA)	Multiplier
E2	0.6	3.4	6.1
E3	1.7	10.0	5.9
E5	1.5	7.5	5.0
E6	1.0	6.2	6.2
E7	1.5	4.5	3.0
E8	2.2	9.0	4.1
E9	1.4	3.3	2.4
E10	1.0	2.5	2.5
E11	1.8	7.2	4.0
E12	1.0	4.5	4.5
E13	1.2	5.6	4.7
E14	1.7	8.2	4.8

Table 6:1 shows the sensory and pain thresholds for all participants and also the multiplier (PT/ST).

6.6.3 SAM activation:

SAM peaks were found in SI at all intensities in all participants with pseudo t values mostly between 1 and 2. Peaks were found in SII, ACC and insula but not as consistently across the group (see Table 6.2). These were not used in further analysis as this study aimed to further explore the oscillatory dynamics seen specifically in SI with regards to results found during previous studies in this thesis.

	SAM peaks in different areas of the pain matrix at different stimulus intensities across all participants															
	SI				**SII**				**ACC**				**Insula**			
	LS	HS	LP	HP	LS	HS	LP	HP	LS	HS	LP	HP	LS	HS	LP	HP
E2	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	Y	Y	Y
E3	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y
E5	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	N	Y	Y	Y
E6	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	N	Y	Y
E7	Y	Y	Y	Y	Y	Y	Y	N	N	Y	N	N	N	Y	Y	N
E8	Y	Y	Y	Y	N	N	N	N	N	N	N	Y	N	N	N	Y
E9	Y	Y	Y	Y	N	N	Y	N	N	N	Y	N	N	N	N	N
E10	Y	Y	Y	Y	Y	Y	N	Y	N	N	Y	Y	Y	N	N	Y
E11	Y	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N	N	Y	N
E12	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	N	Y
E13	Y	Y	Y	Y	Y	N	N	Y	N	Y	N	N	Y	N	Y	Y
E14	Y	Y	Y	Y	N	N	Y	N	N	N	N	N	N	N	N	N
Total	12	12	12	12	8	7	6	7	4	7	7	7	2	5	6	8

Table 6:2 shows whether SAM peaks (pseudo t ≥ 1) were found in key areas of the pain matrix (SI, SII, ACC, Insula) during 4 different intensities (LS=low sensation, HS=high sensation, LP=low pain, HP= high pain) across all 12 participants

Group SAM data can be seen in Figure 6.2. There appeared to be an increase in theta power over the somatosensory cortex during all intensities, predominantly in the contralateral (left) hemisphere. A clear and more focal decrease was seen in both alpha and beta over the somatosensory cortex at all intensities. There was a small focal decrease seen in gamma oscillations over the somatosensory cortex during low sensation, not much activity in that area during high sensation or low pain and a small increase during high pain. SnPM analysis revealed a significant activation in the middle frontal gyrus in the theta band during high pain (see Figure 6.3), however no other significant activations were found.

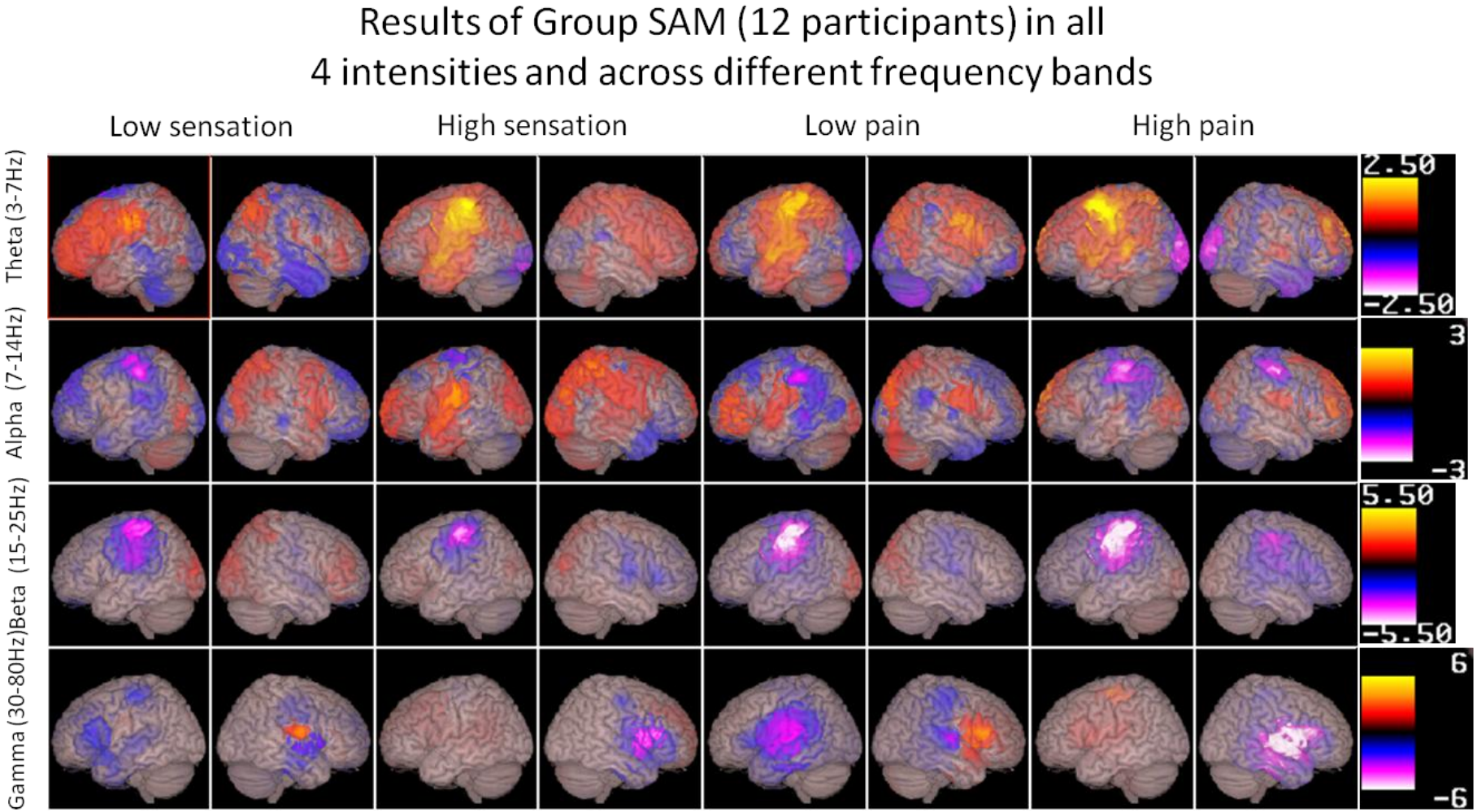


Figure 6:2 shows the Group SAM data across all 4 intensities of electrical stimulation and at 4 different frequency bands.

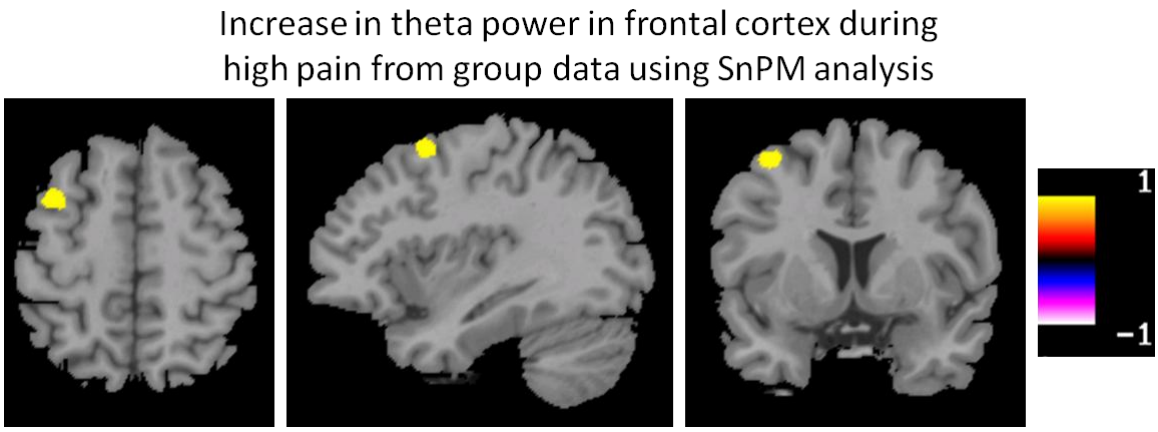


Figure 6:3 shows significant activation found in the middle frontal gyrus in the theta band during high pain from SnPM data.

6.6.4 Evoked fields:

Averaged datasets were created around the electrical train. Weights files were created of the VEs found from SAM peaks in SI. These were loaded into an averaged dataset, to see the evoked response from that location more clearly, as illustrated in Figures 6.4 and 6.5. A strong evoked response was seen in SI in response to high pain in all participants. An example is shown in Figure 6.4 and to the 5s long train in Figure 6.5.

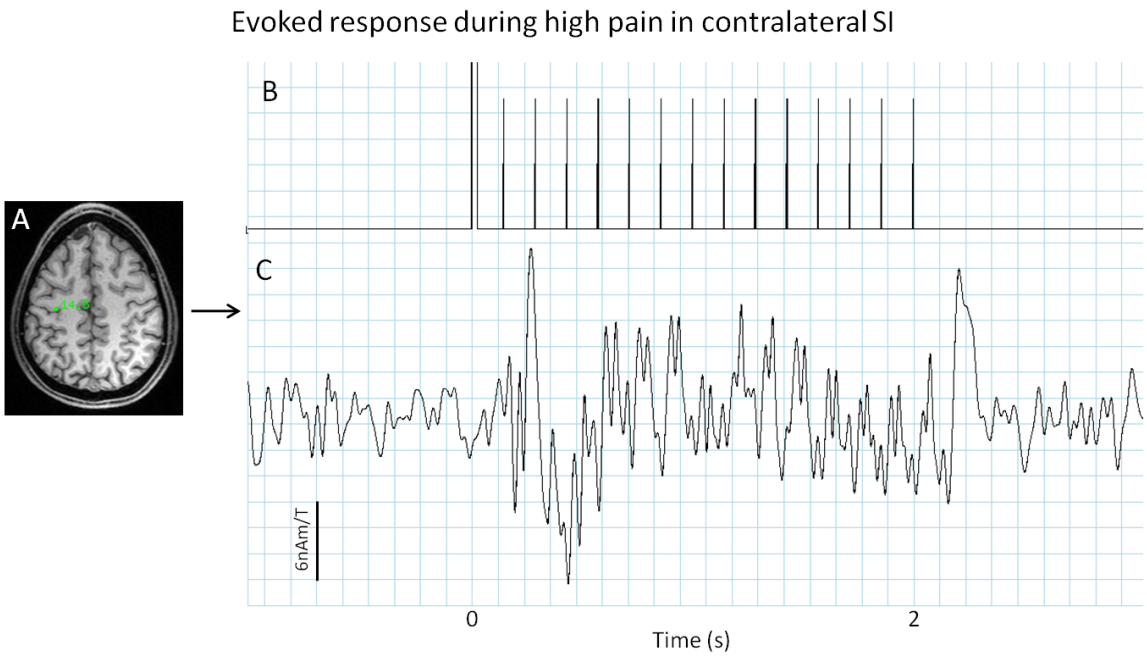


Figure 6:4 shows the evoked profile of a VE (A) taken from the contralateral SI of a representative individual (E8) during high pain. The top line (B) shows the stimulus reference so that it is possible to see when each electrical pulse was administered and the bottom line (C) shows the evoked response to each stimulus in the train.

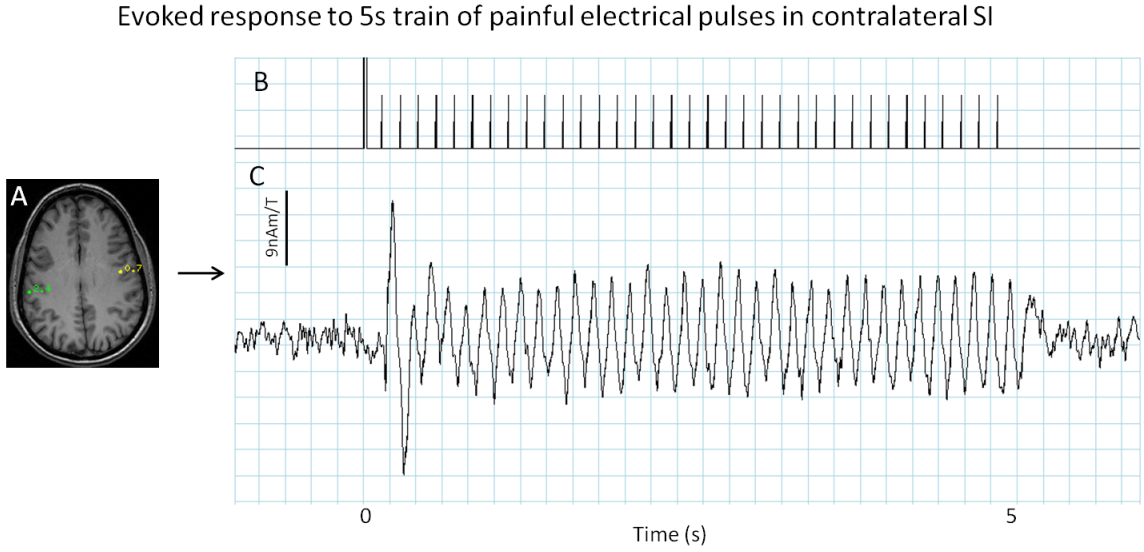


Figure 6:5 shows the evoked profile of a VE (A) taken from the contralateral SI of a representative individual (E8) during the 5s train of painful electrical pulses. The top line (B) illustrates when each electrical pulse was administered and the bottom line (C) shows the evoked response.

The amplitude of the 70ms component of the evoked response changed across the duration of the train as can be seen in Figure 6.4-6.6. This is similar to results found

in Study 1. Amplitude decreased sharply after the first stimulus and then plateaued, in some participants it seemed to gradually increase again towards the end of the 5s train (see Figure 6.6).

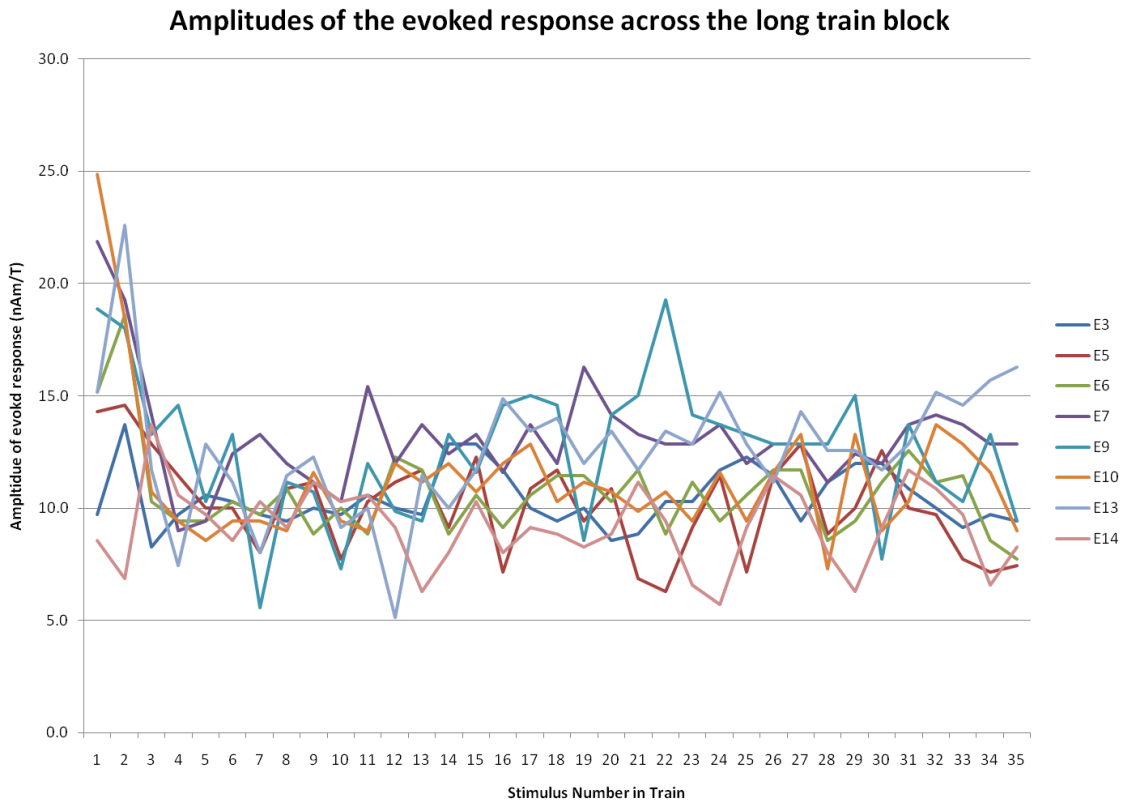


Figure 6:6 shows how the amplitude of the 70ms component of the evoked response from contralateral SI changed across the long train of pulses (5s) in a number of participants. In 4 participants the evoked response was not clear enough to measure the amplitude.

6.6.5 Spectrograms:

Spectrograms were created from SAM peaks in SI for all participants. In 50% of participants, an increase in gamma power could be seen after each electrical stimulus in SI during high pain, an example from a representative individual (E8) is shown in Figure 6.7. A clear evoked response could be seen to each electrical stimulus (Figure 6.7: B) within a similar time window to the gamma increase. The gamma increase was seen in the 140ms between each stimulus. The gamma increase at the beginning of the train had a bandwidth of ~30-75Hz, this then decreased across the

train till the last gamma response was between ~30-55Hz. A beta rebound was observed in 50% of participants at around 500ms after the end of the train of pulses, this can be seen in Figure 6.7: C.

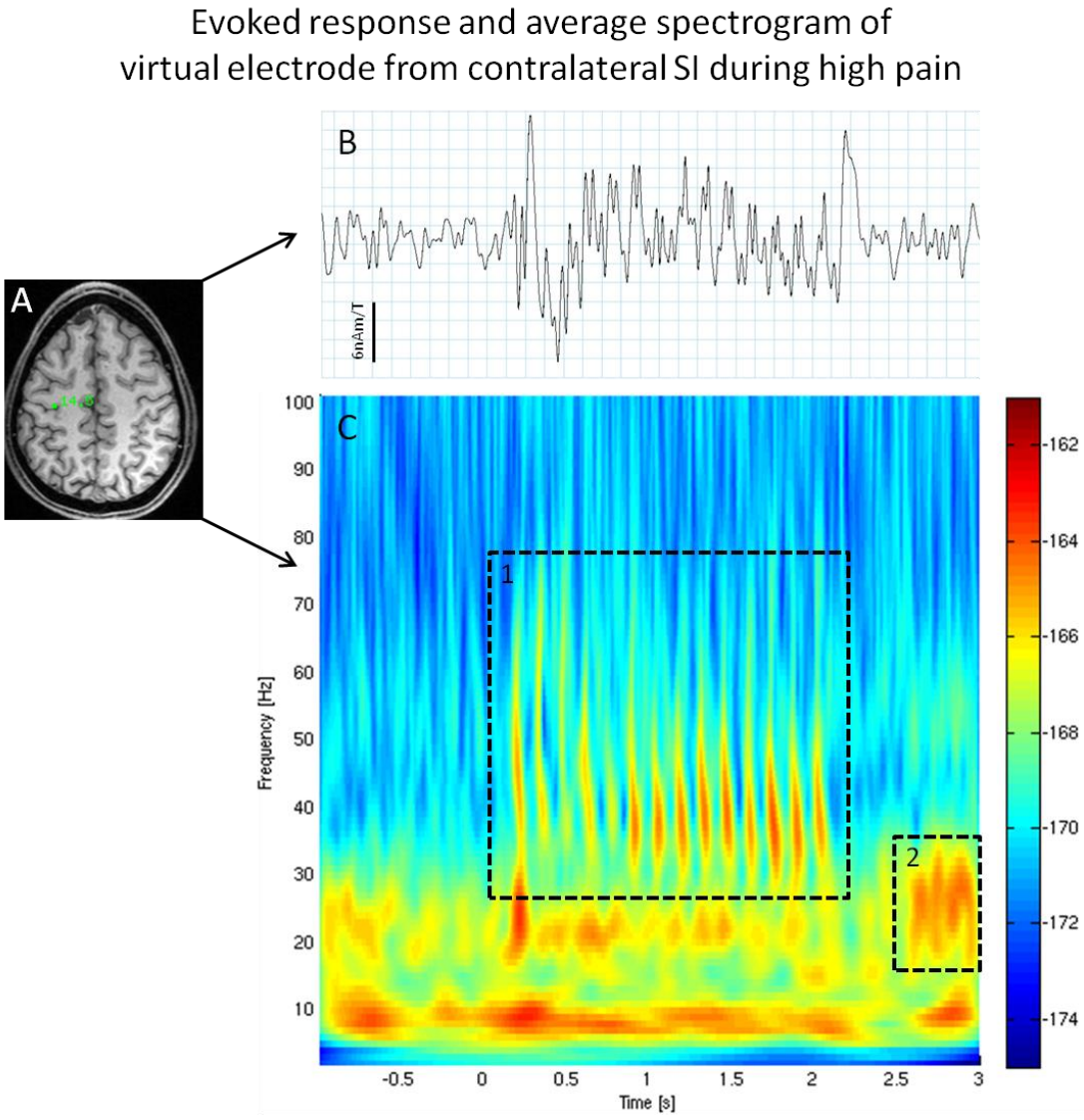


Figure 6:7 shows a VE (A) from contralateral SI during high pain in a representative individual (E8), its evoked profile (B) and an average spectrogram (C) from that location. An increase in the gamma band can be seen in response to each electrical stimulus (Box 1) and a rebound in the beta band can be seen ~500ms after the end of the train (Box 2).

In order to investigate whether any changes in oscillatory dynamics were seen after the offset of the train, bootstrap spectrograms were performed on all participants. If there were only small changes, then a bootstrap spectrogram would be more likely to

show these than an average spectrogram. In 17% of participants, a gamma response could be seen not only during the train (Figure 6.8 Box 1) but also after the offset of the train from around 500ms (Box 2), this can be seen in one of the individuals in Figure 6.8. It is also easier to see the decrease in the frequency of the gamma oscillations across the train in Figure 6.8. No gamma oscillations were apparent at the higher frequencies (>100Hz) in any participants (Figure 6.8).

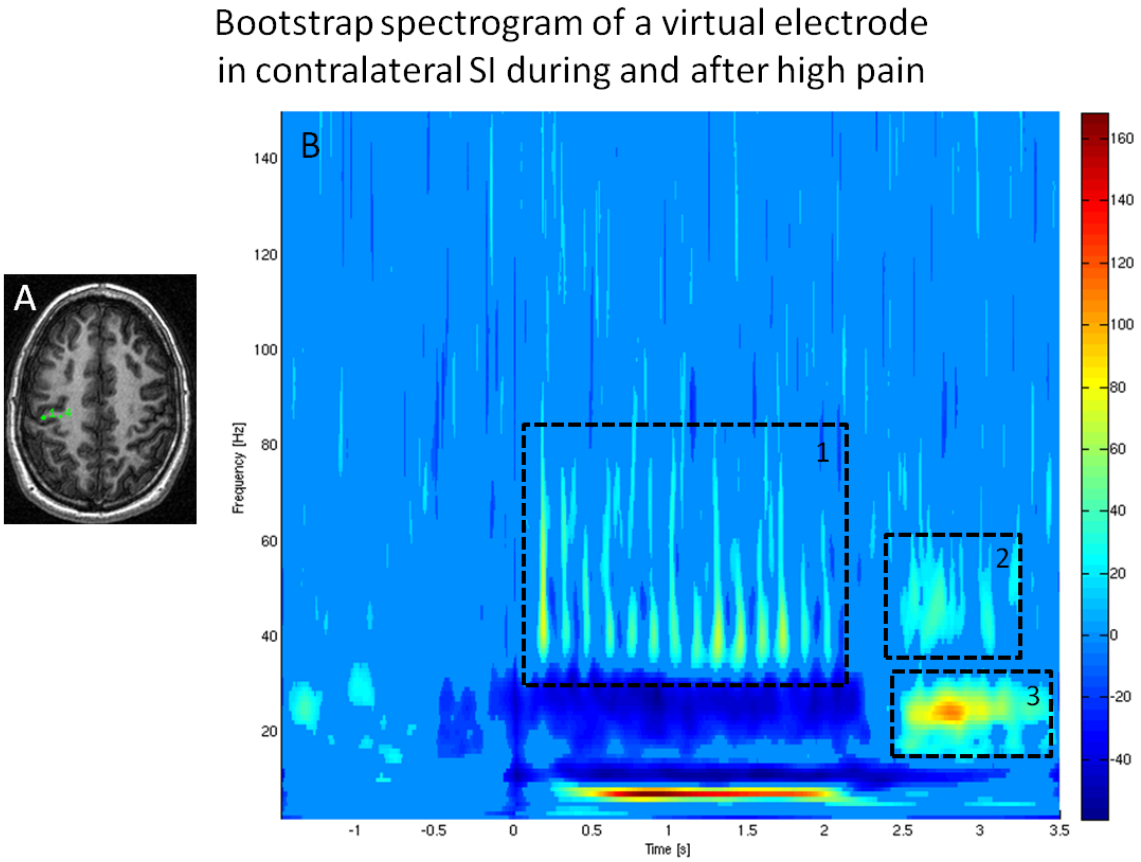


Figure 6:8 shows the percentage change in frequency power during and after high pain compared to a baseline period from a VE in contralateral SI (A) of an individual (E3). An increase in gamma was seen in response to each electrical pulse (Box 1). An increase in gamma was also seen after the offset of the train (Box 2) as well as a beta rebound (Box 3).

The 5s long train was used in order to investigate the change in profile of the gamma oscillations across the train. Figure 6.9 shows the evoked response to each electrical stimulus (B) and the increase in gamma oscillations to each response (C: Box 1). The frequency bandwidth of the gamma oscillations appeared to decrease across the

train. A decrease in beta was seen in all participants, this then rebounded at around 500ms after the offset of the stimulus (C: Box 2). A 7Hz oscillation can be seen that is most likely related to the stimulus being administered at that frequency.

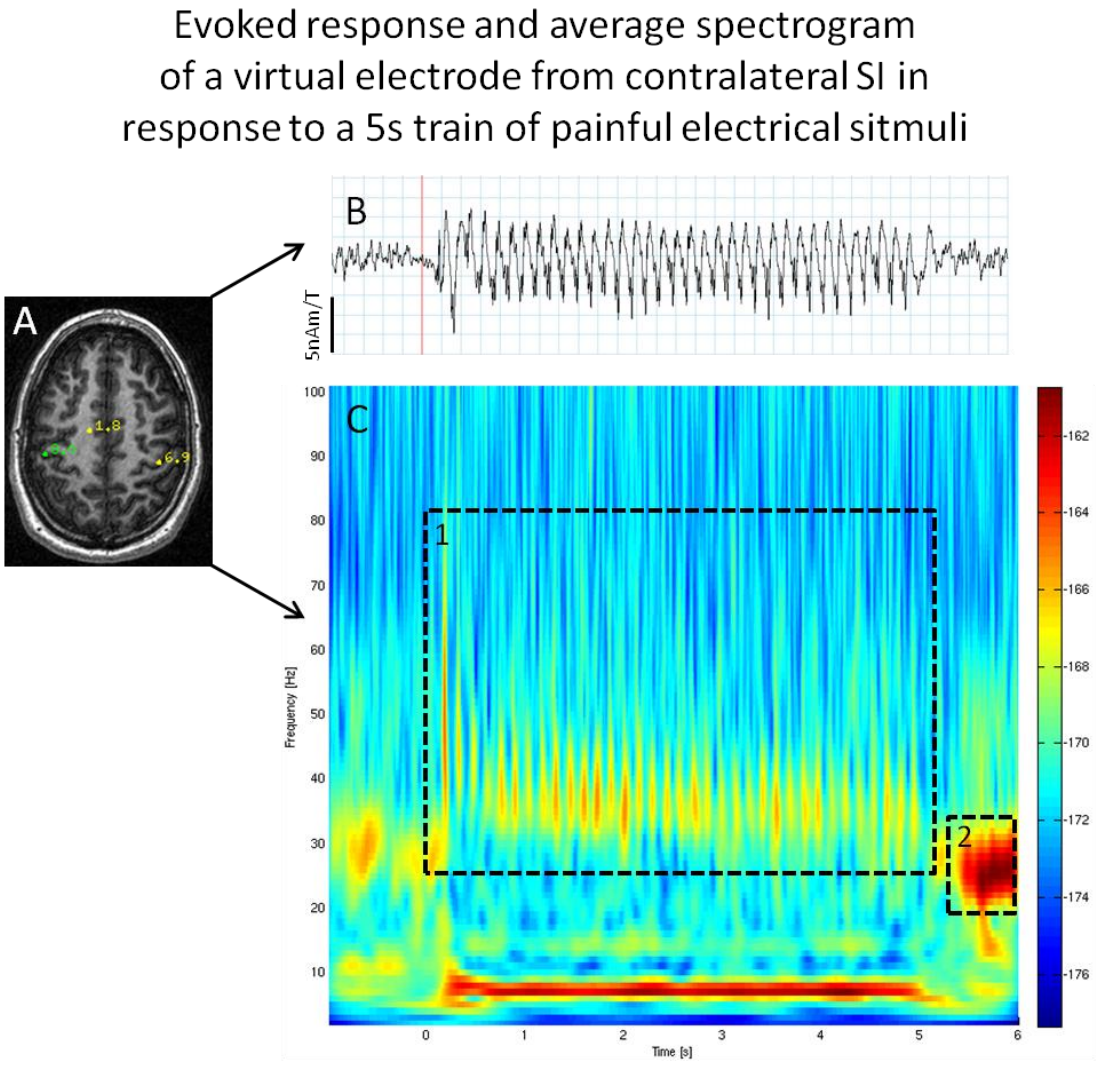


Figure 6:9 shows a VE (A) from contralateral SI in a representative individual (E3) during the longer train (5s) block, its evoked profile (B) and an average spectrogram (C) across the entire trial. An increase in gamma can be seen in response to each stimulus (Box 1). A beta rebound can be seen at around 500ms after the stimulus offset (Box 2).

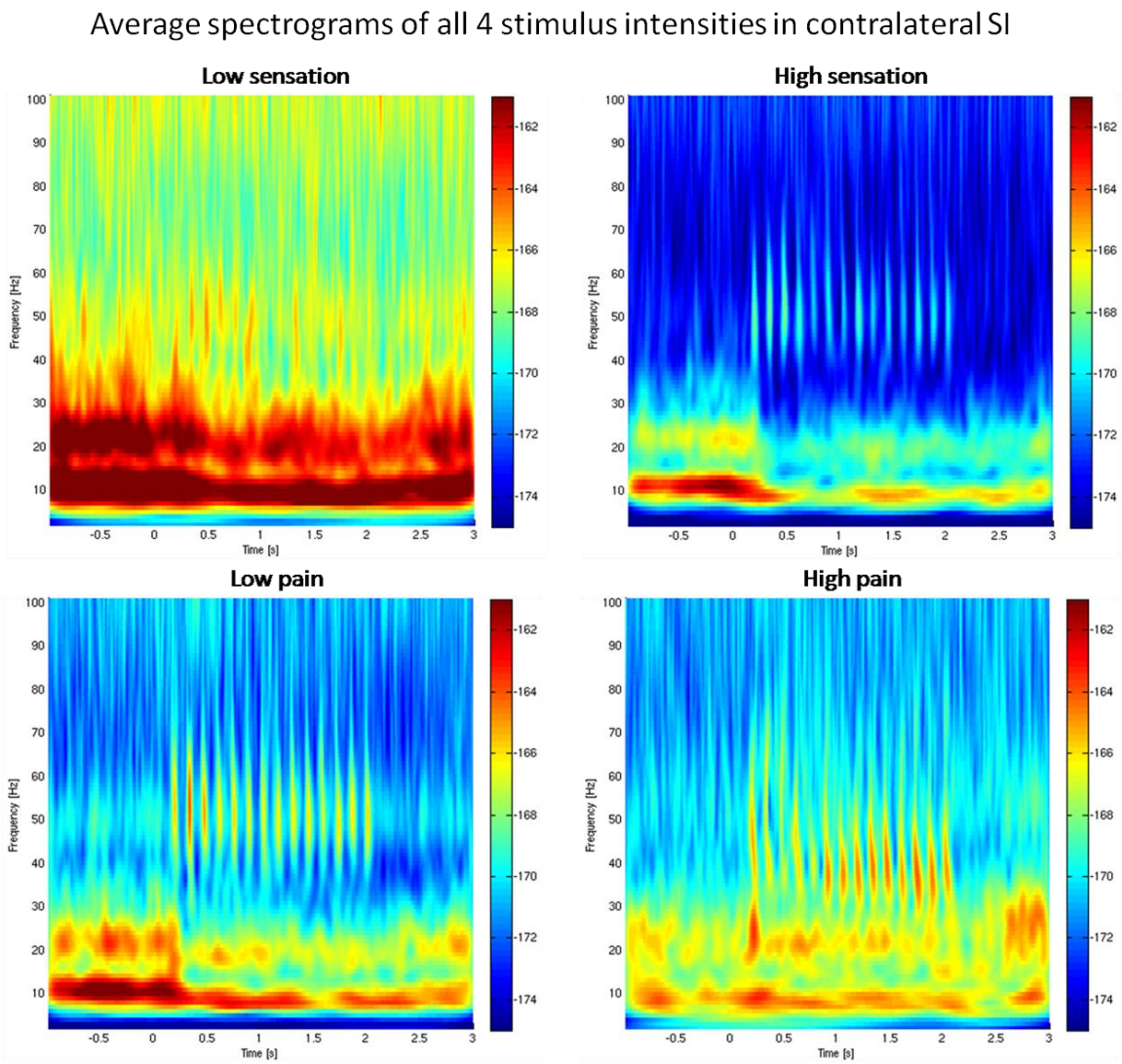


Figure 6:10: This figure shows 4 average spectrograms displaying different stimulus intensities from a VE in contralateral SI of a representative individual (E8) (top left; high-pain, top right; low-pain, bottom left; high-sensory, bottom right; low sensory). The change in gamma appeared to be more intense as the stimulus intensity increased.

The gamma oscillations were not solely present in the painful runs but were also evident in the sensory runs although the increase was not as strong (Figure 6.10). A one-way within-subjects ANOVA was performed on all 6 gamma responders and stimulus intensity was found to be a significant factor in determining the strength of the percentage increase in the gamma response ($F_{(5)}$=7.29, p=0.003). This shows that the strength of gamma power increased with the intensity of the electrical stimulus as can be seen in Figure 6.11.

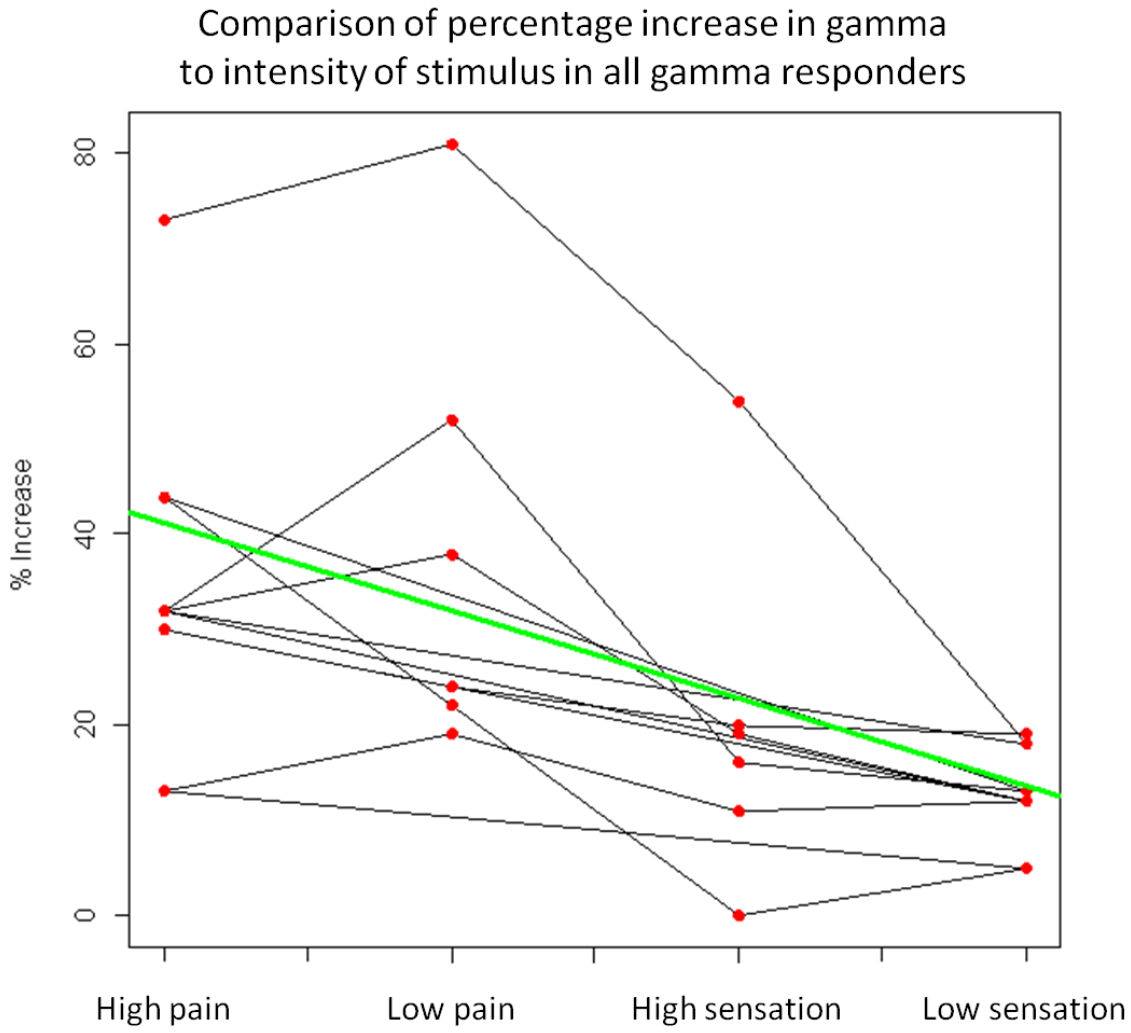


Figure 6:11 shows the percentage increase in gamma in all 6 gamma responders in this study for each stimulus intensity. A line of best fit suggests that the percentage increases with increasing stimulus intensity which is confirmed by the results of a one-way within subjects ANOVA.

Analysis was performed in order to elucidate whether the gamma response was evoked or induced. Figure 6.12 demonstrates that a large component of the gamma response appeared to be evoked i.e. time-locked to the stimulus although induced components were still seen near the onset of the train.

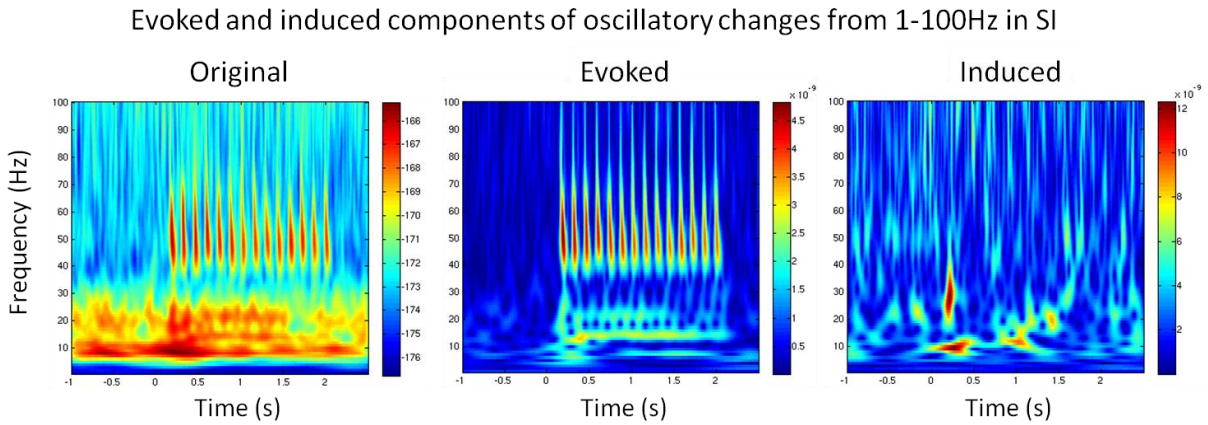


Figure 6:12: This figure shows average spectrograms from 1-100Hz in SI of a representative individual (E8). On the left is a combination of evoked and induced activity (original), in the centre is purely evoked activity and on the right is induced activity. The majority of the gamma increase appeared to be evoked although some aspects of the response were induced.

6.6.6 Correlations

In order to understand in greater depth what factors influenced the presence and strength of gamma power, a Spearmans rho correlation was performed (n=6) comparing percentage increase in gamma oscillations with the amplitude of the first evoked potential (r_s=0.6, p=0.24). The results indicated that there was a positive correlation between percentage increase in gamma oscillations and amplitude of evoked response although this did not reach significance. This fits with the finding that a strong component of the gamma response was evoked (Figure 6.12). A t-test was performed on the pain thresholds of those that showed a gamma response against those that showed no gamma increase, this result was not found to be significant (p=0.55). This suggests that the presence of an increase in gamma oscillations in SI is not related to the individual's pain threshold. It is possible that the results of these statistical tests did not come out as significant due to the small sample size in this study.

6.6.7 Changes in theta in frontal cortex

SnPM analysis on the group data indicated a significant increase in theta during high pain in the frontal cortex (middle frontal gyrus). SAM peaks in this area of cortex were taken from each individual and a group average spectrogram was created (see Figure 6.13). A strong increase in the theta band (~4.5-8.5Hz) could be seen at the onset of the train (~200-700ms). This increased theta continued for the rest of the train of pulses but at a lower strength (see Figure 6.13). The increase in theta is most likely due to the main components of the evoked response occurring at theta frequency, as can be seen in Figure 6.14 which shows the frequency composition of the evoked response in the left frontal cortex of a representative individual (E2).

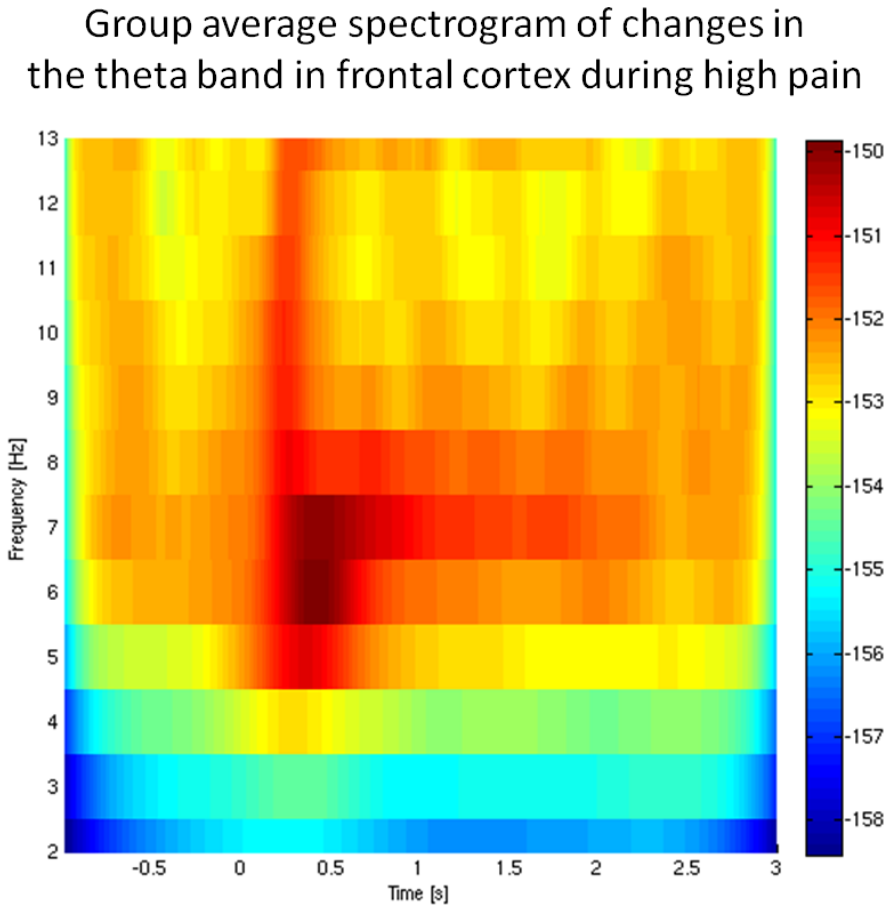


Figure 6:13 shows a group average spectrogram of the increase seen in the theta band in frontal cortex during high pain.

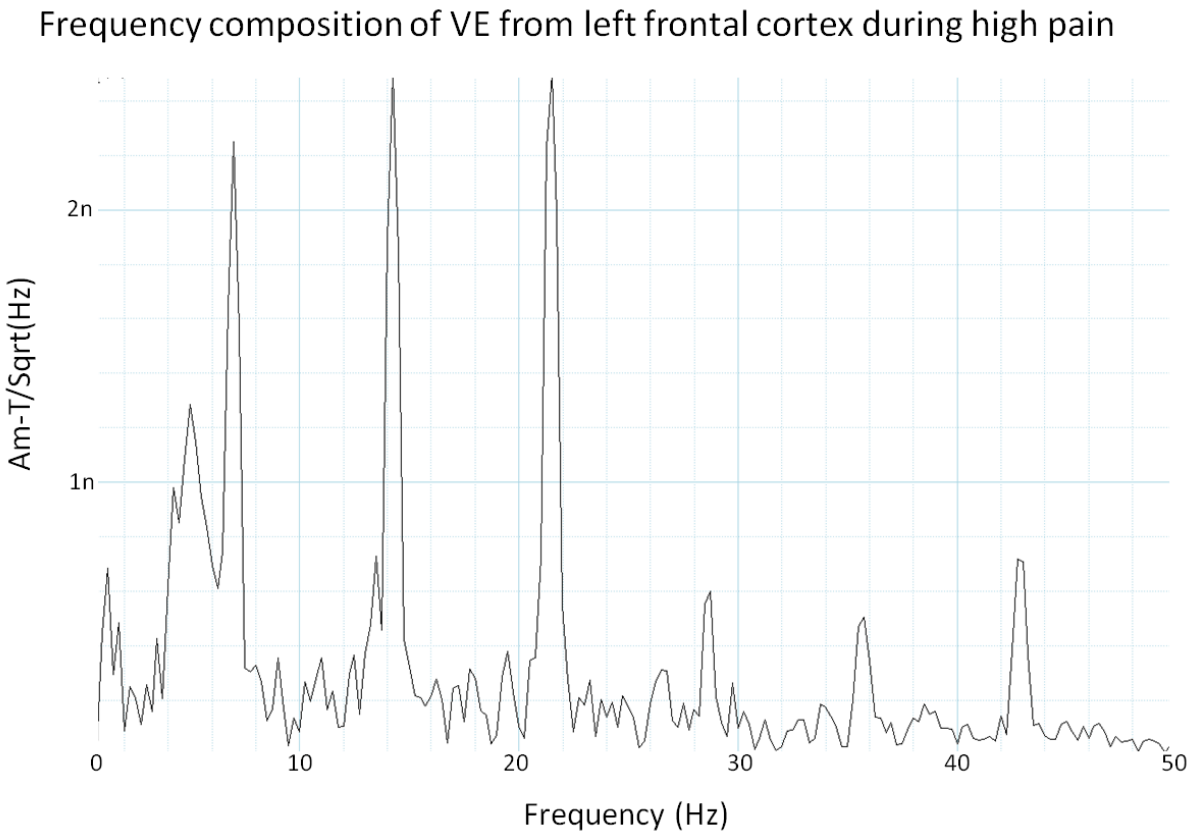


Figure 6:14 shows the frequency composition of the evoked response from a VE in the left fronal cortex of a representative individual (E2). The majority of the evoked response lies around the theta frequency (~3-7Hz).

6.7 Discussion:

6.7.1 Summary of key findings:

This study focused on the oscillatory dynamics in SI with reference to results previously obtained from other studies in this thesis, SAM peaks were found in other key areas of the pain matrix (SII, ACC, Insula) but these were not analysed further (see Table 6.2). However, a significant increase was seen in the theta band in frontal cortex during high pain in SnPM analysis, and a group average spectrogram was created in order to investigate this further (Figure 6.13).

In this study, an increase in gamma oscillations was seen in SI, in response to strong painful stimuli, in 50% of participants. This increase in gamma oscillations was seen

in both the painful and non-painful runs, there was no obvious change in the pattern between painful and non-painful stimulation. The gamma increase appeared to strengthen as the intensity of the stimulus increased (see Figure 6.10). The results of the ANOVA showed that intensity was a significant factor for percentage increase in gamma oscillations ($F_{(5)}$=7.29, p=0.003). Gamma oscillations were not seen in 50% of participants, this was a similar result to that found in Studies 1 and 2. The reason for this remains unclear, and it appears not to be related to pain threshold (see Section 6.6.6). A downward shift in frequency of the gamma response was seen across the train similar to that seen in Study 1. The frequency of gamma oscillations in this study was a lower frequency than Study 1 and crossed over into the beta band in most cases. All participants had SAM peaks in SI during all 4 stimulus intensities.

6.7.2 Evoked response

The amplitude of the ~70ms component of the evoked response decreased across the train of stimuli (Figures 6.4-6.6), as was seen in Study 1 (see Chapter 3: Figures 3.9, 3.10). The first response was much higher than the rest, subsequently they appeared to plateau out (see Figure 6.4), some increased towards the end as can be seen in Figure 6.6. This may be due to some habituation mechanism, either at the peripheral or central level (Greffrath et al., 2007). This has been seen in other studies previously (Huttunen, 2010), a component of the evoked response at 35ms was found to decrease in the first few stimuli of a train. This was linked to a reduction in IPSPs.

6.7.3 Gamma and stimulus intensity

These results, as well as those from the previous studies, suggest that gamma oscillations would not be a suitable biomarker for pain in that they were not observed during distal oesophageal stimulation or other types of somatic stimuli than electrical (CPT). It appears that gamma oscillations were not solely found during pain but also during sensation and there was no clear change when the stimulus changed from high sensation to low pain (see Figure 6.10). This would suggest that gamma

oscillations are not an indicator of the noxious nature of pain. The gamma increase appeared to strengthen as the intensity of the stimulus increased (see Figures 6.10, 6.11). The results of the ANOVA showed that intensity was a significant factor for percentage increase in gamma oscillations ($F_{(5)}$=7.29, p=0.003). This would indicate that, rather than being a biomarker for pain, gamma oscillations in SI encode some type of discriminative information about the stimulus intensity. SI is thought to be involved in the sensory-discriminative properties of somatic stimuli (Treede et al., 1999). SI has been linked to encoding stimulus intensity previously (Coghill et al., 1999, Timmermann et al., 2001, Bornhovd et al., 2002) although these studies did not mention changes in oscillatory dynamics. The strength of the gamma oscillations relating to the intensity of the stimulus could explain a key component of how SI is able to encode stimulus intensity.

6.7.4 Evoked vs Induced gamma

Previous studies investigating the gamma response to painful stimulation indicated that the gamma response seen was induced, not time-locked to the stimulus (Hauck et al., 2007a, Gross et al., 2007). Although others have found it to be time-locked at first and then it became induced later on (Fukuda et al., 2008). The gamma oscillations found in this study were shown to contain evoked components (Figure 6.12). This suggests that it may be a transient increase in gamma synchrony due to the evoked response and is perhaps less involved in higher-cognitive processing such as attentional mechanisms. However, induced gamma oscillations were also seen at the onset of the train as is shown in Figure 6.12. The issue with the train of electrical pulses used is that there was only 140ms between each pulse, the induced gamma oscillations other researchers have found were later than this, however it was possible to investigate this at the offset of the train; an increase within the gamma band was seen at ~500-800ms after the offset of the train of pulses in 17% of participants during high pain using bootstrap spectrograms (Figure 6.8). In Study 2, there was only 1 electrical pulse and gamma oscillations could be seen between 100-250ms after the stimulus.

Both evoked and induced gamma responses have been seen in response to sensory stimuli (Tallon-Baudry and Bertrand, 1999). In response to pain, induced gamma oscillations have been found (Hauck et al., 2007a, Gross et al., 2007). The gamma oscillations that were seen in these studies potentially consisted of both evoked gamma, which was seen in the same temporal window as the evoked response to pain (Figure 6.7, 6.12) and also induced gamma which was seen a few hundred milliseconds after the stimulus had ended (Figure 6.8). It is not clear what the roles of these two gamma responses are although it can be suggested that the evoked gamma response correlates with stimulus intensity (Figure 6.11). The later, induced gamma response may potentially have a role in higher order processing and attention to pain as suggested by Hauck et al (2007).

6.7.5 Gamma frequency shift

The profile of the gamma increase appeared to change across the train of stimuli (see Figures 6.7-6.9), in that the frequency decreased across the train. This has been seen in a number of other studies (Haenschel et al., 2000, Chen and Herrmann, 2001, Fukuda et al., 2008). This could be linked to the decrease in the amplitude of the evoked response as it may form part of the evoked response. Alternatively it could be due to a habituation mechanism (Greffrath et al., 2007).

A shift from gamma down towards beta frequency is a common phenomenon in *in vitro* preparations of rat hippocampal slices in response to tetanic stimulation (Whittington et al., 1997, Traub et al., 1999, Bracci et al., 1999). The interneurons in the cortex fire at gamma frequency and, as a result of their inhibitory effect on the pyramidal cells, entrain the population to oscillate at gamma frequency; this is the signal recorded in MEG (Murakami and Okada, 2006). If stimulated tetanically *in vitro*, there is an increase in the excitatory influence of the pyramidal cells due to an increase in the amplitude of excitatory post-synaptic potentials (EPSPs) and afterhyperpolarizations (AHPs). Pyramidal cells naturally fire at a lower frequency (low-beta) than interneurons and as a consequence of their increased influence,

either directly or indirectly, the field oscillation is slowed to beta frequency (see Chapter 3: Figure 3.18).

The difference between the electrical trains used in Studies 1 and 4 is that Study 1 stimulated at 10Hz and Study 4 stimulated at 7Hz. There was a difference in the frequency of the gamma response seen in these two studies. In Study 1, gamma oscillations were seen between ~65-100Hz at the beginning of the train and decreased to ~45-75Hz at the end whereas in Study 4 they went from ~20-75Hz at the beginning to 20-55Hz at the end which crossed over into the beta range. This suggests that the ISI may have an influence on the frequency of the gamma response. There may be some encoding within the frequency of the gamma oscillations that is able to give information about the ISI between stimuli.

6.7.6 Gamma responders and non-responders

Changes in the gamma band were not very clear from the Group SAM data, this may be due to the focal nature of the gamma increase in that each individual had a different location of gamma oscillations and this meant that they were not found at the same source consistently across the group. The gamma increase in this study crossed over into the beta range so they may have been harder to separate during SAM analysis.

Gamma oscillations were not seen in all individuals; in Studies 1, 2 and 4 it was seen in around 50-67% of participants. This suggests that it does not have an essential role in somatosensory processing as it is completely absent in some individuals. This, however, does not mean that it is of no significance. In other sensory stimuli, for example visual, gamma oscillations have been found to encode specific information about the stimulus (Hadjipapas et al., 2007), this may be what gamma oscillations are doing in these studies, they may encode details of the stimulus intensity within their oscillations.

6.7.7 Other frequency bands

Group SAM data (Figure 6.2) showed an increase in theta band over the somatosensory cortex and frontal areas at all intensities. An increase in theta frequency was found to be significant from SnPM analysis of group data in the left frontal cortex (see Figure 6.3). The group average spectrogram of changes in theta band in the frontal cortex showed a stronger increase at the onset of the train which then lessened but remained throughout the train (Figure 6.13). This is likely to be linked to the evoked response present in the frontal cortex, as it is made up of a similar frequency to theta (see Figure 6.14). Theta has been implicated in chronic pain previously (Sarnthein and Jeanmonod, 2008) and has also been linked to gamma oscillations in that theta is thought to be able to regulate gamma oscillations in some way (Ward, 2003). It has been seen to increase in frontal areas in response to other types of somatic pain such as CPT (Chang et al., 2002, Chang et al., 2005).

A clear and focal decrease in alpha and beta was seen in the Group SAM data (Figure 6.2) however they did not reach significance at the group level. A rebound in the beta band was seen in 50% of participants. The decrease in alpha could be linked to an increase in arousal as alpha is thought to be an 'idling' frequency (Hari and Salmelin, 1997). The change in beta is similar to that seen in Studies 1 and 2 and fits with similar literature on somatosensory stimulation (Raij et al., 2004, Ploner et al., 2006c, Gaetz and Cheyne, 2006). Across all the frequency bands looked at in the Group SAM data, none seemed to have a clear difference between painful and non-painful stimuli, the changes in oscillatory dynamics still appeared whether the stimulus was painful or not.

6.7.8 Methodological Issues:

In Study 1, the onset of the stimulus was jittered in order to ensure that the participant was unaware of exactly when they would receive the stimuli and also so that there would be no conditioned preparatory response which may have lead to muscle

tension before the onset of the stimulus. However in this study, there was no jitter in the protocol, this meant that more trials could be repeated as the trial length was much shorter leading to a better SNR. There is a possibility that there was more of a preparatory response in the muscles or even in the cortex as the onset of the stimulus was predictable and had the same ISI. The baseline used for SAM analysis was 2s before the stimulus onset, this may not have been a true baseline in that it is possible that it was contaminated by a preparatory response.

Using a train of electrical pulses made the stimulus more painful and it had a longer duration than using a single pulse, however when analysing the evoked response and the oscillatory changes, there was only 140ms between each pulse. This allowed us to see the beginning of the evoked response but there may be components of it that were lost as another pulse overlapped with them, this may also be true of the gamma increase. In Study 2, a gamma increase was seen between 150-250ms after the stimulus, the earlier gamma response seen in this study may be a different kind (i.e. evoked or induced) and may signify a different aspect of processing. Only the first 140ms after each pulse could be analysed in this study, however a clear gamma response was still seen within this time frame which is of value to investigate. Also, it was possible to observe these changes at the offset of the train, in 17% of participants, an increase in gamma oscillations was seen at around 500ms after the end of the train (Figure 6.8).

As electrical pain activates both Aδ and C fibres, it cannot be specified which fibre type is responsible for the oscillatory changes seen during these studies. In order to answer this question, it would be necessary to create a stimulus that could specifically activate each type of fibre. Laser stimulation is able to do this; by controlling the surface area and intensity of the stimulus, it is possible to selectively activate Aδ or C fibres (Raij et al., 2004).

6.8 Conclusion

In this study, the strength of the gamma response was seen to increase with increasing intensity. This would suggest that gamma oscillations, rather than encoding a response specific to pain, may encode information about the intensity of the stimulus being received in the strength of its oscillations. The frequency of the gamma oscillations was seen to decrease across the train similar to that seen in Study 1. This may be due to habituation, possibly in the neurons of the cortex. The overall frequency of the gamma oscillations was at a lower frequency than during Study 1 when the stimuli were administered at 10Hz as opposed to 7Hz in this study. These results suggest that there may be some information about the stimuli and the timing between them, within the frequency of the gamma oscillations. Not all participants showed an increase in gamma oscillations to the painful stimulus (33-50%). The presence or absence of gamma oscillations in different individuals may be associated with different personality traits.

7 Discussion and Conclusion

7.1 Introduction

The key regions of the brain involved in pain processing have been revealed from experimental pain studies, especially using PET and fMRI (Derbyshire, 2003, Peyron et al., 2000). The areas most commonly activated are SI, SII, ACC and insula. There are also other areas involved such as PFC which deal with higher cognitive processing (Lorenz et al., 2003). What still eludes pain researchers is a biomarker for pain which would indicate, without any subjective input from the individual, whether they were experiencing pain or not and also whether it was a normal or abnormal response. The temporal dynamics of brain oscillations may provide evidence into how the pain network interacts and how each area processes different aspects of a painful stimulus.

It is the changes in these oscillations as well as the evoked responses that have been investigated in this thesis. The anticipatory response to pain has been explored together with a number of different painful stimuli; median nerve electrical stimulation, digital electrical stimulation, distal oesophageal electrical stimulation and cold ice pack to the hand. Across these different stimuli and protocols, there were similarities in oscillatory dynamics and also some interesting differences which may help to elaborate how oscillations are involved in pain processing and what information they encode. This chapter is split up into areas of the pain matrix and within each area, results of SAM analysis, evoked responses and oscillatory dynamics in different frequency bands (theta, alpha, beta and gamma) are discussed with reference to the current literature.

7.2 Key findings

- A decrease in gamma oscillations was seen in the ipsilateral SI during anticipation of a painful somatic electrical stimulus, this has not been previously reported.
- Gamma oscillations were found in the contralateral SI in response to somatic electrical stimuli in Studies 1, 2 and 4.
- All participants showed clear evoked responses in SI, however gamma oscillations was observed in only 50-67% of participants.
- SAM peaks and clear evoked responses could be seen in SI during oesophageal electrical stimulation, showing that this area was involved in the processing of visceral stimuli, however no change in gamma oscillations was observed.
- No change in gamma oscillations was seen during cold pressor testing.
- During Study 2 (visceral vs somatic), a gamma increase was seen in response to somatic pain between 100-250ms in 64% of participants, which was not coincident with the peak of the evoked response indicating that this gamma response is not purely a transient increase in synchrony caused by the evoked response but is temporally distinct from that.
- Gamma oscillations were not solely seen during painful stimuli but were also seen in response to non-painful stimuli. In Study 4 (stimulus intensity), the strength of gamma increase was found to correlate with the intensity of the stimulus. This data suggests that gamma oscillations in SI may be involved in intensity encoding rather than specifically reflecting pain perception as suggested by Hauck et al (2007a) and Gross et al (2007). This has not previously been characterised.
- The frequency of the gamma response was found to decrease across the train during both Studies 1 and 4.

- Gamma oscillations were found to be at a lower frequency in Study 4 when the ISI between each stimulus of the train was 140ms as compared to Study 1 when the ISI was only 100ms.

7.3 Primary Somatosensory Cortex

SAM peaks with a pseudo t ≥1 were seen in SI in all participants during all blocks, in Study 1 (anticipation), Study 2 (visceral vs somatic), Study 3 (CPT) and Study 4 (stimulus intensity). The main activation was in contralateral SI during somatic pain although peaks of lower value were often seen in the ipsilateral side. The contralateral activation of SI during somatic pain is consistent with the literature in that, the sensory and nociceptive fibres are known to cross the midline before reaching the contralateral thalamus which then projects to SI. Contralateral SI activation has been seen in response to somatic pain in many previous studies (Timmermann et al., 2001, Ploner et al., 1999, Ploner et al., 2000, Bornhovd et al., 2002).

During visceral pain, a significant peak was found in the right SI from group SnPM analysis. Activity was also seen in the left SI but it did not reach significance at the group level. The precise location of the SAM peaks in SI showed considerable inter-individual variability, but were most commonly found slightly lateral to the hand area. Although SI is known to be the primary area for somatosensory processing (Apkarian et al., 2005), it's involvement in visceral processing is debated. Aziz et al (2000a) stated that visceral sensation primarily activates SII whereas SI representation is vague. Schnitzler et al (1999) found bilateral SII responses but no response in SI. However, other studies have found SI activation during visceral stimuli using a variety of neuroimaging techniques (EEG, MEG, fMRI) (Hecht et al., 1999, Hobson et al., 2005, Coen et al., 2007). Hobson et al (2005) found evoked responses in both left and right SI in response to distal oesophageal electrical stimulation although more participants showed a left hemisphere dominance than right or bilateral. Bilateral SI activation was seen using fMRI during balloon distensions of the distal oesophagus

by Coen et al (2007). The results of Study 2 indicate that SI is involved in visceral processing. This may result from direct afferent projections, but it may also be that oesophageal stimulation involves referred pain to the chest wall and therefore SI subsequently is activated.

7.3.1 Evoked responses

In Studies 1, 2 and 4, all participants showed clear evoked responses in SI to somatic electrical stimulation. Study 3 was not an event-related paradigm so evoked responses could not be investigated. In Studies 1 and 4, the amplitude of the 70ms component of the evoked response decreased substantially from the first pulse of the train to the second and then appeared to plateau out (Chapter 3: Figures 3.9, 3.10 and Chapter 6: Figure 6.4-6.6). This may be an indication of habituation across the train, this has been seen previously in response to contact heat stimuli using EEG (Greffrath et al., 2007). In this study most of the habituation was seen in the first few stimuli and then no more was seen after that, this would fit with the data seen in Studies 1 and 4 as the amplitude of the 70ms component to the second stimulus was much lower than the first but then remained consistent in amplitude for the remainder of the stimuli. A similar change in amplitude has been seen *in vitro* to repetitive stimulation and is termed the augmentation response, this was reproduced using MEG in awake human subjects in that a component of the evoked response at a latency of 35ms was seen to decrease in amplitude during the first few stimuli of a 10Hz train of electrical pulses (Huttunen, 2010). This was linked to a reduction in IPSPs. In order to further investigate the possible habituation of the amplitude of the 70ms component of the evoked response, altering the ISI between each electrical pulse (e.g. 10Hz, 5Hz, 2Hz, 1Hz) and monitoring the changes to evoked and induced responses across the train of stimuli would be of value.

In Study 2 (visceral vs somatic), clear evoked responses were seen during somatic and visceral pain in SI. This data indicates that SI is involved in the processing of visceral pain and agrees with studies by Hobson et al (2005) and Coen et al (2007).

The morphology of the evoked response was generally triphasic (Ploner et al., 2000, Hobson et al., 2000a) although there was some variability between participants. The average latency of the first peak of the evoked response in somatic pain was 25±6ms and for distal oesophageal pain was 79±27ms (see Table 4.3). The first peak during somatic pain is consistent with the 20ms component that is well documented in the literature (Kakigi et al., 2000). Distal oesophageal stimulation has been found to have longer latencies than somatic in previous studies (Hobson et al., 2000a, Sami et al., 2006), Hobson et al (2005) found the earliest cortical activity in response to oesophageal electrical stimuli at ~85ms whereas somatic stimuli often trigger evoked responses at a latency ~20ms (Della Penna et al., 2004). The delay seen in visceral evoked responses may be due to a different population of neurons being activated. As the wall of the distal oesophagus contains smooth muscle as opposed to the striated muscle of the proximal oesophagus, it is likely to be less well represented in SI, and it may be that the visceral SI response seen in Study 2 is due to referred pain to the chest therefore explaining in part the delayed evoked response. There was a consistent difference across the group in the amplitude of the evoked response in that it was larger for somatic than visceral pain, as can be seen in Chapter 4: Figure 4.18 from the different scales used. This may be due to the better contact of the electrodes on the skin as compared with the distal oesophageal electrical catheter. It may also be due to the fact that visceral regions have less representation in SI (Aziz et al., 2000a) and therefore the amplitude of the evoked response is smaller. The latencies of the evoked responses suggest that they were mediated by Aδ fibres rather than C fibres (Forss et al., 2005).

7.3.2 Gamma oscillations (~30-100Hz)

Gamma oscillations have been seen in response to painful stimuli in SI previously and have been linked to pain perception (Gross et al., 2007) and attention to pain (Hauck et al., 2007a). The role of gamma oscillations is not fully understood although it has been hypothesised that they have a role in encoding information about sensory stimuli and binding different features of a stimulus together (Engel and Singer, 2001).

Gamma frequency has been found to encode aspects of visual stimuli, such as the spatial frequency of the visual stimulus (Hadjipapas et al., 2007) within its oscillations. It is possible that gamma oscillations provide a similar role for other types of sensory stimuli. This section will discuss the changes in gamma oscillations in SI across all 4 studies. Each issue with the gamma response shall be explored with reference to the relevant literature.

7.3.2.1 Results from Somatic electrical stimulation

During Studies 1, 2 and 4, an increase in gamma power was seen in response to painful electrical stimulation in a proportion of participants. In Study 1 (anticipation), group SnPM analysis found a significant decrease in gamma power in the ipsilateral SI during anticipation of a painful median nerve stimulation to the wrist, followed by a significant increase in gamma power in the contralateral SI during the painful stimulus (in 67% of participants) which consisted of a 2s train of electrical pulses delivered at 10Hz. The increase in gamma power was seen in the range of 30-100Hz. There was 100ms between each electrical pulse in the train and an increase in gamma oscillations could be seen in response to each pulse within this timeframe. An increase in gamma oscillations was also present in 44% of participants during the non-painful block.

During Study 2 (visceral vs somatic), an increase in gamma power was seen in SI in 64% of participants in response to a single painful electrical stimulus to the right index finger, the electrical stimuli were delivered at a rate of 0.2Hz. The increase in gamma oscillations was seen between 60-100Hz and at a latency of 100-250ms. Participants that did not show a gamma increase, still showed clear evoked responses in SI. No change in gamma oscillations was observed in any participants during somatic non-painful sensation. An increase in gamma power was seen in SI in Study 4 (stimulus intensity) in response to a 2s train of electrical pulses delivered at a rate of 7Hz in 50% of participants. The latency of the gamma response in this study was ~20-140ms and was at a lower bandwidth to the previous studies (~25-70Hz), this is discussed

further in Section 7.3.2.8. It was present in both sensory and painful stimuli and increased in strength as the stimulus intensity increased.

7.3.2.2 Results from Visceral electrical and CPT studies

In Study 2, electrical stimuli were delivered to the distal oesophagus, no gamma oscillations were apparent in SI during painful or non-painful oesophageal stimulation despite the presence of clear evoked responses in all participants. During CPT in Study 3 (cold pressor test), no change in gamma oscillations was apparent across the room temperature pack and the cold ice pack.

7.3.2.3 Is gamma pain-specific?

Gamma oscillations have been seen in response to experimental sensory stimuli (Tecchio et al., 2003, Tecchio et al., 2008, Fukuda et al., 2008) and painful stimuli (De Pascalis and Cacace, 2005, Gross et al., 2007, Hauck et al., 2007a) using both electrical and laser stimulation to the finger or hand. Gross et al (2007) found a relation between the strength of the gamma response and pain perception. For stimuli around pain threshold, if the individual rated the stimulus as painful then the gamma response would be stronger than if it was rated as non-painful, when at the same stimulus intensity. De Pascalis et al (2004) found that hypnotic suggestion of analgesia induced a reduction of the phase-ordered gamma patterns in response to an electrical pain stimulus. Hauck et al (2007) found two gamma responses to intracutaneous electrical pain using MEG, one of which was strengthened during focussed attention to the stimulus.

These studies suggest that gamma oscillations may have an important role in the perception of pain. If gamma oscillations were an indicator of whether an individual was perceiving pain or not, then this would be incredibly valuable clinically. If a cortical biomarker for pain could be elucidated then it would diminish the reliance on subjective reports of pain from patients. Objective measures from cortical activity

could be obtained to determine the pain an individual was experiencing. It would also be of great use in testing the efficacy of new drugs and therapies.

The results of Studies 1 (anticipation) and 4 (stimulus intensity) show that the gamma increase was seen during both painful and sensory electrical stimulation. Study 4 demonstrated that there was no apparent change as the sensation went from sensation to pain. This data would indicate that gamma oscillations would not be a suitable cortical biomarker for pain but may encode different features of the stimulus. Gamma oscillations were not present during different modalities of pain, they were not seen during electrical stimulation of the distal oesophagus or during CPT. This suggests that they cannot be generalised to different types of pain and therefore would not make an appropriate biomarker for pain. This is in opposition to a study by Gross et al (2007) who linked gamma oscillations specifically to pain perception, however no sensory comparison was performed in this study or the study by Hauck et al (2007) to determine whether gamma oscillations were also present during non-painful stimuli.

CPT causes a strongly painful sensation that is more akin to second pain mediated by C fibres as opposed to first pain mediated by Aδ fibres (Ploner et al., 2002). The difference in gamma oscillatory dynamics during CPT and electrical stimulation could be due to different fibre activation. However, animal data has suggested that both nociceptive fibre types are activated by cold pain (Simone and Kajander, 1997). The distal oesophagus has different innervations to both the proximal oesophagus and somatic structures in that the vagal afferents are predominantly unmyelinated C fibres (Aziz et al., 2000b) whereas vagal afferents from the proximal gut are mainly myelinated Aδ fibres. However, sensory information from the oesophagus travels via both spinal and vagal afferents and the electrical stimulation of the distal oesophagus is believed to activate a mixture of Aδ and C fibres and the latencies of evoked responses from this region suggest activation of Aδ fibres due to the faster conduction velocities (Schnitzler et al., 1999, Hobson et al., 2000a, Sami et al., 2006).

It is possible that both CPT and visceral electrical stimulation activate a combination of Aδ and C fibres and that perhaps this is why, no gamma oscillations were found during these stimuli. Another possibility is that, a high degree of synchronization is required in order to see this transient gamma response above the noise in the MEG data and CPT may not provide the same degree of neural synchrony as it does not drive the afferents and thus the cortex as strongly in the temporal domain. There is also only one trial in CPT and this stimulus is less time-locked than when using somatic electrical stimuli. Generally the pain threshold level in mA for oesophageal stimulation was much higher than in somatic stimulation, in fact 45% of participants reached the maximum stimulus intensity on the electrical stimulator before reaching their pain tolerance level, and it may be therefore that the oesophageal electrical stimulation was not driving the cortex to the same level of neural synchrony as during somatic pain and therefore did not show any change in gamma oscillations.

7.3.2.4 Gamma and stimulus intensity

Gamma oscillations have been found to encode information about different aspects of a stimulus, such as information about the spatial frequency of a visual stimulus being encoded in the temporal characteristics of gamma oscillations in the visual cortex (Hadjipapas et al., 2007). A one-way within-subjects ANOVA was performed on the 6 gamma responders in Study 4 and intensity was found to be a significant factor in determining the strength of the percentage increase in the gamma response ($F_{(5)}$=7.29, p=0.003). These results suggest that the intensity of a sensory stimulus may be encoded within the strength of gamma oscillations in SI. Changes in SI activation have been found to correlate with stimulus intensity in previous studies (Bornhovd et al., 2002), perhaps the changes seen in this study relate to changes in oscillatory dynamics in the gamma range.

It has been hypothesised that the timing of pyramidal cell firing within the gamma cycle may be able to encode information about sensory stimuli (Fries et al., 2007). It is possible therefore that, intensity information of somatic stimuli are encoded in the

strength of the gamma synchrony. An increase in gamma power, as seen in Study 4, indicates an increase in synchrony of neurons in the gamma range, as intensity increases, more neurons become synchronous. This may be a mechanism for how information about stimulus intensity is processed in SI. If more can be understood about these oscillations and what different aspects of the gamma response encode for then this would be relevant in terms of abnormal sensory processing and how this may be treated.

7.3.2.5 Gamma and anticipation

During anticipation of pain in Study 1, a significant decrease in gamma oscillations was seen in ipsilateral SI at the group level that was spatially consistent with the hand area. This decrease was not apparent in individual bootstrap spectrograms. This suggests that the decrease in gamma oscillations in each individual was small but was consistent across the group in order to become statistically significant at group level. Changes in gamma frequency during anticipation have not been mentioned in the literature previously. Oscillatory changes during anticipation have been noted in other frequency bands, for example, a decrease in alpha in EEG electrodes over contralateral central regions was seen in anticipation of a painful laser stimulation (Babiloni et al., 2006). It is possible that this change in gamma oscillations during anticipation could be linked to attentional processing. Gamma oscillations have been linked to attention previously by Hauck et al (2007), they found that a late, high-frequency gamma oscillation, seen in response to intracutaneous electrical stimulation, was strengthened during focussed attention. It is possible that anticipation stimulates focused attention towards the site of the pain stimulus, altering the gamma oscillations. A decrease in gamma oscillations seen during anticipation may be associated with the activation of inhibitory feedback processes attempting to restrict the pain experienced.

7.3.2.6 Gamma responders and non-responders

Not all participants showed gamma responses in each study; 67% in Study 1 (anticipation), 64% in Study 2 (visceral vs somatic) and 50% in Study 4 (stimulus intensity). It was hypothesised that the presence of gamma oscillations may have been related to pain thresholds. However, t-tests in Studies 1 and 2 comparing pain thresholds of those with and without gamma oscillations, were not found to be significant. From this, it would appear that the individual's pain threshold did not govern the presence of gamma oscillations in response to electrical stimulation. In data from neuroimaging studies, a large amount of inter-individual variability can be seen and it may simply be that the presence or lack of gamma oscillations in these studies is due to the individual differences between participants.

Another possibility for why some individuals show gamma oscillations and other don't is the potential link between the oscillatory dynamics of the cortex and the way an individual's autonomic system responds to stimuli. There appears to be a dichotomy in individuals autonomic responses to pain in visceral experimental pain studies (Paine et al., 2009b, Paine et al., 2009a) in that they can react with a sympathetic nervous system 'fight-or-flight' response or with a parasympathetic reaction. This has been linked to personality traits such as neuroticism and anxiety. Cortical evoked potentials (CEPs) have been found to distinguish between hypersensitive and hypervigilant reactions to pain in that the amplitude of CEPs in hypersensitive individuals is larger than normal for the same stimulus intensity whereas for hypervigilant individuals, the CEP is normal but the pain thresholds are reduced (Hobson et al., 2006). Both autonomic responses and CEPs are able to indicate influences of personality traits and psychological factors such as anxiety on pain processing. It would be interesting to see how the autonomic response to pain and sensation compares with the oscillatory dynamics and perhaps the presence or absence of gamma oscillations across a group as it is possible they may be linked.

7.3.2.7 Evoked vs Induced Gamma

During Studies 1 (anticipation) and 4 (stimulus intensity), an increase in gamma oscillations was apparent in response to each electrical pulse within the train. In Study 1, the train was at 10Hz so there was only 100ms between each pulse, gamma oscillations were seen within this time frame. In Study 4, the pulses of the train were delivered at 7Hz so there was 140ms between each pulse. An increase in gamma oscillations was apparent within this time frame. In Study 2, only one electrical pulse was administered each time and there was 5s between each stimulus. The latency of the gamma response that was seen during this paradigm was later, between 100-250ms in all participants. This is summarised in Table 7.1.

	Latency of gamma
Study 1	~20-100ms
Study 2	~100-250ms
Study 4	~20-140ms

Table 7:1 summarises the different latencies of gamma response in Studies 1, 2 and 4. Studies 1 and 4 used a train of stimuli and gamma was seen in response to each stimulus within this timeframe. Study 2 used one brief electrical pulse and a gamma increase was seen much later.

In Chapter 4: Figure 4.12, the evoked response and the gamma increase are both displayed across the first 500ms after stimulation. From these figures, it is clear that the gamma response observed in this study was not simply part of the evoked response as there was a delay between the peaks of the evoked response and the gamma response. From this, it can be inferred that rather than being a transient synchrony within the gamma range as a function of the evoked response, the gamma increase seen was quite possibly induced, the latency of this gamma response was similar to induced gamma found in response to other sensory stimuli and has been loosely termed a “neural substrate of cognitive awareness” suggesting that it is involved in higher-cognitive processing of stimuli and creating a coherent perception, binding different stimulus features together (Tallon-Baudry and Bertrand, 1999).

In the literature, the latency of the gamma response has varied between studies also. In Fukuda et al (2008), high-frequency gamma oscillations (100-250Hz) were seen

between 15-100ms after a median nerve stimulation at the wrist in intracortical electrodes over the post-central gyrus. These were found to start as phase-locked (evoked) and became non-phase-locked (induced) with time. 'Low-frequency gamma' (30-100Hz) was 30% non-phase-locked at 15ms but became 88% non-phase-locked by 100ms. 'High-frequency gamma' (100-250Hz) was 40% non-phase-locked at 15ms and became 98% non-phase-locked by 55ms. De Pascalis et al (2004) found gamma responses between 0-150ms which they stated were evoked ('phase-ordered') gamma oscillations that are believed to reflect the early processing of a stimulus.

Gross et al (2007) saw gamma oscillations between 60-95Hz at a latency between 100-300ms after painful laser stimuli to the dorsum of the hand which was found to be induced. Hauck et al (2007a) found two different gamma responses; one between 60-80Hz at 50-250ms and also a high-frequency late gamma component (120-140Hz, 400-600ms), both of these were found to be induced. The gamma response seen in Gross et al (2007) and the first gamma pattern in Hauck et al (2007) tie in almost exactly with respect to frequency band and latency of the gamma response seen in Study 2 (visceral vs somatic) in this thesis which may have a strong induced component (see Chapter 4: Figure 4.13). The gamma oscillations in Studies 1 (anticipation) and 4 (stimulus intensity) tie in closer with the gamma responses seen in Fukuda et al (2008) and De Pascalis (2004). The increase in gamma oscillations seen in Study 4 was analysed to separate out the evoked and induced components; in Chapter 6: Figure 6.12 it is apparent that the gamma response had a strong evoked component but may still have had some induced activity at the onset of the train. It may be that the gamma oscillations seen during Studies 1 and 4 were evoked gamma and represent early processing of sensory stimuli and are able to encode stimulus intensity within the strength of its oscillations as shown in Study 4. The gamma oscillations seen in Study 2 may have been a more induced gamma response that could be involved in higher order processing of the stimuli, such as creating an overall perception of the sensory experience and evaluating it.

7.3.2.8 Change in Bandwidth of gamma across train

An interesting phenomenon in the data from Studies 1 and 4 was how the frequency of the gamma oscillations appeared to decrease across the train of electrical pulses (see Chapter 3: Figures 3.12, 3.14, 3.15 and Chapter 6: Figures 6.7-6.9). The amplitude of the evoked response in these studies was also found to decrease across the train of pulses which has been seen in previous studies and linked to a reduction in IPSPs (Huttunen, 2010). It is possible that the decrease in gamma frequency was related to the evoked response in these studies and a change in the IPSPs and therefore the frequency of gamma response may have altered in conjunction with the amplitude of the evoked response (see Section 7.3.1). This decrease in bandwidth could be due to habituation at either the peripheral or central level (Greffrath et al., 2007).

This decrease in bandwidth in the gamma range has been seen in a number of other studies. In a study by Fukuda et al (2008); the gamma response was initially 100-250Hz but gradually slowed to <100Hz from 0-100ms. Chen and Hermann (2001) found gamma oscillations in response to painful median nerve stimulation and found that the frequency of the oscillation slowed across time. It started over the somatosensory cortex around 80Hz at 26ms after stimulation and slowed down through beta to alpha at ~10Hz at 160ms becoming more widespread across central and parietal regions with time. A study by Haenschel et al (2000) administered novel auditory stimuli to humans using EEG and found an evoked gamma response which was then replaced by beta oscillations.

This gamma-to-beta shift can be explained by a cellular mechanism discovered during *in vitro* work in rat hippocampal slices using tetanic stimulation (Whittington et al., 1997, Traub et al., 1999, Bracci et al., 1999). The interneurons in the cortex oscillate at gamma frequency and have an inhibitory effect on the pyramidal cells which provide the signal recorded in MEG. The interneurons in the cortex fire at gamma frequency and, as a result of their inhibitory effect on the pyramidal cells,

entrain the population to oscillate at gamma frequency; this is the signal recorded in MEG (Murakami and Okada, 2006). If stimulated tetanically *in vitro*, there is an increase in the excitatory influence of the pyramidal cells due to an increase in the amplitude of EPSPs and AHPs. Pyramidal cells naturally fire at a lower frequency (low-beta) than interneurons and as a consequence of their increased influence, either directly or indirectly, the field oscillation is slowed to beta frequency (see Chapter 3: Figure 3.18).

In Studies 1 and 4, it was possible to see that the range of gamma frequency was different between the two. During Study 1, the train of stimuli were delivered at 10Hz whereas in Study 4, they were delivered at 7Hz. The bandwidth of gamma change during Study 1 across participants was from ~65-100Hz at the beginning of the train to ~45-75Hz at the end whereas in Study 4 it was ~20-75Hz at the beginning and 20-55Hz by the end of the train which crosses over into the beta range. It is possible that the different ISIs affected the frequency of the gamma response. This could give a clue to the role that gamma oscillations play and the information which they encode. There is an inhibitory feedback network at the cellular level which could control gamma oscillations. The interneurons of the cortex have an inhibitory influence over pyramidal cells. The pyramidal cells are only able to fire during a certain point in this inhibitory cycle creating an oscillation at gamma frequency (Fries et al., 2007). It is plausible that the inhibitory influence of the interneurons is affected by the preceding stimulus and therefore may alter with different ISIs, this may in turn affect the frequency of the gamma oscillations.

7.3.2.9 Gamma and attention

Gamma oscillations have been linked to attention previously by Hauck et al (2007) who stated that the gamma increase they saw between 400-600ms and 120-140Hz was strengthened with directed attention to the stimulus. Other frequency bands have also been linked to attention previously such as alpha (Ohara et al., 2004). In fact it has been suggested that desynchronization of alpha is necessary for the

synchronization in the gamma band (Ward, 2003). From the results of these studies, both a decrease in alpha and an increase in gamma oscillations can be seen but it is not clear whether there is a causal relationship between the two.

As pain is of high behavioural importance, it is logical to expect that oscillatory dynamics will change during attention to and away from a stimulus. It does not appear that the gamma response is solely based on attentional factors as it was only the later, higher-frequency gamma response that was strongly affected by attention in the study by Hauck et al (2007). The gamma oscillations seen in Studies 1, 2 and 4 could be related to attentional processing; due to the high behavioural importance of pain, attention is likely to be strongest during the highest intensity painful stimulus. Also the gamma change seen in anticipation during Study 1 could reflect attention and arousal in preparation for the imminent pain stimulus.

7.3.2.10 High frequency gamma (>100Hz)

High-frequency gamma oscillations were seen over the post-central gyrus in a study by Fukuda et al (2008) between 100-250Hz in response to a non-painful median nerve stimulation at the wrist. Hauck et al (2007) saw an increase in high-frequency gamma oscillations between 120-140Hz in response to intracutaneous painful stimulation of the finger. Spectrograms were created up to 150Hz in Study 1 and up to 200Hz in Studies 2 and 4. No increase in gamma oscillations was apparent at these higher frequencies (see Chapter 3: Figure 3.17 and Chapter 6: Figure 6.8). A disadvantage of wavelet analysis for time-frequency spectrograms is that resolution is not as accurate at high frequencies, there is a possibility that this masked the high-frequency gamma oscillations.

7.3.3 Beta oscillations

During all 4 studies in this thesis, a decrease in beta was seen in SI during visceral and somatic electrical pain and cold pressor pain. During Study 2, a significant decrease was seen in SI in the beta band during both visceral and somatic painful

electrical stimulation in group SnPM analysis, in left (contralateral) SI during somatic stimulation and right SI during visceral stimulation. The decrease in beta was clear in the spectrograms of all participants during somatic pain and then a rebound was evident in 64% of these. During visceral pain, 45% of participants showed a decrease in beta frequency after the stimulus. In Study 3, a trend was seen in individuals suggesting that beta decrease at the onset of the cold ice pack in 71% of participants, and returned back to baseline levels across the course of CPT in 29% of participants. In Study 4, a trend was seen across all participants for beta to decrease and then rebound at around 500ms after the offset of the stimulus. However, no significant activations in SI were seen at the group level using SnPM analysis during Studies 3 (CPT) and 4 (Stimulus intensity).

Beta desynchronization has been seen in response to tactile stimuli in both somatosensory and primary motor cortex, followed by a rebound in the motor cortex (Cheyne et al., 2003, Gaetz and Cheyne, 2006). Beta desynchronization has been seen in response to noxious stimulation in both the motor cortex (Raij et al., 2004) and in primary somatosensory cortex ~400-500ms after stimulation (Ploner et al., 2006a, Ohara et al., 2006).

These results confirm what has been seen in the literature, that beta power is found to decrease during sensory and painful stimuli in the somatosensory cortex, followed by a rebound. The beta decrease was seen in a variety of different stimuli in these studies. It was apparent during electrical stimulation of the median nerve, index finger and distal oesophagus in both pain and sensory blocks, although less strong during sensation. It was also seen during cold tonic pain in Study 3. It is possible that the beta desynchronization seen in these studies facilitates a movement away from the painful stimulus, and the rebound could be due to a recalibration of the motor system (Baker, 2007). Beta has been found to change during CPT previously; in Chang et al (2002), an increase was seen over peripheral bi-temporal regions which they interpreted as a hyperarousal due to the tonic pain. In Chen et al (1994) an increase was seen at a higher beta band (24.5-31.5Hz) over temporal regions and away from

the central gyrus, in the same study a decrease in lower beta bands was seen (13-18Hz and 18.5-24Hz) in the contralateral side above the central gyrus. The beta band used for the studies in this thesis was 15-30Hz, this covers both the low and high beta bands in these CPT studies. It is possible that there were more complex changes at different frequencies of beta in Study 3. It would be interesting to rerun SAM analysis using smaller ranges of beta frequency band in order to investigate exactly which frequencies of beta increased and decreased. It is not clear why beta gradually returned to baseline levels in 29% of participants during CPT. It could be a demonstration of adaptation by receptors at the peripheral level (Stein et al., 2009) or due to the activation of central inhibitory feedback mechanisms controlling the amount of pain perceived (Streff et al., 2009) which may then have had an impact on the cortical oscillations.

7.3.4 Alpha oscillations

Alpha tends to be a widespread phenomenon across the cortex and relates to attentional processing and arousal. Lower frequency oscillations are often found to be more widespread with the high frequency oscillations becoming more focal (Pfurtscheller and Lopes da Silva, 1999). The alpha rhythm is thought of as an idling frequency and is seen during states of rest and when eyes are closed, especially over the occipital cortex (Hari and Salmelin, 1997, Pfurtscheller and Lopes da Silva, 1999). A decrease over central areas during anticipation of a painful stimulus was seen in a study by Babiloni et al (2006), this could be linked to levels of arousal and attention focused on the stimulus.

In Study 1 (anticipation), there was a significant decrease seen in the alpha band in contralateral SI during anticipation of the sensory stimulus from group SnPM analysis (see Chapter 3: Figure 3.6). A decrease in alpha could be seen in the Group SAM image in the other SAM comparisons (anticipation of pain, sensation and pain) (see Chapter 3: Figure 3.3) although it appeared more widespread over the somatosensory cortex. During somatic pain in Study 2 (visceral vs somatic), 42% of

participants showed a clear 10Hz oscillation during the baseline period, in 27% of participants this disappeared at around 200ms and then returned to baseline levels at around 600ms. During visceral pain, a decrease was seen in 45% of participants which rebounded at around 600ms, similar to the beta frequency, these two together could be considered as a mu rhythm which is commonly seen over the somatosensory cortex and is made up of 10 and 20Hz components (Hari and Salmelin, 1997). In Study 2, both 10Hz and 20Hz oscillations decreased ~200ms after the stimulus onset and rebounded at ~600ms post stimulus. In Study 3, a decrease in alpha was seen in 57% of participants. This is likely to indicate a higher level of arousal and attention to the painful stimulus. A similar decrease has been seen in response to CPT previously in the vicinity of the central gyrus (Chen and Rappelsberger, 1994, Dowman et al., 2008) and in posterior regions (Chang et al., 2002). A trend was seen for alpha to decrease during somatic electrical stimulation in Study 4 at the group level (see Chapter 6: Figure 6.2) however this did not reach significance using SnPM analysis.

The suppression of alpha rhythms seen during stimulation in the studies in this thesis could be linked to an attentional arousal due to the high behavioural importance of pain. These results potentially link with previous studies that found that attention to pain accentuated alpha desynchronization (Ohara et al., 2004, Ohara et al., 2006), however a study focussing on altering attentional states to pain would be needed to confirm the link with attentional processing.

7.4 Other areas of the pain matrix

SAM peaks were found in SII, ACC and Insula in a proportion of participants in all studies. This demonstrates that somatic and oesophageal electrical stimulation and CPT involve these areas of the pain matrix. It was thought that due to oesophageal stimuli and CPT providing a more unpleasant emotional response, the affective areas of the pain matrix (ACC, Insula) may have shown a stronger involvement during these stimuli. SAM peaks were found in ACC and insula in all stimuli, and at these locations evoked responses were observed but no clear changes in oscillatory dynamics were seen.

	Percentage of participants showing SAM peaks with pseudo t ≥1			
	SI	SII	ACC	Insula
Study 1				
Anticipation sensory	89	33	78	56
Sensory	100	66	100	56
Anticipation pain	100	56	78	66
Pain	100	56	66	78
Study 2				
Somatic sensory	100	73	27	64
Somatic pain	100	91	55	64
Visceral sensory	100	73	27	64
Visceral pain	100	73	45	55
Study 3				
CPT	100	100	86	86
Study 4				
Low sensory	100	67	33	17
High sensory	100	58	58	42
Low pain	100	50	58	50
High pain	100	58	58	67

Table 7:2 shows the percentage of participants showing activation (SAM peaks with a pseudo t ≥1) in key areas of the pain matrix during different SAM comparisons across all 4 studies.

7.4.1 Secondary Somatosensory Cortex

7.4.1.1 Bilateral SII activation

Table 7.2 demonstrates the percentage of participants who showed SII activation in all 4 studies during the different SAM comparisons. In the literature, SII is most commonly activated bilaterally in response to experimental pain stimuli (Coghill et al.,

1999, Timmermann et al., 2001, Ploner et al., 2002). Only 33% of participants in Study 1 (anticipation) were bilateral. 60% of participants during somatic pain and 25% during visceral pain showed bilateral SII activation in Study 2 (visceral vs somatic). In Study 3, 29% had bilateral activity and in Study 4, 42% had bilateral activity in SII. It is possible that bilateral activation was not found in all participants due to a limitation of SAM analysis. SAM treats any highly coherent sources as originating from a single location, this enables it to eliminate sources of environmental noise but also may mean that bilateral activity is seen in only the dominant hemisphere.

7.4.1.2 SI vs SII: activated in parallel or in series?

There is controversy in the literature as to whether SI and SII are activated in parallel or in series. Ploner et al (1999) found them to be activated in parallel, with latencies of ~131±7ms for SI and ~126±4ms for SII, as did Hobson et al (2005) who found a responses in both SI and SII at around ~85ms following oesophageal stimulation. Others have claimed they are activated in series with SII activation occurring later than SI (SI ~20-35ms, SII ~70-150ms) (Della Penna et al., 2004). All participants that showed SAM peaks in SII during somatic stimulation in Study 1 (anticipation) and 2 (visceral vs somatic) had clear evoked responses. Examples of these can be seen in Chapter 3: Figures 3.11 and 3.19 and Chapter 4: Figures 4.9 and 4.15). In Study 2 (visceral vs somatic), 6 out of the 8 participants that showed SII activation in visceral pain showed clear evoked responses (see Chapter 4: Figure 4.20). During somatic pain in Study 2 (visceral vs somatic), the first peaks in SI and SII were 25±6ms and 76±24ms respectively (see Chapter 4: Table 4.3). The latency of SI seen in Study 2 is similar to the well documented 20ms component of the somatosensory evoked response found in the literature (Kakigi et al., 2000), the latency of the first peak in SII is near to that found in studies by Frot et al (1999) which found the main components of the SII evoked response to be around 60ms and 90ms. This data would agree with data by Frot et al (1999) and Della Penna et al (2004) suggesting that SI and SII are activated in series rather than in parallel. However, evoked responses from distal oesophageal pain are similar for SI and SII. The first peak in SI is 79±27ms and the

first peak in SII is 73±30ms which would suggest that they are activated in parallel, this data is consistent with results from Hobson et al (2005) which also delivered distal oesophageal electrical stimulation. It may be that the SI evoked response to distal oesophageal stimulation is delayed due to different fibre activations or due to it being referred pain to a somatic area.

7.4.1.3 SII vs Insula: functionally different areas?

In Study 1 (anticipation), clear evoked potentials in the insula could be seen in 78% of participants that showed SAM activations. In Study 2 (visceral vs somatic), 100% of participants that showed SAM activation in the insula during somatic pain showed clear evoked responses compared with 83% during visceral pain. During somatic pain in Study 2, the first peak in SII was 76±24ms and for insula was 119±33ms showing that activation in SII is much earlier than in the insula. This data is consistent with data from Frot et al (2007) who were able to tell the two areas apart based on their latencies (SII~140-170ms, Insula~180-230ms). The latencies in this study were based on laser stimulation, however when they performed a similar study with electrical stimulation the latencies for SII evoked responses were comparable with those seen in Study 2 (~60-90ms) (Frot et al., 2001).

Another difference between cortical responses in SII and insula is in the oscillatory dynamics. In Study 1, an increase in gamma oscillations was seen in SII during pain in 22% of participants (see Chapter 3: Figure 3.19) whereas very little change was seen in the insula. In Study 2, during somatic pain, a decrease in both alpha and beta could be seen in SII (see Chapter 4: Figure 4.15) whereas no changes were observed in the insula (see Chapter 4: Figure 4.17).

7.4.1.4 Gamma in SII

In Study 1 (anticipation), an increase in the gamma band was observed during pain in 2 participants (Chapter 3: Figure 3.19) similar to that seen in SI but less strong. It was also seen to decrease in frequency across the duration of the train. This shows

similarities to the gamma oscillations seen in SI. The binding problem (Treisman, 1996) refers to the complex issue of trying to understand how many different areas of the brain coordinate information about a sensory stimulus, whether it be visual, smell, touch, pain and so on. Many different features must combine together in order to form a coherent perception of the stimulus. It is hypothesised that this may happen due to many different neuron populations oscillating in synchrony, potentially at gamma frequency (Engel and Singer, 2001). It is possible that the increase seen in both SI and SII in gamma oscillations in Study 1 demonstrates an exchange of information in these areas via synchronous oscillations. They may be encoding different information about the stimulus and then by oscillating in synchrony, the information can be combined into a coherent perception.

7.4.2 Anterior cingulate and Insular cortex

Both the ACC and the insula are considered to be part of the pain matrix but have an involvement in the more affective side of pain and emotional processing (Apkarian et al., 2005). The ACC has been activated during experimental pain in a number of fMRI and PET studies (Davis et al., 1997, Hsieh et al., 1999, Sawamoto et al., 2000, Buchel et al., 2002). It is believed to be involved in the negative affect of pain, showing activations when observing other people in pain (Benuzzi et al., 2008). Activation in ACC has been found to correlate with pain affect, hypnosis has been used to alter the unpleasantness of a painful stimulus which consequently changed activation in ACC (Rainville et al., 1997). The insula is also commonly activated during experimental pain studies (Derbyshire, 2003), it is thought that it has a role in affective and emotional processing of pain (Apkarian et al., 2005).

Visceral pain is commonly seen to create a more emotional response than somatic and is considered generally as more unpleasant for the same intensity of stimulus (Strigo et al., 2002). Therefore it was hypothesised that there may have been stronger activation in the ACC and insula in response to visceral pain when directly compared to the somatic stimuli. CPT gives a more sustained, ecologically valid pain stimulus as

compared to the brief, stinging pain of electrical stimulation. It was hypothesised that this stimulus may also induce a greater emotional response and therefore stronger involvement of the affective areas. The results of the McGill questionnaires and unpleasantness ratings during Study 2 (see Chapter 4: Figure 4.2 and 4.3) confirm that the visceral stimulus was perceived to be more unpleasant. The percentage of participants that showed SAM activation in each study during each comparison can be seen in Table 7.2. There is no clear difference in the number of people showing activation of ACC and insula between somatic electrical stimulation and visceral electrical or CPT. It is a possibility that SAM activation in the ACC was not seen in all participants due to the fact that currents from the ACC are thought to be predominantly radial which may mean that they are harder to pick up with MEG recordings (Garcia-Larrea et al., 2003, Christmann et al., 2007), although Hillebrand and Barnes (2002) indicated that this is not as significant a problem as once thought.

Evoked responses were found in 75% of those that showed ACC activation from SAM in Study 1 (anticipation). In Study 2 (visceral vs somatic), 100% of participants that showed ACC activation during somatic pain showed clear evoked responses compared to 60% during visceral pain. Examples of these can be seen in Chapter 3: Figure 3.11, 3.20 and Chapter 4: Figure 4.9, 4.16, 4.21. In Study 2 (visceral vs somatic), evoked responses seen in ACC were biphasic with a peak at around 146±46ms in somatic pain and 142±50ms in visceral pain. In previous studies, evoked responses have been recorded from the ACC during experimental pain. Hobson et al (2005) found evoked responses in the cingulate peaking at ~100ms, Ploner et al (2002) found the first peak in ACC at 188ms and a later peak at 782ms using MEG and Christmann et al (2007) saw activation in the ACC at 200ms using EEG. A review by Garcia-Larrea et al (2003) claimed that laser evoked responses to pain in ACC are commonly found later than this at around 325-350ms and are biphasic. The latencies of the evoked responses in Study 2 (visceral vs somatic) correspond more closely to the work of Hobson et al (2005), Ploner et al (2002) and Christmann et al (2007).

Data from evoked responses in Study 2 showed the average latency of the peak amplitude during somatic pain in the insula to be 119±33ms and for visceral pain was 130±39ms. The latencies of evoked responses in the ACC and insula imply that they are likely to be involved in the higher-cognitive tasks and emotional processing as opposed to the sensory-discriminative components in the SI that are found at earlier latencies, this agrees with previous literature (Melzack and Casey, 1968).

7.4.3 Frontal Theta

Theta oscillations (~3-7Hz) have been hard to find in EEG/MEG recordings previously (Ward, 2003) but they have been linked to the encoding and retrieval of memory (Kahana et al., 2001). Theta oscillations have been found to change in response to painful stimuli in CPT over frontal regions (Chang et al., 2002, Chang et al., 2005). Theta has also been associated with pathological oscillations in chronic pain. Levels of theta are found to be higher during resting state in patients than in healthy controls over frontal areas (Sarnthein and Jeanmonod, 2008, Drewes et al., 2008).

There were significant changes found in theta in frontal areas. A significant decrease in theta was seen over right (ipsilateral) frontal cortex during anticipation of the sensory stimulus in Study 1 using SnPM analysis on the group data (see Chapter 3: Figure 3.6). In Study 4, a significant increase was seen in the theta band in the left middle frontal gyrus during high pain from SnPM analysis (see Chapter 6: Figure 6.3). The increase in frontal theta during these two studies may reflect the major components of the evoked response (see Chapter 6: Figures 6.13, 6.14). This matches with data on CPT that sees an increase of frontal theta during pain (Chang et al., 2002), however no changes in theta were observed during CPT in Study 3, nor in Study 2.

7.5 Methodological Issues

7.5.1 Group Analysis

Group Analysis of MEG data, and neuroimaging data more generally, can be misleading. The way Group SAM is calculated, it is possible that a strong response in one participant can have a large influence on the group figure as opposed to equal weighting from each individual, however SnPM does correct for this (Nichols and Holmes, 2002). Taking in to consideration the variance between the anatomy of individual's brains, the process of normalisation used in SPM software does not use anatomical markers such as the anterior and posterior commissure, it merely moulds the activity on to the template brain and therefore activity from SI in one participant may not be in the same anatomical location as another. SnPM is a more statistically robust form of group analysis and if significance is found from this then it is more reliable. However, there is the risk with group data that it does not pick up on details found within the individual data. During the analysis of these studies, the group data was used to get an overall idea of key areas and then the focus went back to the individual SAM peaks and time-frequency data.

7.5.2 Electrical stimulation

Electrical stimulation was used for 3 out of the 4 studies in this thesis. Electrical stimulation has the disadvantage of being less biologically relevant than other experimental pain stimuli such as mechanical pain or cold pain (Babiloni et al., 2007). It is an unfamiliar sensation not experienced during everyday life, it is not thought to be similar to chronic pain (Babiloni et al., 2007). It also has the disadvantage of activating both sensory and nociceptive fibres (both Aδ and C fibres) which means it is impossible to assign the oscillatory changes seen to a particular fibre type as opposed to laser stimuli which is able to selectively activate one or the other (Raij et al., 2004). In Study 2, during distal oesophageal electrical stimulation, the electrical catheter created noise in the data. This was problematic when looking at the raw data

at sensor level. However, SAM analysis was able to localise this noise to a location in the back of the throat and it could then be disregarded. ICA was used on the raw data in order to eliminate as much of the artefact as possible and was found to be effective (see Chapter 4: Figure 4.1) (Hyvarinen et al., 2010). Also, having created VEs in key ROIs, no artefact was obvious at the source level.

7.5.3 Psychophysics and thresholding

Understanding the amount of pain an individual experiences is a difficult task. It relies on their subjective ratings, their preconceptions about what is causing the pain or what treatment will be effective, their psychological state and many other factors. Many have attempted to find ways of quantitatively assessing pain using questionnaires and scales. Examples of this are the McGill questionnaire (Melzack, 1975) and a visual analogue scale (VAS) which requires the individual to rate their pain on a scale of 0-100 with pain anchors at either end such as ‘no pain’ and ‘worst pain imaginable’ (Timmermann et al., 2001).

In Studies 1, 2 and 4 in this thesis, it was necessary to perform thresholding on each participant at the start of the experiment. This involved ascertaining each individual’s sensory threshold, pain threshold and pain tolerance levels. This was achieved by explaining clearly and in a reproducible fashion between participants, what sensation should be felt at each threshold. The instructions given by the experimenter were open to interpretation by the participant and they may have been interpreted differently between different individuals. It is hard to control for this as people have different concepts about what qualifies as pain threshold, ‘worst pain imaginable’, ‘pain tolerance’ etc. The participants were instructed to go as high as they could for pain tolerance before they felt that they could not receive pain any stronger or would not want to. Ethically, it is important for the participants to understand that they must not go past a level of pain that they are comfortable with, however this means that it is likely that people will not reach their maximum tolerance.

After each recording block in Studies 1, 3 and 4, a McGill pain questionnaire was completed by the participant. In Study 2, this was combined with a rating on a 0-10 VAS scale with ‘no pain’ at 0 and ‘worst pain imaginable’ at 10. For Study 3, a Likert scale was used (Cruccu et al., 2004) which is a 0-10 scale with verbal anchors to each number (e.g. 6=mild pain). This seemed the best option as it combined a numerical scale with verbal instructions in order to give guidance as to what sensation correlated with which number. These questionnaires and ratings scales are the best way of quantifying the subjective experience of pain currently. It is the variability in this that demonstrates the importance of finding cortical biomarkers that would be able to measure pain objectively. These methods appeared to be accurate in ensuring pain and sensation were delivered at appropriate levels and that the stimuli were creating the desired amount of pain. However the results did vary between individuals, for example in Study 3, some participants only reached a Likert score of 4 whereas others went up to 9 despite the stimulus being identical.

7.5.4 Train of electrical pulses vs Individual pulse

For Study 1, a train of electrical pulses was used as during pilot studies a 2s train of stimuli at 10Hz created a pain that felt more tonic in nature and as the paradigm was investigating anticipation, it was felt that a strong, longer stimulus would induce greater anticipation than one brief stimulus. The disadvantage of using a train of stimuli was that it was not possible to see the entire evoked response of each stimulus as there was 100ms between each pulse in Study 1 and 140ms between each pulses in Study 4 as the train of stimuli were delivered at 7Hz. Study 2, however did use one brief stimulus as opposed to a train and from this data, it was possible to see clear evoked responses and to investigate the changes in the evoked responses and oscillatory dynamics at a later time window. Hauck et al (2007) used intracutaneous electrical stimulation to create pain and saw oscillatory changes in the high-frequency gamma range between 400-600ms. Study 2 used a single pulse as opposed to a train of electrical stimuli, so in this study it was possible to investigate this higher frequency gamma response. Bootstraps were created up to 200Hz

however no high frequency gamma response was apparent in any participants. The change in high frequency gamma response seen during Hauck et al (2007) showed an increase of only 1% compared to baseline, this was found after averaging all sensors and all participants together. Looking at individual bootstrap spectrograms in Study 2, no consistent change in gamma oscillations could be seen in this frequency band. Although it was not possible to investigate whether this pattern was present in every stimulus in the train, it was still possible to study the offset of the train to investigate oscillatory dynamics beyond 140ms in Study 4 and 100ms in Study 1. The train of electrical stimuli was chosen in Studies 1 and 4 in order to drive the sensory and affective responses to pain as well as anticipation in Study 1.

7.5.5 Non-naïve participants

The majority of the participants in these experiments were colleagues from the labs and postgraduate students. Many of these were very experienced in participating in MEG experiments. This was an advantage in many ways as they were less likely to create movement artefacts and were able to keep still for the duration of the experiment so were generally more compliant than naïve participants would have been. The disadvantage of using non-naïve participants is that they would have had a different level of anxiety to naïve participants and this may have had an impact on the results, especially during Study 1 when investigating anticipation. Also, the non-naïve participants may have experienced electrical stimulation before, electrical stimulation is not experienced in everyday life and is an unusual sensation, therefore if some participants had experienced the stimulus before, they may rate the stimulus differently to those that are unfamiliar with it.

A variety of age ranges was used (21-45 years) and close to 50% male vs female participants. Analysis was not performed comparing male and female responses and different age ranges as the sample size was too small to make any robust findings from this. Studies have found gender differences in response to pain. In a study by Straube et al (2008) the medial PFC was found to have a higher activation during

pain studies in women than men. Also women have been found to have lower pain thresholds than men (Frot et al., 2004) and may respond to pain differently to men potentially resulting in differences in cortical activity.

7.5.6 CPT analysis

SAM analysis works on the basis of averaging over a number of trials, as was done in Studies 1, 2 and 4. The issue with Study 3 (cold pressor test) was that it was one long trial for each participant. In order to localise activity using SAM, smaller trials within different periods of CPT were created and then averaged. 15s from the warm ice pack period, 15s from the first part of CPT, 15s after the highest Likert score and 15s before the end of CPT were used in analysis. Within these 15s periods, markers were placed every 0.5s (30 altogether) which were then used in the SAM analysis comparing each period of CPT to the warm baseline period. This assumes that there were no changes across these 15s periods and the oscillatory dynamics were constant during that time which is not necessarily true, however differences between 15s periods should still have been picked up. Peaks in SI were still found using this method of analysis and spectrograms and envelopes were created from these peaks. In order to understand the change in frequency bands across the whole profile, envelope analysis was performed on this data. Despite the issues with analysis during CPT, it is worth pursuing due to the advantages it has as a pain stimulus such as similarity to chronic pain and its tonic sustained nature.

CPT involved placing the hand on an ice pack. This type of stimulus often causes the participant to tense the muscles of that arm and also possibly the neck. This muscle tension could lead to problems of EMG within the MEG data. In some participants this could be seen in the SAM analysis as peaks were located in the neck region. This caused noise in the data, though was localised by SAM so that the origin of the noise could be determined and removed from further analysis. Dowman et al (2008) claim that the increase in gamma oscillations in the cortex seen in many studies is merely due to EMG artefacts. In their study, a CPT test was administered and, at a separate

time, the participants were asked to contort their faces in order to create EMG artefacts. They saw an increase in gamma in CPT but it was similar to the increase seen during facial wincing and therefore they concluded that the gamma response was merely due to artefact from EMG. This study has shown that although some EMG may be present in the data, it localises to a source outside the brain and no gamma oscillations were seen in the cortex.

7.6 Future Plans

Many interesting findings in both the evoked and oscillatory activity have been found during the studies in this thesis but there are many questions still to be answered. Gamma oscillations are seen in response to somatic electrical pain and sensation but their exact role is still not completely understood. The change in frequency of gamma oscillations across the train of stimuli is an interesting phenomenon which requires further investigation, as is the link between gamma oscillations and attentional processing.

7.6.1 Aδ vs C fibres – Laser stimulation

Electrical stimulation activates both Aδ and C nociceptive fibres. It is therefore difficult to assign different oscillatory patterns to a particular fibre, although the latency of evoked responses gives an indication as Aδ fibres have a much higher conduction velocity than C fibres (5-30m/s for Aδ fibres vs 0.5-2 m/s for C fibres) (Forss et al., 2005). It would be beneficial to perform these experiments using a laser stimulus or using a CHEPS system and preferentially activating Aδ or C fibres using different surface areas and intensities (Raij et al., 2004, Adjamian et al., 2009). This would enable us to confirm whether the oscillatory patterns seen in these studies were present in one particular fibre type and be more specific about what role these oscillations might have in pain mechanisms.

7.6.2 Attentional Paradigm

Distraction and attention to pain are known to affect pain perception (Yamasaki et al., 1999). This is relevant in a clinical setting as attention to clinical pain can be a predictor of disability and distress (Eccleston, 2001). Attention to pain commonly enhances pain perception, and with distraction the pain is felt as less intense (Yamasaki et al., 2000). Changes in oscillatory dynamics have been seen between focussed attention to and distraction from pain, for example in Ohara et al (2004), a decrease in alpha power was stronger and more widespread during attention to the stimulus than distraction. It has also been suggested that gamma oscillations are affected by attention; Hauck et al (2007) found that high-frequency gamma oscillations (120-140Hz) seen in response to pain were strengthened during focussed attention to the stimulus as opposed to distraction. It is possible that the changes in the strength and frequency of gamma oscillations seen in the studies in this thesis may be related to attentional processing. The decrease in gamma oscillations seen in response to anticipation in Study 1 may also be due to attentional factors, it may indicate preparation and attention towards the upcoming painful stimulus. It would be interesting to use a similar protocol to Studies 1 or 4 adding the addition of a distractor task such as a multiplication task (Yamasaki et al., 1999, , 2000) or use an oddball paradigm to see if the gamma oscillations seen in Studies 1, 2 and 4 are affected by attention and distraction from a painful stimulus. Also it would be interesting to see if it is possible to replicate the high-frequency gamma oscillations seen in Hauck et al (2007) and if so to perform source analysis on it in order to determine its spatial location more precisely.

7.6.3 Changing ISI

Study 1 (anticipation) delivered a train of electrical pulses at 10Hz whereas Study 4 (stimulus intensity) used 7Hz. Between the two studies a difference was seen in the profile and bandwidth of the gamma response. The frequency of the gamma response was generally lower (~25-70Hz) in Study 4 than in Study 1 (45-100Hz) and

was closer to the beta range. In Study 1, the decrease in frequency of gamma oscillations across the train was clear and quite substantial (from 65-95 at the start to 45-75Hz at the end of the train) (see Chapter 3: Figure 3.12, 3.17). In Study 4, this decrease in frequency of gamma oscillations across the train was less evident, it appeared to enter into the beta frequency range. The change in bandwidth of gamma oscillations across repeated electrical stimulation is of interest and it would be relevant to repeat the same protocol as seen in Study 4 but alter the inter-stimulus interval (ISI) and observe what changes if any are seen in the profile of gamma oscillations across the train. This may elucidate what information can be encoded within the frequency of the gamma response and how this varies according to different stimulus features.

7.6.4 Proximal vs distal oesophageal stimulation

The proximal and distal oesophagus are known to have different innervations and musculature (Aziz et al., 2000b). The proximal oesophagus is considered more as a somatic structure than visceral, it's wall contains striated muscle as opposed to the smooth muscle in the distal oesophagus and it has a denser spinal innervation (Aziz et al., 2000b). Study 2 of this thesis compared electrical stimulation of the right index finger with the distal oesophagus. It would be interesting to repeat the study using stimulation of both the proximal and distal oesophagus in order to see whether the oscillatory dynamics of the proximal oesophagus are closer to that of a somatic structure like the finger or to the distal oesophagus. If an increase in gamma oscillations was found in response to proximal oesophageal stimulation then it would give us more information about the role that gamma oscillations play in somatosensory and pain processing.

7.6.5 MEG and ANS measures

ANS measures in response to pain have been studied extensively and there are various different types of responses in the autonomic system which have been linked

to particular personality traits (Paine et al., 2009a). There appears to be a dichotomy in individuals autonomic responses to pain seen in visceral experimental pain studies (Paine et al., 2009b, Paine et al., 2009a) in that they can react with a sympathetic nervous system 'fight-or-flight' response or with a parasympathetic reaction, this has been linked to personality traits such as neuroticism and anxiety. It would be interesting to see whether the oscillatory dynamics seen in these studies match up with particular ANS responses.

7.6.6 Oscillatory dynamics and personality traits

Personality traits such as anxiety and neuroticism have been found to affect an individual's response to pain. It would be of interest to obtain information on personality traits from questionnaires such as the Big Five inventory (Paine et al., 2009a) or the Speilberger anxiety score (Spielberger, 1983) and see if these have any relationship with the oscillatory dynamics observed. This would entail the participants completing a number of personality questionnaires about their personality traits and also about their current state of mind such as the state Speilberger anxiety questionnaire and then administering a pain stimulus in the MEG. How aspects of personality correlate with changes in oscillatory dynamics could then be investigated, such as whether a particular personality trait such as anxiety correlates with whether an increase in gamma oscillations is seen in response to pain.

7.7 Conclusion

The results from the studies in this thesis have allowed some interesting observations about gamma oscillations in SI to be made. An increase in gamma oscillations was seen in SI during somatic electrical stimulation in both a train and single pulse. The strength of the gamma oscillations was found to increase with increasing stimulus intensity. This would suggest that the gamma response is able to encode information about the intensity of a somatic stimulus within the strength of its oscillations, this fits

with literature that links gamma oscillations to stimulus feature encoding and binding (Engel and Singer, 2001).

In Studies 1 and 4, the frequency of the gamma response was found to decrease across the train. The amplitude of the 70ms component of the evoked response was seen to decrease across the train (especially in the first few pulses) similar to the augmentation response seen in *in vitro* preparations (Huttunen, 2010), it may be that the frequency of the gamma oscillation has some link with the evoked response amplitude. The decrease in frequency of the gamma response may be due to some form of habituation or inhibitory feedback mechanism involving a reduction of the IPSPs (Huttunen, 2010) or perhaps a change within the interneurons of the cortex which are known to have an influence over the gamma cycle and at what frequency the pyramidal cells are able to fire (Fries et al., 2007). The frequency of the gamma oscillations was lower during Study 4 in which the train of pulses was delivered at 7Hz than in Study 1 when the train was delivered at 10Hz. This may suggest another form of encoding within the frequency band of the gamma oscillations giving information about the timing between different stimuli.

A decrease in gamma oscillations during anticipation of pain was seen in Study 1 at the group level. This may be due to attentional processing and preparation for the upcoming painful stimulus. It may also be involved in some form of inhibitory feedback, attempting to restrict the amount of pain experienced by the individual, which then affects the oscillations in the gamma frequency.

During Study 2, the gamma oscillations seen were not temporally coincident with the main components of the evoked response. Also those that did not show gamma oscillations still had a clear evoked response from the SI. This suggests that gamma oscillations are not simply a transient increase in synchrony due to the evoked response but have a more complex role in somatosensory processing and contain induced components which may be involved in higher order cognitive processing.

Not all participants showed an increase in gamma oscillations in response to somatic electrical stimulation. It is possible that this reflects differences in how individuals respond to pain, it may be linked to certain personality traits as autonomic responses have been found to (Paine et al., 2009a). Gamma oscillations were not seen in response to distal oesophageal electrical stimulation or during a cold ice pack stimulus. It is possible that these stimuli do not drive the cortex to the same degree of synchrony as somatic electrical stimulation. Distal oesophageal stimulation may have less focal SI representation due to the smooth muscle and different innervations in the distal portion of the oesophagus. The cold ice pack stimulus is more similar to that of second pain mediate by C fibre activation, this potential difference in nociceptive fibre activation may explain the lack of gamma oscillations.

Gamma oscillations seen in SI in these studies appears to encode different features of somatic stimuli within its strength and frequency. These findings help to elucidate how somatic stimuli are processed within the cortex which in turn may be used to understand abnormal cases of somatosensory processing.

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