ANALGESIC EFFECTS OF EEG ALPHA-WAVE ENTRAINMENT ON ACUTE AND CHRONIC PAIN

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LIST OF ABBREVIATIONS

ACC	Anterior cingulate cortex
ANOVA	Analysis of variance
BA	Brodmann's area
BCI	Brain–Computer Interface
BF	Biofeedback
CNS	Central nervous system
CO_2	Carbon dioxide
DC	Direct current
DLPT	Dorsolateral Pontine Tegmentum
DRG	Dorsal root ganglion
EEG	Electroencephalography
EOG	Electro-oculogram
ERD	Event-related desynchronisation
ERP	Event-related potential
FIQ	Fibromyalgia Impact Questionnaire
FFT	Fast Fourier Transform
fMRI	Functional magnetic resonance imaging
FM/FMS	Fibromyalgia syndrome
HAQ -DI	Health Assessment Questionnaire – Disability Index
Hz	Hertz
IAF	Individual Alpha Frequency
IASP	International Association for the Study of Pain
ICA	Independent components analysis
KSS	Karolinska Sleepiness Scale
LORETA	Low resolution electromagnetic tomography
LEP	Laser-evoked potential
MCC	Midcingulate cortex
MEG	Magnetoencephalography
NCF	Nucleus Cuneiforms

NRS	Numeric Rating Scale
NSAID	Non-steroidal anti-inflammatory drug
OA	Osteoarthritis
PAG	Peri-aqueductal grey
PASS	Pain Anxiety Symptoms Scale
PCS	Pain Catastrophising Scale
PCC	Posterior cingulate cortex
PET	Positron emission tomography
PHQ - 9	Participant Health Questionnaire - 9
POMS	Profile of mood states
RA	Rheumatoid arthritis
RVM	Rostroventral medulla
SI/S1	Primary somatosensory cortex
SII/S2	Secondary somatosensory cortex
SFC/G	Superior frontal cortex/gyrus
SMR	Sensorimotor Rhythm
STAI	State and Train Anxiety Inventory
VAS	Visual Analogue Scale
WOMAC	Western Ontario and McMaster Universities Arthritis Index

ABSTRACT

Pharmacological treatments for pain show limited analgesic benefits when compared with placebo. Neuro-modulatory approaches, such as mindfulness meditation and neurofeedback training show more promising effects, but are time consuming and difficult to complete. Neural entrainment provides an almost instantaneous increase in EEG power of the stimulated frequency, achieved with minimal effort through visual flicker stimuli or auditory binaural beats. EEG recorded alpha power displays a reproducible inverse relationship with pain perception. Hence, the main objective of this PhD thesis was to develop an analgesic alpha entrainment intervention: increasing alpha power with the aim to reduce the perception of acute pain in healthy volunteers and chronic pain patients.

Prior to attempting to modulate pain, pilot work assessing the ability to entrain alpha power is reported in Chapter 3. A checkerboard stimulus was used to visually entrain frequencies across the alpha band from 7Hz – 14Hz, resulting in a significant power increase at 10Hz and 11Hz. With the goal to reduce behavioural and electrophysiological responses to a moderately painful stimulus, EEG alpha entrainment at 8Hz, 10Hz and 12Hz through auditory binaural beats (in Chapters 4 and 5), and visual flashing LED goggles (in Chapters 4, 6 and 7) was then attempted. A significant reduction of pain ratings was found following both the visual and the auditory alpha stimulation across all three frequencies in Chapters 4,5 and 6. Chapter 5 revealed a significant alpha power increase following 10Hz and 12Hz auditory stimulation. The laser-evoked potential's (LEP) N2 peak reduced significantly following 10Hz auditory entrainment and the P2 peak reduced significantly across all auditory entrainment conditions.

In Chapter 6, alpha power entrained significantly at 8Hz and 10Hz. The P2 peak reduced significantly following the 10Hz visual stimulation. Source analysis showed the precuneus and posterior cingulate cortex might mediate alpha entrainment-induced reductions in LEPs and pain ratings. The paradigm used in Chapter 6 was repeated in osteoarthritic patients in Chapter 7. Significant reductions in pain ratings were observed following all three alpha stimulation sessions, despite a lack of alpha power increase. A significantly reduced response in the P2 peak was also observed following the 12Hz visual stimulation. Decreases in P2 source activity in the posterior insula suggest a functional role in the reduction of pain intensity triggered by alpha stimulation.

A significant reduction in the electrophysiological response and the perception of moderately painful stimuli can be achieved through visual or auditory entrainment across the alpha band range, in both healthy volunteers and osteoarthritic patients. The findings from this PhD thesis provide a solid foundation for further investigation of alpha based neuro-modulation as an analgesic intervention.

DECLARATION

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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DEDICATION

This thesis is dedicated to the 21 osteoarthritic patients from Chapter 7, who regardless of their constant suffering, continue to show an extraordinary amount of strength, optimism and love of life. I thank you all for educating and inspiring me. I hope that in turn my work will be of benefit to those who suffer from pain.

ACKNOWLEDGEMENTS

The completion of this thesis, and the work that led up to it, would not have been possible without the unconditional support of my parents, who have always fully backed my decisions and put trust in my judgements. Their guidance and encouragement has enabled me to realise my own potential.

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PROLOGUE

This PhD thesis has adopted the alternative format approach. Chapters three, four five, six and seven have been written according to the style and guidelines requested by the scientific journals where the chapter is intended to be submitted. As each Chapter represents an independent manuscript, some of the information introduced and discussed may overlap between chapters. Chapter 1 will provide a broader introduction to all the work undertaken in this thesis and will identify the main research aims and hypotheses.

Chapter 3, 4, 5, 6 and 7 are written on the bases of manuscripts prepared for submission in peer reviewed journals

CHAPTER 1

Introduction

1. Overview

The inaugural chapter of this thesis opens by providing a brief outline of the current understanding of essential concepts in pain research. The summarised literature and highlighted gaps in our knowledge sculpt the aims and objectives of this PhD thesis. The introductory chapter is divided into four pain parts.

Initially, an overview of the perception of pain is introduced, accompanied by a concise explanation of the ascending pain pathways. Defining the role and meaning of acute and chronic pain leads to the description of their representation in an assembly of brain structures, known as the pain matrix. The difference in pain matrix structure activation by both acute and chronic pain will be described in moderate detail.

Secondly, neural oscillatory rhythms are introduced with a focus on alpha, sensorimotor, theta and gamma rhythms. A succinct overview of rhythmic changes during the perception of pain is provided for each of the chosen rhythms.

Next, a more detailed description concerning natural modulators of perceived pain is provided. Altered pain perception resulting from ascending and descending changes in the central and peripheral nervous system are discussed. Subsequently, a selection of psychological influences, and emotional factors, manipulating pain intensity are reviewed. Additionally, alterations in neural oscillatory rhythms observed during changes in pain, modulated by psychological effects, are examined.

Finally, the concluding part of the review will provide a summary of the background literature discussing the manipulation of pain through analgesic interventions and neuro-modulatory treatments. Following a brief overview of the effectiveness of analgesic drugs and their limitations, the changes in neural oscillations during successful pharmacological analgesia are highlighted. This leads to a discussion of externally modulating prominent, and pain-related neural activity through an

environmental stimulus. Controlled modulations of oscillatory brain rhythms have been achieved using mindfulness meditation, biofeedback and neurofeedback. The effectiveness for the use of acute and chronic pain for each of these interventions is deliberated, emphasising the main advantages and disadvantages of each technique. This section will conclude with the introduction of an existing brain training therapy, known as entrainment, not yet considered for the alleviation of acute and chronic pain. A discussion of the potential for neural entrainment to overcome the limitations of psychological, pharmacological and neuro-modulatory treatments follows, revealing how frequency entrainment may potentially be used in analgesic pain research. Highlighting the potential use of entrainment as an analgesic intervention for acute and chronic pain will lead to the main aims and objectives of this thesis. Following is a list of hypotheses forming the foundations of the research questions addressed individually in each experimental chapter.

2. The Sensation of Pain

It is deemed impossible to directly compare two individuals' perception of pain. This is not exclusively down to the fact that pain is highly subjective, but furthermore, that the pain intensity felt by an individual can be altered by both physiological processes and psychological factors. This is one of the fundamental reasons why the International Association for the Study of Pain (IASP) defines pain as 'an unpleasant *sensory* and *emotional* experience associated with actual or potential tissue damage, or described in terms of such damage'. The concept of consciousness and awareness of suffering being essential elements of the pain experience has long been established. This becomes apparent when scrutinizing the Latin root of the word pain, known as 'poena', which is believed to have meant punishment, suffering and distress of *body or mind* (Concise Oxford Dictionary). Even without today's technology, it was perhaps always apparent that pain is not a clear-cut phenomenon. It should therefore be no surprise that what we end up perceiving is rarely representative of the physical damage to our bodies (Jones et al., 2003).

3. Nociception and Nociceptive Signal Transmission

Pain requires consciousness and awareness of suffering. Nociception on the other hand, differs from pain as it is defined strictly as '*the neural processes of encoding and processing noxious stimuli*' (Loeser and Treede 2008) . In the healthy body, this noxious stimulus is an actual or potentially tissue-damaging event detected by receptors in the skin called 'nociceptors'. Nociceptors are cutaneous and have a high activation threshold (Sherrington 1906) which may vary according to tissue vulnerability (e.g. the surface of the cornea or tissue which is in the healing process have lower thresholds). Cutaneous afferents can be divided according to their fibre conduction velocity into slow conducting, unmyelinated C, and rapidly conducting, myelinated Aδ, and Aαβ fibres (Figure 1.1)(Djouhri and Lawson 2004). As a generalisation, sharp pricking pain is detected by activation of the more discriminative A-fibre nociceptors, whereas slow burning pain is due to activation of C-polymodals and C-heat nociceptors. C-fibre nociceptors are more abundant and probably have receptive fields in all tissue types (Melzack, 1982). 50-90% of cutaneous afferent C-fibres are polymodal. The remaining fibres selectively transmit noxious mechanical, thermal or chemical stimuli (Fang, Djouhri et al. 2005) from the peripheral to the central nervous system (CNS).

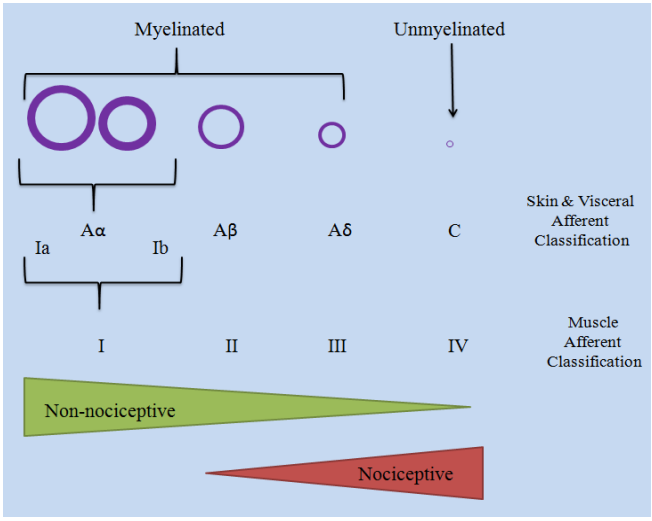


Figure 1.1 C and A - Fibre Afferents. Nociceptive and non-nociceptive cutaneous afferents can be divided according to their fibre conduction velocity into slow conducting, unmyelinated C, and rapidly conducting, myelinated Aδ, and Aαβ fibres. Muscles afferents are divided into group IV, III, II and I fibres (Adapted from Lawson, 2005).

If the threshold is reached at the receptive field, external noxious stimuli are transduced into electrical activity. The consequentially evoked action potentials propagate along the afferent fibre and travel along the peripheral nerve to the sensory cell bodies in the dorsal root ganglion (DRG) (Hunt and Mantyh 2001). Primary afferent neurons have their cell bodies in the DRG, with processes branching peripherally and centrally at the T-junction, after the initial segment. In the dorsal horn of the spinal cord, fibres transducing nociceptive signals synapse with secondary afferent neurones. Out of the 10 Rexed Laminae located in the dorsal horn, centrally projecting Aδ and Aαβ synapses in Rexed's Laminae I and V of the spinal cord, whereas C-fibres transmit nociceptive signals to the nociceptive specific neurons in Laminae I and II (Lawson, 2005).

After decussating, nociceptive signals travel to the higher brain centres along one of two tracts: the spino-thalamic or the spino-reticular tract. As the name suggests, the secondary afferent neurons travelling contralaterally along the more prominent spino-thalamic tract terminate in nuclei located in the thalamus. Third order neurons travel further to the periaqueductal gray (PAG) and the somatosensory cortex. The spino-reticular tract, on the other hand, projects to the reticular formation of the brainstem prior to reaching both the thalamus and hypothalamus, which have further profuse projections throughout the cortex (Price, Kennedy et al. 2006). Only when the nociceptive signal reaches its terminal in the brain, is the conscious perception of pain possible (Figure 1.2). However, it should be noted that work tracing the spino-reticular tract has mainly been completed in animal studies.

Many factors can alter the signal before it reaches its endpoint, hindering perception being an accurate representation of nociceptor activation (Jones et al., 2003). It is important to differentiate the stages of the pain pathway when attempting to modulate isolated property of perception. Acknowledging the complete picture when targeting one aspect of the process facilitates a prediction of the global impact. There can be distinct differences and abnormalities between acute and chronic pain signal transduction, however, these will not be covered in this chapter

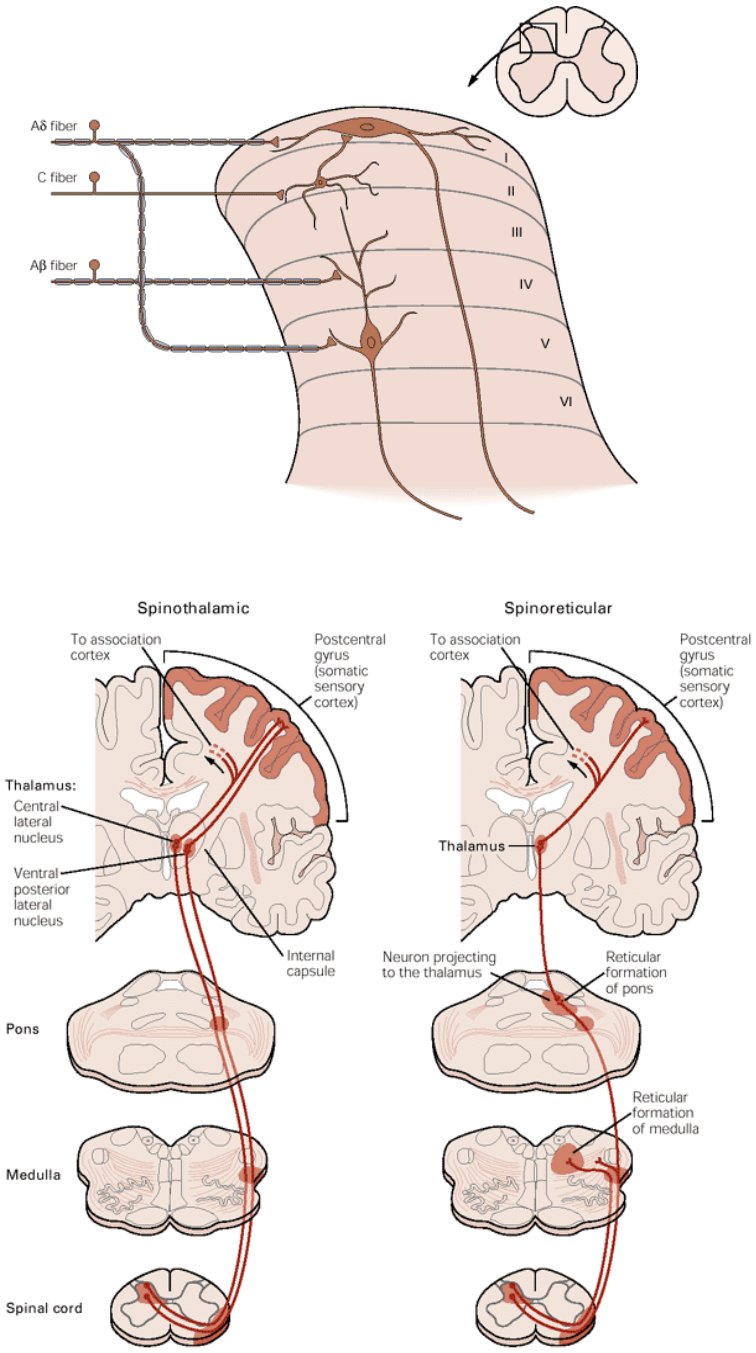


Figure 1.2. Ascending Pathways. *Top Centre*: Nociceptive afferent Aδ and C fibres terminate on projection neurons located in Rexed Laminae I and II of the spinal cord's dorsal horn (adapted from Fields et al 1987). *Bottom Left:* The ascending spino-thalamic pain pathway, travelling contralaterally to the thalamus before reaching higher brain centres. *Bottom Right:* The spino-reticular tract ascends contralaterally, projecting to the reticular formation before reaching the thalamus and hypothalamus. Diagram adapted from (Willis 1985). Image from (Anderson 1989).

4. Acute and Chronic

Albeit unpleasant, acute pain has a protective function in the human body. Acute pain generally provides useful information about the timing, intensity and location of a harmful stimulus. This allows our bodies to react in the most appropriate manner, minimizing harmful consequences. Acute pain either stops with the removal of the harmful stimulus or, when an injury has had time to heal. By contrast, chronic pain often outlasts its healing process and by definition, persists for longer than three months (Gatchel and Okifuji 2006). Unlike acute pain, chronic pain does not always have a protective function, nor is it necessarily the result of a noxious input. Chronic pain does not need to have a peripheral source, but may be maladaptive pain, caused by damage to the central nervous system.

One of the most common causes of chronic pain is the highly prevalent joint disease, osteoarthritis (OA). Affecting an estimated 8.5 million people in the UK alone makes it responsible for a large proportion of medical care costs. Rheumatoid arthritis (RA) is the second most common type of arthritis affecting 106,500 adult men (0.44%) and 297,600 adult women (1.16%) in the UK (Symmons 2001). In total, the direct cost of all arthritis related conditions amounts to £5.5 billion, making arthritis related conditions responsible for around half of the UK's chronic pain costs. Fibromyalgia syndrome (FMS) is a further cause of musculoskeletal pain. FMS was estimated at being the underlying cause for chronic pain of 4.7% of the population (Branco, Bannwarth et al. 2010), making it a leading cause of musculoskeletal pain. Between 10% and 15% of patients who suffer from OA have additionally been diagnosed with FMS, and may also suffer from RA. The chronic pain population selected for investigation in the present thesis all suffered from OA, with a subpopulation additionally fulfilling criteria for RA or FMS.

5. The Pain Matrix

Chronic and Acute pain differ on many levels, including transmission. However, in the brain, where the signal turns into sensation, the encoding is relatively similar. Both acute and chronic pain are represented in a cortical and sub-cortical network, defined by neuro-imaging studies (Jones, Brown et al. 1991, Talbot, Marrett et al. 1991) and known as the pain matrix (Melzack 1991). Taking into account the multitude of sensory and cognitive aspects of pain, it is no surprise that this diverse experience is not controlled by a single distinct pain centre. The pain matrix consists of a collection of structures including midbrain, cerebellum, thalamus, lentiform nucleus, primary (SI) and secondary (SII) somatosensory cortices, insula, cingulate cortex and the prefrontal cortex (Jones, Brown et al. 1991, Talbot, Marrett et al. 1991, Derbyshire, Jones et al. 1997, Coghill, Sang et al. 1999, Peyron, Garcia-Larrea et al. 2000, Peyron, Laurent et al. 2000). The intensity of a pain stimulus is encoded throughout the entire matrix (Derbyshire et al., 1997; Coghill et al., 1999), though distinct intensity coding regions have been unveiled within the cingulate cortex (Buchel, Bornhovd et al. 2002), the Insula (Craig, Chen et al. 2000), and debatably within the SI (Hofbauer, Rainville et al. 2001). However, due to the aforementioned multitude of emotional and cognitive structures involved, the intensity perceived by an individual is rarely an accurate representation of nociceptor activation (Jones et al., 2003). Emotional and cognitive influences cloud the pure sensation of pain by activating descending processes. However, these will not be discussed further in this section.

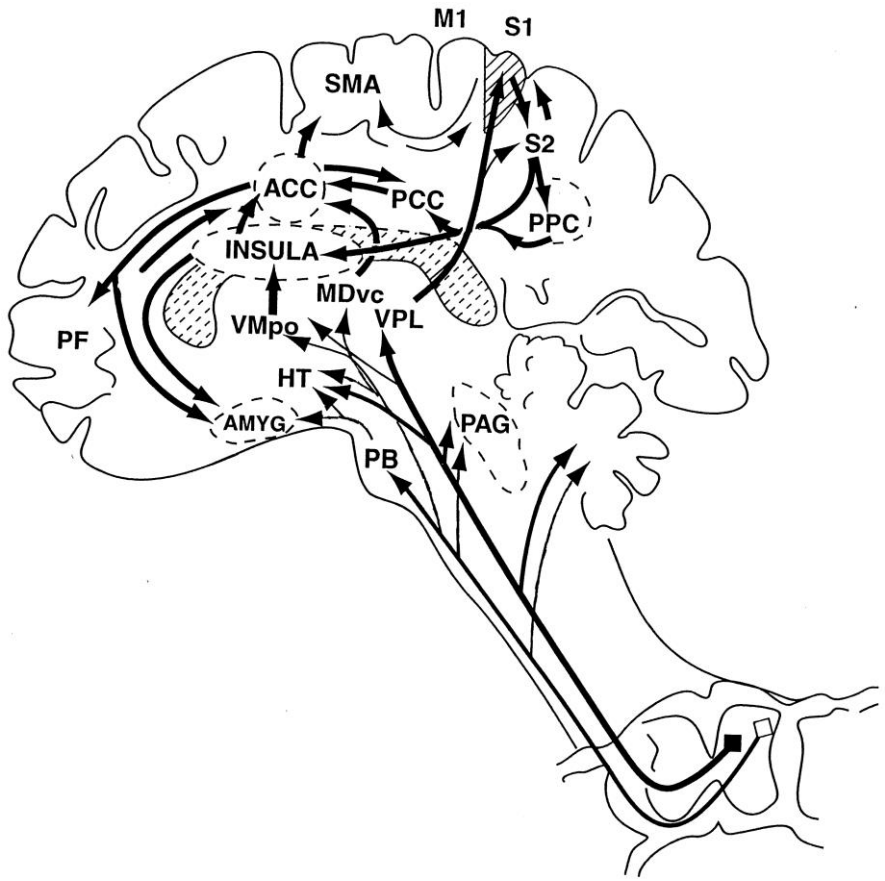


Figure 1.3. Ascending Pathways and the Pain Matrix. PAG, periaqueductal gray; PB, parabrachial nucleus of the dorsolateral pons; VMpo, ventromedial part of the posterior nuclear complex; MDvc, ventrocaudal part of the medial dorsal nucleus; VPL, ventroposterior lateral nucleus; ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; HT, hypothalamus; S1 and S2, first and second somatosensory cortical areas; PPC, posterior parietal complex; SMA, supplementary motor area; AMYG, amygdala; PF, prefrontal cortex. Diagram from (Price 2000).

5.1 Medial and Lateral Pain Matrix

Within the pain matrix, the affective-motivational and cognitive-evaluative components of pain are encoded simultaneously, though independently from the sensory-discriminative characteristic of pain. These aspects are expressed in two separate collections of pain matrix structures known as the medial and lateral pain systems respectively (Albe-Fessard et al., 1985). The medial system consists of projections from medial thalamic nuclei to the anterior insula, amygdala, cingulate cortex, and prefrontal cortex, whilst the lateral system comprises the thalamic nuclei projecting to SI/SII (Peyron et al., 2000; Jones et al., 2003).

Both chronic and acute pain is represented in the lateral and medial pain system. However, it has been observed that medial system is more active in some chronic pain conditions, compared to acute pain. This could be due to the heightened affective-motivational aspects of chronic, compared to acute pain, represented in the medial system. Multiple structures of the medial pain system are furthermore activated in depression. Whether depression in chronic pain patients is a result of a structural overlap is still unclear. It is difficult to evaluate which structures are activated purely by the encoding of chronic pain and which ones affected by the disease side effects.

Although acute pain is represented in both the medial and the lateral system, a dominant activation of the lateral system, especially SI, has been documented in neuro-imaging studies. However, just how much weight this distinction carries is questionable, considering many types of chronic pain consist of reoccurring acute pain (Jones et al., 2003). In the interest of the hypothesis addressed in this thesis, a high structural overlap between acute and chronic pain could be beneficial. A similar underlying pathology could suggest interventions producing analgesic effects in acute pain, may also work as a treatment for chronic pain.

The influence of a pain stimulus can be observed through changes in activation of structures in the pain matrix. Another way of recording cortical changes elicited by pain is through the real-time recording of ongoing neural activity, often allowing the identification of instantaneous changes. The following section will provide an overview of selected neural oscillatory rhythms and how they are altered during the perception of pain.

6. Neural Oscillations

Repetitive or rhythmic activity arising either from within individual neurons or, by the interaction of multiple neurons, results in neural oscillations. Oscillations of a single neuron are generated by cyclic firing of action potentials in the pre-synaptic neuron, which results in an oscillatory activation of the post-synaptic neuron.

Synchronised activity of small groups of neurons can cause local interactions, or interactions between different brain regions arising from synchronous local activity (Laurent et al., 2007). These macroscopic oscillations are detectable with electroencephalography (EEG), a non-invasive recording technique sampling voltage fluctuations through surface electrodes placed on the scalp (Donald L. Schomer 2011). Spontaneous neural oscillations were recorded for the first time using EEG by Hans Berger in 1929 (Berger, 1929). EEG signals trace the activity of specific frequency bands simultaneously throughout the cortex. The spectral analysis of EEG recordings exposes numerous frequency bands: delta (1–4 Hz), theta (4–7 Hz), alpha (7-14 Hz) beta (14–30 Hz) and gamma (30–100 Hz) (Klimesch et al., 1999). Two components that will not be discussed further in this review, include the arch-waved mu rhythm and the third rhythm, detectable using magnetoencephalography (MEG), but not using EEG, the recording technique used throughout this thesis. The following section gives a very brief overview of some of the most common EEG-detectable brain rhythms, and outlines frequently observed changes during pain.

6.1. Alpha

The sinusoidal like alpha activity is the most dominant rhythm in the EEG and hence, was the first to be discovered (Berger, 1929). As alpha rhythms can be detected in 95% of healthy young adults when their eyes are closed (Srinivasan 1999), they are still now the most studied rhythms in the human brain. Alpha rhythms in the adult brain usually fall in the 7Hz - 14Hz range. However, this frequency range varies highly between individuals. The alpha rhythm is generated primarily in the occipital cortex (Lopes Da Silva and Storm Van Leeuwen 1977, Bollimunta, Chen et al. 2008, Spaak, Bonnefond et al. 2012). However, numerous recordings of alpha activity with a parietal (Thut, Veniero et al. 2011, Hanslmayr, Volberg et al. 2013, Jaegle and Ro 2014) and a fronto-central topography (Busch, Dubois et al. 2009) have now been documented.

The EEG-recorded peak alpha frequency of a child increases in unpredictable non-linear fragments until puberty (Basar, Schurmann et al. 1997), when peak alpha frequency slowly starts to decline again, in a linear fashion (Garrick et al., 2004). The lower limit of the alpha range is the upper limit of the theta range. During demanding cognitive tasks, theta activity is more prominent than alpha. The lower limit of the alpha range therefore becomes apparent when the subject switches from a relaxed state of mind to an intellectually demanding task. The flip in activity outlines where the two power spectra intersect, and hence marks the lower limit of the alpha range. The central frequency in the alpha range is referred to as the individual alpha frequency (IAF) and is the frequency peak that desynchronizes during a demanding task. The upper limit of the alpha range commonly defined as being 2Hz higher than the IAF (Klimesch et al., 1999). A simple algorithm for calculating the approximate IAF at baseline was later devised by Pfurtscheller (Kopruner, Pfurtscheller et al. 1984, Klimesch 1999) where:

IAF = 11.95 – (0.053 x subject age)

It should however be noted that numerous transient factors may affect IAF more drastically than age.

6.1.1. Alpha Subdivisions

Alpha rhythms are occasionally subdivided into alpha-1 (lower alpha rhythms 7Hz - 10Hz) and alpha-2 (upper alpha rhythms 10Hz - 12Hz). On an individual level, alpha-1 is calculated as the range between the lower alpha limit and IAF, whereas alpha-2 ranging from IAF to the upper alpha limit (Klimesch et al., 1999). The division of the alpha rhythm is based on distinct cognition-related differences of the two frequency ranges.

Over time, alpha oscillations have been perhaps inaccurately linked with idle or passive brain states. Recent investigations have found strong correlations between alpha activity and cognitive processes. Alpha activity may help modulate processes including attentional tasks, as well as visuospatial and working memory (Osaka 1984, Klimesch 1999). Klimesh et al (2007) developed a theory, which implies a

relationship between low alpha power and active information processing. However, this does not mean that high power is related to low activity. Rather, high alpha may represent inhibitory processes in the cortex. Inhibition may aid the removal of useless information and refine task relevant and important signals. If different cortical locations all coordinate and produce synchronised oscillation, increased excitability can result in the targeted areas. It could be postulated that these synchronised oscillations underlie inhibitory descending processes resulting in reduced pain perception (Klimesch et al., 2007).

It is now apparent that different cognitive demands are often discretely linked to either alpha-1 or alpha-2 activation. The lower peak-frequency alpha-1 activity is thought to be representative of active information processing and most dominant during anticipation and attentional tasks (Moont, Pud et al. 2010). Higher band alpha-2 activity is hypothesised to be representative of inhibitory processes allowing task unrelated information to be filtered out and important signals to be refined. Alpha-2 activity has continuously been recorded as being more prominent during sensorimotor or cognitive processing related to external stimuli (Franciotti et al., 2009).

6.1.2. Alpha Changes in Pain

A suppression of the alpha band, often referred to as alpha blocking, can be observed during the perception of an acute pain stimulus (Chang, Arendt-Nielsen et al. 2002, Chang, Arendt-Nielsen et al. 2003, Ploner, Gross et al. 2006). Furthermore, an increase in behavioural pain ratings of acute stimuli is correlated to a decrease in the alpha rhythm (Huber, Bartling et al. 2006).

Patients suffering from chronic pain due to spinal cord injury have been documented to have lower alpha power and higher theta power than age-matched pain free controls (Jensen, Sherlin et al. 2013). However, no significant correlation between variations in chronic pain intensity in the patient group and alpha power was found. A study by Boord et al found a slowing of alpha peak frequency in

addition to a reduction in alpha power in neuropathic pain patients, relative to those with no pain (Boord, Siddall et al. 2008). Decreases in alpha peak frequency were also observed by Wydenkeller et al in patients with chronic pain from spinal cord injury, although no significant changes in power were documented (Wydenkeller, Maurizio et al. 2009).

Early work by Trifiletti et al. observing that high alpha was associated with intense analgesia, alluded to the idea that this relationship may work both ways (Trifiletti 1984). Since then, numerous EEG studies disclosed a correlation between acute pain relief and an increase in alpha power, predominantly located across posterior and temporal regions of the scalp (Chang, Arendt-Nielsen et al. 2002, Kakigi, Nakata et al. 2005, Saithong, Poolpoem et al. 2012).

A selection of work focussed on separately investigating the relationship of alpha-1 and alpha-2 power with changes in pain perception. Alpha-1 power was found to be negatively correlated with subjective pain ratings (Chang, Arendt-Nielsen et al. 2003). Furthermore, Nir and colleagues extended this study, revealing alpha-1 power during the baseline recordings gave an accurate indication of pain ratings during the tonic noxious condition. Taking into consideration that alpha-1 rhythms become more prominent with increased attention or expectation of external stimuli, it is not surprising the lower alpha-band is correlated with the behavioural pain response. However, the literature to date has not been able to establish whether increases in alpha cause reductions in pain, or whether alpha and pain reduction are independent products of changes in attention and expectancy.

6.2. SMR

Sensorimotor rhythms (SMR) are synchronised oscillatory components of brainwaves found in the 12-15Hz frequency range (Arroyo, Lesser et al. 1993). Although undoubtedly present, the current literature documents practically no inter-individual variations of the frequency range. As the name suggests, this rhythm is most frequently recorded in the sensorimotor cortex using both EEG and

MEG. SMR shares many similarities with the alpha rhythm, one of which is being linked with a 'quiet body and active mind'. Associations between the SMR and attention have repeatedly been made (Egner and Gruzelier 2001). The oscillatory activity itself is believed to be representative of inhibitory somatosensory afferent gating, resultant of ventrobasal thalamic neurons firing in a burst pattern. Any alterations in thalamo-cortical interactions could thus be reflected in SMR oscillatory changes (Howe and Sterman 1972, Howe and Sterman 1972). Consequentially, it is not surprising that alterations in SMR activity can be observed in a large variety of mental states as well as chronic pain conditions. A reduction in SMR activity can be observed in increased states of anxiety, panic, migraine, attention deficit disorders, mood disorders and as well as during acute and chronic pain (Siniatchkin, Gerber et al. 2000, Siniatchkin, Hierundar et al. 2000, Egner and Gruzelier 2001, Monastra, Monastra et al. 2002, Egner and Gruzelier 2004).

6.2.1. Changes in Pain

SMR activity, similarly to the bordering alpha rhythm, is often reduced in chronic pain conditions (Reiner, Sukhotinsky et al. 2008). A transient bilateral suppression of the rhythm can also be observed over the sensorimotor cortices following acute noxious laser stimuli applied to the dorsal surface of the right hand (Ploner 2006). Additionally, EEG recorded SMR activity has been found to be significantly supressed in healthy participants upon observing static images of limbs in painful stimulations. Although the suppression was found bilaterally, observing the pain of others resulted in a more pronounced suppression of SMR over the sensorimotor cortex in the left hemisphere (Whitmarsh 2010).

6.3. Theta

Although it is sometimes challenged whether theta is an oscillatory brainwave, due to its' lack of rhythmic activity, theta is currently still categorised into cortical and hippocampal oscillations. There is no apparent relationship between cortical EEG-recorded theta rhythms, and the not very clearly understood hippocampal theta rhythms, recorded from invasively implanted electrodes in rodents. In primates,

EEG-recorded theta activity is most prominent in the frontal regions, but can be detected all over the skull and will be the activity referred to throughout the remainder of this thesis (Nunez, Wingeier et al. 2001). Current literature suggests that, regardless of scalp recording location, the theta range generally falls in the 3Hz - 8Hz range. There is no official definition of the lower theta limit. The upper limit of the theta range is the lower limit of the alpha-1 range making the inter-individual variance of the ranges dependant on each other. As theta power is inversely proportional to alpha, it is not surprising that, unlike alpha, theta decreases from childhood to adulthood, where it remains stable. It is yet to be determined whether observations of theta power increasing again in the early 60's are related to old age, the decrease in alpha power or neurological diseases. Theta is often found to be increased in disorders such as dementia in adults, or in children suffering from learning disabilities. The most popular theory argues that theta is most dominant during learning and memory formation (Hasselmo 2005). Specifically, recordings of high theta activity during spatial learning and navigation (Buhl and Buzsaki 2005) suggest theta may have a role in encoding sensory stimuli, including noxious stimuli.

6.3.1. Theta Changes in Pain

Inversely to changes in alpha power, increases in theta power are generally linked with higher subjective pain intensity ratings. Phase-locked increases in theta power, usually approximately 150-350ms after the noxious stimulus have been documented. Schultz et al., (2010) revealed a direct correlation between increases in theta power and pain-evoked electrical potentials recorded in the EEG (Schultz et al., 2010).

Moreover, continuous EEG recordings in chronic neurogenic pain patients exhibited higher theta and beta (12.5 – 30Hz) activation compared to pain free controls (Stern, Jeanmonod et al. 2006). Pronounced augmentations in theta and beta activity have been recorded through EEG and localised across the pain matrix, principally, in the insular cortex, anterior cingulate cortex, prefrontal cortex, and

inferior posterior parietal cortices and the primary, secondary and supplementary somatosensory cortices (Stern et al., 2010). As these pain-associated area activations could not be observed in the healthy volunteers, the observed spontaneous oscillatory amplification may provide an indication to the neural processes involved in persistent neurogenic, or other chronic pain conditions.

6.4. Gamma

The gamma rhythm oscillates in the 30-70Hz frequency range, and has been diffusely recorded across the scalp (Schurmann, Basar-Eroglu et al. 1997)(Lynn et al., 2007; Schurmann et al., 1997; Jeffery et al., 1995). Many researches consider gamma rhythm activity a popular candidate for facilitating top down control during voluntary, but not involuntary attention (Lynn et al., 2007). Although the exact role is still unknown, speculations of gamma activity underlying subjective awareness are still popular amongst many researchers.

Recent studies observed an enhancement of gamma activity during both sensory and cognitive processes. Gamma activity can be influenced by both external auditory and visual stimuli. A study by Schurmann et al (1997) revealed an overall increase in gamma band power after visual stimulations. Recordings, performed using inter-cranial implanted electrodes exposed a relationship between visual evoked responses and gamma power (Schurmann, BasarEroglu et al. 1997, Schurmann, BasarEroglu et al. 1997). Results from EEG studies identify similar relationships (Basar et al., 2001).

6.4.1. Gamma changes in Pain

Gamma oscillations follow a similar pattern to the theta rhythm after acute pain; the magnitude of gamma power is directly correlated with subjective pain intensity (Gross et al., 2007; Schulz et al., 2011). Approximately 150-350ms after an acute noxious stimulus, EEG recorded gamma activity has been shown to exhibit a phase-locked increase in power. Similar to theta power, this increase is directly correlated to the electrical pain evoked potential (Schultz et al., 2010). Consequently, a direct

involvement of somatosensory gamma activity in the neural processing of noxious stimulations and the subjective perception of pain has been suggested.

Because gamma oscillations have been associated with attentional processing, the rhythm has previously been linked with the increased salience of painful stimulations, as opposed to intensity. However, recent work by Zhang et al demonstrated the direct correlation with pain intensity holds true even when the noxious stimuli were predictable, repetitive and hence reduced saliency (Zhang, Hu et al. 2012).

7. Altered Pain Perception

Pain can be perceived as more of less severe than the occurring trauma or experimental noxious insult. The intensity of the pain experience can be modulated naturally, by internal and external factors. Internal changes in the central and peripheral nervous system mediated through ascending and descending mechanisms can significantly influence the ultimate sensation of a stimulus. Other naturally occurring manipulations of perception are the psychological and emotional influences on pain. In the following section, increases and decreases in perceived pain intensity as a result of alterations of these naturally occurring modulators of pain will be described. Furthermore, aforementioned changes in neural oscillations (highlighted in the previous section) following psychologically induced increases and decreases in pain will be examined.

7.1. Changes in the Peripheral and Central Nervous Systems

Tissue damage, inflammation and general damage to the peripheral nervous system can alter the activation and, hence, the information transmitted from nociceptors. The activation threshold, excitability and transmission properties of nociceptors can be distorted. These changes are predominantly due to post-translational changes, which ultimately could significantly influence hypersensitivity of receptors and trigger spontaneous pain. These changes can vary from being general shifts in membrane properties in specified locations on the peripheral

terminals (peripheral sensitisation), the site of axonal injury or central synapses (Svensson and Yaksh 2002).

Nociceptors are not the only receptors capable of producing painful experiences. Low threshold sensory fibres that normally detect innocuous stimuli such as light touch can be modified to produce painful sensations. Such modifications may arise following tissue damage to the peripheral nervous system, where a shift in the excitability of the CNS causes hypersensitivity of non-noxious stimuli. In this case, even though pain is not representative of a damaging external stimulus, it may still have a protective function during recuperation.

Central sensitisation is caused by increased transmission in the dorsal horn from activity-dependant phosphorylation. Trafficking of receptors and ion channels can result in these changes happening almost instantaneously. Transcriptional changes altering the excitability of neurons results in maintenance of increased activity for an indefinite period of time. A fixed state of central sensitisation can be obtained from structural changes in the CNS including loss of inhibitory inter-neurones as well as alterations in synaptic connections of low threshold afferents with nociceptive neurons. This is one example of non-useful pain and is one of the suggested causal mechanisms of chronic pan, in particular, allodynia.

Acute and chronic pain experiences can be altered by descending supra-spinal control of spinal nociception. Many chronic pain states are influenced or maintained by descending modulation of the midbrain PAG. The PAG plays a key role in both the descending inhibition and facilitation of nociceptive input. It is a structure heavily interconnected with the hypothalamus and the limbic forebrain structures. After receiving spino-mesencephalic input, the PAG operates though caudal medullary structures as well as projecting directly to the rostral ventromedial medulla (RVM), which in turn, projects to the dorsal horn laminae. The PAG is known to play a vital role in integrating intrinsic and extrinsic stressors to develop adequate coping strategies. Furthermore, the PAG is universally acknowledged as a central site of action of analgesics such as opioids,

cyclooxygenase inhibitors and cannabinoids (Leith et al., 2007; Yaksh et al., 1976; Hohmann, 2005).

Although descending control arises from numerous supraspinal sites, the PAG/ rostral ventromedial medulla (RVM) is the most studied system (Reynolds, Waters and Lumb, 1997, Mayer et al., 1971, Fields and Heinricher, 1985). This system exerts tonically active descending control, where the dynamic balance between inhibition and facilitation is easily disrupted by diverse behavioural, emotional and pathological influences (Fields and Heinricher, 1985). Behavioural influences such as during fearful or intensely stressful situations a shift towards descending inhibition may result in hypoalgesia. A shift towards facilitation is indicative of inflammation or nerve injury and often distinguished by hyperalgesia. Descending facilitation also underlies the development of secondary hyperalgesia by being heavily involved in central sensitisation. These findings may be an indication that a shift in the balance towards facilitation may contribute to the transformation of acute to chronic pain (Ren and Dubner, 2002; Zhuo and Gebhart, 1997; Zhuo and Gebhart, 1992).

Over time, evidence of a complex integrated system which modulates our perception of pain through descending interactions, has been accumulated. Amongst the structures that have been identified in playing key roles in the descending modulation of pain, are not only the PAG and RVM, but additionally, the frontal lobe, aCC, insula, amygdala, hypothalamus and nucleus cuneiformis (NCF), depicted in figure 1.3 (Tracey and Mantyh 2007). However, this review will not discussing descending modulation of pain in more detail.

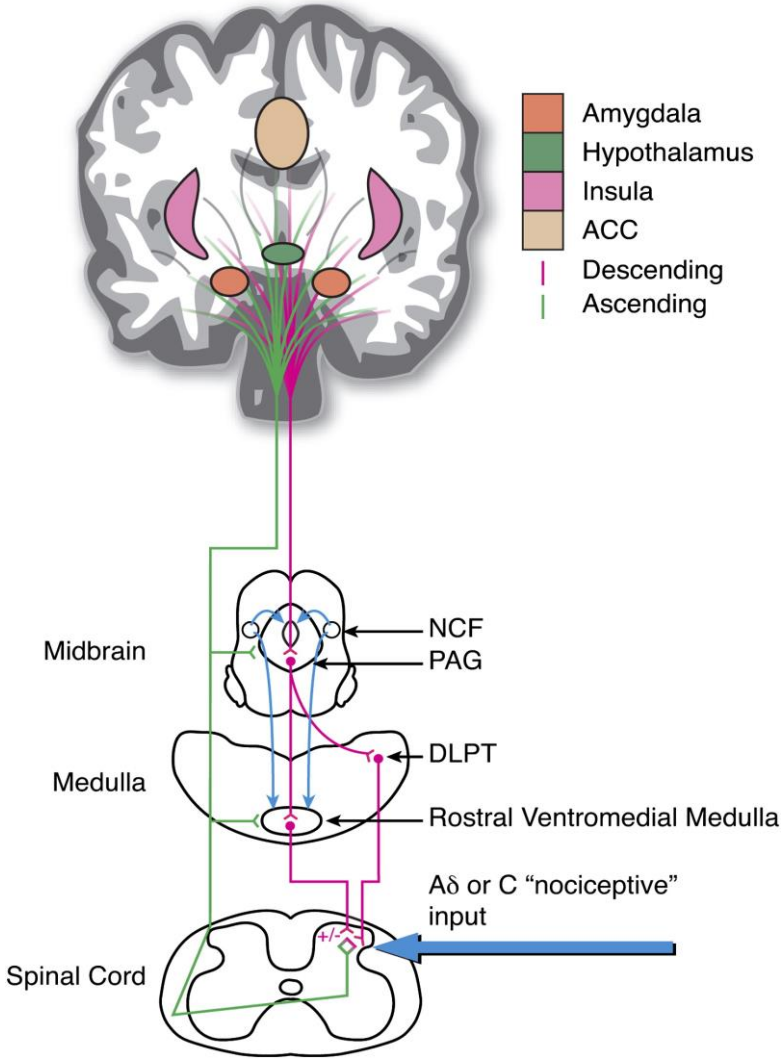


Figure 1.4 Descending Modulation of Pain. NCF (nucleus cuneiforms); PAG (periaqueductal gray); DLPT (dorsolateral pontine tegmentum); aCC (anterior cingulate cortex); +/- represents the pro and anti- nociceptive influences

7.2. Psychological Modulation of Pain and Pain Control

Physiological factors are not alone in modulating pain perception. Psychological and emotional influences contribute to the multifaceted, highly subjective ultimate experience of pain (Jones, Kulkarni et al. 2003). Additionally, even patients who suffer from the same disease may experience different pain, and may not have equal responsiveness to treatments (Coghill et al., 2003).

Targeting only the physiological factors, whilst ignoring the psychological, social and cultural influences in the understanding, treatment or management of pain, does not produce the same results as when multiple factors are considered (Gatchel and Okifuji 2006). The sheer magnitude of psychological influences is often underestimated. In psychogenic pain, for example, the severe and prolonged pain experience is the only cause of complaint, inconsistent with activity in the nervous system (Valdes, Garcia et al. 1989). In chronic pain states, psychological influences have been shown to be the dominant factors in determining perception of pain severity, tolerance, sensitivity, pain-associated disability, and effectiveness of treatments compared to other physical or pathophysiological factors (Vranceanu et al., 2009; Bobey and Davidson, 1970).

A vast body of literature has studied different psychological factors that may contribute to the definite experience of pain, including optimism (Scheier et al. 2007), depression (Terhaar et al. 2009), attention (Bentley et al., 2004), anxiety (Tang and Gibson 2005), anger (Greenwood et al. 2003), catastrophising (Turner et al. 2004), and fear (Bradley et al. 2008).

Psychological factors can be categorised into cognitive-evaluative factors such as self-efficacy, expectations as well as attention, and affective-motivational factors including depression, anxiety, and anger (Chen et al., 2001). Psychological factors such as optimism, or pain relief expectancies tend to decrease the painful sensation whereas negative influences, such as anxiety or fear can produce and increase the pain experience (Keefe et al. 2004). Negative beliefs do not only exacerbate painful sensations but furthermore have been shown to reduce response to pain treatment (Vranceanu et al. 2009).

Patients suffering from chronic pain vary extensively in their judgement and coping strategies. In general, patients who use ‘positive coping strategies’ (e.g. distraction from pain, exercise), find that their pain has less influence on their daily lives. By contrast, patients who dwell on their own pain and adopt negative coping strategies (e.g. restricting movement and activity) will perceive an enhanced pain

experience (Moreno et al., 1999). Such cognitive factors have been shown to dramatically influence not only response to therapy but also additionally the rate of recovery after surgery (den Boer et al., 2006).

The influence of these cognitive factors can be measured quantitatively through changes in activation of the pain matrix. For example, studies investigating the effect of attention (Seminowicz et al. 2004) and noise distraction (Boyle et al 2008) have demonstrated precisely this: whilst subjects focused their attention on a cognitive demanding task the pain was reduced and the activation of the primary somatosensory, secondary somatosensory, and anterior insular cortices were reduced. Similarly, decreased activity in the aCC but increased activity in the SI and occipital pole was enhanced during selective modulation resulting from noise distraction from pain.

How painful we expect a stimulus to be dramatically influences our subsequent perception of that stimulus. Expectancy can both enhance and diminish the perceived painful experience. Positive, non-painful beliefs about an incoming stimulus will result in a reduced pain experience, whereas negative beliefs about a painful stimulus will exacerbate the painful sensation (Sawamoto, Manabu Honda et al. 2000; Koyama, John G. McHaffie et al. 2005; Keltner. J., A. Furst et al. 2006; Brown, Seymour et al. 2008). Both positive and negative expectation, leading to the placebo and nocebo effects respectively, are clinically relevant (Colloca et al. 2008; Petrovic, 2008). The less-studied nocebo effect causes the exacerbation of symptoms after an intervention as a result of negative beliefs about the procedure or its effects. The nocebo effect can be represented in both the number of and the intensity of symptoms (Petrovic, 2008). Negative emotions such as anxiety, fear and catastrophising are induced by negative expectation of a painful intervention, and further enhance painful sensations (Wiech and Tracey, 2009). The effects of negative expectations on pain are not limited to the immediate experience but may influence fear of future painful sensations. This may result in further disabilities such as restricting movements, or in the form of fear avoidance behaviours, which can lead to symptom chronicity (Boersma and Linton, 2006).

7.2.1. Neural Oscillations in the Psychological Modulation of Pain

As previously mentioned, gamma oscillations are thought to play a key role in the top-down control of attention (Lynn et al., 2007). Depending on the cortical location of the rhythm, gamma activity produced by attended stimuli display increases and decreases in power. Alpha activity is also believed to be involved in facilitating modulatory processes of attentional tasks (Osaka, 1984; Klimesch, 1999). Specifically, attention-demanding conditions, like alertness and expectancy, are associated with changes in the lower alpha-1 activity.

Brainwave rhythms have been observed to undergo alterations during expectations of painful stimuli (Babiloni et al., 2003). Franciotti et al 2009 investigated the effect of expectation of a painful stimulus on alpha rhythm oscillations in the anterior insular cortex. Additionally, to examine at which point during expectation the insular cortex is most active. The results demonstrated reduced alpha power before and after painful stimulation. These results support the findings of low-band alpha coding for expectancy, and high-band alpha rhythms represent sensorimotor or cognitive processes that relate to other external events (Franciotti et al., 2009). Reduced alpha power in the anterior insula could be representative of a preparatory state, allowing the sensory system to adjust to the expected incoming stimulus. This is not surprising as alpha power reductions have been observed in motor preparatory states, as well as during the anticipation of emotionally negative images (Onoda et al., 2007).

8. Manipulating Pain

Although the perception of pain can be altered, often unintentionally and unknowingly, by natural modulators of pain, the perception of pain can be modulated with a purpose. The following section discusses different ways of inducing analgesia, firstly, through pharmacological manipulation, and secondly through a selection of neuro-modulatory techniques i.e. modifying neural oscillations with the aim to evoke analgesia.

8.1. Pharmacological modulation of Pain Perception

Analgesic drug treatments tend to be the first solution patients seek when suffering from chronic pain. Although paracetamol (Jordon et al 2003), opioids (Pavelka 2000) nonsteroidal anti-inflammatory drugs (NSAIDs) (Kadman et al., 2004) antidepressants (esp. tricyclic antidepressants) are all individually effective at relieving different symptoms, many patients complain about the numerous side effects of these drugs. Opioid treatments have recorded unwanted side effects in over half the patients, varying tremendously between individuals. Physical interventions like operations for osteoarthritic patients replacing sections of the knee and hip do not always result in long-term alleviation of pain. In fact, there is no correlation between pain relief and success of the operation (Nilsdotter et al., 2003).

8.1.1. Neural Oscillations in Pharmacologically induced Analgesia

Osteoarthritic pain relief resulting from the administration of opioid-like agent tramadol is paired with an increased measure of cortical oscillatory alpha power (7-14Hz), as measured by EEG (Freye and Levy 2006). Tramadol-provoked reductions behavioural pain ratings were also coupled with a significant increase in beta power (13‑30 Hz). Due to its limited side effects, tramadol is perceived an appropriate drug for the older generation, despite its intermediate potency (Osipova, Novikov et al. 1991, Moore and McQuay 1997, Liu, Zhou et al. 1999). Supplementary to chronic pain analgesia, tramadol also produced ameliorations of mood and cognitive impairment. It was not possible to extrapolate from the results whether this was a direct effect of the tramadol, or the product of the altered oscillatory rhythms.

Opioids, and opioid-like agents are consistently termed the most effective analgesic for chronic pain. However, a recent meta-analysis disclosed surprisingly minor ameliorations when compared with the effects of placebo interventions (Moore and McQuay 1997). As previously, stated, physical and pharmacologically induced interventions are not the only way to treat chronic pain. Although effective,

psychological therapies require patient motivation, time and often rely heavily on the ability of altering the patient's perception of their pain. Instead, disrupting the neural encoding of pain by manipulating pain-associated oscillatory patterns may result in a more controlled and consistent analgesic effect.

9. Analgesic Neuro-modulation

Although pharmacological treatment alone is rarely sufficient for a substantial reduction in pain, psychological or neuro-modulatory interventions are commonly only considered, if at all, succeeding the failure of a large range of analgesic drugs. This is surprising considering our knowledge of supra-spinal role in the transformation of nociception to the perception of pain. A direct manipulation of the neural signature of pain may thus result in superior pain relief, eliminating drug related side effects. Interventions targeting the neural oscillatory activity altered in pain already exist. In the following section, a selection of neuro-modulatory treatments, and their potential or already existing ability to act as analgesics will be discussed. The neuro-modulatory treatments selected for discussion in the following section include mindfulness meditation, biofeedback, neurofeedback and neural entrainment.

9.1. Mindfulness Meditation

Like all other neuro-modulatory interventions, mindfulness-based meditation is exclusively implemented in addition to pharmacological treatments. Consequentially, all data sets assessing the benefits of mindfulness meditation compare the pain relief of patients receiving analgesic drugs, to those receiving a combination of medication and mindfulness training. Positive effects on both the sensory and affective components of persistent pain are a common benefit observed in patients, who additionally practice mindfulness (Grant 2014). Chronic pain patients who regularly practice mindfulness furthermore show a reduction in pain catastrophizing, pain associated disabilities, and an increase in pain acceptance (Grossman, Niemann et al. 2004, Teixeira 2010, Fjorback, Arendt et al. 2011, Keng, Smoski et al. 2011). However, even inexperienced meditators can benefit from the

analgesic effects of mindfulness. Significant reductions in pain ratings and deactivation in associated cortical areas (insula, SI, SII) is achievable following a succinct mindfulness meditation training of 20 minutes a day, over only 4 days (Zeidan, Grant et al. 2012).

Mindfulness meditation is believed to alleviate pain by removing the psychologically exacerbating aspects of the sensation, and allowing the practitioner to focus on the raw nociceptive input. This theory was supported by Grant and colleagues who observed meditators experiencing an acutely painful stimulus, display a lower activation in brain areas associated with evaluation and emotion PFC, amygdala and hippocampus), and higher activation in areas of the pain matrix associated with intensity (CC, thalamus and insula) (Grant, Courtemanche et al. 2011). This result was paired with a higher pain threshold in regular mindfulness meditators compared to inexperienced individuals.

9.1.1. Neural Oscillations in Mindfulness Meditation

Alpha power increases occur in line with augmentations in analgesia. This affiliation is in line with studies recording alpha frequencies during mindfulness meditation, which have unanimously found an increase in alpha power paired with a slowing of the alpha rhythm (Taneli and Krahne 1987). Although other neuro-modulatory studies have produced modulation of alpha activity in both directions, volunteers appear to find it more pleasant, but also more difficult to increase, rather than decrease alpha band activity. Mindfulness elicited increases in alpha power are not dependant on, or correlated with the experience of the meditator (Fell, Axmacher et al. 2010). Additionally, meditative increases in alpha are believed to play a fundamental role in reducing feelings of anxiety and depression, as well as in the positive and calm emotions experienced by those who practice (Cahn and Polich 2006). Behavioural results have demonstrated that laser induced acute heat pain is perceived as less painful by mindfulness meditators, compared to controls. Furthermore, an inverse correlation between meditation experience and pain ratings has been revealed as well as reduced anticipatory processing in the insula

and increased processing in the dorso-lateral prefrontal cortex (Brown and Jones 2010). If we are to presume mindfulness meditators have increased alpha power, an inverse relationship between pain ratings and alpha power as a result of mindfulness, could be postulated.

Modulations in the alpha band are not the only oscillatory changes that occur during meditation, nor is it the only rhythm more prominent in experienced meditators, compared to controls. Theta rhythm activity (3-8Hz), generally most prominent in the transition from the awake to sleep state, has been found to be increased in meditators compared to the untrained population. However, theta has not been successfully correlated to a specific phase of meditation. Furthermore, inexperienced meditators tend to find it harder to reach a state of continuous, steady theta activity. Increased theta activity is often associated with an advanced meditation, whose practitioners already have a stable control over alpha during meditation.

Basic changes in alpha oscillations are easily accomplished by inexperienced meditators. One reason may be that neural patterns of alpha oscillations are closely related to common, non-meditative tasks. An easy and common way to increase alpha activity is by simply increasing internal attention. This involves focussing the mind on bodily sensations at that exact moment in time, without influences from the past or expectations of the future. In relation to pain, this would result in decreased attention and expectation of pain from an impending stimulus. Only during the actual noxious stimulation would a meditator focus on the pure sensation of pain. The affective-motivational aspect of pain is a major influence on the perceived experience. A combination of reduced expectation and removal of emotional aspects of pain, consequently results in an overall reduced pain sensation (Brown and Jones 2010). It could hence be postulated that meditation reduces activity in the areas of the pain matrix, such as the aCC, involved in attention and expectation of pain.

A study by Brown et al (2010) demonstrated that the less meditators perceived the pain, the lower the activation in areas involved in expectation of pain. More specifically, lower activity in the midcingulate cortex was directly correlated with lower unpleasantness ratings. Additionally, well-practiced meditators demonstrated a lower activity in the S2 and insula during noxious stimulations. It has yet to be determined whether changes in alpha activity are as a result of mindfulness meditation, expectancy or simply representative of reduced perception of pain.

Finally, mindfulness meditators show a shift in alpha activity towards the left anterior hemisphere (Chiesa and Serretti 2010). This is a change often associated with feelings of joy and overall positive emotions (Davidson, Ekman et al. 1990). By contrast, patients suffering from depression, common in chronic pain patients, displayed increased alpha activity in the left frontal region as well right parietal (Debener, Beauducel et al. 2000). However, it cannot be determined how depression, chronic pain and changes in alpha activity are interlinked purely from this observation.

9.2. Biofeedback

Biofeedback (BF) is a mental training therapy where the patients' autonomic or neuromuscular activity is measured, processed and fed back to the patients through visual or auditory signals (Dursun et al., 2009). The aim of the therapy is to allow the patient to become more aware and consequentially have more conscious control over their physiological activity. BF therapy has already been successfully applied in a number of conditions including motor weakness (Wissel et al.1989; Intiso et al. 1994), neurogenic bladder dysfunctions (Middaugh et al. 1989), bowel dysfunctions (Chiarioni et al. 2005; Ho and Tan 1997), as well as speech (Gentil et al. 1994) and swallowing problems (Reddy et al. 2000; Denk and Kaider 1997).

In recent years, BF has started to become a popular idea as a therapy for treating different types of chronic pain conditions. Thus far, pain conditions which have

shown to be successfully improved, or become more easily manageable for the patients include temporo-mandibular joint dysfunctions (Crider et al. 2005) and patellofemoral pain syndrome (Dursun et al. 2001, Yip and Ng 2006).

Exactly what cortical changes take place for BF to work is still unknown. Suggested theories include the activation of silent synapses (Wolf 1983) as well as recruitment and development of new cerebral pathways (Basmajian et al., 1982). It could be postulated that improvement through the repetitive practice of BF training is underlined by synaptic plasticity. However, recent data obtained through EEG-BF, also known as neurofeedback, shows no evidence of synaptic modulation (Ros et al. 2010).

9.3. Neurofeedback

Neurofeedback is a personalised therapy whereby participants are trained to control and modulate their own brain activity, associated with targeted mental states. Neurofeedback is a real-time training procedure: a chosen activation property is recorded, processed using a brain-computer interface (BCI) and fed back to the volunteers by means of a visual, tactile or auditory representation of their ongoing brain activity (Dursun et al., 2009).

BCI is a method of translating recorded neural activity into a computer-based output, which accurately communicates ongoing real-time cortical activity (Heetderks et al., 2000). The activity presented by the BCI can be recordings obtained from electroencephalography (EEG), Magnetoencephalography (MEG) as well as functional magnetic resonance imaging (fMRI).

9.3.1. Learning through EEG-Neurofeedback

Although neurofeedback has been done using real-time fMRI (Weiskopf et al., 2011), and research is starting to evolve towards using MEG based neurofeedback (Folds et al et al., 2011), EEG is still the more popular recording technique for neurofeedback. EEG works effectively in combination with a BCI to create a

paradigm encompassing one of the most important aspects of neurofeedback training: a short feedback time delay. The brain takes into account the time delay between response and reward. The less time between response (data acquisition) and reward (feedback), the larger the learning capacity. This is the foundation of operant learning, which relies on a specific reinforcement schedule (Ferster and Skinner 1957). As a result, the high temporal resolution of EEG and its user-friendly nature makes it a preferred candidate to the slower real-time fMRI.

The efficacy of neurofeedback training can also be accredited to the more modern prediction error learning theory (Rescorla 1972, Ploghaus, Tracey et al. 2000, Schultz 2002). Prediction error is the difference between the actual outcome and the outcome hypothesised by the subject. With practice, prediction errors decrease until the hypothesised outcome matches the actual outcome. In terms of neurofeedback training, this type of learning is best demonstrated when participants are instructed to manipulate visual or auditory representations of their cortical activity. Targeted activity is represented to the subject as a picture on a screen, commonly in the form of a thermometer. Participants training to increase or decrease their brain activity (by increasing or decreasing the temperature of the thermometer), will find that with practice, the task will not only get easier, but manipulations of their visually represented brain activity, will become more precise.

9.3.2. Neurofeedback Training for Chronic Pain

Data from past EEG-neurofeedback studies suggests that the alpha rhythm is the frequency volunteers are able to enhance or reduce most effectively, with the least training, compared to other frequency rhythms. Increasing alpha activity through neurofeedback training has a more pronounced analgesic effect on persistent headache than standard care, following only 20 training sessions (Holmes and Burish 1983). Although this preliminarily result of analgesic alpha enhancement is promising for the alleviation of headache pain, this study only contained 12 subjects. Larger participant numbers are needed to test the solidity of these initial findings.

An EEG-neurofeedback study on FMS patients by Kayıran et al. (2010) demonstrated a significant increase in SMR/theta ratio at the end of the four-week therapy. However, no significant changes in the recorded average SMR or theta amplitudes were found following the training. Nevertheless, significant improvements were observed in all recorded outcome measures: clinical pain, fatigue, anxiety and depressive symptoms. Twenty-four weeks post completion of the neurofeedback-training patients persistently displayed significantly lower scores in both pain and fatigue questionnaires than the control group. It could be postulated that these long lasting improvements could be attributed to neurofeedback stimulated alterations of the thalamo-cortical inhibitory pathways (Sterman 2000).

Reductions in chronic pain and pain unpleasantness from spinal cord injury were observed following EEG-neurofeedback sessions. Patients were trained to increase alpha and SMR power, and decrease theta power (Jensen et al 2013). Ameliorations in perceived pain were maintained three month post neurofeedback training. However, alterations in neural oscillations were not preserved, and had returned to baseline. Increases in alpha and SMR power may initiate a cascade of events, leading to a long-term analgesic effect.

Although it may seem like a promising candidate for the treatment of chronic pain, attempting analgesic neuro-modulation through neurofeedback training has several limitations. The narrow selection of neurofeedback studies completed on chronic pain only show weak analgesic effects. Furthermore, to the knowledge of the author, none have revealed long term benefits following the intervention. In order for neurofeedback training to be successful, persistent, long-term involvement of the patient is required. As the mechanisms of neurofeedback are not fully understood, completing months of training is an inefficient way to establish which frequency to target with the hope to reduce chronic pain. As neurofeedback equipment is costly, it would be more effective to work with a paradigm that instantaneously increases selected frequencies, without conscious effort of the participants. This would allow the testing of specific frequencies not only on chronic

pain, but also on acute pain perception. One way of establishing whether selected frequencies play a role in the perception of pain is by directly altering them through frequency entrainment.

9.4. Entrainment

Brain oscillatory rhythms that fall in the 1 – 30Hz frequency range can be modulated by an external stimulus (Donaldson 1998, Donaldson 2003). Brainwave activity will naturally start to adapt to the oscillatory frequency of the stimulus, making that frequency more prominent over background neural oscillations throughout the cortex. For example, listening to a steady metronome at 4Hz will result in an increased brain activity in the 4Hz range. Power enhancement of a naturally occurring rhythm, by phase-locking to a remote frequency (Adrian and Matthews 1934, Walter and Walter 1949, Compston 2010), defined as entrainment, may be driven by environmental visual, tactile or auditory stimuli oscillating in a repetitive or sinusoidal manner (Rosenfeld, Reinhart et al. 1997, Collura and Siever 2009, Goodin, Ciorciari et al. 2012, Halbleib, Gratkowski et al. 2012, de Graaf, Gross et al. 2013, Spaak, de Lange et al. 2014). Variations in the definition of entrainment exist, with some claiming true entrainment occurs when other regions of the brain fall into lockstep with the stimulated cortex and the increase in power outlasts the stimulus (Halbleib, Gratkowski et al. 2012, Thut, Miniussi et al. 2012, de Graaf, Gross et al. 2013, Spaak, de Lange et al. 2014).

Touch, vision and hearing all access the cerebral cortex via the highly innervated thalamus. Activating these senses through auditory, visual or tactile stimulation allows us to influence brainwave activity. However, a large area of skin must be stimulated to alter neural oscillatory activity through tactile stimulation. Visual and auditory stimulations have a superior profile for successful entrainment of an external pulse with a consistent frequency oscillating in the 1 – 30Hz range (Collura and Siever 2009). At the time of writing, no work has been completed on frequency entrainment and acute or chronic pain relief.

9.4.1. Visual Entrainment

Entrainment through visual stimulations such as flashing light primarily affects the primary visual cortex in occipital lobe of the brain. Nevertheless, perhaps facilitated by its size, entrainment in the visual cortex can elicit changes in cortical activity widely distributed throughout the cortex (Timmermann, Lubar et al. 1999). The visual cortex displays the most powerful resonance at 10Hz (Herrmann 2001), a frequency in the middle of the alpha range, and often close to the individual alpha frequency of a healthy young adult (Klimesch 1999). The alpha band may be more readily entrained though visual stimuli than other frequencies (Herrmann 2001, Ding, Sperling et al. 2006, Shang, Dan et al. 2011), as rhythmic activity in the 7Hz-14Hz range is more spontaneously prominent in the parieto-occipital regions of the cortex (Klimesch 1999, de Graaf, Gross et al. 2013).

Although resting EEG records a maximal alpha amplitude over the occipital regions (Cantero, Atienza et al. 2002), entrainment through auditory binaural beats proves just as effective at increasing alpha power (Schwarz and Taylor 2005, Karino, Yumoto et al. 2006). Both visual and auditory alpha entrainment may thus prove promising candidates for acute pain relief.

9.4.2. Auditory Entrainment

The human hearing range lies between 20Hz – 20,000Hz, making frequencies in the 1 – 30Hz range either difficult to hear, or inaudible to the human ear. Auditory entrainment is thus best performed through the use of binaural beats. Binaural beats occur when two sinusoidal waves, close in frequency, are played separately to each ear, generating a beat frequency equal to the difference in frequency of the two tones (Oster 1973, Wahbeh, Calabrese et al. 2007). For example, listening simultaneously to a 400Hz tone played through one earphone, and 410Hz in the other, would result in a subsonic beat frequency of 10Hz. Binaural beats are known to be entrainment most readily with a carrier frequency ranging from 300Hz to 600 Hz (Reedijk, Bolders et al. 2013) Reedijk, Bolders et al. 2013) with the greatest effect between 450-500Hz (Perrott and Nelson 1969, Oster 1973).

Binaural beats have demonstrated widespread entrainment across frontal and central sites of the scalp (Schwarz and Taylor 2005). Although the auditory cortex is much smaller than the visual cortex, it is located much closer to the targeted structures in the pain matrix (Wahbeh, Calabrese et al. 2007). Binaural beat entrainment may therefore be just as effective at reducing behavioural pain ratings as visual entrainment.

10. Thesis Aims and Objectives

To summarize the main points so far, pharmacological treatments for pain show limited analgesic benefits when compared with placebo. Neuro-modulatory approaches show a more promising overall effect, targeting acute and chronic pain with fewer side effects. The analgesic effects of neuro-modulatory treatments on chronic pain have predominantly been examined in combination with pharmacological treatments, meaning their independent effect has not yet been determined. Current neuro-modulatory treatments applied in acute and chronic pain relief research all require motivation and a prolonged time commitment from the participant to complete the oscillatory rhythm training. Neural entrainment, on the other hand, provides an almost instantaneous increase in power of the stimulated oscillatory frequency, and can be achieved with minimal effort from the participant through a visual flicker stimulus or auditory binaural beats.

The thesis introduction scrutinises various neural oscillatory frequencies and their power fluctuations during pain. Although numerous rhythms prove promising candidates for analgesic neuro-modulation, the increases in alpha power distinctively displays the greatest reproducible correlation with chronic and acute pain relief. The alpha rhythm is the most prominent rhythm detectable using EEG. According to neurofeedback training studies, participants find it not only the rhythm they are able to manipulate most effectively, with the least training, but furthermore, the most pleasant to modulate compared to other rhythms. Alpha power was therefore selected as the stimulation frequency for analgesic visual and

auditory neural oscillatory entrainment. Therefore, the **main aim** of this PhD thesis is *to develop an analgesic alpha entrainment intervention for acute pain in healthy volunteers and chronic pain patients.*

In order to quantify the magnitude of alpha entrainment triggered analgesia, changes in alpha power as well as reductions in both behavioural and electrophysiological pain responses will be recorded. The **main objective** for this thesis is therefore *to investigate the effect of alpha entrainment on the pain evoked electrophysiological responses and the behavioural pain ratings*.

11. Thesis Hypotheses

Multiple factors will affect the efficacy of neural alpha entrainment. In addition to testing different entrainment modalities (auditory and visual), the effectiveness of frequencies across the alpha range will be compared. Furthermore, as outlined in the introduction, psychological factors influence pain perception and are additionally correlated with changes in alpha power. To investigate whether alpha entrainment modulates pain independently of, or via the manipulation of psychological factors, selected behavioural questionnaires will be employed. After establishing a robust entrainment paradigm, initial analgesic testing of entrainment modalities and frequencies will be completed in healthy volunteers, prior to attempting analgesic modulation of acute experimental and chronic osteoarthritic pain in patients.

The following main hypotheses will underline the focus of the experimental studies completed in the subsequent chapters of this PhD thesis. Each experimental chapter includes a discrete justification of the hypotheses addressed, and the paradigms selected to do so.

1) Alpha power can be significantly increased from spontaneous baseline activity following frequency entrainment in healthy volunteers.

2) Auditory and visual alpha entrainment can significantly reduce behavioural ratings of acute pain in healthy volunteers

   2.1) The largest decrease in behavioural pain ratings will be apparent following the 10Hz entrainment in healthy volunteers

3) Auditory entrainment can significantly increase alpha power and significantly reduce pain-evoked potentials in healthy volunteers

   3.1) The Largest reduction in pain will be observed following the 10Hz entrainment.

4) Visual alpha entrainment can significantly increase alpha power and significantly reduce pain-evoked potentials in healthy volunteers

   4.1) The largest reduction in pain will be observed following the 10Hz entrainment.

5) Visual entrainment can significantly reduce electrophysiological and behavioural responses to experimental pain and can significantly reduce chronic pain in osteoarthritic patients

CHAPTER 2

Methodology

1. Introduction

This chapter will provide a background and the justifications for the methodology used in the experimental chapters of this thesis. The methodological concepts needed to address the main aims and objectives highlighted in Chapter 1, are introduced here, starting with the use of experimental pain. Initially, an outline of the different modalities (mechanical, electrical and thermal) for the induction of experimental pain is presented and their individual advantages and limitations discussed. Next, this chapter will present the quantitative and qualitative techniques used for the assessment of pain throughout this thesis. The qualitative assessments will consist of behavioural ratings of pain, whereas the quantitative assessment will focus on electrophysiological brain responses to pain; namely, the previously introduced EEG. Neurophysiological measures will additionally be introduced as a method for the quantitative evaluation of alpha entrainment. Psychological assessments, including monitoring the affect of alpha entrainment and pain on negative moods, sleepiness and anxiety will remain consistent throughout the experimental chapters. Thus, a background and validations of the questionnaires will be included in this chapter. Details of questionnaire administration will be covered individually in the experimental chapters themselves. This chapter will conclude with a sample size calculation and an introduction to the statistical approaches used throughout the experimental chapters of this thesis. Details of EEG recording and analysis, as well as modes of alpha entrainment, will be addressed individually in each experimental chapter, as they vary according to the chapter objectives and hypotheses.

2. Experimental Pain

The perception of pain in a non-experimental setting, whether chronic or acute, is often complemented by emotional, cognitive and autonomic responses, distorting the intensity of the noxious stimulus (Melzack 1975, Jones et al., 2003). Reducing one of these factors, for example, depression during an analgesic treatment, can result in significant pain alleviation, making it difficult to differentiate the efficacy of the two influences (Bjordal et al., 2004). Applying controlled noxious stimuli in an

experimental setting may minimize certain environmental variables, making it a preferable option for the investigation of analgesic treatment effects, as well as for the study of pain mechanisms.

A reliable experimental paradigm requires controlled assessment and stimulus parameters, including stimulation intensity, frequency, duration, reproducibility and location. Furthermore, the noxious stimulus must elicit a sensation of pain that is perceived as natural, and can be related to a previous experience. Additionally, in order for a recording to obtain an accurate measure of when noxious stimulation is occurring, rapid stimulus onset and offset times are needed; locking responses to recordings. To limit other sensory inputs from contaminating the results, a stimulus that selectively activates the noxious system under examination must be selected (Iannetti, 2013). This can be achieved by selectively activating Aδ and C nociceptors (Plaghki and Mouraux, 2003).

Human experimental pain models fulfilling the specified criteria exist for different modalities and different tissues: skin, muscle, and viscera. However, for the purpose of this thesis, the following section will focus on electrical, chemical, mechanical and thermal pain models applied to the skin, highlighting the benefits and limitations of each modality.

2.1. Mechanical Stimulation

The mechanical stimulation of the skin is a popular method for experimental pain due its simplicity of use and reproducibility of results, allowing a quantitative assessment of pain. Three of the main existing methods of mechanical stimulation, touch, pinprick and pressure, are described in the following section.

Touch: In patients with allodynia or pinprick hyperalgesia, thin von Frey hairs, cotton buds and brushstrokes can be used to elicit a sensation of pain (Curatolo et al., 2000). However, these incidents additionally activate low threshold mechanoreceptors, which do not cause pain or unpleasantness in healthy participants (Le Bars et al., 2001).

Pinprick: A painful pricking sensation can be produced by the activation of nociceptive A-fibres, by pressing down on the skin with the needle of a thick von Frey filament (Curatolo et al., 2000; Le Bars et al., 2001). Unfortunately, the stimulus onset is not fast or brief enough to produce a synchronous activation of signal transmitting nerve fibres (Staahl and Drewes, 2004).

Pressure: Painful pressure can be applied to the skin through pressure algometers. Aδ and C fibres are activated as the algometer deforms the skin (Curatoloet al., 2000). Similarly to pinprick pain, pressure pain often also activates non-nociceptive mechanoreceptors, polluting the evoked signal. Furthermore, the slow onset and variability caused by this difficult to regulate technique, makes it difficult to detect evoked potentials in the electrical recordings (Handwerker and Kobal, 1993).

2.2. Electrical Stimulation

Noxious cutaneous electrical stimulation can be achieved by placing surface electrodes on the skin, connection to an intensity controlling stimulation device (Handwerker and Kobal, 1993). This easy-to-use device allows precise temporal control of the rapid stimulus onset and offset times. Electrical stimulation has been extensively used as a reliable method of painful cutaneous stimulation (Handwerker and Kobal, 1993). The main limitation with electrical stimuli is that information from especially peripheral receptors is lost. This is due to the fact that electrical stimuli often bypass some of the exclusively peripherally located nerve endings. Unfortunately, electrical stimulation is not nociceptive specific. Bar the bypassed nerve endings in the periphery, electrical stimulation excites all nerve type endings (including Aβ-fibres). Moreover, electrical stimulation directly excites afferent fibres in a synchronised manner, which would not be observed outside of an experimental setting. Electrical stimulation is hence often classed as ‘unnatural’ (Handwerker and Kobal, 1993).

2.3. Thermal Stimulation

Thermal stimulation can be divided into 2 groups: contact stimulation (hot and cold) and non-contact.

Contact – Cold: Cold pain can be administered through ice, gel bags and cooling thermodes. Aδ fibres are believed to mediate the perception of a cold stimulus. However, recordings of C-fibres reveal they transmit noxious cold sensations (Fowler et al., 1988). The limitations of cold contact as a pain stimulus is the large variability in the cold pain threshold and subjective pain ratings (Blasco and Baye´s,1988). There furthermore appears to be a lack of standardisation in measuring cold pain responses, and in how long the cold stimulus is applied. Finally, time-locking the instant the stimulus turns from cold to noxious-cold is difficult due to the slow onset of the stimulus. The use of noxious cold stimulation is therefore inappropriate in paradigms investigating pain-evoked potentials, including neuroimaging studies (Olesen et al., 2012).

Contact – Hot: High heat contact activation of nociceptors can be achieved through a heat thermode stimulus. Rapid heating of the skin initially excites the fast conducting Aδ fibres (mean conduction velocity 14 m/s), followed by a delayed activation of slower conducing C-fibres (0.8 m/s). A slow temperature increase of the heat thermode of less than 1°C/second, preferentially activates C-fibres (Handwerker and Kobal, 1993), giving rise to a slow burning, poorly localised sensation of a long duration (Olesen et al., 2012). A slow onset of pain as described here does not elicited neural evoked responses detectable in neuroimaging studies with high temporal resolutions (Le Bars et al., 2001). Additionally, as thermode heating requires cutaneous contact, activation low threshold, non-nociceptive mechanoreceptors is unavoidable, contaminating the nociceptive signal (Iannetti, 2013). Moreover, due to the differences in surface texture and contour of human skin and the thermode, there is a non-ideal and very variable contact between the two (Watson, 2009).

LASER: Painful non-contact heating of the skin can be achieved through light amplification by stimulated emission of radiation (i.e., LASER). The pain evoking radiation pulses can be emitted by a selection of light amplification mediums. Experimentally, the most commonly used lasers are CO_2 lasers, thulium lasers and diode lasers (Plaghki and Mouraux, 2003; Frahm et al., 2010). As no direct physical contact is made with the skin, mechanoreceptors are not activated, resulting in a purely thermal stimulation (Staahl and Drewes, 2004; Tzabazis et al., 2011). Laser radiation only heats the superficial layer of the dermis. Nociceptive Aδ and C fibre are located superficially (Plaghki and Mouraux, 2003), resulting in a nociceptive specific, and simultaneous activation of both Aδ and C-fibres, perceived as a 'pricking' sensation by participants (Bromm and Treede, 1991). Heat lasers are a reliable and reproducible, high-energy noxious stimulus. Pulses are brief (microseconds to milliseconds), and well controlled with fast onset and offset times, making them ideal for paradigm requiring a stimulus time-locked to brain potentials (Plaghki and Mouraux, 2003; Frahm et al., 2010).

There is some minor within and between subject variability in stimulus transmission and absorption of the dermis (Bromm and Treede, 1991). However, the main disadvantage of the laser stimulus is that it is more difficult to operate and therefore requires some technical understanding and experience compared to other stimulation techniques (Olesen et al., 2012).

3. Pain Assessment

3.1. Behavioural Pain Assessment

The two most commonly used behavioural measures of pain, the visual analogue and the numeric rating scale; both depend on a truthful and subjective verbal rating of the experimental pain. Verbal ratings scales are minimally intrusive and deemed easy to understand and implement by the volunteer and researcher respectively. Their popularity arises from their accessibility, efficiency, low cost, and consistent correlation with pain responses in neuroimaging studies (Morton et al., 2010, Brown et al., 2008, Watson et al., 2009, Katz and Melzack, 1999).

Visual Analogue Scale (VAS): Pain specific visual analogue scales (VASs) are the most popular choice in experimental pain studies. VASs always consist of a 10 cm long, horizontal line alone which participants rate their clinical or experimental pain (Katz and Melzack, 1999). This consistency in length is essential for result reproducibility and comparisons across paradigms (Johnson, 2005). Apart from the 'no pain' labelled left end, and 'maximum pain' labelled right end, the un-segmented line has no labels or markings. This allows the participants to mark a vertical line, or cross, where on the scale their perceived pain is, without the potential influence of a number associated with that location. The score is later measured as cm (or mm) from the 'No Pain' end of the line. The main limitation with the VAS scale is that it does not allow the rating on non-painful sensations, below the pain threshold. There is no documentation of the stimulation intensities, gradient and the varying degrees of perception from the sensory threshold to the pain threshold (Brown, 2007).

Numeric Rating Scale (NRS): The numeric rating scale includes values starting from the sensory threshold, to the maximum pain tolerable, including painful and non-painful scores (Salomons et al., 2007). The NRS is a segmented scale with whole numbers labelled from 0 to 10. Additionally, the NRS sometimes has a distinct level, frequently level 4, where the pain threshold is marked. An example of an NRS can been seen in figure 2.1 The main disadvantage of both the VAS and the NRS is that they do not accurately measure the multidimensional aspects of pain on their respective one-dimensional scales (Katz and Melzack, 1999).

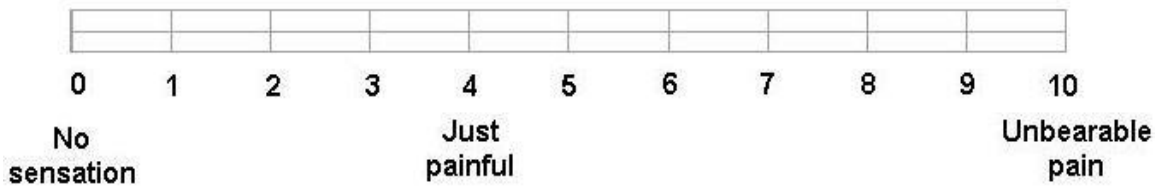


Figure 2.1. Numeric Pain Scale. A 0-10 numerical pain rating scale, in which pain threshold is anchored at number 4 on the scale.

3.2. Neurophysiological Measures of Pain

The most popular techniques for imagine pain include positron emission tomography (PET), functional magnetic resonance imaging (fMRI), electroencephalography (EEG) and magnetoencephalography (MEG). fMRI and PET have a higher spatial resolution than EEG, potentially allowing a more accurate identification of activity sources. However, both fMRI and PET have a clearly inferior temporal resolution to EEG and MEG. High temporal resolution is a property that is essential for the brief noxious stimuli investigated in this thesis. The main disadvantage of MEG is that unlike EEG, it is an expensive and technically demanding technique. Furthermore, MEG is only available in selected specialist centres, not accessible for the purpose of this PhD.

3.2.1. Electroencephalography (EEG)

As previously mentioned, EEG is the measure of macroscopic neural oscillations of larger groups of neurons, firing simultaneously. This electrical activity can be detected through electrodes placed on the surface of the scalp, amplifying and plotting the activity in units of voltage over time (Handy 2004). EEG recordings are used to investigate ongoing neural activity, as well as brief sensory events. Due to the small amplitude of a single evoked potential, signal processing techniques are commonly implemented to accurately detect the magnitude of the response. Frequently, the magnitude of an evoked response is calculated by averaging across trials, and hence reducing the signal to noise ratio (SNR) (Rugg. and Coles., 1995). The amplitude of such electrophysiological responses, evoked by noxious stimuli, has been correlated with subjective pain responses.

Event-related Potentials (ERP): Following a noxious stimulus, a time locked response appears on the millisecond scale, revealing the location of pain as it occurs. (Handy 2004). Averaging multiple stimuli reveals distinct event-related potentials (ERPs) above spontaneous neural oscillations. The main limitation averaging ERPs over background EEG, or noise, is that variations of single events in individual trials cannot be analysed (Rugg and Coles 1995). Furthermore, individual waveform of single trials may not resemble the shape and size of the final averaged waveform.

The analysis of averaged ERPs focuses mainly on the latency and amplitude of the waveform, treating peaks and troughs as individual events.

Laser-evoked Potentials (LEP): ERPs evoked by a laser stimulus, normally above the pain threshold, are referred to as laser-evoked potentials (LEPs). LEPs are believed to represent the activation of nociceptive Aδ fibres (Garcia-Larrea et al., 2003). Nevertheless, they can be manipulated by sensory, emotional and cognitive aspects of cortical activity. LEPs can be modulated by analgesics (Banoub et al., 2003; Staahl et al., 2011) and correlate with subjective ratings of noxious stimuli (Morton et al., 2010, Wager, 2005, Watson et al., 2009, Brown et al., 2008). LEPs are grouped according to the time it takes them to appear on the EEG trace. LEP latency classifications are universally defined as early (<60ms), mid (60-200ms), late (200-700ms), and ultra-late (>700ms) (Chen et al., 1998).

Components of the LEP: LEPs incorporate four major peaks at the vertex; two negative (N1, N2) and two positive (P1, P2) peaks in a W-shaped, tri-phasic waveform (N1, P1, N2, P2) (Chen et al., 1998). Recorded properties of each of these peaks (incidence, latency, amplitude and topography) can be modulated independently and may be influenced by subjective differences, laser properties and experimental settings (Watson, 2009). LEP waveform components can be influenced by a multitude of affective and cognitive factors, as well as environmental factors (stimulus duration, location and frequency) (Garcia-Larrea et al., 2003). The N2 and P2 peaks of the LEP waveform have independently been correlated to both the intensity of a stimulus and subjective ratings of pain (Ohara et al., 2004). Additionally, both components exhibit reduced amplitudes associated with pain relief (Beydoun et al., 1997). We will hence be focussing on the N2P2 waveform for the remainder of this thesis.

N2P2 Peaks: Although the N2 and P2 components of the LEP waveform have many overlapping features, the negative N2 peaks, found 100-200ms post-stimulus (Brown et al., 2007), are believed to be modulated more readily by the sensory aspects of pain (Bentley et al., 2004). N2 peak amplitudes can nevertheless still be

significantly influenced by cognitive factors, including expectation and attention paid to a noxious stimulus (Brown 2007). The generation of N2 peaks has been linked to multiple neural sources including the caudal mid-cingulate cortex, insular cortex, bilateral SII and contralateral SI (Brown, 2007). The positive P2 peak appears on the trace after the N2 peak, approximately 300-600ms following the noxious stimulus (Garcia-Larrea et al., 2003). The P2 amplitude is thought to be modulated primarily by cognitive factors, particularly, attention (Garcia-Larrea et al., 1997). The sources generating this LEP component have been identified as the mid-cingulate and the posterior cingulate cortex (Brown, 2007). The relevance of the different influences on the individual peaks will be discussed in more detail in the experimental chapters.

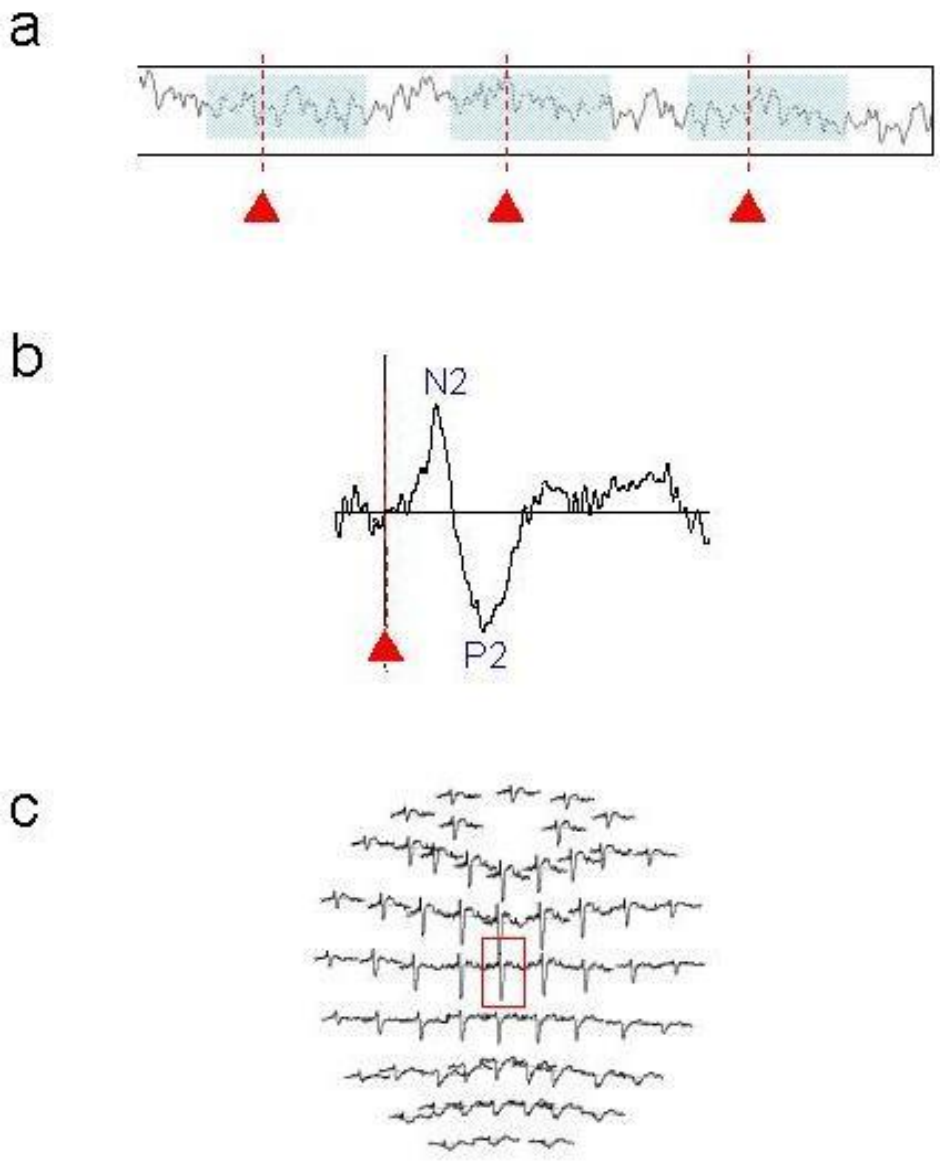


Figure 2.2 Generating evoked-potential responses from continuous EEG data. (**a**) Continuous EEG is shown, measured at one electrode, during the presentation of laser stimuli (represented by the red arrows/dashed lines). Discrete time segments ('epochs') are extracted from the continuous EEG trace either side of stimulus presentation. These are then averaged. (**b**) The resulting laser-evoked potential (LEP), showing a large negative (N2) and positive (P2) deviation, at electrode Cz. (**c**) A representation of LEPs recorded from 61 scalp electrodes, with the LEP at electrode Cz highlighted in the red box. The P2 LEP peak has the largest amplitude at central (e.g. Cz) electrodes.

Fast-Fourier Transform (FFT): The Fast-Fourier Transform (FFT) is a time-frequency, or spectral analysis where peaks and troughs are not treated as separate entities. The FFT is generally performed on continuous data, or over the entirety of a trial, to

calculate the dominant neural oscillatory rhythm of the selected frequency range. The transform disassembles the EEG time scale into a voltage by frequency spectra plot. The FFT plot depicts the power (y-axis) of the different neural oscillatory frequencies (x-axis), simultaneously active during the time of recording. The power of the frequencies displayed on the graph is representation of the EEG magnitude squared, i.e. the average peak-to-trough amplitude of the EEG signal, across the selected time frame, squared. The length of the segment chosen for analysis, defines the frequency resolution of the algorithm output. For example, segmenting the data into 1-second epochs, produces an output with a 1Hz resolution. The FFT will be used to calculate the power of the frequency bands of interest in chapters 3,5,6 and 7 of this thesis. The Fourier transform required for quantitative EEG analysis can be defined as follows:

$$f(\xi) = \int_{-\infty}^{\infty} f(x) e^{-2\pi i x \xi} \, dx$$

where ξ = frequency.

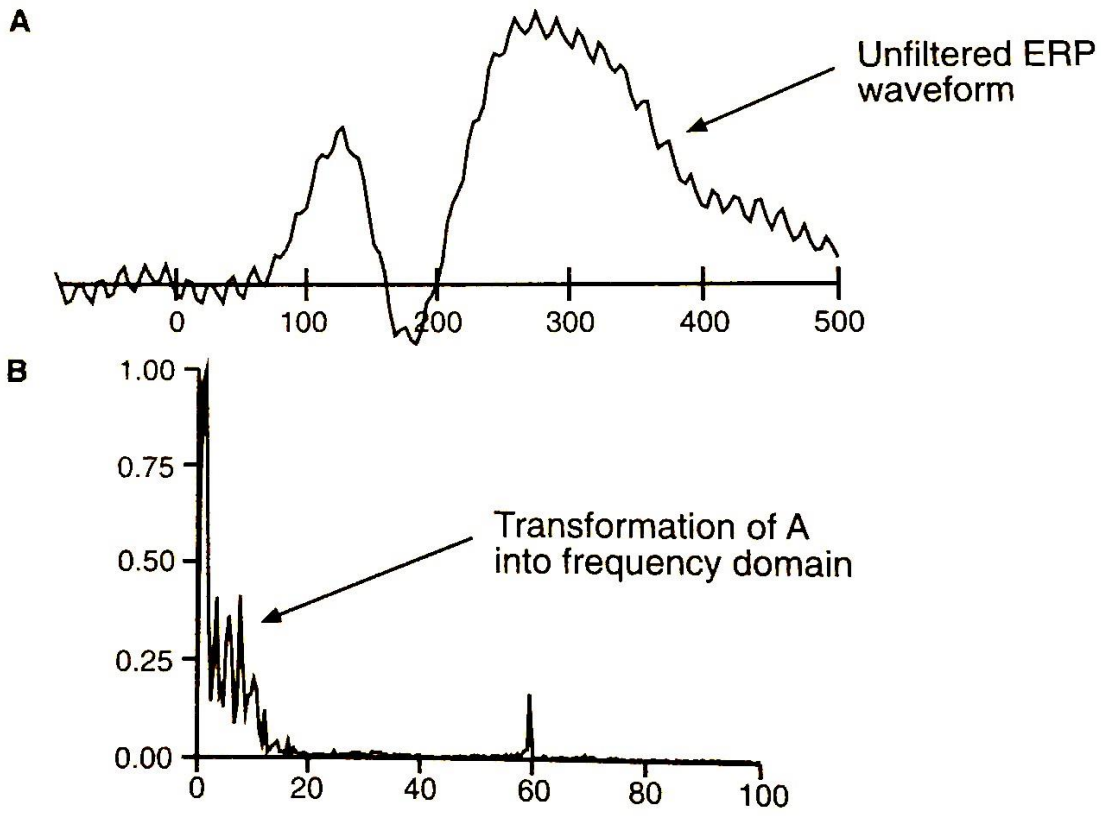


Figure 2.3 Frequency Domain Transformation. a) An example of an unfiltered Event-related potential (ERP) waveform, contaminated by noise at 60Hz. The *y*-axis is the amplitude (mV), *x*-axis is time (ms). **b)** The transformation of diagram a) into the frequency domain, with a clear peak at 60Hz. The *y*-axis is power (μV^2), *x*-axis is hertz (Hz) from (Luck, 2005).

Limitations of EEG: EEG is the most appropriate choice for this thesis due to its relatively low cost and high temporal resolution necessary for ERP analysis. Nevertheless, this technique has several limitations. Primarily, EEG has a low spatial resolution compared the PET and fMRI, because EEG electrodes on the scalp record a summation of neural source signals that pass through, and are affected by, several surfaces of different conductivities (Iannetti et al., 2013). The estimation of the locations of neural sources is confounded by the underdeterminacy of the problem (many more potential sources to estimate than channels measured; i.e., the inverse problem), and the difficulty in accurate modelling of the relevant head compartments and their conductivities (i.e., the forward problem).

Inverse problems can be solved, to some extent, by inverse solutions. EEG recordings do not accumulate sufficient information concerning the sources of activity to create an error-free localisation, and is therefore an estimation of sources. Inverse solutions rely on creating a three-dimensional approximation of active sources from the two-dimensional array of scalp electrode EEG recordings. However, as the ratio of scalp electrodes to potential neural generators is very low, many uncertainties arise when attempting to solve the problem (Baillet et al., 2001).

Various inverse modelling algorithms have overcome this limitation to some extent. Tomographic inverse solutions calculate the current source density with, on average, 3000-4000 voxels throughout the entire cortical volume. Low-resolution electromagnetic tomography (LORETA) was the first three-dimensional topographic inverse solution for EEG (Pascual-Marqui et al., 1994), and will be applied in experimental chapters 5, 6 and 7. LORETA limits its computed sources by assuming adjacent or neighbouring neurons are simultaneously and synchronously active. Evidence from single cell recordings form the foundation for this assumption (Pascual-Marqui et al., 1994). Therefore, the spatially 'smoothest' solution is selected as the source of activity.

4. Behavioural Associations with Pain and Alpha Activity

Throughout experimental Chapters 4 to 7, a selection of behavioural questionnaires are implemented to assess various psychological states that have previously been correlated with changes in acute laser pain (Brown, Seymour et al. 2008, Morton, Watson et al. 2009), or are believed to be modulated by changes in alpha power (Melzack and Perry 1975, Ossebaard 2000). These consisted of the Profile of Mood States (POMS), the State-Trait Anxiety Inventory (STAI), the Karolinska Sleepiness Scale (KSS), Pain Catastrophizing Scale (PCS), Patient Health Questionnaire - 9 (PHQ-9) and the Pain Anxiety Symptoms Scale (PASS). Each of these is described in more detail below.

4.1. Profile of Mood States (POMS)

The profile of mood states (POMS) is a questionnaire used to assess six moods experienced by the participant at the time of completing it. These are anxiety, sadness/depression, anger, fatigue, vigour and confusion (McNair et al., 1971). As previously stated, an inverse relationship between high alpha activity and negative emotions has been extensively documented (Petrovic, Dietrich et al. 2005, Morton, Watson et al. 2009, Chiesa and Serretti 2010, Watson, Power et al. 2012, Haddad, Walters et al. 2013) In Chapters 4 to 7, where the effects of alpha entrainment were investigated, nine items representing negative moods were taken from the POMS to determine participants' degree of emotional distress, as had been previously done (Sullivan, Rodgers et al. 2001, Brown, Seymour et al. 2008). The nine emotions were divided to represent the three different negative mood categories: (1) sadness (sad, discouraged, hopeless); (2) anger (angry, hostile, irritable); and (3) anxiety (anxious, tense, worried). Participants were asked to rate the intensity of each of the 9 adjectives on a 5-point Likert scale with 0 representing 'not at all' and 4 'very much', in relation to their current emotional state. A composite score of emotional distress was computed by taking the sum of all nine items on the mood scale.

4.2. State-Trait Anxiety Inventory (STAI)

Feelings of anxiety have been correlated to both pain intensity and alpha power (Sharma, Parnian et al. 1983, McCracken, Zayfert et al. 1992, Petrovic, Dietrich et al. 2005, Watson, Power et al. 2012) The state-trait anxiety (STAI) consists of 20 items assessing trait anxiety and 20 items assessing state anxiety and is implemented following alpha entrainment and the acute laser pain in Chapters 4-7. The state and trait assessments of the inventory are presented to the volunteers separately. The State anxiety inventory evaluates temporary nervousness, fear, discomfort etc. (i.e. the arousal of the autonomic nervous system), whereas the trait inventory measures prolonged feelings of stress, worry or discomfort. The items on both inventories are rated on a 4-point Likert scale (from "Almost Never" to "Almost Always"). A higher overall score indicates greater anxiety (Spielberger et al., 1983).

4.3. Karolinska Sleepiness Scale (KSS)

As changes in alpha activity are also observed during the different stages of falling asleep (Cantero, Atienza et al. 2002), a test to monitor whether alpha activity as influencing sleepiness, the Karolinska Sleepiness Scale (KSS), was used in Chapters 4 to 7. The 9-point KSS was used where 1=very alert, 3=alert, 5=neither alert nor sleepy, 7=sleepy (but not fighting sleep) and 9=very sleepy (fighting sleep)(Akerstedt and Gillberg 1990). Participants were asked to rate the scale once, preceding the alpha entrainment and once after each pain assessment trial. This was done to control for any changes in the volunteers' alertness, and subsequent potential influence on pain perception.

4.4. Pain Catastrophising Scale (PCS)

The Pain Catastrophising Scale (PCS) assesses a participants' degree of pain related catastrophic thinking. The PCS test focuses on the three primary qualities people who are prone to pain related catastrophic thinking tend to possess; pain related rumination (e.g. "I can´t stop thinking about how much it hurts"), pain magnification/ exacerbation (e.g. "I´m afraid that something serious might happen"), and helplessness in terms of managing their pain (e.g. "There is nothing I can do to reduce the intensity of my pain"). The PCS is a 13-item, five-point Likert-scale response questionnaire, ranging from zero ('not at all') to five ('all the time'). The PCS differs from many other questionnaires assessing pain-related catastrophizing, as the participant does not need to be experiencing pain whilst scoring the questionnaire. The questions refer to pervious experiences, assessing the rumination, magnification and helplessness. The internal consistency of the scale has been confirmed by Osman and colleagues Osman et al., 1997). Participants who score in the upper quartile are classed as high catastrophisers, and participants scoring in the lowest quartile fall into the low catastrophisers category (Sullivan, Bishop et al. 1995).

4.5. The Pain Anxiety Symptoms Scale (PASS)

The development of and persistence of chronic pain behaviour is fortified by the fear of pain. As previously stated, feelings of anxiety and fear of pain have been correlated to both pain intensity and alpha (Ploghaus, Tracey et al. 1999, Phan, Wager et al. 2002, Kulkarni, Bentley et al. 2007). The Pain Anxiety Symptoms Scale (PASS) measures the fear of pain across cognitive, overt behavioural and physiological domains (McCracken, Zayfert et al. 1992). The PASS has been validated through significant correlations with measures of anxiety and disability. Following the administration of the PASS in 104 consecutive referrals of a multidisciplinary pain clinic, a regression analysis (which controlled for pain and emotional distress) revealed that the PASS made a unique contribution to the prediction of disability due to fear of pain, and the development of pain behaviours. Participants are not required to be in pain when completing the PASS. The PASS was therefore presented to the participants prior to the start of the experiment in Chapters 4 to 7 to assess the participants' fear of pain.

4.6. The Patient Health Questionnaire 9-item (PHQ-9)

The PHQ-9 is a self-administered depression questionnaire with nine criteria score from '0' (not at all) to '3' (nearly every day). The questionnaire consists of 8 questions assessing symptoms of depression and one question assessing functional impairment. It has been validated for the use in primary care (Cameron, Crawford et al. 2008). The PHQ-9 is used to monitor the severity of depression and can be used to make tentative diagnosis of depression (de Man-van Ginkel, Gooskens et al. 2012, Haddad, Walters et al. 2013). Feelings of depression have previously been negatively correlated with alpha power. The PHQ-9 was hence presented to participants prior to the experiment in Chapters 4 to 7.

5. Statistical Approaches

All statistical analysis in Chapters 3 to 7 was performed using SPSS version 20. A *p* value of less than 0.05 was considered significant. In Chapters 4 to 7, the average

pain rating for each subject and trial was calculated. For the behavioural analysis a mixed linear model was implemented to assess the size of change in pain ratings following the entrainment of different frequencies compared to control. The model incorporated baseline pain ratings as a covariate and included the frequency entrained, order of entrainment sessions, and order of the control/ entrainment visit as factors. Using each alpha frequency entrained as a reference category, the model was refitted with a Bonferroni correction to assess the significant differences in pain ratings following each of the frequency entrainment sessions. The equivalent linear mixed model was applied to the N2 and P2 amplitudes (average values taken +/- 10ms of the peak from each subject) with baseline N2 and P2 amplitudes as a covariate and condition, session order and visit order as factors. The same model was applied separately to the POMS, STAI and KSS scores, which were recorded following each of the frequency entrainment sessions, taking into account the same respective covariates and factors. The PHQ-9, PCS, Participant Sleep Questionnaire and PASS scores were only recorded at the beginning of each study. These were correlated to the changes in pain ratings from baseline following each of the frequency entrainment sessions.

Throughout Chapters 3 to 7, comparisons in alpha power were made across all the electrodes across multiple conditions (the different alpha entrainment frequencies vs. the matched control). In order to control for multiple comparisons, a repeated measures analysis of variance (ANOVA) was used to test within subject effects of condition (control vs. entrainment) and the location of the electrodes. The EEG scalp electrodes were divided into nine scalp regions. The electrodes were divided into 9 scalp regions: Left Anterior, Central Anterior, Right Anterior, Left Middle, Central, Right Middle, Left Posterior, Central Posterior and Right Posterior, averaging the electrodes' activity in each region. For each condition, a repeated-measures ANOVA was completed comparing the activity of the stimulated frequency band, to activity of the same frequency band in the control condition. For each condition the ANOVA was run with factors Left/Central/Right x Anterior/Middle/Posterior x Control/Condition. A two-tailed t-test was then used to follow-up the main effects by calculating alpha power differences between the

entrainment condition frequency bands and matching control condition frequency bands, separately for each scalp region.

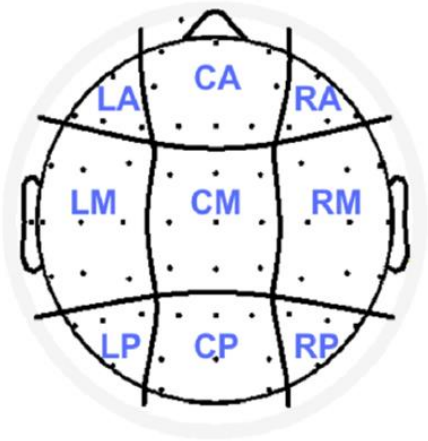


Figure 2.4 The 9 EEG Electrode Regions of the Scalp
Schematic representation of the division of electrodes into the 9 scalp regions: Left Anterior (LA), Central Anterior (CA), Right Anterior (RA), Left Middle (LA), Central Middle (CM), Right Middle (RM), Left Posterior (LP), Central Posterior (CP) and Right Posterior (RP).

6. Sample Size Calculation

In order to detect a significant change in pain ratings, a sample size calculation was completed to calculate the number of participants needed to show a significant effect. From previous data from the lab, mean (SD) pain scores before and after a placebo intervention in the control group were 5.63 (1.01) and 6.16 (0.61) respectively, so that the mean change in pain observed was 0.53. The baseline standard deviation in sample size calculations is used, with the following reasoning: the standard deviation in the change scores will be less than that observed at baseline, due to the correlation between baseline and follow up measures in each individual. As such, estimates based on the baseline standard deviation will be conservative, and will prevent underestimation of the sample size required on the basis of an underestimated standard deviation in the change score.

We begin by assuming a difference in change scores of 0.5 represents the smallest effect size that we are interested in being able to detect. To have 46% power to detect an improvement in change scores from 1.03 in the control group to 0.53 in the treatment group with a two-sided 5% significance level requires 10 participants, each acting as their own control. An analysis at 30 participants would give around

89% power to detect a difference as small as 0.5. With a two-sided 5% significance level, we need 32 participants to have 90% power for the study. Thus, this is the number aimed for in the analgesic alpha entrainment studies. In Chapter 7, due to time constraints, only 21 patients were recruited for the study. This resulted in a power of 72.1%, which must be taken into consideration when interpreting the results. No sample size calculation was completed for the pilot study (Chapter 3), as this pilot did not include a pain stimulus and hence, no power calculation for the change in pain ratings was needed. The sample size for the pilot study was chosen based on previous visual entrainment studies (Teplan, Krakovská et al. 2006, Caro and Winter 2011, Abeln, Kleinert et al. 2014).

CHAPTER 3

Pilot Study: Neural Entrainment of an Alpha Rhythm Flicker

This Chapter is based on a manuscript that is being prepared for submission for peer review.

1. Abstract

Resting-state alpha EEG activity can be entrained by an external rhythmic stimulus oscillating in the 7 Hz to 14 Hz frequency range. Visual stimuli can induce frequency sensitive entrainment across the spectrum, increasing the power in the stimulated frequency band. In the present study, a rhythmic pattern reversing checkerboard stimulus was implemented to visually entrain frequencies across the alpha band. The analysis of 13 volunteers' recordings revealed a significant increase in power in the 10 Hz and 11 Hz frequency bands, following the 10 Hz and 11 Hz checkerboard entrainments respectively. In the 10 Hz condition, there was a significant anterior-to-posterior scalp region interaction with entrainment condition. Additionally a significant main effect could primarily be observed in the anterior scalp regions. Following the 11 Hz entrainment, significant main alpha power effects were observed anteriorly and in the right hand side scalp regions. These significant increases from baseline activity suggest rhythmic 10 Hz and 11 Hz flickers can successfully entrain spontaneous neural oscillations, locking the timing of cycles, and thus increasing power.

2. Introduction

Electroencephalography (EEG) is able to detect large-scale oscillatory activity generated by groups of neurons. EEG signals trace oscillatory activity of specific frequency bands simultaneously throughout the cortex. The multitude of frequency bands: delta (1–4 Hz), theta (4–7 Hz), alpha (7-14 Hz) beta (14–30 Hz) and gamma (30–100 Hz), highlights the broad spectral content of EEG signals. Detectable in 95% of adults with their eyes closed (Srinivasan 1999), the large amplitude alpha rhythm is the most prominent neural phenomenon, visible in the raw EEG recordings (Berger 1929). The modulation of sinusoidal waveforms is made possible by selectively enhancing one frequency over the background neural oscillations. The phase of spontaneous, resting-state electrical activity can be locked to an external rhythmically oscillating stimulus in the 1-30 Hz frequency range (Adrian and Matthews 1934, Walter and Walter 1949, Compston 2010). Power enhancement of naturally occurring alpha, by phase-locking to a remote frequency, defined as alpha entrainment, may be driven by environmental visual, tactile or auditory stimuli oscillating in a sinusoidal manner (Rosenfeld, Reinhart et al. 1997, Collura and Siever 2009, Goodin, Ciorciari et al. 2012, Halbleib, Gratkowski et al. 2012, de Graaf, Gross et al. 2013, Spaak, de Lange et al. 2014). Resting alpha activity is predominately located in the parietal-occipital regions of the cortex (Klimesch 1999, de Graaf, Gross et al. 2013), and has previously been entrained by 10Hz visual stimuli (Herrmann 2001, Ding, Sperling et al. 2006, Shang, Dan et al. 2011).

With the aim to amplify oscillatory alpha activity, a visual stimulus was selected to target the primary visual cortex, where resting alpha power is thought to be maximal (Cantero, Atienza et al. 2002). The visual cortex is furthermore believed to have the strongest resonance (Herrmann 2001). Entrainment of the large occipital lobe may hence facilitate surrounding cortices to fall in lockstep, allowing the effect to propagate, resulting in a global increase in alpha power.

In the present study, a rhythmic visual checkerboard stimulus with repetitive reversing patterns was selected. Checkerboards flickering above a rate of 4 Hz can

precisely synchronise evoked brain responses, with a precision measurable to ±0.1 Hz (Bakardjian, Tanaka et al. 2010, Tomita, Vialatte et al. 2014). Visual stimuli can induce frequency sensitive entrainment across the spectrum (Pastor, Artieda et al. 2002, Pastor, Artieda et al. 2003). We therefore attempted visual entrainment from 7 Hz – 14 Hz, across the alpha range. By accurately measuring alpha power in each frequency band at rest and during rhythmic stimulation, we aimed to obtain an accurate estimate of visual entrainment.

We hypothesized that rhythmic visual stimuli will entrain ongoing alpha oscillations across the entire frequency band. Although we expected all frequencies to display entrainment, the largest effect was predicted around the central 10 Hz frequency, where the visual cortex exhibits the strongest resonance (Herrmann 2001).

3. Materials and Methods

3.1.1. Ethics Statement

The experimental protocol was ethically approved by the University of Manchester Ethics Committee. Prior to study participation, all volunteers were obliged to provide written informed consent.

3.1.2. Participants

Thirteen (5 Female, 8 Male) self-reported right-handed volunteers over the age of 18, with a mean age of 21.85 (SD 4.81) were recruited for the study. In order to participate, volunteers must not have been diagnosed with a neurological or psychiatric condition nor have a history, or family history of epilepsy. Due to the nature of the study, participants were obliged to have normal, or corrected normal vision.

3.1.3. Procedure

During the experiment, participants were situated in a dark, soundproof room, sitting comfortably 75 cm away from a 30x40 cm CRT monitor displaying the visual stimulus. The stimulus consisted of a checkerboard with alternatively flashing fields, which subtended circa 30° of the participants visual field. The stimulus was generated with a Cambridge Research Systems ViSaGe MKII Stimulus Generator (Cambridge Research Systems, Rochester, UK), and programmed using the CRS toolbox in Matlab v.7.10 (The Mathworks, Inc., Natick, MA). Following the placement of the EEG-cap, a 5-minute baseline recording was completed. Participants were then presented with the checkerboard stimulus flashing at 7Hz, 8Hz, 9Hz, 10Hz, 11Hz, 12Hz, 13Hz and 14Hz, in a randomised order, for a total of 4 minutes each. Each 4-minute flashing frequency trial was followed by a 5-minutes rest period, where the stimulus screen displayed a black fixation cross on a grey background. Participants were asked to keep their eyes naturally open (with normal blinking) during this time. To ensure participants did not fall asleep, or close their eyes for a prolonged period of time, the grey background would flash randomly 19 – 26 times (median 23). Participants were required to acknowledge they had seen the screen flash by pressing a button within 2 seconds of the flash. The average response time across subjects was 0.458s (SD 0.087). If participants failed to acknowledge more than 75% of the background flashes, the data were eliminated from the analysis.

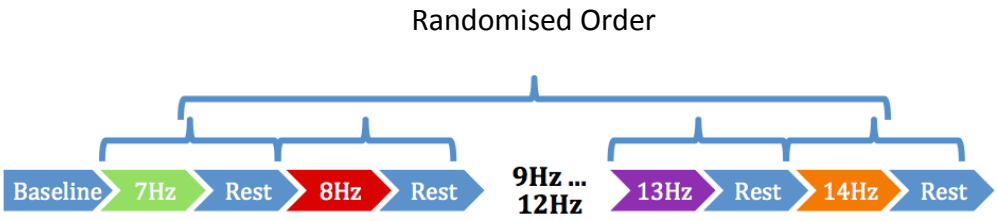


Figure 3.1 Experimental Entrainment Procedure. The procedure commenced with a 5-minute baseline EEG recoding. Participants were exposed to four minutes of visual entrainment at 7Hz, 8Hz, 9Hz, 10Hz, 11Hz, 12Hz 13Hz and 14Hz by means of a flashing checkerboard stimulus. Each 4-minute entrainment session was separated by a 5-minute rest period.

3.1.4. Electrophysiological Recordings

Continuous EEG recordings were completed throughout the experiment. Data were recorded using a 64-channel Ag/AgCl electrode, ActiveTwo BioSemi system (Amsterdam, The Netherlands), in the extended 10-20 layout. Eye-movement and blink artefacts in the EEG were detected using an additional two horizontal and two vertical electro-oculograms (EOG). A sampling rate of 512Hz was used and an open pass band from DC to 150 Hz was applied throughout the recording. EEG activity was recorded with Actiview acquisition software (BioSemi).

3.1.5. Alpha Power Analysis

Spectral oscillatory power was calculated using Brain Vision Analyzer 2.0 (Brain Products GmBH, Germany). After re-referencing the data to a common average, low and high cut off filters at 0.05Hz (12 dB/oct) and 35 Hz (48 dB/oct) were applied to the whole data. For each entrainment condition, the data were segmented from 0s to 240s past the stimulus marker, where 0s indicated the start of the flashing visual stimulations from 7Hz-14Hz. At baseline, the data were segmented to 240s past the marker indicating the start of the recording. A 25-component Independent Component Analysis (ICA) was implemented on the segmented data to isolate eye-movement and muscle artefacts. The median number of components removed was 4 with a range of 0 to 5. Following reconstruction using only the clean components, data in each condition were segmented into 1-second epochs. Spectral analysis was carried out through the use of a Fast-Fourier Transformation algorithm with a 10% Hanning window. Activity was averaged over the 1-second epochs. In each condition, the average power of the frequency bands had a 0.5Hz resolution, expressed in log units ($10*\log10(\mu V^2/Hz)$), providing a measure of frequency density.

Spectral power was averaged into 9 scalp regions; Left Anterior (AF7, F9, F7, F5, F3) Central Anterior (Fp1, Fpz, Fp2, AF3, AFz, AF4, F1, Fz, F2), Right Anterior (AF8, F4, F6, F8, F10), Left Middle (FT7, FC5, FC3, T7, C5, C3, TP7, CP5, CP3), Central Middle (FC1, FCz, FC2, C1, Cz, C2, CP1, CPz, CP2), Right Middle (FC3, FC6, FT10, C4, C6, T8, CP4, CP6, TP8), Left Posterior (P7, P5, P3, PO7), Central Posterior (P1, Pz, P2, PO3,

POz, PO4, O1, Oz, O2) and Right Posterior (P4, P6, P8, PO8). For each of the 9 regions, and for each entrainment condition, the power of the frequency stimulated (±1Hz), was compared to the matching frequency band at baseline. For example, comparing power at 7Hz (±1Hz), following the 7Hz visual stimulation, to 7Hz (±1Hz), at baseline.

A repeated measures analysis of variance (ANOVA) was used to test within subject effects of condition (baseline vs. visual stimulation) and scalp location. An ANOVA was completed comparing activity of each stimulated frequency band to the corresponding baseline activity with the following factors: Left/Central/Right x Anterior/Middle/Posterior x Baseline/Condition. Significant main effects and interactions were followed up with paired two-tailed t-tests to compare individual scalp regions across conditions. All statistical analysis was performed in SPSS (v. 20), with a *p* value of less than 0.05 considered statistically significant.

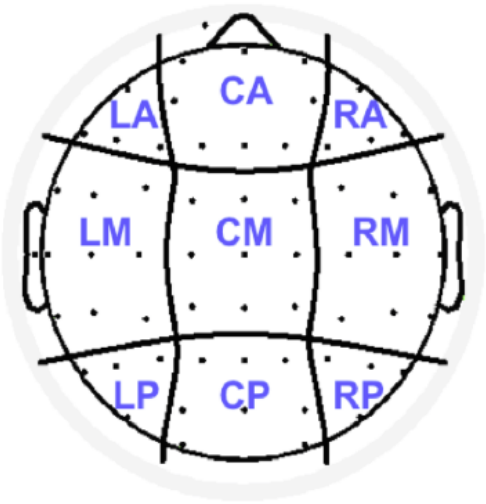
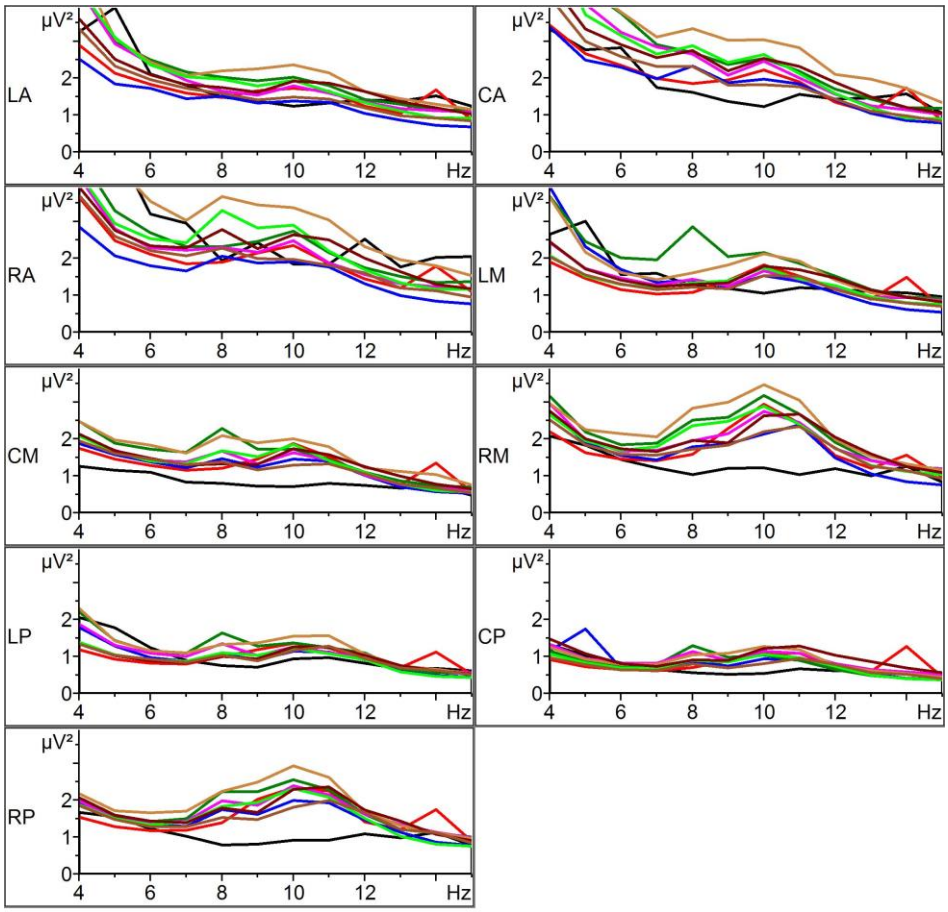


Figure 3.2 Electrode Scalp Regions

Schematic representation of the division of electrodes into the 9 scalp regions: Left Anterior (AF7, F9, F7, F5, F3) Central Anterior (Fp1, Fpz, Fp2, AF3, AFz, AF4, F1, Fz, F2), Right Anterior (AF8, F4, F6, F8, F10), Left Middle (FT7, FC5, FC3, T7, C5, C3, TP7, CP5, CP3), Central Middle (FC1, FCz, FC2, C1, Cz, C2, CP1, CPz, CP2), Right Middle (FC3, FC6, FT10, C4, C6, T8, CP4, CP6, TP8), Left Posterior (P7, P5, P3, PO7), Central Posterior (P1, Pz, P2, PO3, POz, PO4, O1, Oz, O2) and Right Posterior (P4, P6, P8, PO8)

4. Results

The repeated measures ANOVAs revealed that a significant main effect of condition (stimulation power vs. baseline power) could be found following the 10Hz and 11Hz entrainment.



Key of Entrainment Frequencies (Hz):

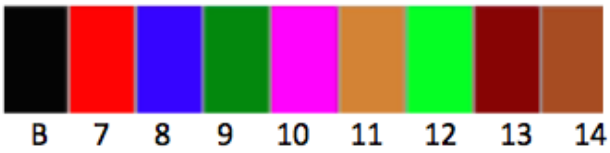
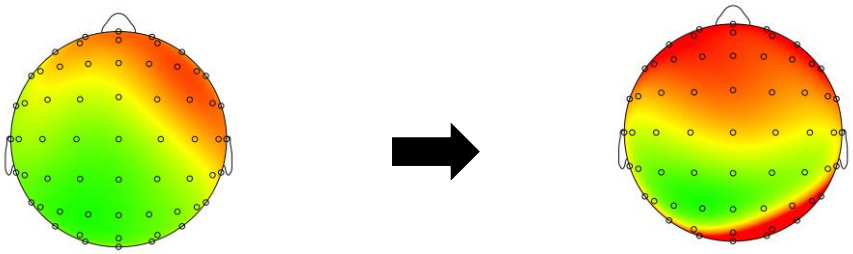
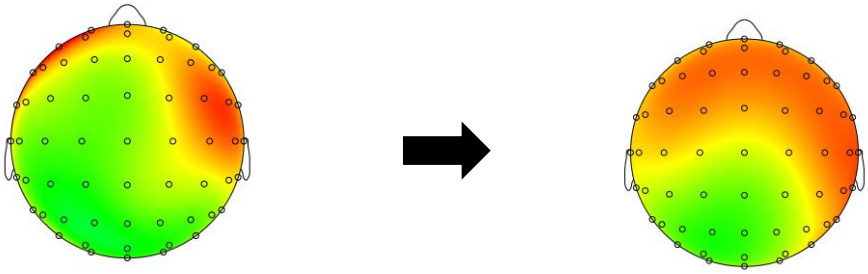


Figure 3.3 Alpha Power. Electrophysiological power shown separately in the nine scalp regions: Left Anterior (LA), Central Anterior (CA), Right Anterior (RA), Left Middle (LM), Central Middle (CM), Right Middle (RM), Left Posterior (LP), Central Posterior (CP) and Right Posterior (RP). The key indicates the frequency stimulated.



10 Hz Band at Control	10 Hz Band at 10 Hz Entrainment



11 Hz Band at Control	11 Hz Band at 11 Hz Entrainment

Figure 3.4 Topographical Maps. Electrical activity in the 10Hz (±1Hz) band and the 11Hz (±1Hz) band averaged across all subjects, following 10Hz and 11Hz entrainment, respectively.

4.1.1. 10Hz Alpha Entrainment

Following the 10Hz entrainment, power in the 10Hz (±1Hz) band was significantly higher than in the 10Hz (±1Hz) band at baseline ($F(1,31) = 7.75$, $p<0.05$). A significant interaction between condition and anterior-to-posterior effects was found ($F(1,31) = 4.463$, $p<0.05$). Figure 3.5 reveals that, although baseline anterior alpha power is greater, increases in posterior alpha power from baseline are much larger than anterior increases. No significant effect for the right-left vs. condition interaction was found. Furthermore, a significant anterior to posterior scalp region main effect can be detected ($F(1,31) = 5.26$, $p<0.05$), with higher alpha power in the anterior regions, again depicted in Figure 3.5. Upon viewing Figure 3.6 a trend can

be observed from the Left to Right regions, with seemingly higher alpha power in the Right scalp regions, however, this effect was not significant (p=0.103)

Condition Effect

Condition n= 13	Mean Power	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
10Hz	1.748	.265	1.068	2.429
Baseline	1.169	.186	.691	1.647

Anterior to Posterior Effects

Ant. - Post.	Mean Power	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Anterior	1.676	.248	1.038	2.315
Middle	1.445	.196	.940	1.949
Posterior	1.255	.203	.732	1.777

Table 3.1 Condition Effects of Scalp Electrode Region Entrainment at 10Hz. The above tables display the main condition (entrainment vs. baseline) effect and the anterior to posterior scalp region effect results of the repeated measures ANOVA when comparing all nine scalp regions of the 10Hz and baseline alpha activity.

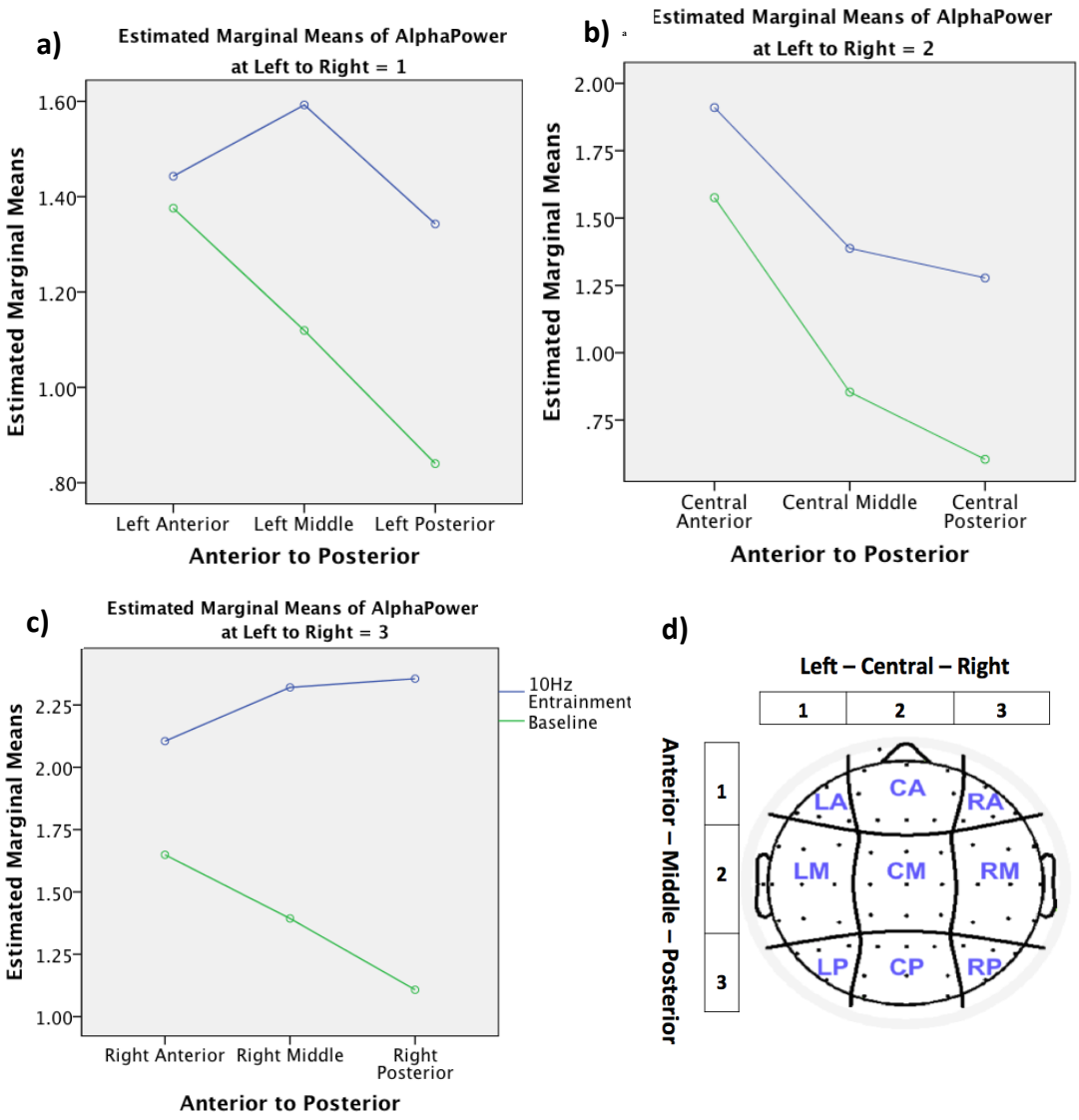


Figure 3.5 Anterior to Posterior Effects. A significant effect of condition (entrainment vs. baseline) was observed following 10Hz stimulation. A significant anterior effect could be observed, also visible in figure 3.3. **a)** Alpha power in the left electrodes from anterior to posterior represented as LA (1,1), LM (2,1) and LP (3,1) in figure d). **b)** Alpha power in the central electrodes from anterior to posterior represented as CA (1,2), CM (2,2) and CP (3,2) in figure d). **c)** Alpha power in the right electrodes from anterior to posterior represented as RA (1,3), RM (2,3) and RP (3,3) in figure d). **d)** Diagram of scalp electrode regions.

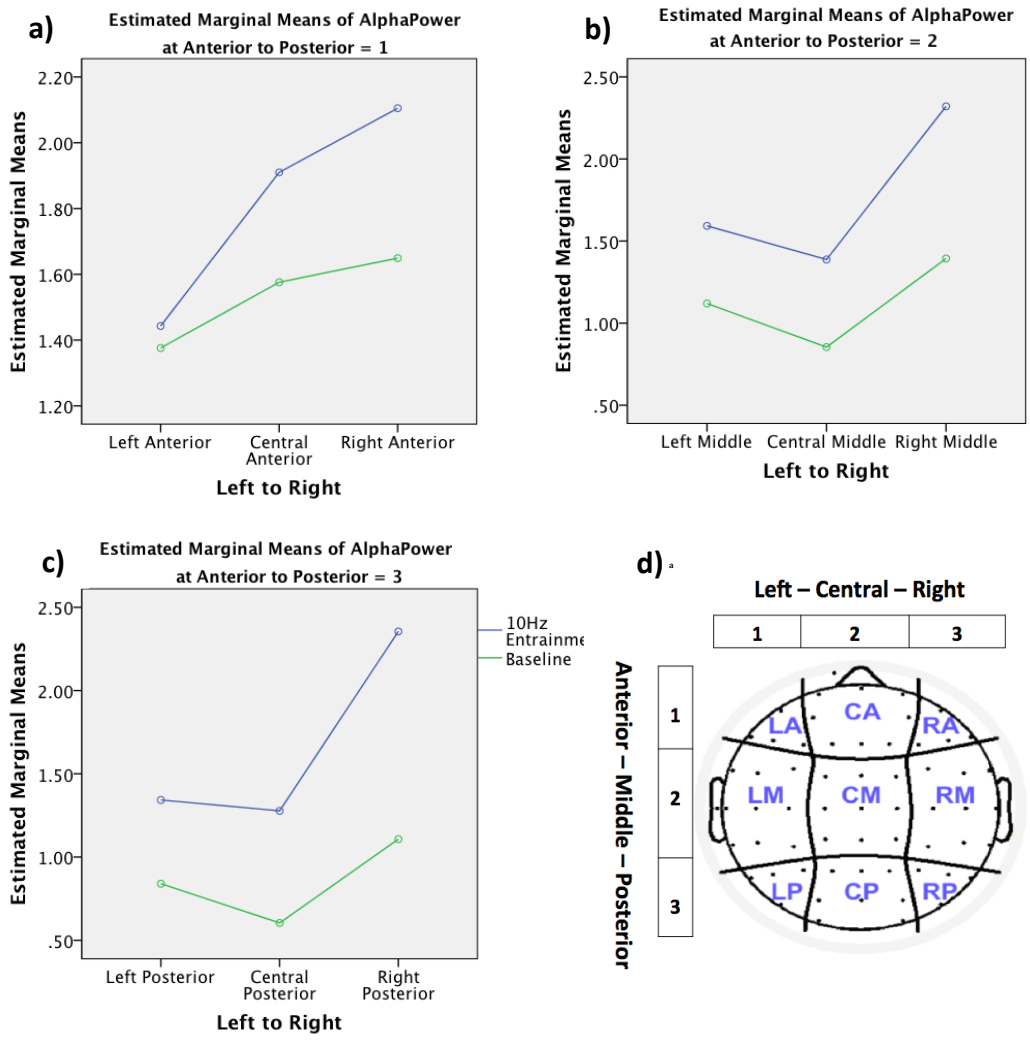


Figure 3.6. Left to Right Effect. Following 10Hz entrainment, no significant left to right effect was detected by the ANOVA. A trend suggested higher power in the right hemisphere could be observed. **a)** Alpha power in the anterior electrode scalp regions from left to right represented as LA (1,1), CA (1,2) and RA (1,3) in figure d). **b)** Alpha power in the middle electrode scalp regions from left to right represented as LM (2,1), CM (2,2) and RM (2,3) in figure d).**c)** Alpha power in the posterior electrode scalp regions from left to right represented as LP (3,1), CP (3,2) and RP (3,3) in figure d). **d)** Diagram of scalp electrode regions.

The significant anterior-to-posterior interaction calculated through the ANOVA was followed-up with paired t-tests comparing the average 10Hz activity in the anterior, middle and posterior regions, across the baseline and entrainment conditions. Following the 10 Hz entrainment, middle and posterior scalp regions showed a significant increase in average 10 Hz band power compared to baseline (p<0.05).

Table 3.2 Follow-up Paired t-test of Anterior-to-Posterior Interaction following 10Hz Entrainment

Paired Samples Test								
n=13	Paired Differences					t	df	Sig. (2-tailed)
	Mean Power	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Ant. Baseline – Ant. 10Hz	.28556	1.14885	.46902	-.92009	1.49120	.609	9	.569
Mid. Baseline – Mid. 10Hz	.64556	.44967	.18358	.17366	1.11746	3.517	9	.017
Post. Baseline – Post. 10Hz	.80722	.64247	.26229	.13300	1.48145	3.078	9	.028

4.1.2. 11Hz Alpha Entrainment

The power in the 11 Hz (±1Hz) band following the 11 Hz stimulation was significantly higher than in the same band at baseline ($F(1,31) = 8.35$, $p<0.05$). Although there was significant interaction effect between regions (Anterior to Posterior and Left to Right ($F(1,31) = 4.709$, $p<0.05$), no significant interaction effect between condition and location was observed. However, a significant anterior to posterior scalp region effect was detected ($F(1,31) = 15.57$, $p<0.01$), again, with higher alpha power in the anterior regions. A significant left to right effect at $F(1,31) = 8.36$, $p<0.05$ was also observed, with significantly higher alpha power in the right scalp regions.

Condition				
n = 13			Lower Bound	Upper Bound
11 Hz	2.726	.473	1.414	4.038
Baseline	1.278	.085	1.043	1.513

Anterior to Posterior				
Ant. – Post.	Mean Power	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Anterior	2.444	.291	1.637	3.250
Middle	1.941	.200	1.386	2.495
Posterior	1.622	.235	.969	2.274

Left to Right				
Left – Right	Mean Power	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Left	1.866	.332	.943	2.789
Central	1.611	.212	1.022	2.199
Right	2.530	.237	1.873	3.187

Anterior to Posterior X Left to Right					
Ant. to Post	Left to Right	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1) Anterior	1) Left	2.083	.349	1.114	3.051
	2) Central	2.338	.274	1.578	3.099
	3) Right	2.910	.319	2.026	3.795
2) Middle	1) Left	1.917	.311	1.053	2.780
	2) Central	1.428	.228	.794	2.062
	3) Right	2.477	.243	1.803	3.152
3) Posterior	1) Left	1.598	.380	.543	2.653
	2) Central	1.065	.170	.592	1.538
	3) Right	2.202	.254	1.497	2.906

Table 3.3 Condition Effects of Scalp Electrode Region Entrainment at 11Hz. The above tables display the main condition (entrainment vs. baseline) effect, the main anterior to posterior scalp region effect, the main left to right effect and the anterior to posterior x left to right interaction results of the repeated measures ANOVA when comparing all nine scalp regions of the 11Hz and baseline alpha activity.

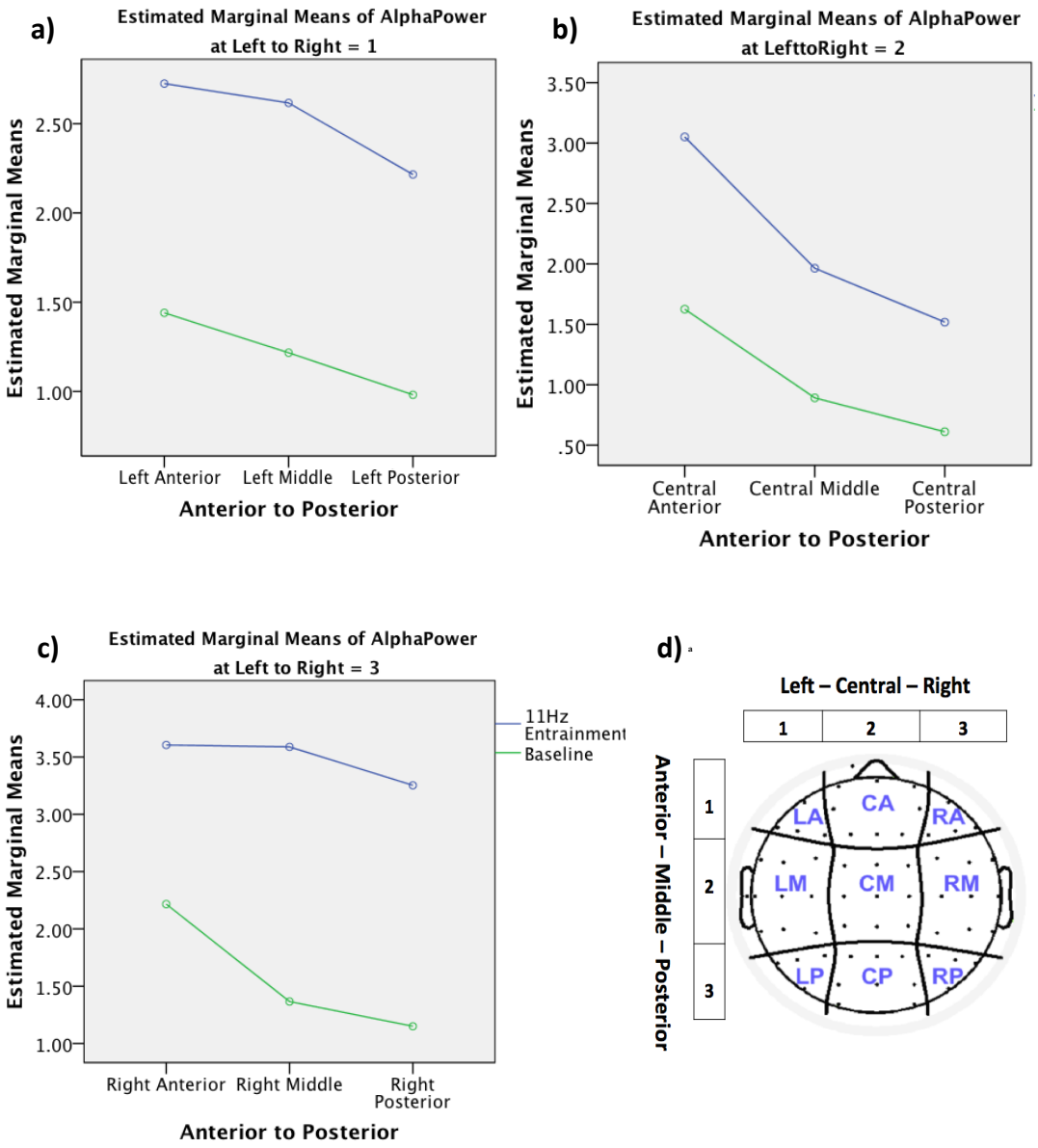


Figure 3.7 Anterior to Posterior Effects. A significant effect of condition (entrainment vs. baseline) was observed following 11Hz stimulation. Visible in figure 3.3, a significant anterior within subject effect could be observed ($p<0.05$). **a)** Alpha power in the left electrodes from anterior to posterior represented as LA (1,1), LM (2,1) and LP (3,1) in figure d). **b)** Alpha power in the central electrodes from anterior to posterior represented as CA (1,2), CM (2,2) and CP (3,2) in figure d). **c)** Alpha power in the right electrodes from anterior to posterior represented as RA (1,3), RM (2,3) and RP (3,3) in figure d). **d)** Diagram of scalp electrode regions.

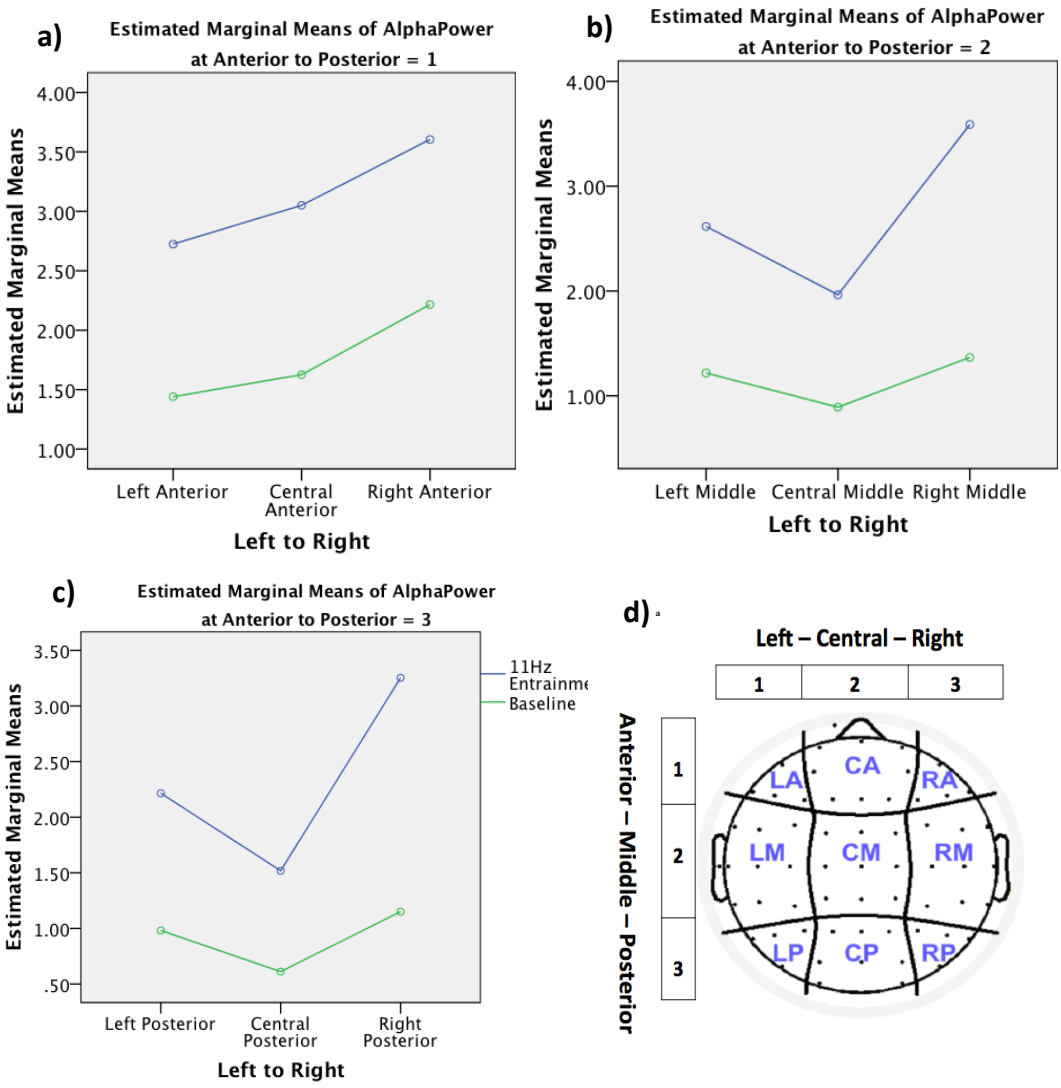


Figure 3.8 Left to Right Effects. A significant left to right within subject effect could additionally be observed following the 11Hz entrainment ($p<0.05$). Alpha power in the right hemisphere was larger than in the left hemisphere. . **a)** Alpha power in the anterior electrode scalp regions from left to right represented as LA (1,1), CA (1,2) and RA (1,3) in figure d). **b)** Alpha power in the middle electrode scalp regions from left to right represented as LM (2,1), CM (2,2) and RM (2,3) in figure d). **c)** Alpha power in the posterior electrode scalp regions from left to right represented as LP (3,1), CP (3,2) and RP (3,3) in figure d). **d)** Diagram of scalp electrode regions.

To identify differences in scalp regions, the ANOVA was again followed-up with paired t-tests comparing the electrode regions between baseline and the 11Hz stimulation condition. Significant differences at $p<0.05$ were found in the Central Anterior, Central Middle and Right Middle scalp regions.

5. Discussion

The present study investigated the effectiveness of a visual checkerboard stimulus, flickering in the alpha rhythm, at entraining frequencies in the 7 Hz – 14 Hz range. The analysis revealed that 4 minutes of visual alpha stimulation at 10 Hz and at 11 Hz resulted in a significant promotion of naturally occurring alpha power. These significant increases from baseline activity suggest rhythmic 10 Hz and 11 Hz flickers can successfully entrain spontaneous neural oscillations, locking the timing of cycles, and thus increasing power.

Alpha Topography

Although we hypothesised that, all frequencies would significantly increase in power following entrainment, the only significant increases were observed in the 10 Hz and 11 Hz conditions. This suggests entrainment is more effective in frequencies in the central alpha range compared to in frequencies located peripherally in the range. Similar results have previously been observed in visual entrainment studies (Herrmann 2001, Ding, Sperling et al. 2006, Shang, Dan et al. 2011). Ensuing visual stimulation, increases in alpha activity around 10 Hz have been linked to the natural resonance of the primary visual cortex (Herrmann 2001).

In line with this, our results reveal an interaction effect between condition and anterior to posterior scalp regions in the 10 Hz entrainment condition. Furthermore, follow-up tests exposed that alpha power was significantly higher in the posterior and middle scalp regions in the 10 Hz entrainment condition compared to control. Although alpha power displayed higher activity anteriorly at baseline, following the 10 Hz entrainment, alpha power increases were significant posteriorly, but not anteriorly, and may be a reflection of the known resonance of the primary visual cortex.

Although alpha rhythm generators are predominately believed to be located in the occipital cortex (Lopes Da Silva and Storm Van Leeuwen 1977, Bollimunta, Chen et al. 2008, Spaak, Bonnefond et al. 2012), ample evidence of additional parietal alpha

sources has been presented (Thut, Veniero et al. 2011, Hanslmayr, Volberg et al. 2013, Jaegle and Ro 2014). These may have facilitated the entrainment of alpha power following the 10 Hz stimulus in the middle scalp electrode regions. Alternatively, middle scalp areas may have been activated by the propagation of the alpha rhythm through the cortex, again, due to the strong resonance and the large size of the visual cortex (Herrmann 2001).

Our results reveal a main anterior electrode effect following the 10Hz entrainment and an anterior and right hand side electrode effect following the 11Hz entrainment, suggesting alpha power was higher in these regions across conditions. Although no visual stimulus was present during the baseline recording, throughout the experiment, volunteers were focussed on the screen in front of them. Evidence of right hemisphere superiority when processing visual stimuli has been documented (Rubino 1970, Beaumont 2008), and could explain the right scalp region effect. Furthermore, although unilateral, parietal sources of alpha may have contributed to the right electrode scalp-region effect observed following 11Hz entrainment.

Additional work on perception by Busch et al., describes neural oscillatory activity with a fronto-central topography, when focusing on visual stimuli (Busch, Dubois et al. 2009). In the current study, high levels of concentration on the visual stimulus may have led to an anterior topographical effect across conditions. Frontal, parietal and posterior alpha power locations may reflect corresponding mechanisms, or may interact in the processing visual inputs. However, no source analysis was performed in the present study. The perceived topography does not necessarily reflect the engagement of posterior, parietal or anterior sources. Additional work needs to be completed to further our understanding of the relationship between cortical alpha sources and oscillatory entrainment.

Rhythmic Entrainment

It has formerly been argued that visual steady-state responses during rhythmic stimulation are exclusively responsible for augmentations in power, rationalized by

the superposition of transient evoked potentials (Capilla et al., 2011). If this were the case, we would expect an increase in alpha power solely in the targeted visual cortex, which was not the case in the present study. Additionally, we would expect frequency matched increases following each of the visual stimulations, not merely following 10 Hz and 11 Hz visual stimulation. The greatest power increase may have been recorded at 10 Hz, due to the previously discussed maximal resonance of the visual cortex (Herrmann 2001). Nevertheless, this theory does not provide an adequate justification for the significant increases observed in both the 10 Hz and 11 Hz stimulation condition.

Efficacy of frequency entrainment has been linked to ongoing baseline neural activity. A study by Mathewson et al disclosed entrainment-induced alpha power augmentation was related to the amount of spontaneous alpha power prior to the start of the experiment. Additionally, increases at 12 Hz following a 12 Hz visual stimulation, were dependant on the power of 12 Hz at the start of each trial. Baseline ongoing alpha frequency of a young healthy adult, as sampled in this experiment, is believed to be around the 10 Hz – 11 Hz mark (Klimesch 1999). A simple algorithm for calculating the approximate baseline peak alpha frequency is 11.95 – (0.053 x age), which in the current study, reveals an estimated peak baseline frequency of 10.79 Hz. A more plausible explanation for the exclusive significant increase in power at 10 Hz and 11 Hz may thus be the entrainment of spontaneous alpha activity, as opposed to steady-state responses.

Additional Interactions and Limitations

Promotion of 10 Hz and 11 Hz power was not limited to the targeted visual cortex. The significant increase in alpha power in the middle scalp regions suggests the stimulus was successful at propagating the effect and entraining neighbouring cortices. Nevertheless, it must be considered what hindered the entrainment of 100% of the recorded scalp regions. It could be postulated that entrainment was not as globally powerful as expected due to the nature of the checkerboard stimulus. To create an overall 10 Hz stimulation effect, each square constructing the board only flickers at 5 Hz, or 5.5 Hz in the 11 Hz entrainment condition. It is

possible that additional entrainment at lower frequencies, 3.5 Hz to 7 Hz, may have depreciated the strength of the alpha entrainment.

When visually inspecting the shape of the bell-shape alpha frequency graphs, it becomes apparent that the peaks in each condition are not in line with the frequency stimulated. The majority of the peaks lie around the 10 Hz mark. Although entrainment is believed to be frequency specific, it has been documented that frequencies in the EEG spectrum may increase oscillatory alpha activity at 10 Hz. The cumulative power increase resulting from alpha stimulations across the alpha band may have resulted in a significant increase at 10 Hz, as opposed to entrainment alone.

Conclusion

The present study revealed four minutes of visual stimulation in the alpha band is sufficient to significantly increase oscillatory power at 10 Hz and 11 Hz. Due to the spread of the activity throughout the cortex, this increase is believed to be through the process of entrainment. Further investigation into the type of stimulus, and activated sources must be completed.

CHAPTER 4

Visual and Auditory Alpha Stimulation reduces Pain Perception

This Chapter is based on a manuscript that is being prepared for submission to Plos One.

1. Abstract

Alpha power is believed to have an inverse relationship with the perception of pain. Increasing alpha power through an external stimulus may therefore induce an analgesic effect. Here, we attempt to modulate the perception of a moderately painful acute laser stimulus by separately entraining three frequencies across the alpha band: 8Hz, 10Hz and 12Hz. Visual and auditory alpha stimulation was applied separately to two groups of participants at the three alpha frequencies and a control frequency. We collected verbal pain ratings of laser stimuli from participants following ten minutes of flashing LED goggle stimulation and ten minutes of binaural beat stimulation across the alpha range. Alterations in sleepiness, anxiety and negative moods were recorded following each auditory or visual alpha stimulation trial. A significant reduction in pain ratings was found after both the visual and the auditory alpha stimulation across all three frequencies compared to the control condition. In the visual group, a significantly larger reduction was recorded following the 10Hz stimulation than succeeding the 8Hz and 12Hz conditions. The present study suggests a short presentation of auditory and visual stimuli, oscillating in the alpha range, have an analgesic effect on acute laser pain, with the largest effect following the 10Hz visual stimulation. Pain reductions following alpha stimulation are independent of sleepiness, anxiety and negative moods.

2. Introduction

Berger was the first to observe that power in the alpha band (7-14Hz) is reduced when participants open their eyes (Berger 1929). This phenomenon, referred to as alpha blocking, can also be observed during the perception of acute pain (Chang, Arendt-Nielsen et al. 2002, Chang, Arendt-Nielsen et al. 2003). Chang and colleagues demonstrated that a reduction in absolute alpha-1 power, which they defined as 8.0-10.8 Hz, is negatively correlated with subjective pain ratings (Chang, Arendt-Nielsen et al. 2003). Several electroencephalogram (EEG) studies revealed acute pain relief is associated with an increase in alpha power and a decrease in beta activity (20-25Hz), predominantly across posterior and temporal regions of the scalp (Chang, Arendt-Nielsen et al. 2002, Kakigi, Nakata et al. 2005, Saithong, Poolpoem et al. 2012).

Early work by Trifiletti et al, observing that high alpha was associated with intense analgesia, alluded to the idea that this relationship may work both ways (Trifiletti 1984). The concurrent presence of high alpha power during analgesia could indicate there is a causal relationship. Alpha EEG rhythms are believed to arise from the thalamus, and subsequently transmitted through thalamo-cortical tracts to the cortex. Alpha rhythms can hence be influenced via inputs to the thalamus, synchronising or desynchronizing alpha oscillations (Schmidt, Gottwald et al. 1985). Recent neurofeedback studies have developed this idea and confirmed brain training to increase alpha power can lead to a long-term reduction in chronic pain (Jensen, Gertz et al. 2013). The main disadvantage of neurofeedback is that it takes concentration and often weeks of training to be effective and thus is currently ineffective for acute pain (Kayiran, Dursun et al. 2010).

Visual and auditory entrainment enables almost immediate increases in cortical alpha power through an external pulse with a consistent frequency oscillating in the alpha range (Frederick, Timmermann et al. 2005, Spaak, de Lange et al. 2014). Entrainment occurs when other regions of the brain fall into lockstep with the stimulated cortex, eliciting a broader increase in alpha power (Halbleib, Gratkowski

et al. 2012, Thut, Miniussi et al. 2012, de Graaf, Gross et al. 2013, Spaak, de Lange et al. 2014).

While visual alpha entrainment primarily affects the primary visual cortex with the strongest resonance at 10Hz (Herrmann 2001, de Graaf, Gross et al. 2013), literature suggests that modulations in cortical activity are widely elicited throughout the cortex (Timmermann, Lubar et al. 1999, de Graaf, Gross et al. 2013). Although resting EEG records a maximal alpha amplitude over the occipital regions (Cantero, Atienza et al. 2002), entrainment through auditory binaural beats proves just as effective at increasing alpha power (Schwarz and Taylor 2005, Karino, Yumoto et al. 2006). Both visual and auditory alpha entrainments thus prove promising candidates for effortless acute pain relief.

In the current study, we entrained three different alpha frequencies (8Hz, 10Hz and 12Hz) using both auditory and visual stimulation separately, with the aim to decrease the perception of acute pain. We hypothesized that we would observe the largest reduction in pain ratings after the 10Hz alpha stimulation in both the visual and auditory studies, as this would be closest in frequency to the average individual alpha frequency (IAF) of the participants' age group. We additionally aimed to examine whether one type of entrainment (visual or auditory) would result in a larger pain rating reduction compared to the other.

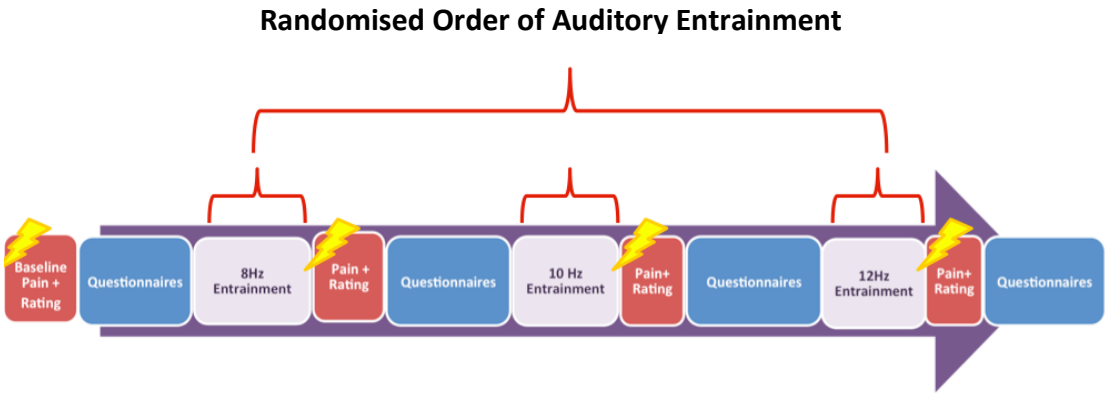
3. Methods

Participants were divided into two experimental groups: auditory and visual. Participants allocated to the auditory entrainment group were asked to visit Salford Royal NHS foundation Trust on two separate visits. The auditory group's visits consisted of a separate entrainment visit and a control visit, the order of which was randomised. A minimum of two weeks was left between the visits to allow the skin to fully recover in case of mild sensitivity outlasting the first visit. Due to time constraints, the visual entrainment study was condensed into one visit with fewer

overall pain sessions. A single control condition was included in the one visit. A diagram of the experimental procedure can be seen in Figures 4.1 and 4.2.

Experimental procedure:

Auditory Entrainment – Entrainment Visit



Auditory Entrainment – Control Visit

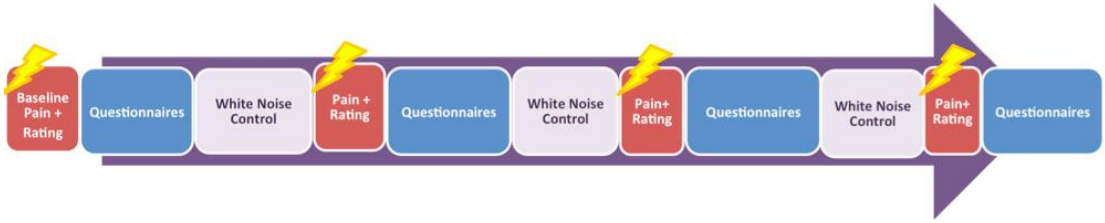


Figure 4.1 Procedure in the Auditory Entrainment Group. Participants in the auditory group attended two visits (control and entrainment), in a randomised order. Both visits were initiated with baseline questionnaires (Sleep Questionnaire, PCS, PHQ-9, PASS, POMS, STAI, KSS) and the rating of 30 laser pulses at 'level-7' pain. In the entrainment visit, participants were subjected to 10 minutes of auditory entrainment at 8Hz, 10Hz and 12Hz in a randomised order. Following entrainment, participants rated 30 pulses, and completed the POMS, KSS and STAI-state questionnaires. The control visit was identical to the entrainment visit, but the stimulus was 10 minutes of white noise, three times, instead of alpha entrainment.

Visual Entrainment – Single visit

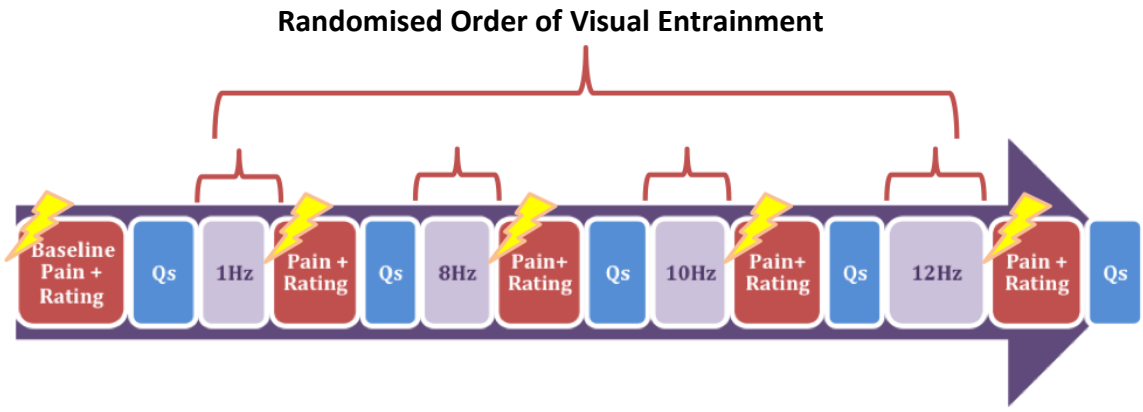


Figure 4.2 Procedure in the Visual Entrainment Group. A single visit was required for participants in the visual entrainment group. Participants completed baseline questionnaires (Sleep Questionnaire, PCS, PHQ-9, PASS, POMS, STAI, KSS) and 30 pulses of their 'level-7' pain. Participants were subjected to four visual (flashing LED goggle) entrainment sessions at 8Hz, 10Hz, 12Hz and 1Hz (control), each 10 minutes long, in a randomised order. Following entrainment, volunteers were subjected to 30 'level-7' pulses and were asked to rate these on a 0-10 numerical rating scale. Following each pain rating session, volunteers completed the KSS, STAI-state and POMS. **Qs** = Questionnaires

3.1.1. Ethics statement

All volunteers provided written, informed consent according to the International Conference on Harmonisation Good Clinical Practice guidelines, before participating in the study. The study obtained ethical approval from the NRES Committee North West – Liverpool Central (reference number 13/NW/0007).

3.1.2. Participants

Sixty-four healthy, (33 male, average age 24.65 ± 8.2 SD), self-reported right-handed volunteers were invited to Salford Royal NHS foundation Trust to participate in the study. All participants volunteered for the study after contacting the group through advertisements placed on the University of Manchester website and throughout Salford Royal NHS Foundation Trust. Volunteers in both groups were provided with a participant information sheet a minimum of 24 hours prior to

the first visit. Participants were provided with a verbal and written explanation of the laser pain applied during the study, without revealing the aims and objectives of the study. All volunteers were aged 18 years or older. Participants self-reported themselves as free from chronic pain, psychiatric illnesses (e.g. major depression, bipolar disorder, schizophrenia), ischemic heart disease, uncontrolled high blood pressure, peripheral vascular disease, chronic skin disease (e.g. eczema, psoriasis), hypertension not controlled by medication and free from a history, or family history of epilepsy. After providing written and verbal consent, volunteers were randomly assigned to either the auditory or the visual entrainment group. 32 Volunteers were allocated to the auditory entrainment group (16 Male, mean age 23.25 ± 7.9 SD) and 32 to the visual entrainment group (17 Male, mean age 25.82 ± 8.6 SD).

3.1.3. Pre-experimental Psychophysics procedure

It has been demonstrated that a contactless activation of nociceptors related to Aδ and C fibres can be achieved through the use of a brief CO_2 laser stimulus (Meyer, Walker et al. 1976). In this study, the pain stimulus consisted of a CO_2 laser stimulus of 150ms duration and a beam diameter of 15mm. This laser was applied to the dorsal surface of the volunteers' right forearm, firing once every 10 seconds. The laser beam was moved to a new location after every pulse to avoid sensitisation, habituation or damage to the skin. It was obligatory for participants to wear a pair of safety goggles whenever the laser was in use.

Each visit was initiated with the calculation of the participants' moderately painful level with the aid of a 0-10 numeric rating scale. Level 0 on the scale was marked as 'no sensation', level 4 represented the pain threshold, and level 10 was marked as the maximum amount of pain they believed they could tolerate. Participants were told to regard the sensation halfway between pain threshold and their tolerance level as 'moderately painful', identified as the number 7 on the pain scale. A ramping procedure with increasingly powerful laser stimuli was initiated, during which the participants were asked to verbally rate each pulse until their level 7 was attained. This entire procedure was repeated three times. Ratings of the laser

intensity levels were then tested by repeating a series of laser pulses at the volunteers' predetermined level 7. The laser voltage was readjusted if a level 7 was not consistently attained.

3.1.4. Pre-experimental Questionnaires

After determining volunteers' level 7 on the 0-10 numeric rating scale, participants were asked to complete a set of behavioural questionnaires previously demonstrated to correlate with changes in acute laser pain (Brown, Seymour et al. 2008, Morton, Watson et al. 2009) or believed to be modulated by changes in alpha power (Melzack and Perry 1975, Ossebaard 2000). These consisted of the Profile of Mood States (POMS), the State-Trait Anxiety Inventory (STAI), the Karolinska Sleepiness Scale (KSS), Participant Sleep Questionnaire, Pain Catastrophizing Scale (PCS), Patient Health Questionnaire - 9 (PHQ-9) and the Pain Anxiety Symptoms Scale (PASS). The Participant Sleep Questionnaire, PCS, PHQ-9 and PASS were used to determine the volunteers' quality of sleep, degree of pain related catastrophic thinking, depression and pain specific fear and anxiety respectively. These were given once at the beginning of each visit. The POMS, STAI – state, and KSS were repeated during the experiment after each of the pain assessment trials and are explained in more detail below. As the time taken to complete the questionnaires varied between individuals, the POMS, STAI – state and KSS were presented to the participants only once they had completed rating all 30 of the heat laser pulses. This was done to ensure each participant would receive the first and last laser pulse following an equal amount of time post alpha entrainment. Participants were hence asked to relate the POMS, STAI and KSS to the preceding alpha entrainment session, as opposed to the pain assessment trial.

3.1.5. Profile of Mood States (POMS)

Nine items representing negative moods were taken from the Profile of Mood States (POMS; McNair et al., 1971) to determine participants' degree of emotional distress, as previously described (Sullivan, Rodgers et al. 2001, Brown, Seymour et al. 2008). The nine emotions were divided to represent three different mood

categories: (1) sadness (sad, discouraged, hopeless); (2) anger (angry, hostile, irritable); and (3) anxiety (anxious, tense, worried). Participants were asked to rate the intensity of each of the 9 adjectives on a 5-point Likert scale with 0 representing 'not at all' and 4 'very much', in relation to their current emotional state. A composite score of emotional distress was computed by taking the sum of all nine items on the mood scale. Participants received the POMS before the start of the experiment and after each of the pain assessment trials.

3.1.6. State-Trait Anxiety Inventory (STAI)

The STAI consists of 20 items assessing trait anxiety and 20 items assessing state anxiety. The state and trait assessments of the inventory were presented to the volunteers separately. The State anxiety inventory evaluates temporary nervousness, fear, discomfort etc. (i.e. the arousal of the autonomic nervous system), whereas the trait inventory measures prolonged feelings of stress, worry or discomfort. The items on both inventories are rated on a 4-point Likert scale (from "Almost Never" to "Almost Always"). A higher overall score indicates greater anxiety (Spielberger et al., 1983). Participants received the trait inventory once in each visit, at the start of the experiment. The state inventory was given after each of the pain stimulus assessment trials. The Inventory was used to assess whether visual or auditory alpha entrainment influenced participants' present state of anxiety, and if so, whether it correlated with changes in pain perception.

3.1.7. Karolinska Sleepiness Scale (KSS)

A 9-point KSS was used where 1=very alert, 3=alert, 5=neither alert nor sleepy, 7=sleepy (but not fighting sleep) and 9=very sleepy (fighting sleep)(Akerstedt and Gillberg 1990). Participants were asked to rate the scale once, preceding the experiment and once after each pain assessment trial. This was done to control for any changes in the volunteers' alertness, and subsequent potential influence on pain perception.

3.1.8. Pre-experimental trial

Participants were asked to rate 30 pulses on the pain rating scale, at 10 second intervals, of their predetermined 'moderately painful' level 7, in order to document their average baseline pain ratings.

3.1.9. Auditory entrainment

Volunteers allocated to the auditory stimulation group attended two visits in a randomised order, one control and one entrainment visit. During the entrainment visit, volunteers were subjected to 10 minutes of auditory entrainment at 8Hz, 10Hz and 12Hz in a randomised order. Binaural Beats were employed to enable auditory alpha entrainment. The hearing range for a healthy adult is between 20Hz-20,000Hz, thus frequencies at 8Hz, 10Hz and 12Hz are not audible to the human ear. Binaural beats are produced when two tones close in frequency generate a beat frequency equal to the difference in frequency of the two tones (Wahbeh, Calabrese et al. 2007). For example, volunteers in the present study listened simultaneously to 445Hz played in one earphone, and 455Hz played in the other, producing a binaural beat frequency of 10Hz. Binaural beats are believed to originate in the brainstem's superior olivary nucleus, where the contralateral auditory input is integrated (Oster 1973).

Binaural beats were produced using BrainWave Generator software version 3.1.12 (http://www.bwgen.com/) as used by Goodin and colleagues (Goodin, Ciorciari et al. 2012). As binaural beats are known to be entrained most readily with a carrier frequency ranging from 300Hz to 600 Hz (Reedijk, Bolders et al. 2013) with the greatest effect between 450-500Hz (Perrott and Nelson 1969, Oster 1973), all entrainment sessions utilised a 450Hz carrier frequency. Presenting 446Hz and 454Hz, 445Hz and 455Hz, 444Hz and 456Hz tones in the left and right ears created 8Hz, 10Hz and 12Hz binaural beats respectively. All frequencies were presented to participants at 70 dB SPL, as previously done by Stevens and colleagues (Stevens, Haga et al. 2003).

After each randomised 10 minute auditory entrainment session, participants were asked to rate 30 painful heat laser pulses, and subsequently requested to complete the POMS, KSS and STAI-state, in relation to the entrainment session. The control visit was identical to the entrainment visit, except that the volunteers listened to white noise for 10 minutes, three times, instead of 8Hz, 10Hz and 12Hz binaural beats.

3.1.10. Visual entrainment

Participants in the visual entrainment group were subjected to four randomised visual entrainment sessions at 8Hz, 10Hz, 12Hz and 1Hz (control), each lasting a total of 10 minutes. The visual stimulus consisted of a pair of in-house made flashing LED goggles. Volunteers kept their eyes closed throughout the stimulation, as this is just as effective, but more pleasant for the volunteer (Collura and Siever 2009, Spaak, de Lange et al. 2014). After each one of the visual entrainment sessions, volunteers were subjected to 30 moderately painful laser pulses at their predetermined level 7, and asked to rate these on the 0-10 numerical rating scale. Following all four pain rating trials, volunteers were asked to complete the KSS, STAI-state and POMS. Instructions included attempting to relate the questionnaires to the preceding entrainment session, rather than post pain rating trial.

On completion of the study volunteers in both the auditory and visual entrainment groups were asked if they were able to differentiate between the 8Hz, 10Hz and 12Hz frequency entrainment. None claimed to be able to do so.

3.1.11. Behavioural Data Analysis

Statistical analysis was performed using SPSS version 20. A *p* value of less than 0.05 was considered significant. The average pain rating for each subject and trial was calculated. We applied a mixed linear model to pain ratings of the 8Hz, 10Hz, 12Hz and control condition of both groups to assess the size of the change in pain ratings compared to control. This model took into account the baseline pain ratings as a covariate and the frequency entrained, the order of the entrainment session, and for the auditory group, the order of the control/ entrainment visit as a factor. Using

each condition as a reference category, the model was refitted with a Bonferroni correction to assess the significant differences. The same model was applied separately to the POMS, STAI and KSS scores, taking into account the same respective covariates and factors. The baseline scores of the PHQ-9, PCS, Participant Sleep Questionnaire and PASS were correlated to the changes in pain ratings from baseline in the 8Hz, 10Hz, 12Hz and control condition.

4. Result

The largest reduction in pain ratings from baseline (level -7) could be observed after the 10Hz entrainment session in both the auditory and visual groups, followed by the 8Hz then the 12Hz condition. Visual 10Hz entrainment resulted in a larger pain reduction than the 10Hz auditory entrainment. There were no significant changes or correlations observed in the questionnaire scores.

4.1.1. Pain Ratings – Auditory Entrainment Group

Taking into account baseline (level 7) pain ratings and covariates, the mixed linear model calculated that pain ratings succeeding the 8Hz, 10Hz and 12Hz entrainment conditions were all significantly different from all three control conditions ($t(31) = 4.90$, $p<0.001$; $t(31) = 5.61$, $p<0.001$; $t(31) = 4.85$, $p<0.001$, respectively). Adjusted mean pain ratings following entrainment were respectively 0.51(SE 0.10), 0.58 (SE 0.10) and 0.5 (SE 0.10) points lower than the control on the numeric ratings scale (Figure 4.3). No significant difference was detected between the three auditory entrainment conditions when refitting the model with a Bonferroni correction.

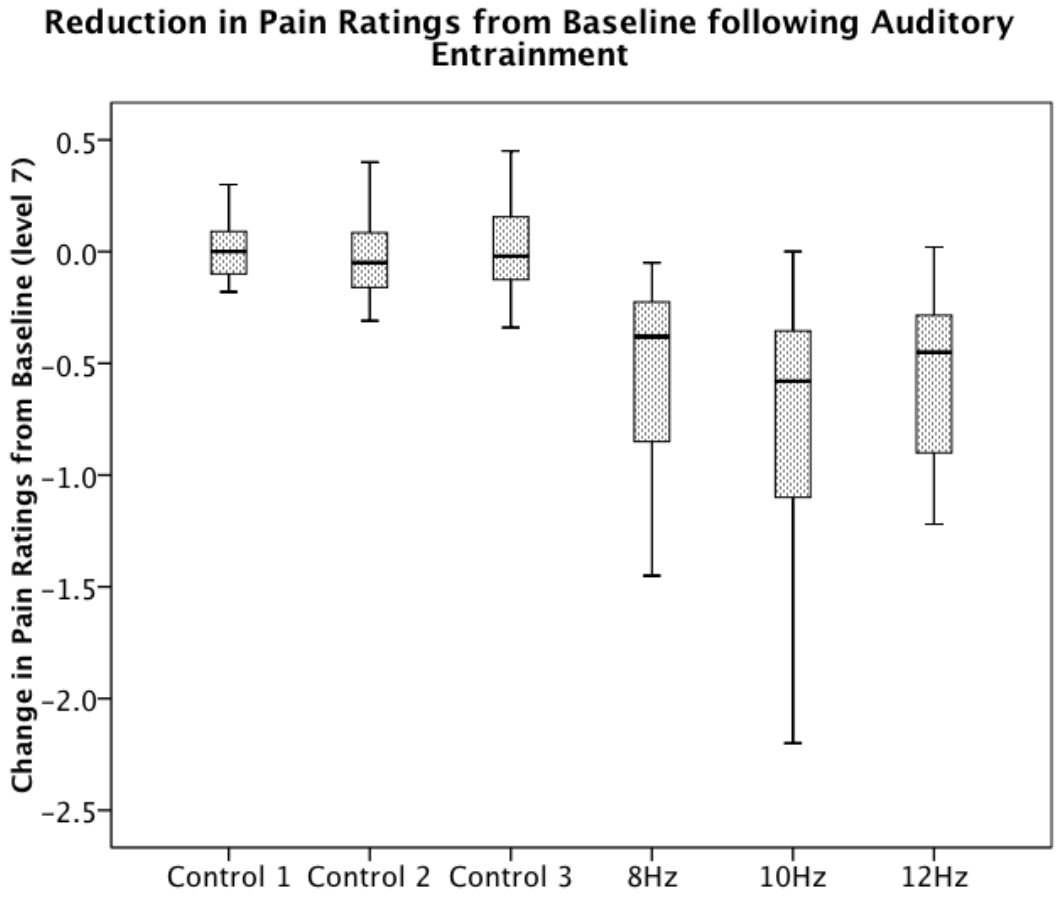


Figure 4.3 Auditory Group - Change in Pain Ratings Graph displaying the average change in pain ratings from baseline (level 7), across subjects in each condition. Applying the mixed linear model revealed that the largest change from control was observed succeeding 10Hz entrainment. Model covariates are not included in the graph.

4.1.2. Pain Ratings – Visual Entrainment Group

The mixed linear model established that, accounting for covariates and factors, the pain ratings in the entrainment conditions (8Hz, 10Hz and 12Hz) were all significantly different from control ($t(31)=2.28$, $p<0.01$; $t(31)=5.32$, $p<0.001$; $t(31)=2.59$, $p<0.01$ respectively). The model-corrected pain ratings of the 8Hz, 10Hz and 12Hz conditions were on average 0.6, 1.1 and 0.3 points lower on the pain rating scale than the control, respectively. Additionally, when refitting the model, pain ratings were significantly different in the 10Hz condition compare to the 8Hz ($t(31)=2.22$ $p<0.01$) and 12Hz ($t(31)=4.04$, $p<0.001$) condition. The 8Hz and the 12Hz conditions did not differ from each other (p=0.287) (Figure 4.4).

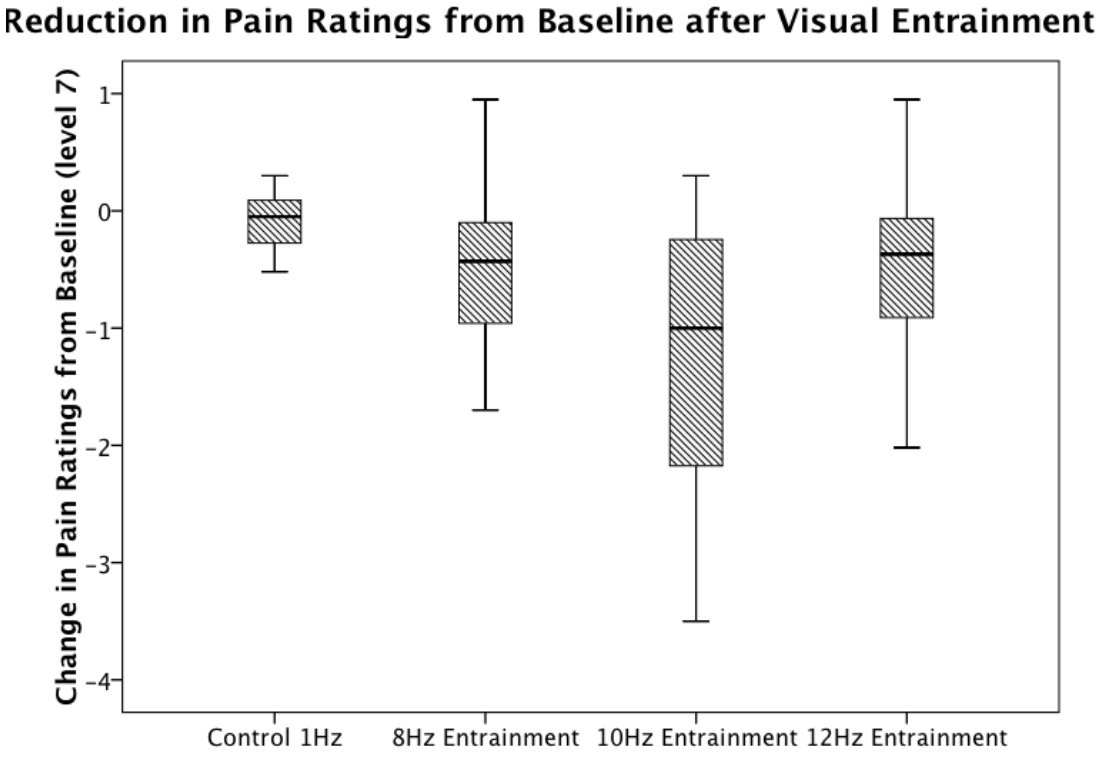


Figure 4.4 Visual Group - Change in Pain Ratings. Graph displaying the change in ratings of pain from initial baseline (level 7) ratings. Applying the mixed linear model revealed that the largest change from control was observed succeeding 10Hz entrainment. Model covariates are not included in the graph.

4.1.3. Questionnaire Results

A mixed linear model was applied to the KSS, POMS and STAI–State scores of the 8Hz, 10Hz, and 12Hz condition comparing them to the control scores. The model revealed no significant score changes compared to the control condition in both the visual and auditory groups. The pre-experimental questionnaires showed no significant correlation to the change in pain ratings from baseline across all three conditions, in both groups.

5. Discussion

The present study investigated the effect of visual and auditory entrainment (at 8Hz, 10Hz and 12Hz) on the perception of a moderately painful stimulus. In both the auditory and visual groups, all three entrainment conditions resulted in pain ratings significantly lower than control. As hypothesized, we found the largest reduction in pain ratings after the 10Hz entrainment, reducing by an average of 1.1 points on the pain scale after the visual and 0.58 points after the auditory entrainment. In both groups the 8Hz condition was more effective than the 12Hz. However, the visual group demonstrated a reduction after the 10Hz entrainment that was significantly larger than the 8Hz and 12Hz condition, which was not the case in the auditory group. Overall, the visual group demonstrated much larger reductions in pain ratings in the 8Hz and 10Hz conditions (0.6, 1.1) compared to the auditory group (0.51, 0.58). This was not the case in the 12Hz condition, where the reduction in the visual and auditory group was 0.3 and 0.5 respectively.

We did not see any significant correlations between the pre-experimental questionnaires and the change in pain ratings. Additionally, the STAI, POMS and KSS scores did not display significant differences between the three conditions. This implies that visual and auditory alpha entrainment alone could be enough to reduce pain ratings. Alpha entrainment may affect cortical processes independently of the measures considered in this study.

Entrainment Conditions

Across both modalities, the largest reduction followed the 10Hz entrainment (although inter-condition differences where only significant in visual entrainment). It could be postulated that the largest reduction in pain occurs when the frequency closest to the volunteers' individual alpha frequency (IAF) is increased in power. It has been previously suggested that entrainment can be more effective if the driving stimulus is close in frequency to the IAF, rather than using the midpoint of the traditional alpha band (Frederick, Timmermann et al. 2005). The IAF is determined by the membrane properties of the thalamic neurons projecting to the cortex, and

lies at around the 10Hz mark in the healthy adult (Steriade, Gloor et al. 1990, Klimesch, Doppelmayr et al. 1997, Klimesch 1999), but can vary marginally between individuals (Klimesch 1999). The IAF is known to be lower in children, and decreases again with normal aging (Kopruner, Pfurtscheller et al. 1984, Klimesch 1997, Posthuma, Neale et al. 2001). As the population sampled for this study is relatively young in age, the IAF of a subset might be slightly lower. This would explain why 8Hz had the second largest effect on pain reduction in both the auditory and visual groups. If the ability to reduce acute pain perception is related to the IAF, repeating the current experiment in an elderly population might result in the largest pain reduction occurring after the 8Hz entrainment. Identifying participants' IAF and entraining that frequency could result in more robust entrainment, and consequentially lower acute pain ratings. Further work acquiring electrophysiological data would be able to tell whether an increase in alpha power closest in frequency to the individual's IAF or an increase in 10Hz, unrelated to IAF, results in the largest alpha analgesia.

We cannot be certain that 10Hz or IAF specifically produce the largest reduction of pain perception. Frequencies in the entire alpha band may have equivalent analgesic properties, but may simply not entrain as well as 10Hz and hence remain undetected. Previous work on visual entrainment has shown a maximal entrainment at 10Hz when assessing visual performance (Romei, Gross et al. 2010, de Graaf, Gross et al. 2013). Additionally, it has been shown that frequencies other than 10Hz, as well as non-rhythmic external stimulations can result in increased cortical oscillations at 10Hz (VanRullen and Macdonald 2012). Reductions in pain ratings could thus be related to magnitude of alpha rhythms, as opposed to frequency.

Visual vs. Auditory

As visual and auditory entrainment paradigms were not equivalent, changes in pain ratings have not been statistically compared. Nevertheless, visual entrainment resulted in a numerically larger decrease in pain ratings in the 8Hz and 10Hz conditions than the auditory entrainment. A study by Frederick et al., 1999 found

auditory stimulation resulted in greater entrainment at the vertex than visual stimulation (Frederick, Lubar et al. 1999). If this concept were applied to the current results, it would imply that even though entrainment is greater in the auditory group, visual entrainment is more effective at reducing pain. It must be noted that the study conducted by Frederick et al. compared entrainment at 18.5Hz, which may not be representative of alpha entrainment. Additionally, increased alpha power at the vertex may be irrelevant to the processing of pain perception.

Binaural beats have demonstrated widespread entrainment across frontal and central sites of the scalp (Schwarz and Taylor 2005). Visual entrainment primarily affects the primary visual cortex. Nonetheless, research has shown that, perhaps due to its size, entrainment in the visual cortex can elicit changes in cortical activity widely distributed throughout the cortex (Timmermann, Lubar et al. 1999). Furthermore, the visual cortex has the strongest resonance at 10Hz (Herrmann 2001), where the largest analgesic effect in the visual group was observed. Although alpha power is most commonly generated in thalamocortical feedback loops of excitatory and inhibitory nerve cells (Steriade, Gloor et al. 1990, Lopes da Silva 1991), in the visual cortex, alpha power is also believed to be generated by cortico-cortical networks (Lopes Da Silva and Storm Van Leeuwen 1977, Steriade, Gloor et al. 1990, Bollimunta, Chen et al. 2008, Spaak, Bonnefond et al. 2012), that are modulated by attention (Yamagishi, Callan et al. 2003, Bollimunta, Mo et al. 2011). It could be postulated that this can contribute to a global analgesic entrainment.

Although auditory stimuli have been shown to entrain better than visual, one of the reasons they may not be as effective for reducing pain is that entrainment does not last as long. Wahbeh and colleagues saw no lasting effects after 30mins of 7Hz auditory alpha entrainment (Wahbeh, Calabrese et al. 2007). The 7Hz entrainment was however conducted with 133 and 140 pure tone carrier frequencies. Numerous studies have demonstrated that carrier frequencies between 450-500 are much

more effective than those around 100Hz (Perrott and Nelson 1969, Oster 1973). Nevertheless, this study questions the reliability of auditory entrainment.

Previous studies have attempted to use visual-audio entrainment, however, Frederick et al. 1999 observed that combining both modalities did not result in a greater increase than applying them in isolation (Frederick, Lubar et al. 1999). This may be because simultaneous stimulation of both the visual and auditory cortex may interfere rather than reinforce the entrainment effect.

A potential contribution to the larger effect in the visual study could be owed to the effect of distraction. Distraction can have a significant influence on the perception of pain (Boyle, El-Deredy et al. 2008). The bright flashing LED lights of the visual entrainment are much more distracting than the soothing sound of the binaural beats. Even though by the time the volunteers rate the pain, the entrainment session is over, there might be lasting effects that disorientate the volunteers, and are hence unable to fully attend to the pain stimulus. However, it seems unlikely that the effect of distraction can produce significant differences between different alpha stimulation frequencies, as found in this study. Furthermore, it is presently not possible to differentiate between the effects of alpha entrainment and distraction. They may insofar be separate mechanisms, but may also interact; increasing alpha power may increase distractibility.

Emotional Influences of Visual and Auditory Entrainment

It has previously been observed that visual entrainment at 10Hz allows participants to enter a hypnogogic state (Richardson and McAndrew 1990). Being halfway between fully awake and asleep, it is often percieved as being a dream-like state. Although the present study saw no change in sleepiness, entering a hypnogogic state might not have been detected by the sleepiness scale (KSS), due to its mild hallucinations. It could be postulated that this is a contributing factor to the larger decrease in pain ratings after the visual entrainment.

A study by Lane et al states that auditory entrainment, by contrast, induces increased cognitive vigilance. However numerous methodological flaws can be identified in the study's paradigm (Lane, Kasian et al. 1998). The consensus from the preponderance of work suggests high alpha power is incompatible with states of high arousal and hence, may interfere with the perception of pain.

Anxiety, Sleepiness and Negative Moods

STAI, POMS and KSS scores did not change after visual or auditory entrainment. This finding suggests that alpha influences pain perception independently of anxiety, wakefulness and negative moods. Ossebaard et al., 2000 demonstrated that after 35 minutes of visual alpha entrainment, a significant reduction in STAI scores could be observed (Ossebaard 2000). In the present study, there was no evidence to suggest any of the three conditions influenced the volunteer's anxiety scores. However, in the study conducted by Ossebaard et al., the initial anxiety scores were on average 42.16, and reduced significantly to 33.4. The STAI scores recorded at the beginning of the present study were on average 28.5, and as the STAI ranges from 20-80 points, it suggests volunteers were already feeling relatively at-ease at the start of the experiment. Hence, a decrease in STAI would have been difficult to detect. Additionally, Ossebaard and colleagues entrained their volunteers for 35 minutes. A larger reduction in anxiety might have been observed if the entrainment sessions were longer and a more anxious population had been sampled. Whether this reduction in anxiety would have amplified the reduction in pain ratings is worth further study.

Melzack and Perry (1975) claimed that increases in alpha power could only influence the perception of pain when coupled with relaxation and suggestion (Melzack and Perry 1975). The present study demonstrates that even without any changes in negative moods, anxiety or sleepiness, alpha power alone has the ability to alter the perception of acute pain. Reducing negative moods may amplify the analgesic effects of alpha entrainment due to ensuing increased relaxation, rather than the other way round. A recent study by Spaak et al. (2014) supports this notion by demonstrating alpha rhythms are not an epiphenomenon of attentional

processes, instead, they influence perception independently (Spaak, de Lange et al. 2014).

Limitations of the Design

The STAI, POMS and KSS questionnaires were always provided following the pain trials to ensure the first and last pain stimulus was delivered at identical post-entrainment times. This resulted in the participants starting the questionnaires 5 minutes post-entrainment. Although participants were asked to relate the questionnaires to the entrainment session, the entrainment effect would have diminished, and potentially contaminated by the intersecting pain trial. Furthermore, the average time to complete all three questionnaires varied significantly between volunteers, and consequentially, post-entrainment to questionnaire time window also varied accordingly.

A further limitation was the obvious dissimilarity of the control condition in the visual group. Although undisclosed, the difference in flickering velocity potentially could have resulted in an identification of the control condition by participants, consequentially introducing a placebo effect. However, this is unlikely as participants were not aware of the aims of the experiment and would not have had any particular expectations regarding the analgesic properties of the different experimental conditions.

Very little work has been completed investigating the similarity between naturally occurring alpha and entrained alpha rhythms. Whether entrained alpha accurately mimics cortical rhythms is still unknown. It is presumed they rely on the same mechanisms as entrained activity influences subsequent spontaneous cortically generated activity, outlasting the stimulation period. Literature suggests that changes in neural activity during rhythmic stimulations are similar to the neural changes observed when the stimulation finishes (Halbleib, Gratkowski et al. 2012). Spaak et al reported visual 10Hz entrainment outlasting the stimulation by multiple cycles. This suggests a 10Hz flicker induced alpha oscillations intrinsic to the cortex (Spaak, de Lange et al. 2014).

Concluding Remarks

The present study provides new evidence that visual and auditory entrainment in the alpha range can influence the perception of acute pain independently of arousal and negative emotional influences. The results reveal that 10Hz stimulation has a significantly larger analgesic effect than 8Hz and 12Hz following the visual entrainment, and non-significantly following the auditory entrainment. Overall, visual entrainment produced a larger effect than auditory entrainment in the mid- and lower alpha frequencies. This provides further evidence that external stimulation can modulate pain perception and requires further study to ascertain its relevance to clinical pain states.

CHAPTER 5

Alpha Analgesia: modulating Pain Perception through Binaural Beat Entrainment

This Chapter is based on a manuscript that is being prepared for submission for peer review.

1. Abstract

The inverse relationship between the pre-stimulus alpha power and the subsequent pain perceived has been well documented in the literature. Increasing pre-stimulus alpha power may therefore have an analgesic effect on acute pain perception. Resting cortical alpha power can be increased through binaural beat neural oscillatory frequency entrainment.

In the present study, 32 volunteers entrained alpha at 8Hz, 10Hz and 12Hz using binaural beats, prior to rating pulses of moderately painful acute laser stimuli. A significant increase in alpha power from control was found following the 10Hz and 12Hz binaural beat stimulations. Verbal pain ratings were reduced significantly after binaural beat entrainment at all frequencies, compared to control. The P2 peaks of the EEG recorded laser-evoked potentials, revealed a significant reduction, with the peaks following the 10Hz and 12Hz entrainment additionally being significantly smaller than following the 8Hz entrainment. The N2 peak showed a significant reduction after the 10Hz entrainment, but not following 8Hz and 12Hz.

Source analysis of alpha and P2 peak results suggests a modulatory role of frontal sources, believed to reflect alterations in ongoing brain states and psychological influences that co-occur with the electrophysiological pain response. Auditory entrainment of the alpha rhythm thus appears to exhibit a robust analgesic effect on acute pain perception, and electrophysiologically recorded laser-evoked responses.

2. Introduction

A significant body of evidence suggest cortical alpha power plays a pivotal role in the cognitive aspects of pain processing. Alpha activity is believed to represent important aspects of cognitive processing, including top-down regulation of sensory inputs (Klimesch, Sauseng et al. 2007). Consistently, reported decreases in alpha power have been coupled with painful stimuli (Babiloni, Brancucci et al. 2003, Babiloni, Brancucci et al. 2006, Franciotti, Ciancetta et al. 2009, Nir, Sinai et al. 2012). Also, resting alpha activity preceding a noxious stimulus may potentially be functionally relevant to the cortical processing of pain. There appears to be an inverse relationship between the pre-stimulus alpha power and the subsequent pain perceived (Babiloni, Brancucci et al. 2006, Nir, Sinai et al. 2012). It has previously been suggested that resting-state or anticipatory alpha power influences noxious and non-noxious stimulus processing (Basar, Schurmann et al. 1997, Damoiseaux, Rombouts et al. 2006, De Luca, Beckmann et al. 2006). Increasing the pre-stimulus alpha power may therefore have an analgesic effect on pain perception.

Virtually immediate increases in cortical alpha activity can be achieved through cortical auditory entrainment. As frequencies in the alpha range are inaudible to the human ear, binaural beats are employed. Binaural beats occur when two sinusoidal waves close in frequency are played separately to each ear, generating a pulse at a frequency equal to the difference in frequency of the two waves (Oster 1973, Wahbeh, Calabrese et al. 2007). For example, listening simultaneously to a 400Hz tone played through one earphone, and 410Hz in the other, would result in a beat frequency of 10Hz. Binaural beat stimulation is defined as entrainment when further regions of the brain fall into lockstep with the targeted cortex (Halbleib, Gratkowski et al. 2012, Thut, Miniussi et al. 2012, de Graaf, Gross et al. 2013, Spaak, de Lange et al. 2014).

The present study thus investigates whether increasing cortical alpha activity by means of binaural beats, before an acute noxious stimulus, alters the volunteers'

perception of pain. A range of low to high alpha (8Hz, 10Hz and 12Hz) entrainment was implemented to explore discrepancies in the range. Electroencephalography (EEG) is used to record on-going alpha power during the entrainment, as well as event-related potentials (ERPs) following acute laser pain. We hypothesised that binaural beat entrainment at 10Hz would result in the largest reduction in behavioural pain ratings, accompanied by a mirrored reduction in ERPs. We predicted the source analysis to identify the involvement of pain matrix structures when comparing entrainment to control conditions.

3. Methods

3.1.1. Ethics statement

All volunteers provided written, informed consent according to the International Conference on Harmonisation Good Clinical Practice guidelines, before participating in the study. The study obtained ethical approval from the NRES Committee North West – Liverpool Central (reference number 13/NW/0007).

3.1.2. Participants

Thirty-two healthy volunteers (16 Male, mean age 23.25 ± 7.9 SD) were recruited for the study through advertisements placed on the University of Manchester website and throughout Salford Royal NHS Foundation Trust. In order to participate in the study, volunteers needed to be 18 or older, right-handed and free from chronic pain, morbid psychiatric illness (e.g. major depression, schizophrenia, bipolar disorder), neurological illness, ischemic heart disease, uncontrolled high blood pressure, peripheral vascular disease, chronic skin disease (e.g. eczema, psoriasis), hypertension not controlled by medication and a history, or family history of epilepsy.

3.1.3. The Pain Stimulus

The pain stimulus consisted of a CO_2 heat laser, applied to the dorsal surface of the participants' right forearm. Brief CO_2 laser stimuli are able to activate nociceptors

related to Aδ and C fibres without contact (Meyer, Walker et al. 1976). The laser stimulus lasted a total of 150ms, had a beam diameter of 15mm firing once every 10 seconds. The laser beam was relocated following every pulse to avoid habituation, sensitisation or skin damage. All participants were obliged to wear safety goggles whenever the laser was in use.

3.1.4. Pre-experimental Psychophysics procedure

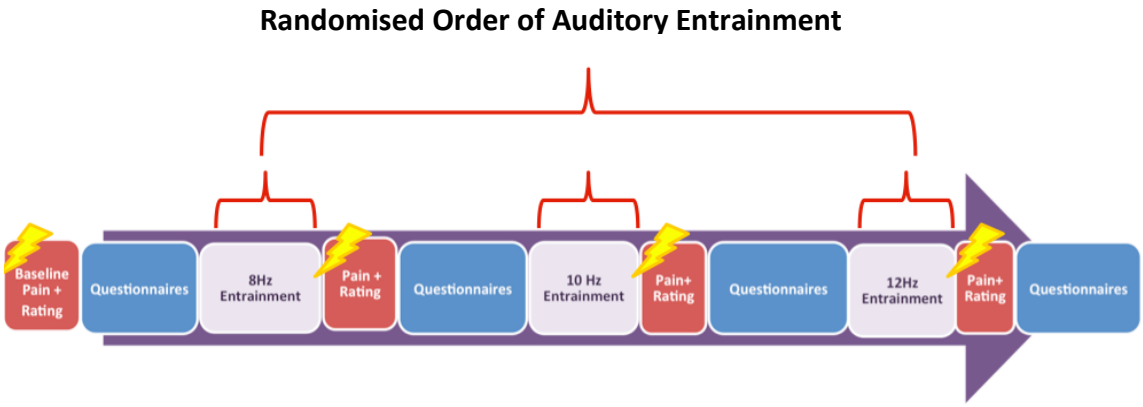
At the beginning of each visit, all participants underwent the psychophysics procedure to establish their 'moderate pain' level with the aid of a 0-10 numeric rating scale. The scale was labelled as 'no sensation' at point 0, 'Just Painful' at the level 4 pain-threshold and 10 was identified as the maximum amount of pain they believed they could tolerate. Level 7, half way between the pain threshold and tolerance was defined as 'moderately painful'. Participants were asked to rate laser stimuli with increasingly powerful pulses, ending once the level 7 was attained. This ramping procedure was completed three times. After completing a series of laser pulses at the volunteer's level 7, the voltage was adjusted where necessary to evoke a consistent moderately painful sensation across the right forearm.

3.1.5. Pre-experimental Questionnaires

Prior to the start of the experiment, volunteers were asked to complete a set of questionnaires. These were the Profile of Mood States (POMS), the State-Trait Anxiety Inventory (STAI), the Karolinska Sleepiness Scale (KSS), Participant Sleep Questionnaire, Pain Catastrophizing Scale (PCS), Patient Health Questionnaire - 9 (PHQ-9) and the Pain Anxiety Symptoms Scale (PASS). The Participant sleep questionnaire, STAI-trait, PCS, PHQ-9 and PASS were used to assess the volunteers' quality of sleep, trait anxiety, degree of pain related catastrophic thinking, depression and pain specific fear and anxiety respectively. These five questionnaires were provided once at the beginning of each visit. The POMS, STAI – state, and KSS were given once at the start and then repeated during the experiment after each of the pain rating trials. A nine-item 'negative moods' short form of the POMS was used, as had been done previously by (Sullivan, Rodgers et

al. 2001, Brown, Seymour et al. 2008). The short form POMS, the STAI–state and the KSS enabled the assessment of the participants' degree of emotional distress, present anxiety and wakefulness respectively.

Entrainment Visit:



Control Visit:

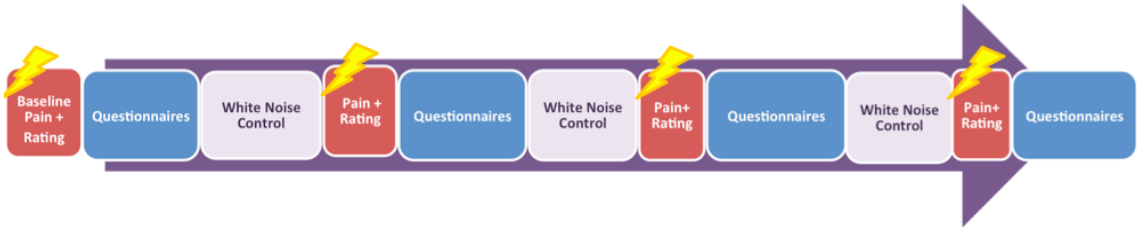


Figure 5.1 Experimental Paradigm of Auditory Binaural Beat Entrainment. Study participants attended two visits (control and entrainment), in a randomised order. Both visits were initiated with baseline questionnaires (Sleep Questionnaire, PCS, PHQ-9, PASS, POMS, STAI, KSS) and the rating of 30 laser pulses at 'level-7' pain. During the entrainment visit, participants were subjected to 10 minutes of auditory entrainment at 8Hz, 10Hz and 12Hz in a randomised order. Following each entrainment session, participants rated 30 pulses, and completed the POMS, KSS and STAI-state questionnaires. The control visit was identical to the entrainment visit, but the auditory stimulus consisted of 10 minutes of white noise, repeated three times, instead of alpha entrainment.

3.1.6. Pre-experimental trial

In order to record participants' baseline pain response, they were asked to rate 30 pulses at 10-second intervals of their predetermined level 7 (moderately painful).

3.1.7. Entrainment

Participants were asked to attend one control and one entrainment visit in a randomised order. Volunteers were exposed to 10 minutes of randomised auditory stimulation at 8Hz, 10Hz and 12Hz in the entrainment session, and three times 10 minutes of equal intensity white noise in the control session. In the entrainment visit binaural beats were employed as the human hearing range lies between 20Hz-20,000Hz, making frequencies in the alpha range inaudible to the human ear. Binaural beats are the results of two tones, close in frequency, generating a beat equal to the difference in frequency of the two tones (Wahbeh, Calabrese et al. 2007). As exemplified in the current study, listening simultaneously to 445Hz in one ear and 455Hz in the other produces a binaural beat of 10Hz.

BrainWave Generator software version 3.1.12 (http://www.bwgen.com/) as used by (Goodin, Ciorciari et al. 2012), was used to create the binaural beats played in this study. The entrainment of binaural beats most readily occurs with a carrier frequency ranging from 300Hz to 600 Hz (Reedijk, Bolders et al. 2013) with the greatest effect between 450-500Hz (Perrott and Nelson 1969, Oster 1973). Alpha entrainment in the present study utilised a 450Hz carrier frequency, playing 446Hz and 454Hz, 445Hz and 455Hz, 444Hz and 456Hz tones in the left and right ears created 8Hz, 10Hz and 12Hz binaural beats respectively. All tones were presented at 70 dB SPL to volunteers, previously done by Stevens and colleagues (Stevens, Haga et al. 2003).

Following each 10-minute binaural beat and white noise entrainment session, participants were requested to rate 30 heat laser pulses. In addition, participants were asked to complete the POMS, KSS and STAI-state, in relation to the preceding entrainment session (Figure 5.1).

3.1.8. EEG Recording

EEG was recorded during the entrainment, as well as during the pain sessions using 64 Ag/AgCl surface electrodes fixed in a cap according to the extended standard 10-

20 system (Brain Vision Acticap and BrainAmp, Brain Products GmBH, Germany). This included 4 electrodes measuring horizontal and vertical electro-oculograms (EOG) in order to isolate eye-movement and blink artefacts. All electrodes were referenced to the right mastoid electrode. Electrode AFz acted as the ground electrode in all recordings. A sampling rate of 500Hz was used and band-pass filters were set at DC – 100Hz. A 50Hz notch filter was applied to the recording to minimize electrical interference. EEG signals were recorded using BrainVision Recorder 1.10 (Brain Products GmBH, Germany).

3.2. EEG Data Analysis

3.2.1. Event-Related Potentials

EEG recordings were analysed using Brain Vision Analyzer 2.0. The analysis approach was consistent with previously published work (Brown, Seymour et al. 2008, Watson, Petrakis et al. 2009, Morton, Brown et al. 2010, Huneke, Brown et al. 2013). The recording was initially down-sampled to 125 Hz. Epochs around the ERP were segmented 1000ms prior to the laser stimulus (marked as 0ms), to 1500ms after the stimulus. A baseline correction was performed from -500ms to the stimulus at 0ms. Using the first 500ms and the last 100ms of the epoch, a DC Detrend was performed to remove linear from the data. An Independent Component Analysis with classic sphering was implemented with an Infomax (Gradient) Restricted Biased algorithm to remove horizontal and vertical eye movement artefacts. Data were split into 25 components, removing a median of 6 components, with a minimum of 0 and a maximum of 11 components removed. The epochs were then re-referenced to the common average of electrodes across the scalp for analysis. The laser-evoked potential was quantified using the N2 and P2 peak amplitudes. For each subject the negative apex between 200ms and 300ms was identified as N2, and the largest positive deflection in a period between 350ms and 500ms after the laser stimulation was identified as P2. Data were pooled around the nine electrodes surrounding the central electrode (Cz CPz, Cz, Fcz, CP1, CP2, C1, C2, FC2, FC1), where the N2-P2 peak topographies were maximal. Peak

pooled values (+/-10ms either side of the peak) were exported for each subject, across all conditions. A grand average was produced from the analysed LEP trials.

3.2.2. Spectral Analysis

Continuous alpha power analysis was performed on data recorded throughout entrainment, in Brain Vision Analyzer 2.0 consistent with (Huneke, Brown et al. 2013). The data was re-referenced to the average voltage over all electrodes (i.e. the common average). The data were filtered using a low cut off of 0.05Hz (12 dB/oct), a high cut off of 35 Hz (48 dB/oct). An Independent Components Analysis was performed with 25 components, eliminating significant artefacts such as muscle activity and eye-blinks. Data were then re-constructed from the residual components and segmented into 1-second epochs. Following a manual inspection, any epochs still containing artefacts were then discarded. A power spectrum was obtained applying Fast-Fourier transformation of the remaining data. A 10% Hanning window was applied to the data before the FFT. As the sampling rate was kept at the original 500Hz the subsequent frequency resolution was 0.5Hz. This provided values for the average power of each frequency band expressed in log units ($10*\log10(\mu V^2/Hz)$, a measure of frequency density, or activity. On this occasion, we looked at each entrainment frequency (8Hz, 10Hz and 12Hz) +/- 1Hz and compared the average power of that frequency band in the matching control condition.

3.2.3. Source Localisation Analysis

Cortical sources of N2 and P2 peaks were estimated independently on averaged data from each subject with low-resolution electromagnetic tomography (LORETA), using the LORETA-KEY software (Lantz, Michel et al. 1997). LORETA was used to contrast the N2 and P2 components of the control condition with the same components in the condition with the largest reduction in pain ratings. Additionally, in the entrainment condition with the largest analgesic effect, alpha power sources of the entrained frequency band were compared to alpha sources in the control condition. LORETA was used to estimate the spatially smoothest source compatible

with the recorded EEG activity across all scalp electrodes. Electrical activity was mapped onto 2394 voxels in a three-dimensional space with each voxel representing a potential activity zone.

3.2.4. Statistical Analysis

Statistical analysis was performed using SPSS version 20. A *p* value of less than 0.05 was considered significant. For the behavioural analysis a mixed linear model was implemented to assess the size of change in pain ratings of the 8Hz, 10Hz and 12Hz conditions compared to control. The model incorporated baseline pain ratings as a covariate and included the frequency entrained, order of entrainment sessions, and order of the control/ entrainment visit as factors. The model was refitted with a Bonferroni correction using each condition as a reference category in order to quantify the effect of each condition to that of the other two. The equivalent model was applied to the POMS, STAI and KSS scores, taking into account the same respective covariates and factors. Baseline PHQ-9, PCS, Participant Sleep Questionnaire and PASS scores were correlated to the change in pain ratings from baseline in the 8Hz, 10Hz, 12Hz and control condition.

A linear mixed model was applied separately to the N2 and P2 amplitudes (average values taken +/- 10ms of the peak from each subject) with baseline N2 and P2 amplitudes as a covariate and condition, session order and visit order as factors.

The power in each entrainment condition band (eg. 8Hz ±1Hz) was compared to the same frequency band in the control condition. The electrodes were divided into 9 scalp regions: Left Anterior (AF7, F9, F7, F5, F3) Central Anterior (Fp1, Fpz, Fp2, AF3, AFz, AF4, F1, Fz, F2), Right Anterior (AF8, F4, F6, F8, F10), Left Middle (FT7, FC5, FC3, T7, C5, C3, TP7, CP5, CP3), Central Middle (FC1, FCz, FC2, C1, Cz, C2, CP1, CPz, CP2), Right Middle (FC3, FC6, FT10, C4, C6, T8, CP4, CP6, TP8), Left Posterior (P7, P5, P3, PO7), Central Posterior (P1, Pz, P2, PO3, POz, PO4, O1, Oz, O2) and Right Posterior (P4, P6, P8, PO8), averaging the electrodes' activity in each region (Figure 5.2). For each condition, a repeated-measures ANOVA was completed comparing the activity of the stimulated frequency band, to activity of the same frequency band in the

control condition. For each condition the ANOVA was run with factors Left/Central/Right x Anterior/Middle/Posterior x Control/Condition. A two-tailed t-test was then used to follow-up the main effects by calculating alpha power differences between the entrainment condition frequency bands and matching control condition frequency bands, separately for each scalp region. Global alpha power comparing the activity across all electrodes was implemented across conditions using a one-way ANOVA with a Bonferroni correction.

The control condition, and the condition with the largest analgesic effect were selected for LORETA analysis. A direct comparison between the two conditions was made by creating statistical maps in performing voxel-wise binary t-tests using non-parametric randomisation a voxel-wise t-test. A threshold (t) was calculated to identify significant differences in activation of brain areas. Source analysis of N2 and P2 peaks, as well as alpha power, was completed separately in LORETA.

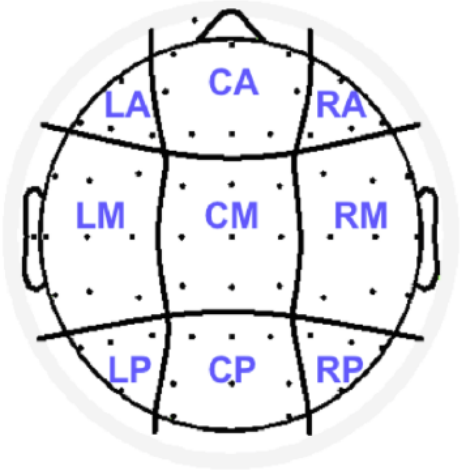


Figure 5.2 Map of Electrode Scalp Regions. Schematic representation of the division of electrodes into the 9 scalp regions: Left Anterior (LA), Central Anterior (CA), Right Anterior (RA), Left Middle (LM), Central Middle (CM), Right Middle (RM), Left Posterior (LP), Central Posterior (CP) and Right Posterior (RP).

4. Results

4.1.1. Pain Ratings

The linear mixed model revealed all three entrainment conditions (8Hz, 10Hz and 12Hz) resulted in significantly lower behavioural pain ratings than the white noise control ($t(31) = 4.90$, $p<0.001$; $t(31) = 5.61$, $p<0.001$; $t(31) = 4.85$, $p<0.001$, respectively). The pain ratings after the 8Hz, 10Hz and 12Hz entrainment sessions were an average of 0.51 (SE 0.10), 0.58 (SE 0.10) and 0.5 (SE 0.10) points lower than

the control conditions respectively. When refitting the model to each condition as a reference category with a Bonferroni correction, no significant difference between the entrainment conditions could be observed.

4.2. EEG Results

4.2.1. Laser Evoked Potential (LEP)

A late, high-amplitude negative-positive complex (N2-P2) was detected in the pooled electrode recordings. The N2 and P2 components of the complex were initially inspected separately. The decrease from the N2 control condition peak was 1.39μV (SE 0.73), 1.49μV (SE 0.75) and 0.39μV (SE 0.73) after 8Hz, 10Hz and 12Hz respectively, estimated by the linear mixed model. The reduction in N2 peak amplitude following 10Hz entrainment was the only condition that was significantly different from control ($t(31) = 1.99$, $p<0.01$). Refitting the model with a Bonferroni correction revealed that the reduction N2 peak in the 10Hz condition was significantly more than following the 8Hz and 12Hz stimulation ($t(31) = 1.09$, $p<0.01$). The 12Hz and 8Hz N2 peaks did not vary significantly from the control.

The P2 peaks following 8Hz, 10Hz and 12Hz alpha entrainment all displayed a significant reduction compare to the white noise control condition ($t(31) = 3.23$, $p<0.01$; $t(31) = 5.89$, $p<0.001$; $t(31) = 6.1$, $p<0.001$ respectively). The P2 amplitude reduction after the 8Hz entrainment was not as large as after the 10Hz and 12Hz, resulting in the 8Hz P2 peak being significantly different from the 10Hz and 12Hz condition at $p<0.01$ in addition to the control condition ($t(31) = 0.94$, $p<0.01$). With the aid of the linear mixed model it was possible to identify that the average reduction of the P2 peak after the 8Hz entrainment was 1.34μV (SE 0.75), significantly less than the 2.28 μV (SE0 0.74) reduction and 2.29μV (SE 0.74) reduction observed after the 10Hz and 12Hz entrainment respectively.

4.2.2. N2-P2 Components

When assessing the complete N2-P2 complex it becomes apparent that all three entrainment conditions diminished the amplitude of the complex. The largest reduction of the combined peaks succeeded the 10Hz entrainment, being an overall 3.77µV smaller than the control (compared to the 2.73µV and 2.68µV reduction from control ensuing the 8Hz and 12Hz binaural beats respectively). This led to the 10Hz entrainment session and the individual N2 and P2 components of the 10Hz condition being used for source analysis.

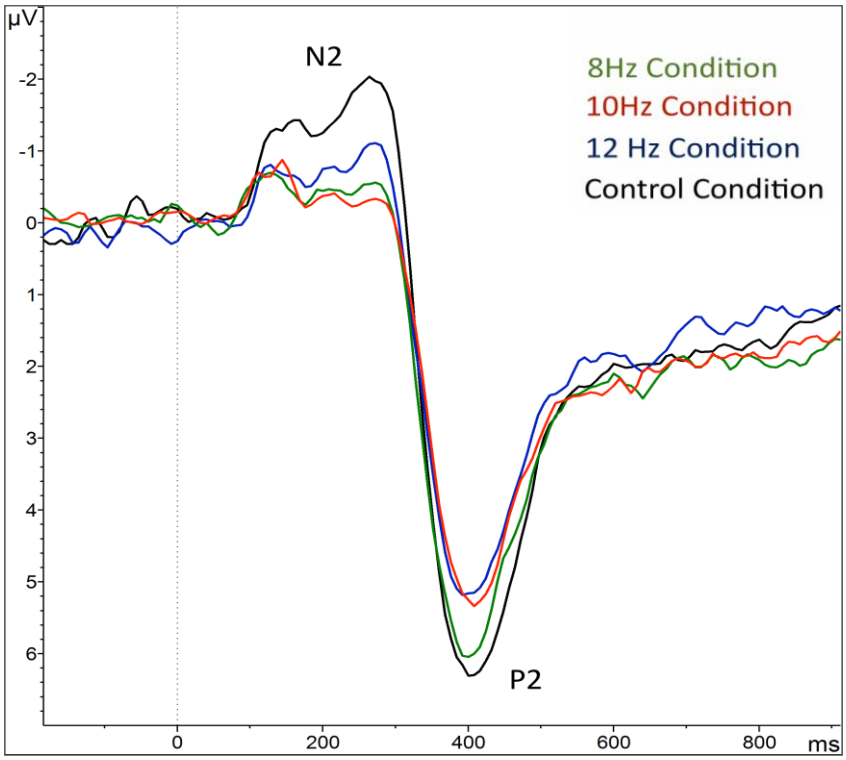
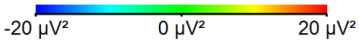
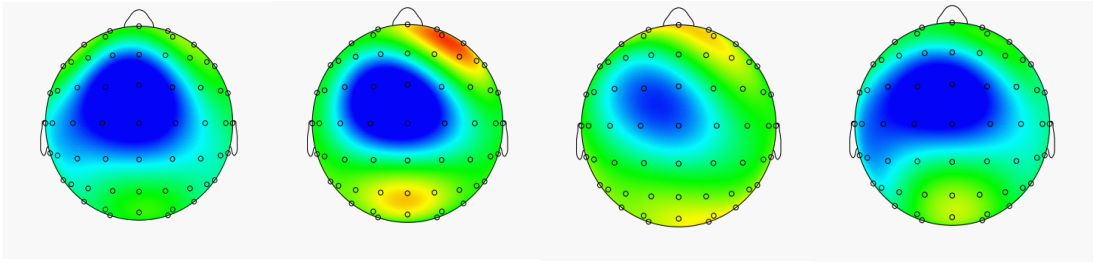


Figure 5.3 Laser-Evoked Potential (N2-P2 Complex) over Middle Central Electrodes. Graph shows the average LEP amplitude across all subjects. Nine electrodes (FC1, FCz, FC2, C1, Cz, C2, CP1, CPz, CP2) were pooled around the central Cz to produce an average N2P2 complex. The LEP following the control stimulation is depicted in black, the LEP in the 8Hz condition is represented in green, the 10Hz condition in red and the 12Hz condition in blue. Following all three alpha entrainment conditions, P2 reduced significantly from control. Reductions following the 10Hz and 12Hz entrainment were significantly larger than following the 8Hz entrainment ($p<0.01$).

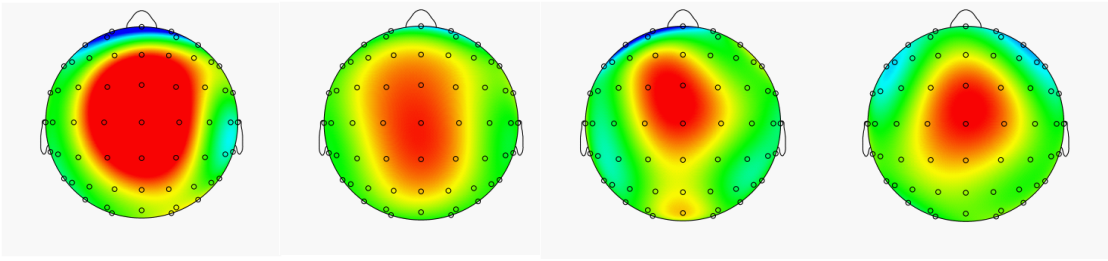


N2 Peak



Control N2 Peak	8Hz N2 Peak	10Hz N2 Peak	12Hz N2 Peak

P2 Peak



Control P2 Peak	8Hz P2 Peak	10Hz P2 Peak	12Hz P2 Peak

Figure 5. 4 N2P2 Topographies. Topographies of the negative N2 (top) and positive (P2) peaks following auditory stimulation at Control, 8Hz, 10Hz and 12Hz. The P2 peak reduced significantly from control following the 8Hz, 10Hz and 12Hz entrainment. The N2 peak reduced significantly from control following the 10Hz entrainment.

4.2.3. Alpha Power

Alpha power at each stimulation frequency (+/- 1Hz) was compared to the matching band of the control across the nine scalp regions. A repeated-measures ANOVA with the factors Left/Central/Right x Anterior/Middle/Posterior x Control/Condition was performed to see whether alpha power in each frequency band had changed from control in each of the pooled electrode groups (Figure 5.4).

Following the 8Hz entrainment, a significant anterior-to-posterior region effect could be observed across entrainment and control conditions, with higher alpha power in the posterior scalp regions ($F(2,31) = 3.903$, $p<0.05$). No significant condition effect could be observed ($F(1,31) = 1.26$, $p=0.271$).

Following ten minutes of 10Hz binaural beat stimulation; there was a significant condition effect, confirming the entrainment of alpha power ($F(1,31) = 10.72$, $p<0.01$). A significant interaction between condition and left-to-right was also detected ($F(2,31) = 3.69$, $p<0.05$), with higher alpha power in the left scalp regions. Similarly to following the 8Hz condition, a main anterior to posterior effect was observed across entrainment and control conditions, with higher posterior alpha power ($F(2,31) = 10.32$, $p<0.001$).

To determine which scalp electrode regions drove the condition x left scalp region interaction, a two-tailed paired t-test was used to follow-up the result. As the effect was higher in the left scalp regions, the activity in each of the three left regions (Left Anterior (LA), Left Middle (LM) and Left Posterior (LP)) was compared across control and entrainment conditions. A significant difference could be observed in the Left Anterior ($t(31) = 3.58$, $p<0.01$) and Left Middle ($t(31) = 2.67$, $p<0.05$) scalp regions.

Table 5.1 Paired t-test comparing activity in LA, LM and LP regions to control at 10Hz

Paired Samples Test								
	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
LA: Control vs. 10 Hz	-6.03	8.58	1.68	-9.50	-2.56	-3.58	31	.001
LM: Control vs. 10 Hz	-1.70	3.13	0.64	-3.02	-.39	-2.67	31	.014
LP: Control vs. 10 Hz	-3.44	8.57	1.75	-7.06	0.18	-1.97	31	.061

Binaural beat entrainment with a beat frequency of 12Hz, resulted in a significant condition effect with higher alpha power following stimulation ($F(1,31) = 4.6$, $p<0.05$). Furthermore, an interaction between condition and anterior-to-posterior scalp regions was calculated at $F(2,31) = 5.36$, $p<0.01$. Main interactions across both the entrainment and control conditions were observed from left to right ($F(2,31) = 6.72$, $p<0.01$) and anterior to posterior scalp regions ($F(2,31) = 18.94$, $p<0.001$) with higher alpha power posteriorly and higher alpha power in the left scalp regions.

A two-tailed paired t-test was used to follow up the condition and anterior-to-posterior interaction. As posterior alpha was higher across the control and entrainment condition, alpha power was compared across the three posterior scalp regions (Left Posterior (LP), Central Posterior (CP) and Right Posterior (RP) to detect which region was driving the interaction.

Table 5.2 Paired t-test comparing activity in LA, LM and LP regions to control at 10Hz

Paired Samples Test								
	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
LP: Control vs. 12Hz	-5.01	9.96	1.95	-9.03	-0.99	-2.57	31	.017
CP: Control vs. 12Hz	-7.46	15.04	2.95	-13.53	-1.38	-2.53	31	.018
RP: Control vs. 12Hz	-6.15	13.77	2.70	-11.72	-0.59	-2.28	31	.032

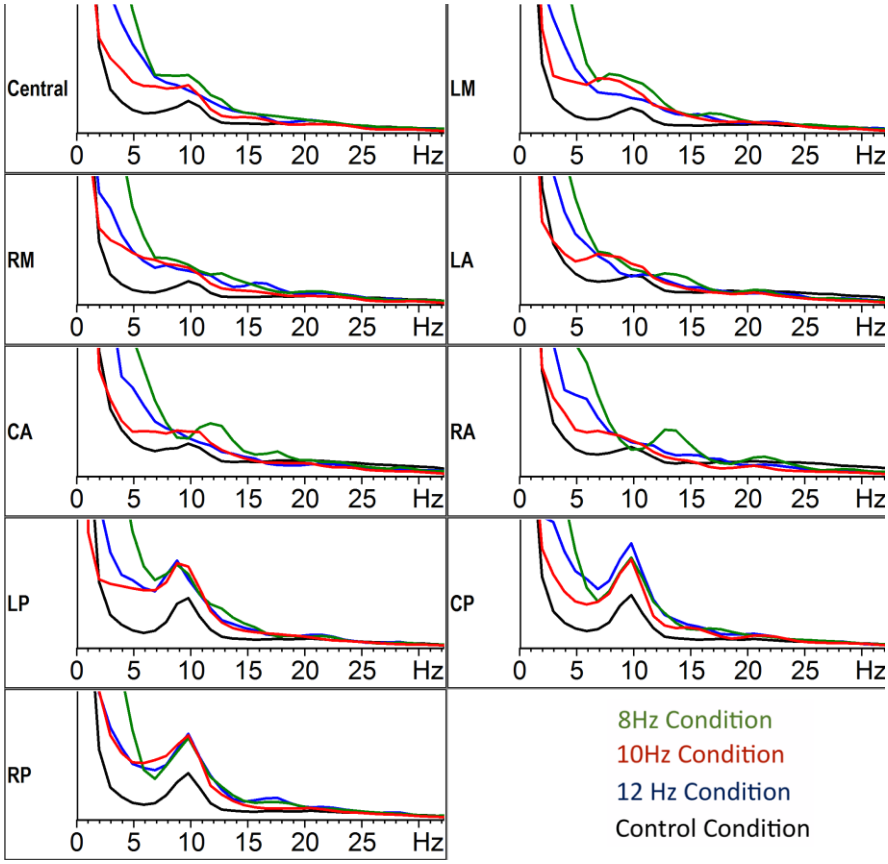
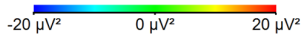
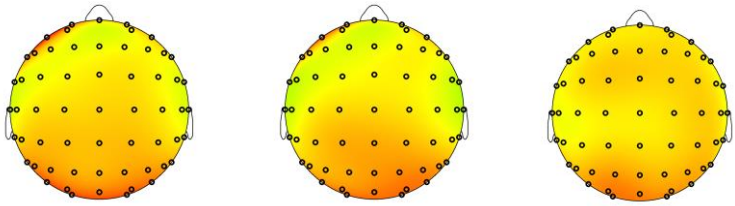


Figure 5.5 Alpha Power across Scalp Regions. The average alpha power was calculated for each scalp region Left Anterior (LA) (AF7, F9, F7, F5, F3) Central Anterior (CA) (Fp1, Fpz, Fp2, AF3, AFz, AF4, F1, Fz, F2), Right Anterior (RA) (AF8, F4, F6, F8, F10), Left Medial (FT7, FC5, FC3, T7, C5, C3, TP7, CP5, CP3), Central Medial (Central) (FC1, FCz, FC2, C1, Cz, C2, CP1, CPz, CP2), Right Medial (RM) (FC3, FC6, FT10, C4, C6, T8, CP4, CP6, TP8), Left Posterior (LP) (P7, P5, P3, PO7), Central Posterior (P1, Pz, P2, PO3, POz, PO4, O1, Oz, O2) and Right Posterior (RP) (P4, P6, P8, PO8). In black is the alpha activity observed during the white noise control entrainment. Activity during 8Hz, 10Hz and 12Hz auditory entrainment is represented in green, red and blue respectively.

Finally, the global alpha power across all electrodes was compared across all three entrainment conditions. This exposed a significantly larger alpha power after the 10Hz entrainment compared to 8Hz ($t(58) = 2.93$, $p<0.05$) and 12Hz ($t(58) = 5.82$, $p<0.001$).

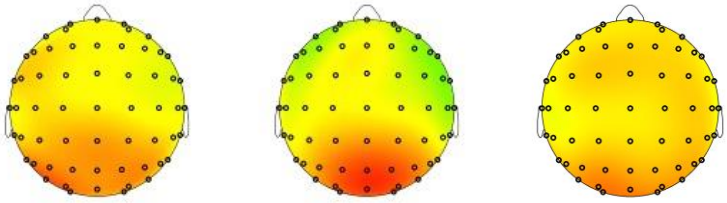


Control



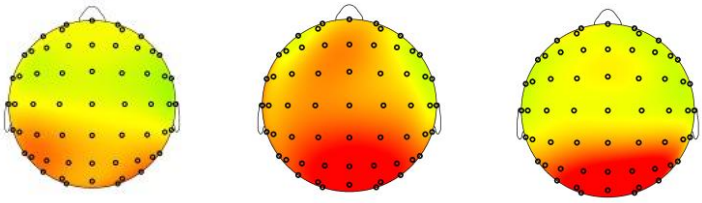
8 Hz Band	10 Hz Band	12 Hz Band

8Hz Condition



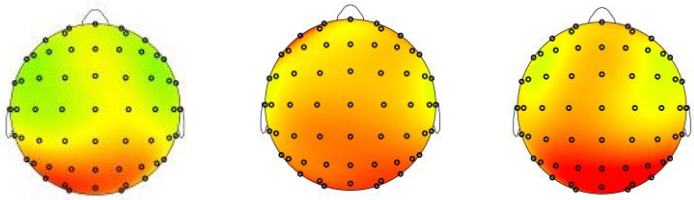
8 Hz Band	10 Hz Band	12 Hz Band

10Hz Condition



8 Hz Band	10 Hz Band	12 Hz Band

12Hz Condition



8 Hz Band	10 Hz Band	12 Hz Band

Figure 5.6 Alpha Band Topographies Topographies of alpha bands 8Hz (+/- 1Hz), 10Hz (+/- 1Hz), 12Hz (+/- 1Hz) following 1Hz control, 8Hz, 10Hz and 12Hz alpha stimulation.

4.2.4. Source Analysis (LORETA)

Source localisation identified the Brodmann's area 10, Superior Frontal Gyrus (X= -24, Y= 66 , Z= 8) when assessing the significant difference in activation between the P2 peak in the 10Hz and control condition. No significant brain activation was detected when contrasting the N2 of these conditions. P2 LORETA results are displayed in figure 5.7.

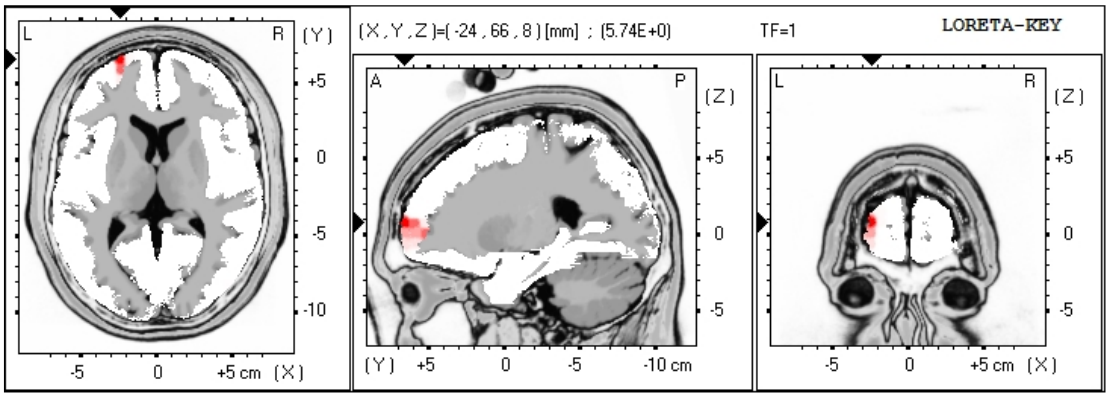


Figure 5.7 LORETA P2 Results Significant sources following a paired t-test of P2 peak values following the 10Hz and Control stimulation.

Source localisation of the neural activity contributing to the measured difference between the 10Hz (+/- 1Hz) band power in the control and 10Hz condition was found to be maximal in Brodmann's area 10, Superior Frontal Gyrus (X= -38 , Y= 52 , Z= 22). Figure 5.8 presents the alpha band power LORETA results, highlighting the area with significantly higher activation following the 10Hz entrainment.

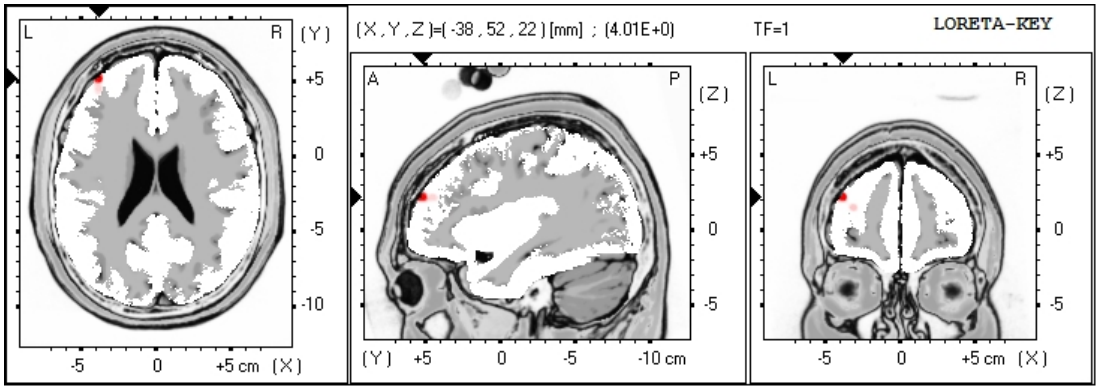


Figure 5.8 LORETA Alpha Results Sources showing significant activation when comparing power in the 10Hz alpha band in the 10Hz entrainment condition and the control condition

5. Discussion

In the present study, verbal ratings of moderate pain were reduced significantly after alpha entrainment compared to white noise control. Although there was no significant difference between the three conditions, the largest reduction could be observed after 10 minutes of 10Hz binaural beat entrainment. An independent decline from control could be observed in the N2 and P2 peak amplitudes following the entrainment sessions. The N2 peak showed a significant reduction after the 10Hz entrainment, but not following 8Hz and 12Hz. All P2 peaks revealed a significant reduction compared to control, with the peaks after the 10Hz and 12Hz entrainment additionally being significantly smaller than following the 8Hz entrainment. Ensuing the medium 10Hz alpha entrainment, a more dramatic reduction of the N2-P2 complex was recorded than following the remaining conditions.

A significant increase in alpha power from control was found following the 10Hz and 12Hz binaural beat entrainment, where the largest reductions in P2 peaks were observed. Across the 8Hz, 10Hz, 12Hz and the control conditions, posterior alpha power was consistently higher than anterior alpha power. Following the 10Hz entrainment, a significant interaction between the left scalp regions and entrainment condition was found. Follow-up t-test revealed that this interaction

was driven by the left anterior and left middle scalp electrode regions. This result was in line with the source analysis, where both changes in P2 amplitude and 10Hz alpha entrainment source activation were found in Brodmann's Area 10, also located left anteriorly. No significant correlation was found with the pain response and changes in behavioural questionnaires. This suggests the analgesic effects of alpha entrainment either occurred independently of the behavioural measures considered, or the selected questionnaires and their method of implementation were inappropriate for accurately measuring behavioural influences.

Laser-Evoked Potentials

N2-P2 peak amplitudes and behavioural pain ratings both displayed the largest reduction following the 10Hz entrainment. However, the significant differences detected between stimulation frequencies in the electrophysiological results, were not replicated behaviourally. Although numerically the largest reduction in verbal pain ratings also occurred following the 10Hz stimulation, these ratings did not statistically differ from the 8Hz and 12Hz conditions. This disconnect emphasises that results obtained from pain scales uncover but a narrow selection of cortical changes evoked succeeding painful cutaneous stimulation. N2-P2 amplitudes have been found to reduce without a corresponding decrease in behavioural pain ratings (Colloca, Tinazzi et al. 2008), suggesting the LEP waveform represents more complex integrated cortical activity, rather than simply the magnitude of a noxious stimulus.

As aforementioned, the complete N2-P2 complex amplitude exhibited the most drastic reduction following the 10Hz entrainment. Overall, the 8Hz entrainment had the second largest effect on the complete LEP complex amplitude. A theoretical explanation for this pattern could be that the largest reduction in LEPs occurs following entrainment of the frequency closest to the volunteers' individual alpha frequency (IAF), commonly around 10Hz (Steriade, Gloor et al. 1990, Klimesch, Doppelmayr et al. 1997, Klimesch 1999). The IAF varies substantially between individuals as it is controlled by the membrane properties of the thalamic neurons projecting to the cortex. Furthermore, IAF fluctuates across the lifespan, with lower

frequencies observed in children and elderly (Kopruner, Pfurtscheller et al. 1984, Klimesch 1997, Posthuma, Neale et al. 2001). The strong LEP complex reduction following 8Hz entrainment may hence in part be due the sampled population being relatively young.

Nevertheless, it should be noted that this theory only holds true when investigating the combined N2-P2 complex. Upon scrutinizing the individual peaks, it becomes apparent that they do not follow the same pattern as the combined complex. When focussing on the P2 peaks alone, a significantly larger reduction can be observed following the 10Hz and 12Hz auditory stimulation. This result is in line with the entrainment results, which displayed significant entrainment only in the 10Hz and 12Hz conditions, supporting the alpha analgesic literature.

Positive and negative LEP peaks are believed to provide a measure of neural processing and are modulated by a combination of sensory-nociceptive, affective and cognitive components (Clark, Brown et al. 2008, Iannetti and Mouraux 2010). The early N2 component of the LEP waveform is believed to be influenced more by somatosensory-specific activity (i.e. activity resulting from both nociceptive and non-nociceptive somatosensory stimuli) and activity elicited from selectively attending to the spatial location of pain (Bentley, Watson et al. 2004, Mouraux and Iannetti 2009). On the other hand, only part of the early N2, but the majority of the P2 component, is believed to be influenced by activity elicited by stimuli of other sensory modalities, i.e. multimodal activity. Previous studies have established that attending to a painful sensation increases solely the P2 peak amplitude, whilst distractions cause a reduction of that positive peak at the vertex (Bentley, Watson et al. 2004, Boyle, El-Deredy et al. 2008). These results underline the general accepted notion that P2 amplitudes are exclusively sensitive to changes in the cognitive and affective components of pain (e.g. attention), as well as the intensity of pain (Siedenberg and Treede 1996, Zaslansky, Sprecher et al. 1996, Bentley, Watson et al. 2004, Brown, Seymour et al. 2008). A preponderance of work suggests that accumulating alpha power is incompatible with states of high arousal. It could thus be postulated that fluctuations in P2 amplitude in the current study

were influenced by deviations in attention, facilitated by alpha-wave entrainment. Hence, this provides an explanation for why only P2 peaks, and not the entire LEP complex matched the predicted effects of alpha entrainment: higher alpha, lower P2 peak amplitude.

As indicated previously, variations in LEPs might not be specific to nociception. Instead, recent evidence suggests N2-P2 amplitudes could be reactions to salience, and notable adverse events, including but not limited to pain (Garcia-Larrea, Peyron et al. 1997, Legrain, Guerit et al. 2002, Iannetti and Mouraux 2010). If salience were purely defined as stimulus novelty, this would not explain why LEPs decreased in amplitude succeeding all three alpha entrainment conditions, and not following the control. Laser stimuli following entrainment were not less novel than those ensuing white noise stimulations, as the order of conditions and visits was randomised. This implies that condition-specific differences in the amplitude of the LEP waveform were not controlled by attentional demands, but rather the analgesic properties of alpha entrainment. However, if salience were defined as a measure of attention attracted by a stimulus, salience could modulated and dependent upon the participant's state. Therefore, if alpha power reduces attention to painful stimuli, these stimuli may be perceived relatively less salient following entrainment.

Changes in Alpha Power

Following 10Hz entrainment global alpha power was significantly higher than after 8Hz and 12Hz entrainment. Our results demonstrated that the 10Hz entrainment resulted in a significantly larger increase that the 8Hz and 12Hz entrainment. Maximal power at 10Hz has previously been observed following entrainment (Romei, Gross et al. 2010, de Graaf, Gross et al. 2013). Furthermore, numerous other frequencies in the 1-30 Hz range, as well as certain non-rhythmic external stimuli can elicit a 10Hz power increase (VanRullen and Macdonald 2012). Entrainment at 8Hz and at 12Hz could therefore additionally promote oscillatory power at 10Hz.

Across all three entrainment conditions, significantly higher alpha power could be observed in the posterior scalp regions. However, posterior alpha power was also higher in the control condition, where sources of alpha rhythm are believed to be (Palva and Palva 2007). As posterior alpha power did not display a proportionally larger increase following entrainment than anterior or middle regions, significant differences in posterior electrode activity and source activation was not expected. Following the 10Hz entrainment, a significant interaction between condition and the left scalp regions, driven by the left anterior and left middle regions, was detected. This is in line with the LORETA P2 peak and alpha power results, which show activation in the superior frontal gyrus when comparing activity in the control and 10Hz condition.

Cognitive Influences

Source analysis of P2 peaks did not reveal the activation of areas commonly identified as contributing to the LEP peak amplitudes (Garcia-Larrea, Frot et al. 2003). The LORETA analysis merely identifies differences in brain activity between conditions, rather than absolute activation in each condition. A high variance in alpha power between volunteers may therefore have hindered the detection of differences in activation in LEP source associated structures. The influence of pain-associated structures may therefore be difficult to quantify. Changes in activation of frontal sources may be reflecting alterations in ongoing brain states that co-occur with the electrophysiological pain response. Rather than revealing the sources contributing to the changes in P2 amplitude, they may be revealing sources associated with the cognitive manipulation of pain.

The prefrontal cortex, in which BA 10 lies, is directly involved in the top-down cognitive modulation of pain (Petrovic, Dietrich et al. 2005, Seifert, Bschorer et al. 2009, Watson, El-Deredy et al. 2009). Evidence suggests the superior frontal gyrus is activated during self-awareness, coordinating with sensory system activity (Goldberg, Harel et al. 2006), and additionally plays a fundamental role in pain expectancy (Ploghaus, Tracey et al. 1999, Porro, Baraldi et al. 2002). P2 source results may not be revealing the sources contributing to changes in LEP amplitudes,

but may be detecting activity related to pain expectancy, or post-stimulus updating of expectations. Investigating prefrontal activity may therefore provide a solid foundation for assessing the different cognitive and emotional influences on the pain response.

Results in the present study reveal that LEP activity in the superior frontal gyrus was significantly larger in the control compared to succeeding entrainment conditions. When a noxious stimulus is unexpected, activity in the superior frontal gyrus has been documented to be greater compared to when the pain is expected (Ploghaus, Tracey et al. 2000). Changes in superior frontal gyrus activation may therefore reflect common processes underlying the differential, expected (vs. unexpected) pain and post-alpha-entrainment (vs. post-white-noise) pain perceived.

Increased alpha activity in the superior frontal gyrus might have resulted in augmented internally-directed attention and self-referential thought, as described previously (Cooper, Croft et al. 2003, Cooper, Burgess et al. 2006, Knyazev, Slobodskoj-Plusnin et al. 2011). Although alpha oscillations mainly originate in thalamo-cortical feedback loops of excitatory and inhibitory nerve cells (Steriade, Gloor et al. 1990, Lopes da Silva 1991), alpha power is additionally modulated by cortico-cortical networks (Lopes Da Silva and Storm Van Leeuwen 1977, Steriade, Gloor et al. 1990, Bollimunta, Chen et al. 2008, Spaak, Bonnefond et al. 2012), that are believed to be controlled by attention (Yamagishi, Callan et al. 2003, Bollimunta, Mo et al. 2011).

We therefore consider in our study the higher alpha power allowed volunteers to diminish the cognitive and emotional aspects of pain and focus on the physical aspects, resulting in less cognitively influenced P2 peaks and pain ratings following entrainment. By contrast, reducing alpha activity has been suggested to reflect alertness to external inputs (Klimesch 1999) allowing the mind to be occupied with numerous inputs, and hence allowing the pain stimulus to feel more salient.

Brodmann's Area 10 and Thermal Pain

Although BA 10 is not believed to be a direct target of the spino-thalamo-cortical pathway, nor is it believed to contribute to peak amplitudes of LEPs, it nevertheless has been activated in previous studies related to thermal pain (Becerra, Breiter et al. 1999, Lorenz, Cross et al. 2002, Zambreanu, Wise et al. 2005).

A recent study by Atlas et al (2014) investigating the neural networks contributing to the generation of pain, found noxious heat-related increases activity in BA 10 (Atlas, Lindquist et al. 2014). The study revealed increases in BA 10 were both temperature and pain related, and may mediate temperature effects on pain. As the heat stimulus in the present study did not change in intensity, BA 10 activation may modulate heat specific pain perception. Alternatively, BA 10 may play a role in how intense heat is perceived, rather than providing an accurate representation of the external thermal input intensity. Work completed by Casey and colleagues supports this notion, detecting higher activation of the contralateral BA 10 during 'just painful' cutaneous heat stimulation, than in higher pain stimulations (Casey, Minoshima et al. 1996). BA10 may therefore have displayed a higher activation in the control condition as the stimulus was experienced as, or expected to be experienced as, hotter compared to in the 10Hz entrainment condition. However, this theory cannot be confirmed by our results as stimulus heat ratings were not recorded.

It should be noted that these conclusions should be made with caution as the results obtained by Casey et al., are contradictory to the work completed by Becerra et al., 1999 where contralateral activation of BA 10 was found in both 'just painful' and 'moderately painful' conditions, with a significantly higher activation in the more painful condition (Becerra, Breiter et al. 1999). These differences could be accredited to a difference in imaging techniques used by the groups.

Conclusion

Alpha binaural beats effectively decrease the perception of acute laser pain with beat frequencies across the alpha range. The central beat frequency, 10Hz, resulted

in a significantly larger reduction in laser-evoked potential amplitude compared to the other frequencies. Changes in alpha activity and LEP peak amplitude, appear to be influenced by activity arising from the prefrontal cortex, suggesting alpha elicited analgesia.

CHAPTER 6

Visual Alpha-Band Entrainment reduces the Behavioural and Electrophysiological Pain Response

This Chapter is based on a manuscript that is being prepared for submission to PAIN.

1. Abstract

Acute noxious stimuli induce a suppression of cortical alpha activity, yet it is still unknown whether increasing alpha power causes a reciprocal suppression of acute pain processing and perception. Here, we attempted to increase alpha activity through visual entrainment at 8Hz, 10Hz and 12Hz to investigate the influence on the behavioural and electrophysiological pain responses. Verbal and electrophysiological pain ratings of a moderately painful stimulus were recorded following 10 minutes of visual entrainment across the alpha range. Alpha power increased significantly relative to the 1Hz control condition following 8Hz and 10Hz visual stimulation. A significant reduction in pain ratings was found across all three frequencies with a significantly larger reduction following the 10Hz stimulation than in the 8Hz and 12Hz conditions. Significant reductions in the P2 peak amplitude of the laser-evoked potentials were found following visual entrainment at 10Hz. Changes in the precuneus and posterior cingulate cortex activity appear to play a key role in mediating the alpha entrainment-induced reductions in laser-evoked potentials and behavioural pain ratings, following the 10Hz stimulation. The findings of the present study provide evidence revealing the experimental induction of alpha power suppresses the cortical processing and perception on acute pain.

2. Introduction

The alpha rhythm (7-14Hz) is the most studied frequency band in the human brain as it can be detected in 95% of healthy young adults with their eyes closed (Srinivasan 1999). The alpha rhythm has historically been described as an 'idling' rhythm and was believed to represent low information processing. However, more recent work suggests a central role in cognitive processing, specifically the top-down control of sensory information (Klimesch, Sauseng et al. 2007). Alpha power suppression over the contralateral sensorimotor and occipital cortices has been repeatedly found to be correlated with the intensity of a painful stimulus (Mouraux, Guerit et al. 2003, Ohara, Crone et al. 2004, Raij, Forss et al. 2004, Ploner, Gross et al. 2006, Hu, Peng et al. 2013, Peng, Hu et al. 2014). The suppression of oscillatory activity in the alpha band as a result of a painful stimulus has been linked to cortical excitability. A global alpha suppression may permit alerting the cortex of the pain, to allow the processing of relevant stimuli, through the opening of sensory and motor system gates (Downar, Crawley et al. 2000). Importantly, behavioural pain intensity ratings have also been correlated significantly with decreases in alpha power (Mouraux, Guerit et al. 2003, Babiloni, Brancucci et al. 2006, Gross, Schnitzler et al. 2007).

The inverse relationship between cortical alpha activity and pain perception appears to work both ways. Mindfulness meditation, which has been shown to have a significantly positive impact on both the affective and cognitive aspects of pain (Grant 2014), increases alpha power over the primary somatosensory cortex (Kerr, Jones et al. 2011). The use of neurofeedback training to increase alpha power has also been associated with a significant reduction in chronic pain perception in fibromyalgia patients (Kayiran, Dursun et al. 2010, Jensen, Gertz et al. 2013). Furthermore, increases in resting alpha activity have also been reported during experimental placebo analgesia using acute laser pain in healthy participants (Huneke, Brown et al. 2013). Alpha activity may therefore play a fundamental role

in regulating the cognitive processes of analgesia. Attempting to directly influence alpha activity may hence provide an effective way of altering pain perception.

Oscillatory rhythms in the 1-30Hz frequency range, such as the alpha rhythm, can be modulated by an external stimulus. In a process known as entrainment, brainwave activity naturally adapts to the frequency of the stimulus, making that frequency more prominent throughout the cortex. Visual entrainment has the strongest resonance at 10Hz and predominantly affects the primary visual cortex (Herrmann 2001, de Graaf, Gross et al. 2013). However, changes in cortical activity following visual alpha stimulation can be observed widely throughout the cortex (Timmermann, Lubar et al. 1999, de Graaf, Gross et al. 2013). Visual alpha entrainment may thus prove a promising candidate for the modulation of nociceptive processes at the cortical level, enabling acute pain relief.

The present study investigates the effect of visual entrainment at 8Hz, 10Hz and 12Hz on the behavioural perception of acute pain induced by a laser stimulus and its evoked electrophysiological response. Electroencephalography (EEG) was used to record increases in alpha power and the laser-evoked potential (LEP) of painful heat laser stimuli. As the 10Hz flicker is believed to have the strongest resonance (Herrmann 2001), we hypothesised that visual entrainment in the 10Hz condition would result in the largest reduction in behavioural pain ratings and LEP amplitude.

3. Materials and Methods

3.1.1. Ethics Statement

All volunteers provided written, informed consent according to the International Conference on Harmonisation Good Clinical Practice guidelines, before participating in the study. The study obtained ethical approval from the NRES Committee North West – Liverpool Central (reference number 13/NW/0007).

3.1.2. Participants

32 healthy volunteers (17 Male, mean age 25.82 ± 8.6 SD) attended a session at Salford Royal NHS foundation Trust after having contacted the lab through advertisements placed throughout the Trust and on the University of Manchester website. In order to participate in the study, all volunteers needed to be right-handed, over the age of 18, and not possess any of the following exclusion criteria: chronic pain, morbid psychiatric illness (e.g. major depression, schizophrenia, bipolar disorder), neurological illness, ischemic heart disease, uncontrolled high blood pressure, peripheral vascular disease, chronic skin disease (e.g. eczema, psoriasis), hypertension not controlled by medication and a history, or family history of epilepsy.

3.1.3. The Painful Stimulus

A CO_2 heat laser was applied to the dorsal surface of the participants arm to elicit a moderately painful sensation. The laser stimulus had a total duration of 150ms with a 15mm beam diameter. The laser beam was relocated across the forearm after each stimulation occurred (every 10 seconds), in order to minimise sensitisation, habituation or damage to the skin. Previous studies have shown evidence of contactless activation of nociceptors related to Aδ and C fibres using brief CO_2 laser stimuli (Meyer, Walker et al. 1976). All participants were obliged to wear safety goggles whenever the laser was in use.

3.1.4. Psychophysics Procedure (Pre-experimental)

Prior to the start of the experiment, each volunteer's 'moderately painful' level on the 0-10 numeric rating scale was calculated by means of the psychophysics procedure. 'No sensation' was marked next to level 0 on the scale, 4 was marked as the 'pain threshold', 7 as 'moderately painful' and 10 was labelled the 'maximum tolerance level'. The psychophysics consisted of a ramping procedure with increasing stimulus intensity whereby the subjects were ask to verbally rate each pulse until their level 7 (moderately painful level) was reached. The procedure was completed a minimum of three times, until a consistent voltage for moderate pain was established. The intensity was tested and adjusted by repeating a series of laser pulses across the forearm, with the participant rating each pulse.

3.1.5. Questionnaires (pre-experimental)

All volunteers completed a set of behavioural questionnaires before the start of the experiment. These were the Profile of Mood States (POMS; selected negative mood items only, see Brown et al., 2008), the State-Trait Anxiety Inventory (STAI), the Karolinska Sleepiness Scale (KSS), Participant Sleep Questionnaire, Pain Catastrophizing Scale (PCS), Patient Health Questionnaire - 9 (PHQ-9) and the Pain Anxiety Symptoms Scale (PASS). The Participant sleep questionnaire, PCS, PHQ-9 and PASS were used to measure quality of sleep, degree of pain related catastrophic thinking, depression and pain specific fear and anxiety respectively. They were completed once, following the psychophysics procedure. The POMS (short form), STAI – state, and KSS were given once following the psychophysics, and again after each entrainment conditions' pain rating trial. These three questionnaires were used to assess the degree of negative moods, state anxiety and present sleepiness. Participants were asked to attempt to relate these questionnaires to the preceding alpha entrainment session, as opposed to the 30 pulses of pain.

Experimental Procedure

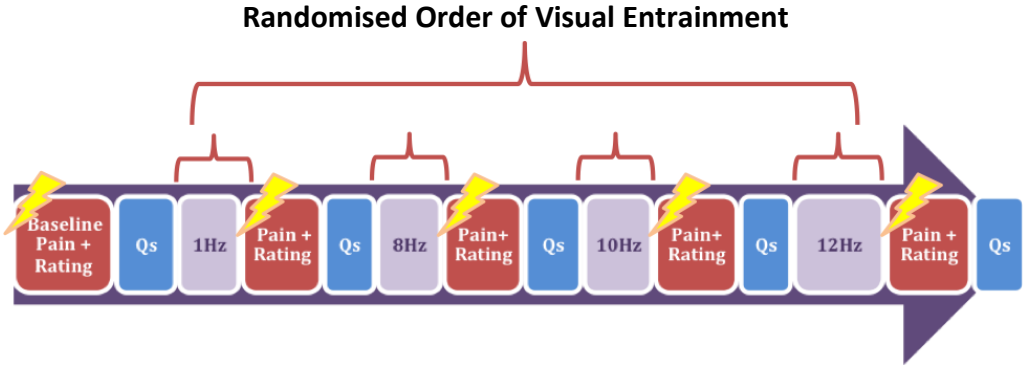


Figure 6.1 Experimental Procedures. After obtaining written consent, all study participants were subjected to 30 pulses of their 'level-7' pain (Baseline Ratings). Participants were then asked to fill out a set of baseline questionnaires (Qs: Sleep Questionnaire, PCS, PHQ-9, PASS, POMS, STAI, KSS). Participants were presented with four visual (flashing LED goggle) stimulation sessions at 8Hz, 10Hz, 12Hz and 1Hz (control), of 10 minute duration, in a randomised order. Participants were asked to rate 30 pulses of 'level-7' pain following each visual stimulation session. Following each pain rating session, volunteers were asked to complete the KSS, STAI-state and POMS. Qs: Questionnaires

3.1.6. Baseline Pain Ratings

Prior to the first entrainment session, all participants were asked to rate 30 pulses of their 'moderately painful' level 7 pain, with a 10 second resting period between each pulse. The ratings were averaged to determine each volunteer's baseline pain rating.

3.1.7. Visual Entrainment

There were a total of three visual alpha entrainment sessions, and one control condition, presented in a randomised order to participants. Each entrainment or control session lasted a total of 10 minutes. Frequency entrainment was performed at 8Hz, 10Hz, 12Hz and 1Hz (control) with a pair of flashing LED goggles. Participants were requested to keep their eyes closed whilst wearing the goggles, as entrainment is just as effective, but perceived as more pleasant with closed eyes. Following each entrainment and control stimulation volunteers were asked to rate 30 pulses of their predetermined 'moderately painful' level 7.

3.1.8. Acquisition of EEG Data

EEG was recorded continuously throughout the entrainment session and throughout the pain rating session using 64 Ag/AgCl surface electrodes attached to a cap according to the extended standard 10-20 system (Brain Vision Acticap and BrainAmp, Brain Products GmBH, Germany). In order to isolate eye-movement and blink artefacts, four electrodes in the set measured the horizontal and vertical electro-oculograms (EOG). The right mastoid electrode was used as a reference electrode and AFz acted as the ground electrode for all other electrodes. For all recordings a sampling rate of 500Hz was implemented and band-pass filters were set at DC – 100Hz. To minimise the amount of electrical interference in the recording, a notch filter at 50Hz was applied. Recordings were made using BrainVision Recorder 1.10 (Brain Products GmBH, Germany).

3.2. Quantitative Electrophysiological Analysis

3.2.1. Laser-Evoked Potentials (LEPs)

EEG analysis was completed with Brain Vision Analyzer 2.0. Data were analysed consistently with procedures in previously published work (Brown, Seymour et al. 2008, Watson, Petrakis et al. 2009, Morton, Brown et al. 2010, Huneke, Brown et al. 2013). Initially, EEG recording were down sampled from 500Hz to 125Hz. A 50Hz notch filter was applied to all data sets to minimise artefacts from electrical equipment. Segments containing the LEPs were epoched 1000ms pre-stimulus and 1500ms post. The laser stimulus was marked as time 0ms. A baseline correction was completed at -500ms to 0ms. A DC detrend was applied on the first 500ms and the last 100ms of the epoch to remove linear trends from the segment. To remove horizontal eye movement and blink artefacts an Independent Component Analysis (ICA) with classic sphering was performed with an Infomax (Gradient) Restricted Biased algorithm. Data were split into 25 components. The median number of components removed was 4 with a range of 0 to 10. The data were reconstructed with the remaining components and manually re-inspected for any remaining artefacts. Epochs were then averaged across all data sets, separately for each condition. Data were then re-referenced to the common average of electrodes.

In every subject and each condition LEPs were measured separately using the most negative post-stimulus point between 200ms and 300ms, identified as N2 and the largest positive peak between 350ms and 500ms after the stimulus, labelled as P2. The data from nine electrodes (CPz, Cz, Fcz, CP1, CP2, C1, C2, FC2, FC1) surrounding the electrode with the largest global N2P2 complex at baseline: the central Cz electrode, was pooled for statistical analysis for each subject and condition. Values of N2 and P2 peaks of the pooled elected were exported +/-10ms either side of the peak, for each subject, across all conditions. A grand average was produced to create an image of the LEPs for all conditions.

3.2.2. Spectral Analysis

Analysis of alpha power was performed using Brain Vision Analyzer 2.0 consistent with previous work by (Huneke, Brown et al. 2013). The data were initially re-referenced to the common average of electrodes. A low cut off filter at 0.05Hz (12 dB/oct), and a high cut off filter at 35 Hz (48 dB/oct) were applied. In order to eliminate artefacts including eye-movement and muscle activity, a 25 component ICA was performed, removing all the bad segments before reconstructing the clean data. Data were segmented into 1-second epochs, with any epoch still containing artefacts being removed after manual inspection. A Fast-Fourier Transformation with a 10% Hanning window was applied to the remaining data. With a sampling rate of 500Hz, this resulted in a frequency resolution of 0.5Hz. The average power was measured in frequency density, or activity expressed in log units ($10*\log10(\mu V^2/Hz)$). In the present study the power in the frequency bands 8Hz ±1Hz, 10Hz ± 1Hz, 12Hz ± 1Hz was compared to the activity in the matching frequency band of the control condition.

3.2.3. Source Localisation

Cortical sources of the N2 and P2 components of the LEP were estimated independently using low-resolution electromagnetic tomography (LORETA), using the LORETA-KEY software (Lantz, Michel et al. 1997, Pascual-Marqui, Esslen et al. 2002). The LORETA software uses a three-shell spherical head model, which is registered to the Talairach anatomical brain atlas (Talairach, Tournoux et al. 1988). However, the coordinates used for electrodes were calculated from a co-registration between spherical and realistic head geometry, and thus creating the best-fitting sphere, relative to cortical anatomy (Towle, Bolanos et al. 1993). Sources of alpha and LEPs in grey matter volume are estimated in LORETA to a $7mm^3$ resolution (2394 voxels) (Mazziotta, Toga et al. 2001).

The condition with the largest reduction in pain ratings was selected for LORETA analysis. The sources of the amplitude of N2 and P2 peaks in the entrainment condition were compared to the control condition. Furthermore, the cortical

sources of the differences in alpha activity between control and the entrainment condition with the largest analgesic effect were identified. The spatially smoothest source compatible with the electrophysiological data was estimated across all electrodes. The EEG data were plotted onto the previously mentioned map of 2394 voxels in a three-dimensional space in with each voxel represented a single potential activity zone.

3.3. Statistical Analysis

Statistical analysis was completed in SPSS version 20, with a p value of <0.05 regarded as significant. A mixed linear model was used to analyse the differences in pain ratings between entrainment conditions and control. The model considered the baseline ratings as a covariate and accounted for the condition (8Hz, 10Hz or 12Hz) order of entrainment. The model output revealed amplitude of change from control for each condition, and the significance of this difference with the mentioned factors and covariate taken into account. In order to calculate the differences between conditions the mixed linear model was refitted with a Bonferroni correction using each condition as a reference category. A separate mixed linear model was also applied to each of the behavioural questionnaires repeated after each condition (POMS, STAI and KSS scores), taking into account order and condition as in the previous model. The questionnaires completed prior to the baseline pain (PHQ-9, PCS, Participant Sleep Questionnaire and PASS scores) were correlated to the change in pain ratings from baseline across all four conditions (entrainment and control).

The equivalent model was applied independently to the N2 and P2 peak amplitudes, with respective baseline peak values as covariates and condition and session order as factors. Change in alpha power averaged across all scalp electrodes was compared between the three conditions using a one-way ANOVA with a Bonferroni correction to assess the global influence of entrainment. The power in each entrainment condition band (eg. 8Hz ±1Hz) was compared to the same frequency band in the control condition. The electrodes were then divided into 9

scalp regions: Left Anterior (AF7, F9, F7, F5, F3) Central Anterior (Fp1, Fpz, Fp2, AF3, AFz, AF4, F1, Fz, F2), Right Anterior (AF8, F4, F6, F8, F10), Left Middle (FT7, FC5, FC3, T7, C5, C3, TP7, CP5, CP3), Central Middle (FC1, FCz, FC2, C1, Cz, C2, CP1, CPz, CP2), Right Middle (FC3, FC6, FT10, C4, C6, T8, CP4, CP6, TP8), Left Posterior (P7, P5, P3, PO7), Central Posterior (P1, Pz, P2, PO3, POz, PO4, O1, Oz, O2) and Right Posterior (P4, P6, P8, PO8), averaging the electrodes' activity in each region (Figure 6.2). For each condition, a repeated measures ANOVA was completed comparing the activity of the stimulated frequency band, to activity of the same frequency band in the control condition. For each condition the ANOVA was run with factors Left/Central/Right x Anterior/Middle/Posterior x Control/Condition. A two-tailed t-test was then used to follow-up the main effects by calculating alpha power differences between the entrainment condition frequency bands and matching control condition frequency bands, separately for each scalp region.

The LORETA analysis compared the control condition to the entrainment condition with the largest analgesic effect. To compare the two conditions, LORETA created statistical maps by performing voxel-wise binary t-tests using non-parametric randomisation. A threshold (*t*) was calculated to identify significant areas of activation. A LORETA analysis was completed for N2 peaks, P2 peaks and global alpha activity for the selected conditions.

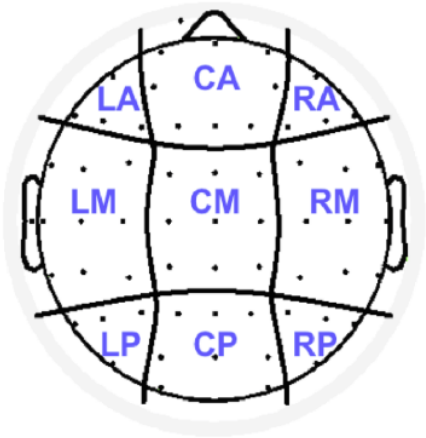


Figure 6.2 Map of Scalp Electrode Regions. Schematic representation of the division of electrodes into the 9 scalp regions: Left Anterior (AF7, F9, F7, F5, F3) Central Anterior (Fp1, Fpz, Fp2, AF3, AFz, AF4, F1, Fz, F2), Right Anterior (AF8, F4, F6, F8, F10), Left Medial (FT7, FC5, FC3, T7, C5, C3, TP7, CP5, CP3), Central Medial (FC1, FCz, FC2, C1, Cz, C2, CP1, CPz, CP2), Right Medial (FC3, FC6, FT10, C4, C6, T8, CP4, CP6, TP8), Left Posterior (P7, P5, P3, PO7), Central Posterior (P1, Pz, P2, PO3, POz, PO4, O1, Oz, O2) and Right Posterior (P4, P6, P8, PO8)

4. Results

4.1.1. Behavioural Pain Ratings

The behavioural pain ratings following the 8Hz, 10Hz and 12Hz entrainment all reduced significantly from control ($t(31)=2.28$, $p<0.01$; $t(31)=5.32$, $p<0.001$; $t(31)=2.59$, $p<0.01$ respectively). Changes in pain rating following entrainment were -0.55 (SE 0.24; Confidence Interval (CI) -1.024 to -0.0712) following 8Hz, -0.99 (SE 0.24; CI -1.46 to -0.52) following 10Hz, and 0.35 (SE 0.24; CI -0.82 to 0.12) following 12Hz. Furthermore, pain ratings following 10Hz entrainment were significantly lower than succeeding 8Hz ($t(31)=2.22$ $p<0.01$) and 12Hz ($t(31)=4.04$, $p<0.001$) entrainment. The 8Hz and the 12Hz condition did not differ significantly from each other (p=0.287).

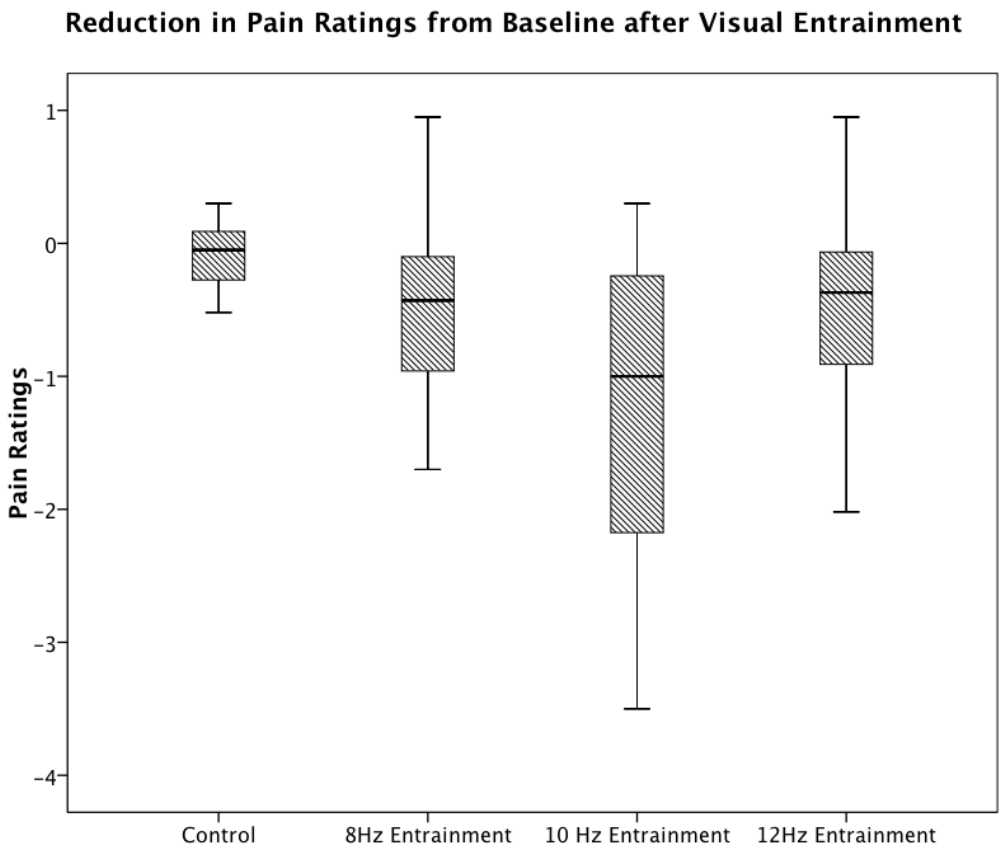


Figure 6.3 Change in Pain Ratings from Baseline. Following alpha entrainment at 8Hz, 10Hz and 12Hz, behavioural ratings of the moderately painful stimulus decreased significantly more from baseline than control (p<0.001). Reduction from baseline following 10Hz entrainment was additionally larger than following 8Hz (p<0.01) and 12Hz (p<0.001)

4.1.2. Correlation Analysis

No significant correlation was observed between the change in the pain response (both behavioural and electrophysiological) and the baseline questionnaires (PCS, STAI- trait, PHQ-9, PASS)

4.1.3. Alpha Activity

For each entrainment frequency, a repeated-measures ANOVA was completed comparing the alpha power across conditions (entrainment vs. control) and across the nine scalp regions. Following the 8Hz stimulation, there was a significant main condition effect ($F(1,31) = 5.6$, $p<0.05$) with significantly higher alpha power following stimulation, confirming entrainment was successful. A significant main anterior-to-posterior effect, with higher posterior alpha power was found across control and entrainment conditions ($F(2,30) = 8.71$, $p<0.01$).

Visual entrainment at 10Hz also resulted in a significant increase in alpha power, made apparent by the main condition effect found across scalp regions ($F(1,31)= 6.0$, $p<0.05$). Again, a significant main anterior to posterior effect was observed ($F(30,2) = 5.33$ $p<0.01$). Following the 12Hz stimulation, no significant effect of condition was observed, suggesting no entrainment occurred in the selected frequency band. Similarly to the 8Hz and 10Hz stimulations, alpha power was significantly higher posteriorly across conditions ($F(2,30) = 6.05$, $p<0.05$) following the 12Hz stimulation. As there was no interaction between condition and anterior-to-posterior alpha power following any of the frequency stimulations, it appears that alpha power was consistently higher posteriorly, and increases in alpha power in the 8Hz and 10Hz conditions occurred globally, across multiple scalp regions. Namely, high posterior alpha power increased proportionally to other scalp regions. To test this hypothesis, follow-up two-tailed paired t-tests were completed separately over the nine scalp regions comparing activity of the frequency entrained, to the matching band in the control condition. Following the 8Hz entrainment, activity in all 7/9 scalp regions was higher than in the matching control (at p <0.05). Regions that were not significantly different were the Right

Anterior and the Right Posterior. In the 10Hz condition, all regions except for the Left Anterior were significantly higher than in the control condition (at $p<0.05$). No regions were significantly different than control following the 12Hz stimulation, as expected by the lack of condition effect.

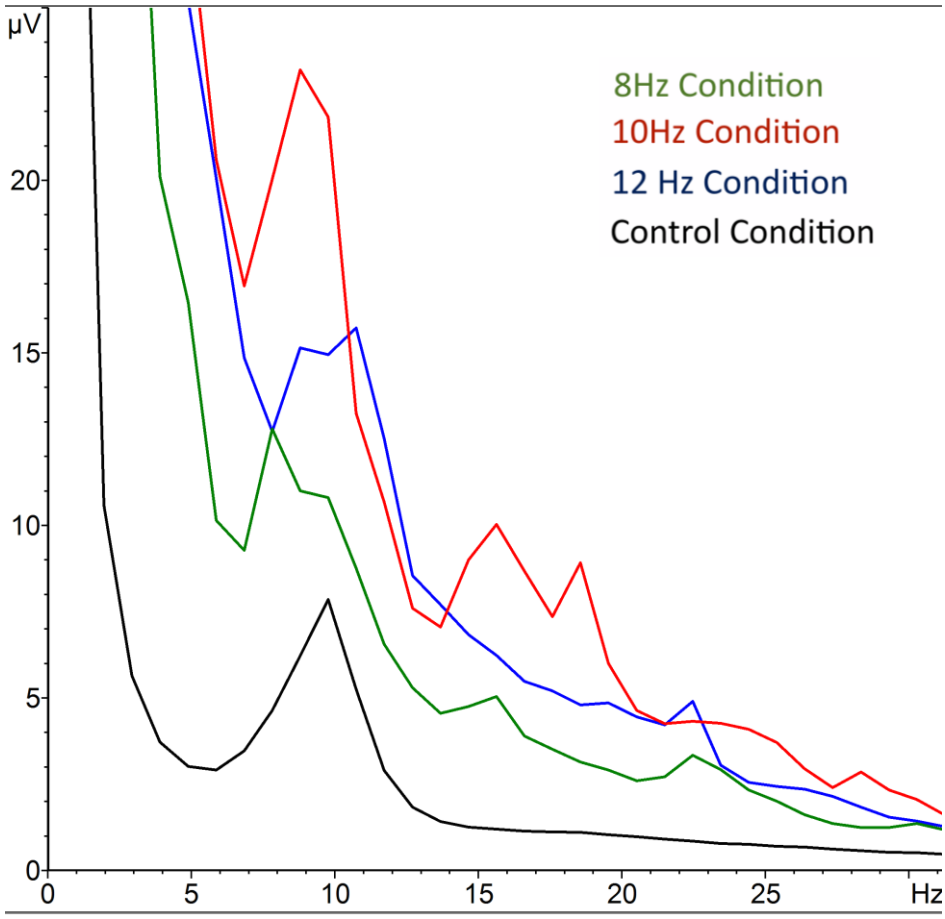


Figure 6.4 Global Alpha Power Post Visual Entrainment. Global alpha power averaged over all 62 scalp electrodes. Increases in alpha power from control (black) can be observed in the 8Hz condition (green), the 10Hz condition (red) and the 12Hz condition (blue). Increases in alpha power were significant in 7/9 scalp regions in the 8Hz condition, 8/9 regions in the 10Hz condition and 0/9 in the 12Hz condition.

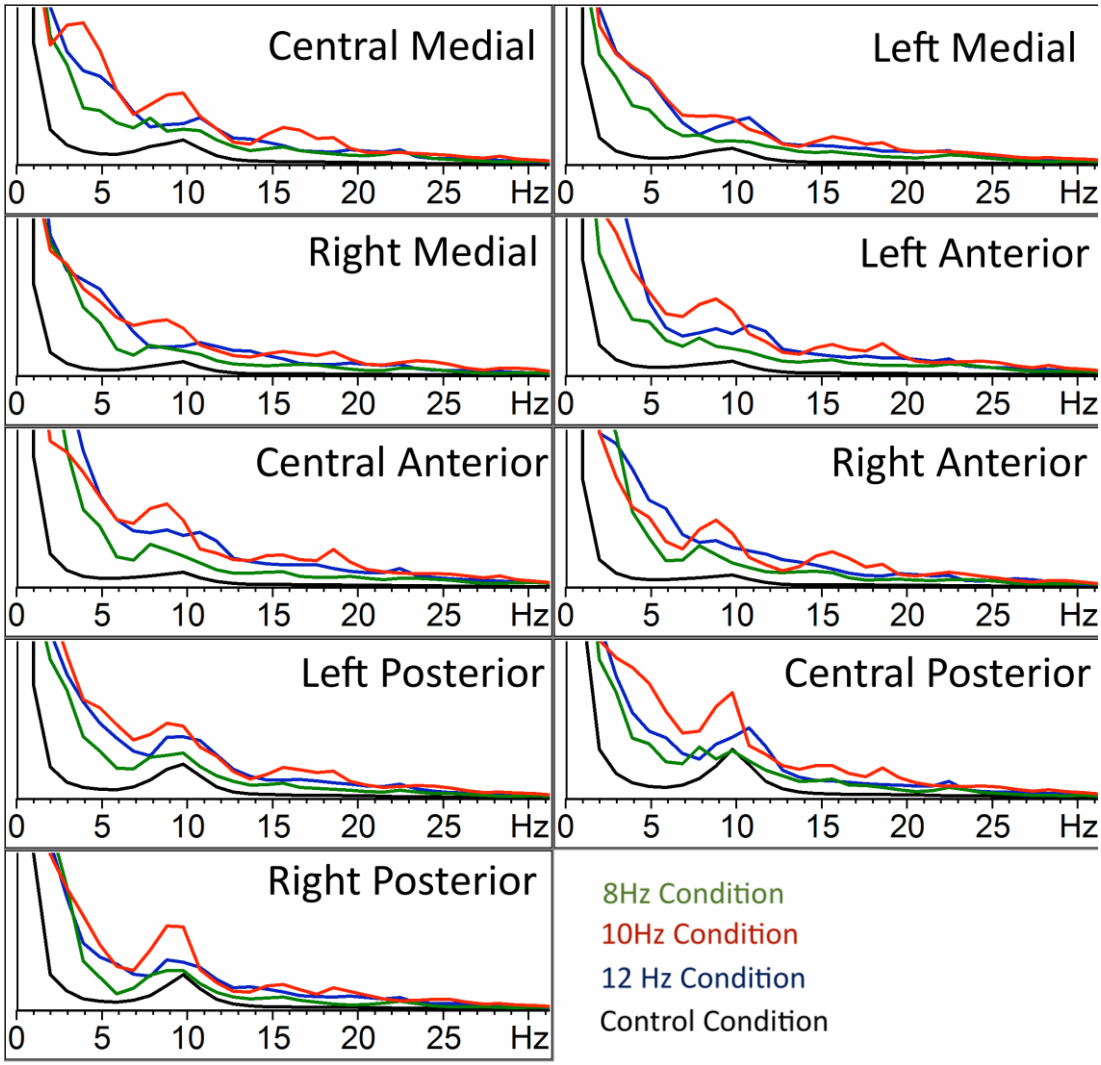
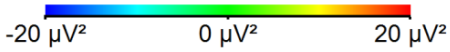
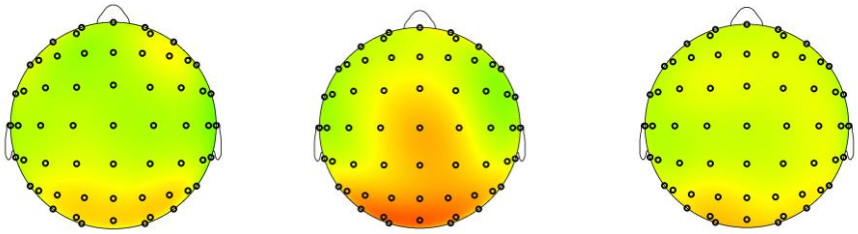


Figure 6.5 Alpha Power across Scalp Regions. The average alpha power was calculated for each scalp region Left Anterior (AF7, F9, F7, F5, F3) Central Anterior (Fp1, Fpz, Fp2, AF3, AFz, AF4, F1, Fz, F2), Right Anterior (AF8, F4, F6, F8, F10), Left Medial (FT7, FC5, FC3, T7, C5, C3, TP7, CP5, CP3), Central Medial (FC1, FCz, FC2, C1, Cz, C2, CP1, CPz, CP2), Right Medial (FC3, FC6, FT10, C4, C6, T8, CP4, CP6, TP8), Left Posterior (P7, P5, P3, PO7), Central Posterior (P1, Pz, P2, PO3, POz, PO4, O1, Oz, O2) and Right Posterior (P4, P6, P8, PO8). In black is the alpha activity observed during the 1Hz control entrainment. Activity during 8Hz, 10Hz and 12Hz visual entrainment is represented in green, red and blue respectively.

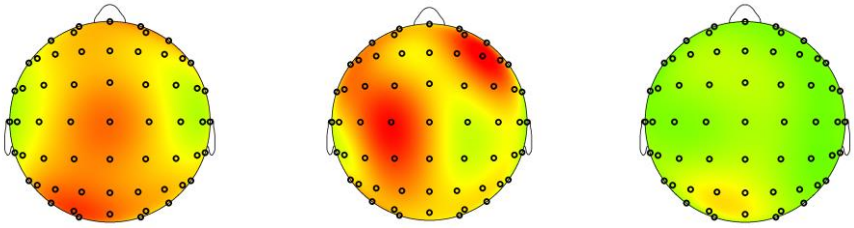


1Hz Control:



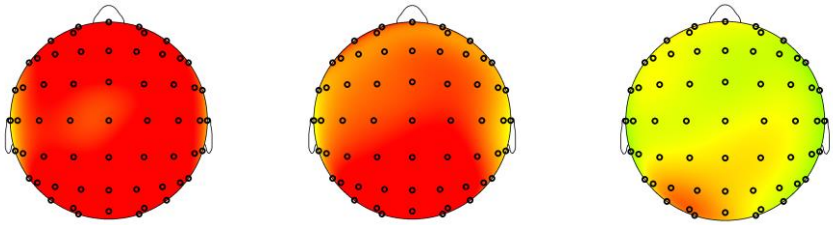
8Hz Band	10 Hz Band	12Hz Band

8Hz Stimulation:



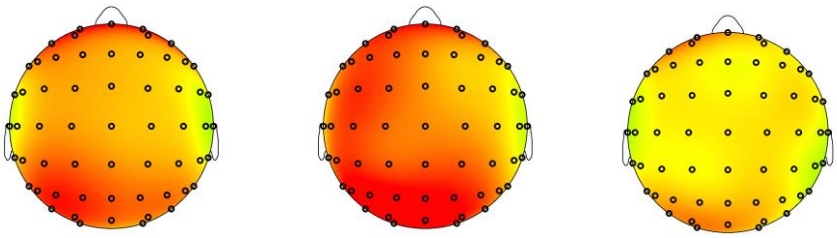
8Hz Band	10Hz Band	12Hz Band

10Hz Stimulation:



8Hz Band	10Hz Band	12Hz Band

12Hz Stimulation:



8Hz Band	10Hz Band	12Hz Band

Figure 6.6 Alpha Band Topographies. Topographies of alpha bands 8Hz (+/- 1Hz), 10Hz (+/- 1Hz), 12Hz (+/- 1Hz) following 1Hz control, 8Hz, 10Hz and 12Hz visual alpha stimulation.

4.1.4. Laser-Evoked Potentials

The mixed linear model revealed that there were no significant differences between the N2 peaks following alpha entrainment and control ($t(31) = -0.56$, $p=0.58$; $t(31) = -0.47$, $p=0.638$; $t(31) = -0.22$, $p = 0.823$ for 8Hz, 10Hz and 12Hz respectively). Changes in P2 were found to be significant after 10Hz entrainment ($t(31) = -3.81$, $p<0.001$), but not following 8Hz and 12Hz ($t(31) = -1.35$, $p = 0.180$ and ($t(31) = -0.171$, $p = 0.864$, respectively). The average reduction in P2 peak from control was -.89mV (SE 0.98; CI -2.84 to 1.05) following the 8Hz entrainment, -2.33mV (SE 0.99 CI; -4.29 to -0.37) following 10Hz and -0.085mV (SE 0.99; CI -2.04 to 1.87) following 12Hz stimulation. Refitting the model with a Bonferroni correction revealed that the average P2 peaks following 10Hz entrainment were significantly smaller than following the 8Hz ($t(31) = -3.52$, $p<0.01$) and 12Hz ($t(31) = -4.5$, $p<0.001$) entrainment sessions. Average peak amplitude following 8Hz and 12Hz did not differ significantly from one another ($t(31) = -1.39$, $p = 1.74$).

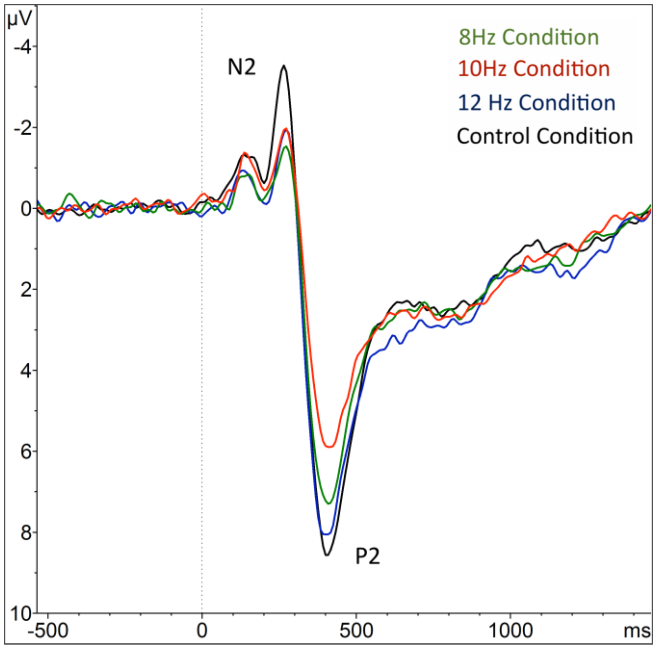
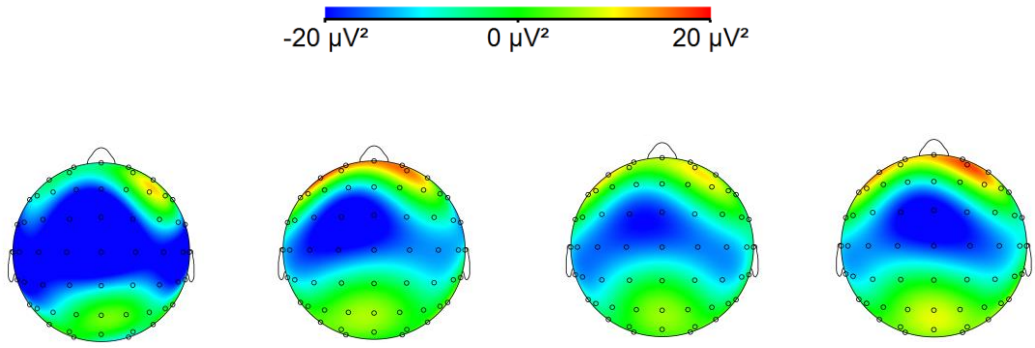
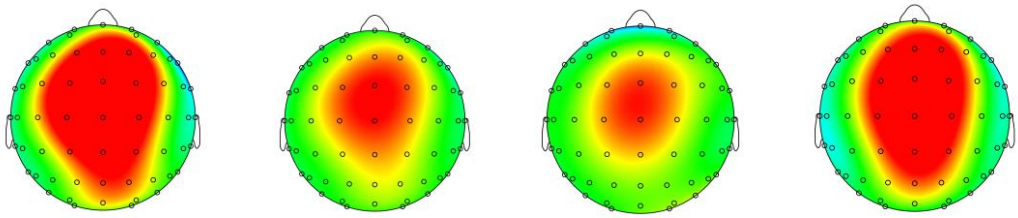


Figure 6.7 Laser-Evoked Potentials over Middle Central Electrodes. Graph shows the average LEP amplitude across all subjects. Nine electrodes (FC1, FCz, FC2, C1, Cz, C2, CP1, CPz, CP2) were pooled around the central Cz to produce an average N2P2 complex. The LEP following the control stimulation is depicted in black, the LEP in the 8Hz condition is represented in green, the 10Hz condition in red and the 12Hz condition in blue. Only the P2 peak in the 10Hz condition (red) showed a significant reduction from control ($p<0.001$)



Control N2 Peak	8Hz N2 Peak	10Hz N2 Peak	12Hz N2 Peak



Control P2 Peak	8Hz P2 Peak	10Hz P2 Peak	12Hz P2 Peak

Figure 6.8 N2 and P2 Peak Topographies. Topographies of the negative N2 (top) and positive (P2) peaks following visual stimulation at 1Hz (Control), 8Hz, 10Hz and 12Hz. Following 10Hz entrainment only, the P2 peak reduced significantly from control ($p<0.001$)

4.1.5. Sources of Laser-Evoked Potentials

The LORETA analysis identified 11 Brodmann areas that showed a significant difference in the P2 peak between the 10Hz and the control conditions ($p<0.05$). These areas are listed in the table below (Table 6.1). The main activity could be found in the Temporal Lobe, the Parietal Lobe, the Limbic Lobe, the Insula and the Occipital Lobe, with higher P2 source activity in the more painful control condition. There was no significant difference in the sources of the N2 peak between the 10Hz and the control condition.

Brodmann Area	Coordinates	Area
Brodmann area 39	X= 53 , Y= -74 , Z= 15	Middle Temporal Gyrus, Temporal Lobe
Brodmann area 30	X= 25 , Y= -67 , Z= 8	Posterior Cingulate, Limbic Lobe
Brodmann area 18	X= 4 , Y= -88 , Z= 22	Cuneus, Occipital Lobe
Brodmann area 31	X= 4 , Y= -74 , Z= 22	Precuneus, Parietal Lobe
Brodmann area 20	X= 53 , Y= -32 , Z= -20	Fusiform Gyrus, Temporal Lobe
Brodmann area 22	X= 67 , Y= -46 , Z= 8	Superior Temporal Gyrus, Temporal Lobe
Brodmann area 37	X= 60 , Y= -60 , Z= -6	Middle Temporal Gyrus, Temporal Lobe
Brodmann area 13	X= 39 , Y= 3 , Z= 1	Insula
Brodmann area 13	X= 32 , Y= 24 , Z= 1	Insula
Brodmann area 22	X= 53 , Y= 10 , Z= -6	Superior Temporal Gyrus, Temporal Lobe
Brodmann area 22	X= 67 , Y= -18 , Z= 1	Superior Temporal Gyrus
Brodmann area 7	X= -31 , Y= -60 , Z= 43	Inferior Parietal Lobule, Parietal Lobe
Brodmann area 7	X= 4 , Y= -74 , Z= 50	Precuneus, Parietal Lobe
Brodmann area 7	X= 18 , Y= -67 , Z= 29	Precuneus, Parietal Lobe
Brodmann area 37	X= -45 , Y= -46 , Z= -27	Inferior Temporal Gyrus, Temporal Lobe
Brodmann area 37	X= -52 , Y= -46 , Z= -27	Fusiform Gyrus, Temporal Lobe

Table 6.1 LORETA P2 Results. Brain regions showing cortical differences in current density when comparing P2 in the 10Hz entrainment and the 1Hz control conditions, quoted in Talairach coordinates. For all results, $t(p<0.05) = 3.54$ for single voxels. Negative X coordinates signify left hemisphere activation.

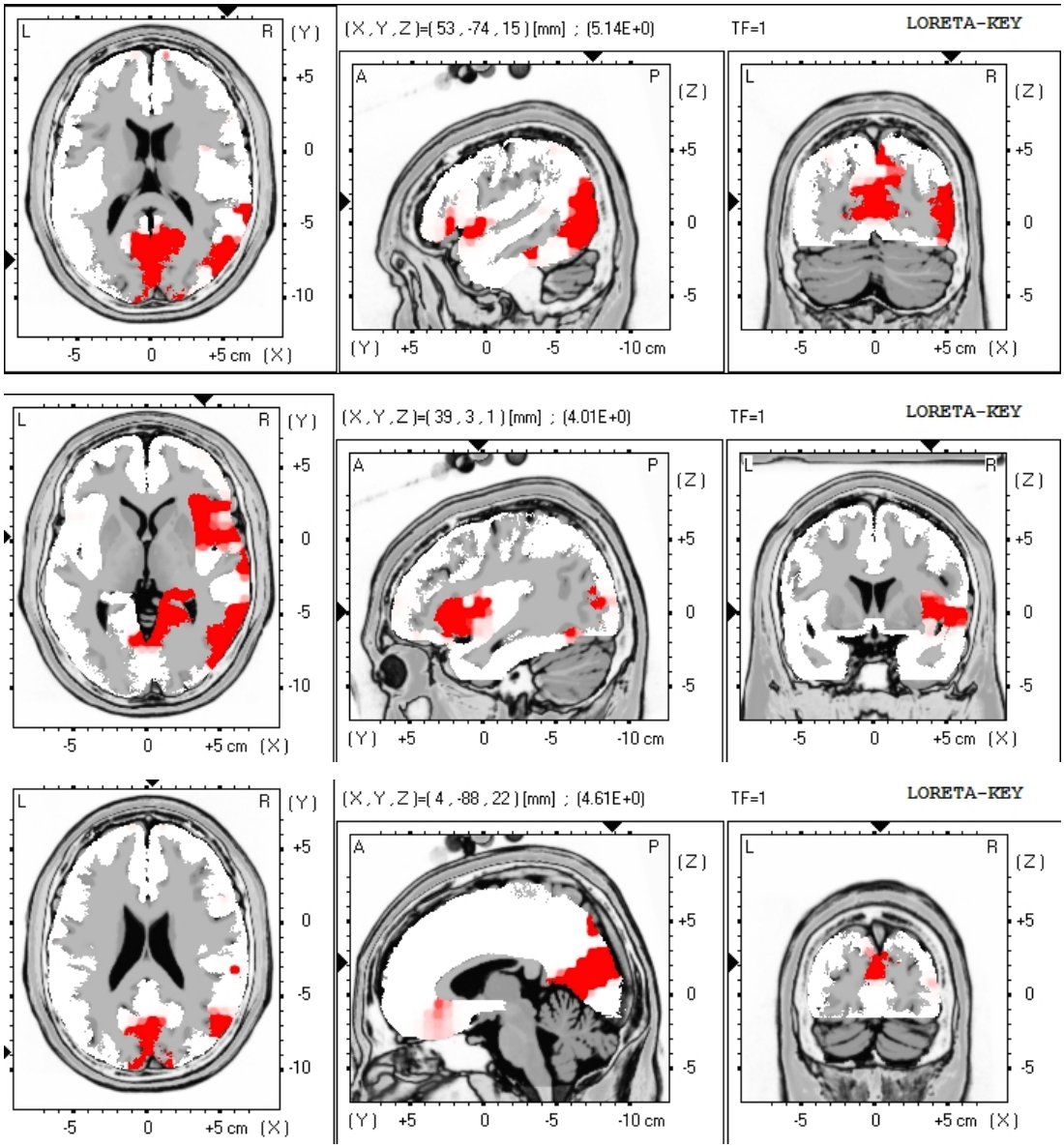


Figure 6.9 LORETA P2 Results. LORETA estimated brain regions displaying significant differences in P2 in the 10Hz alpha entrainment and 1Hz control condition. Significantly higher P2 peak activation, displayed in red, was observed in the temporal lobe, parietal lobe, occipital lobe and the limbic lobe in control condition, compared to the less painful 10Hz entrainment condition

4.1.6. Source Analysis of Alpha Activity

Significant changes in current density were found using LORETA when comparing the 10Hz alpha entrainment condition to the 1Hz control (p<0.05). The source analysis showed that 10Hz entrainment resulted in a significant increase in alpha power in the posterior cingulate and the precuneus when compared to control.

Brodmann Area	Coordinates (X,Y,Z)	Area
Brodmann area 23	X= 4 , Y= -60 , Z= 15	Posterior Cingulate, Limbic Lobe
Brodmann area 19	X= -3 , Y= -88 , Z= 36	Precuneus, Parietal Lobe

Table 6.2 LORETA Alpha Power Results. Brain regions showing cortical differences in current density when comparing the 10Hz entrainment and the 1Hz control condition, quoted in Talairach coordinates. For all results, t($p<0.05$) = 3.08 for single voxels. Negative X coordinates signify left hemisphere activation.

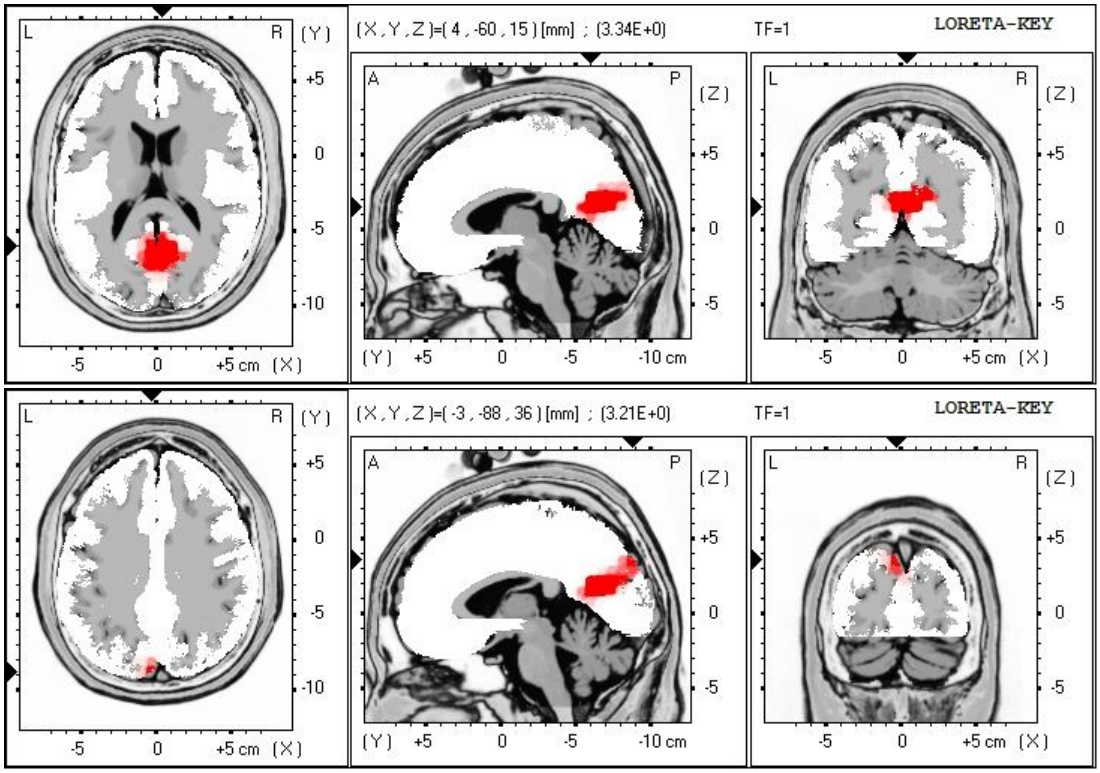


Figure 6.10 LORETA Alpha Power Results. Brain regions displaying significant differences in activation when comparing alpha power in the 10Hz and 1Hz condition, estimated with LORETA. Increased activation of the posterior cingulate (above) and the precuneus (below) can but observed following 10Hz entrainment compared to control.

5. Discussion

The study revealed that ten minutes of visual alpha stimulation at 8Hz, 10Hz and 12Hz significantly reduced verbal pain ratings, with the largest effect following the 10Hz entrainment. The electrophysiological results demonstrate a significant reduction of the positive P2 peak following the 10Hz entrainment session. The majority of electrode scalp regions showed a significant power increase from the matching control band ensuing the 8Hz and 10Hz entrainment. As the 10Hz entrainment resulted both in the largest behavioural and electrophysiological reduction, it was selected for source localisation analysis. Widespread sources of alpha power and LEP peak amplitude displayed a significantly different activation between the entrainment and control condition. An overlap of alpha power and P2 peak source results in the posterior cingulate cortex and precuneus suggests these areas may mediate the effects of visual alpha stimulation on pain suppression.

Alpha entrainment and Behavioural Pain Ratings

A significant reduction in pain ratings was found following all three entrainment conditions, confirming alpha entrainment across the range was successful at eliciting an analgesic response. When considering the entrainment data, a significant condition effect in both the 8Hz and the 10 Hz condition can be observed, confirming visual stimulation was successful at entraining not only the targeted cortex, but also numerous scalp electrode regions. Behavioural pain ratings were still reduced in response to the 12Hz driving frequency even though no evidence that the brain was entrained by visual stimulation at this frequency was found. This could imply that stimulation in the alpha range is enough to reduce the perception of pain, rather than entrainment itself. It could be postulated that pain ratings after alpha entrainment are lower than in the control as the slower 1Hz flicker may have less of a disorientating or distracting effect outlasting the stimulus, than the faster alpha frequencies. However, attributing the diminished perception of the stimulus to distraction, caused by the more intense, higher frequency stimuli, does not explain the 10Hz condition having a larger analgesic effect than the 12Hz stimulation.

Another possible explanation for the disconnect between power increase and acute pain analgesia in the 12Hz condition, is that 12Hz stimulation may result in an increase in the 10Hz (+/- 1Hz) alpha band, and not the 12Hz (+/- 1Hz) band. Previous studies have shown non-rhythmic stimulations and frequencies other than 10Hz can result in increased power at 10Hz (VanRullen and Macdonald 2012). It is possible that the reduction in pain ratings following the 12Hz stimulation is less than following the 10Hz entrainment, as 12Hz stimulation does not increase the 10Hz frequency band as effectively as the 10Hz flicker, and analgesia is linked to a mid-alpha power increase. Work by Nir and colleagues suggest that only lower alpha power (7Hz- 10Hz) has an inverse relationship with pain ratings. Our results may potentially be in line with these findings, as the reduction in pain ratings after the 12Hz stimulation may not have been caused by a 12Hz entrainment, but conceivably by an increase in 10Hz (Nir, Sinai et al. 2012).

Entrainment and Laser-evoked Potentials

Numerically, reductions in P2 peaks matched the entrainment results; with more wide spread entrainment resulting in larger decreases in P2 peak amplitude. The statistically significant and numerically largest reduction in P2 occurred following the 10Hz stimulation, which had a significant condition effect entraining a total of 8 of the 9 scalp regions. The numerically second largest reduction in P2 peak amplitude was observed following the 8Hz stimulation which successfully entrained 7/9 scalp regions, two less than following the 10Hz stimulation. The numerically smallest P2 amplitude reduction was observed following the 12Hz stimulation, were no significant entrainment was recorded (0/9 scalp regions). These results are in line with our hypothesis that a greater entrainment of alpha power is paired with reduced electrophysiological responses.

Reductions in P2 amplitude following the 12Hz stimulation may not have been statistically significant due to the lack of entrainment. However, P2 peak amplitude succeeding 8Hz stimulation also did not display a significant reduction, despite having significantly entrained 7/9 electrode scalp regions. It is thus vital to not place

too much weight on the numeric P2 peak reductions, as following the 8Hz and 12Hz stimulation, these were not significantly different from control. Although we can state with confidence, that 10Hz entrainment reduces the laser-evoked responses significantly through entrainment, from the present study it is not possible to determine whether P2 amplitudes in the 8Hz and 12Hz conditions would have reduced significantly had entrainment been more successful following the 8Hz and 12Hz stimulation.

Laser-Evoked Potentials and Pain Ratings

The numeric reduction of P2 amplitudes followed a similar trend to the behavioural pain ratings with the largest reduction ensuing the 10Hz entrainment, followed by 8Hz and then the 12Hz stimulation. Taken together with the observed changes in alpha power, these results are in line with our hypotheses. Nevertheless, discrepancies between the behavioural ratings and the electrophysiological responses should not be overlooked. Although following all three entrainment conditions a significant reduction in pain ratings was observed, N2 peak amplitudes and P2 peak amplitudes following the 8Hz and 10Hz entrainment were not significantly different from control peak amplitudes. These inconsistencies emphasise that cortical changes liable for a reduction in pain ratings are but a fraction of the changes evoked succeeding noxious stimulation. Both N2 and P2 peak amplitudes have been found to change independently of pain ratings (Colloca, Tinazzi et al. 2008). The LEP waveform is influenced by multiple cortical and external inputs, rather than being an unpolluted measure of pain perception.

The reason for a selected effect of alpha entrainment of P2 peak amplitude is unclear, but it is known that N2 and P2 peaks may be influenced independently by sensory, affective and cognitive factors (Clark, Brown et al. 2008, Iannetti and Mouraux 2010). The N2 component of the LEP complex is believed to be affected by both nociceptive and non-nociceptive somatosensory stimuli (Bentley, Watson et al. 2004, Mouraux and Iannetti 2009). Multimodal activity (i.e. activity elicited by stimulus of other sensory modalities) also influence part of the N2 components but predominantly influence the P2 peak amplitude. The P2 peak can be increased

independently of N2 when attending to a stimulus, and is reduced by distractions (Bentley, Watson et al. 2004, Boyle, El-Deredy et al. 2008). Cognitive and affective components of pain, including attention, are believed to exclusively manipulate P2 peak amplitude, regardless of pain intensity (Siedenberg and Treede 1996, Zaslansky, Sprecher et al. 1996, Bentley, Watson et al. 2004, Brown, Seymour et al. 2008). High levels of alpha activity appear to be incompatible with high states of arousal. The reductions in P2 amplitude in the current study might therefore be a result of pain attention deficiency, generated by alpha band entrainment, which did not affect the earlier N2 peak.

Source Localisation

Cortical sources showing differences in P2 peak activation between the 10Hz and the control condition are spread over the posterior regions of the brain including the occipital lobe, the limbic lobe, the temporal lobe and the parietal lobe. Occipital changes were not unexpected as this region was being entrained by the visual stimulus. Additionally, spontaneous alpha activity is found to be maximal in the occipital region (Palva and Palva 2007), making it a likely candidate for influencing the pain response.

Differences in activation in the insular cortex were not unexpected. The insula plays a vital role in producing an emotionally relevant context for noxious and non-noxious sensations. Functional imaging data confirms the insula's involvement in the perception of pain and pain-associated emotions including fear, anger and sadness (Phan, Wager et al. 2002). Additionally, magnitude of pain perceived from a noxious stimulus is believed to be assessed in the insular cortex (Baliki, Geha et al. 2009). As the noxious input in the present study did not alter, but the pain ratings did, it could be postulated that activity in the insular cortex may have contributed to the reduction in the pain response, following entrainment.

The insular cortex is activated through pain-related activity in the thalamus, distinguishing pain from other homeostatic emotions (Sanfey, Rilling et al. 2003). Alpha activity is largely generated in thalamocortical feedback loops of excitatory

and inhibitory nerve cells (Steriade, Gloor et al. 1990, Lopes da Silva 1991). Changes in thalamo-cortical alpha activity may in turn influence the activity of the insula, regulating the behavioural and electrophysiological pain response.

Both the P2 amplitude and alpha activity at 10Hz showed a significantly higher activation of the precuneus and posterior cingulate when comparing the control to the 10Hz entrainment condition. The precuneus is known to be involved in shifting attention to different spatial locations (Wenderoth, Debaere et al. 2005). Changes in activity between the two conditions might imply that changes in attention caused by alpha entrainment, reduce sensitivity to the spatial location of the painful stimulus, and hence making it harder to distinguish sensory characteristic of the stimulus such as its intensity. However, as the early negative component of the LEP waveform is believed to be modulated by selectively attending to the spatial location of pain (Bentley, Watson et al. 2004, Mouraux and Iannetti 2009), it is surprising no changes in N2 peak were detected, and questions the validity of this theory.

Activations of the posterior cingulate cortex have been extensively found in both experimental and clinical pain literature (Nielsen, Balslev et al. 2005). The posterior cingulate is believed to be activated by Aδ fibres via the spino-thalamic tract (Bromm, Scharein et al. 2000). The latencies of LEPs recorded in the present study are consistent with the conduction velocity of Aδ fibres, which can be activated by heat lasers (Bromm, Jahnke et al. 1984, Bromm and Treede 1987).

Using dipole source localisation of LEP data, Bentley et al. demonstrated a consistent activation of the posterior cingulate along with anterior cingulate, suggesting an involvement in visuospatial processing and aversive conditioning (Bentley, Youell et al. 2002). Thermal pain specific signal changes in the posterior cingulate have also been observed in fMRI studies (Gelnar, Krauss et al. 1999). Gelnar and colleagues interpreted this result to reflect a direct spinothalamic input to the posterior and caudal ‘visuospatial’ regions (Vogt, Derbyshire et al. 1996), and proposed a somatosensory role for the posterior cingulate cortex (Gelnar, Krauss et

al. 1999). A PET study by Tolle et al described a correlation between increases in blood flow to the posterior cingulate and pain intensity (Tolle, Kaufmann et al. 1999). Our results display greater activity in the posterior cingulate in the high P2 amplitude, high pain control condition. Higher P2 activation of the posterior cingulate in the control condition may hence reflect a more intense perception of the noxious heat laser stimulus. Higher activation of the posterior cingulate could also be observed during the 10Hz alpha entrainment compared to control. Although these two results appear to contradict each other, the posterior cingulate may play a role in interpreting changes in pain intensity, regardless of the direction of change.

The most caudal part of the posterior cingulate has consistently been associated with salience of experimental stimuli (Maddock 1999). P2 peak sources revealed higher activity in the posterior cingulate in the more painful control condition, compared to the 10Hz entrainment condition. Changes in LEP amplitude have in addition to pain intensity also been linked to salience and non-painful adverse events (Garcia-Larrea, Peyron et al. 1997, Legrain, Guerit et al. 2002, Iannetti and Mouraux 2010). As alpha power is commonly accepted to be incompatible with high states of arousal, it is possible that entrainment at 10Hz reduces the attention paid to the intensity of the painful stimuli, making them seem less salient. Further research investigating the relationship between the posterior cingulate, saliency and alpha activity is needed to provide a more comprehensive overview of the underlying mechanisms of attention in pain perception.

Limitations and Further Research

Several limitations within the present study should be the initial focus in future investigations. Only the perception of acute pain, as a result of a heat laser stimulus was investigated. Whether our results are specific to the perception of pain, or whether changes in alpha power influence the perception of other sensory modalities calls for further investigation. Furthermore, as the volunteers selected for the study were all healthy young adults; future research should concentrate on expanding current findings to the older and chronic pain population. The present

study focuses exclusively on frequencies in the alpha range, in part, as it is known to be entrained more readily than other frequencies (Romei, Gross et al. 2010, de Graaf, Gross et al. 2013). However, additional bands have shown promising preliminary results (Gross, Schnitzler et al. 2007, Zhang, Hu et al. 2012), and should be explored for entrainment to assess subjective experiences and electrophysiological responses to acute pain.

In conclusion, current findings extend prior research regarding the characteristics of alpha power entrainment, and its effect on the behavioural and electrophysiological pain response. The present study reveals frequencies central to the alpha range provided the largest analgesic effect, and resulted in significant decreases in the electrophysiological response, upon successful entrainment. Whether these modulations are specific to the frequency entrained, or the magnitude of alpha-band power increase, requires further research.

CHAPTER 7

Modulating Pain through Visual Alpha Stimulation in Osteoarthritis Patients: a role for the Insula Cortex

This Chapter is based on a manuscript that is being prepared for submission to PAIN.

1. Abstract

Reductions in clinical and experimental pain are correlated with an increase in power of the oscillatory alpha band. In an attempt to modulate the perception of a moderately painful stimulus in osteoarthritic patients, a flickering visual stimulus was presented at 8Hz, 10Hz and 12Hz, with the aim to increase the analgesic cortical alpha power. Verbal numeric pain ratings and electrophysiological recordings were collected in order to quantify the magnitude of change of the pain response. A significant reduction in pain ratings was observed following all three alpha stimulation sessions compared to a 1Hz control, despite a lack of alpha power increase. A significantly reduced response in the laser-evoked potential was also observed following the 12Hz visual stimulation. Laser-evoked activity in the posterior insula proved to be significantly reduced following alpha stimulation, suggesting a functional role in the reduction of pain intensity triggered by alpha stimulation. We have shown that in osteoarthritis patients, a significant reduction in the perception of moderately painful experimental pain can be achieved through exposure to a visual stimulus oscillating across the alpha band range.

2. Introduction

Chronic pain resulting from Osteoarthritis (OA) is an increasingly common health problem. More than 6 million people in the UK have painful OA in one or both knees (Lacey, Thomas et al. 2008), and over 8.5 million have X-ray evidence of OA in the spine (Pye, Reid et al. 2004). As the population ages, chronic pain as a result of OA is likely to increase. Despite the increasing prevalence, only a limited number of medications currently alleviate the symptoms of chronic pain (Goldenberg 2007, Clauw 2010). As the severity of chronic pain is rarely correlated with the extent of tissue damage (Frymoyer, Newberg et al. 1984, Jensen, Brant-Zawadzki et al. 1994, Bedson and Croft 2008), the inefficacy of drugs can in part be attributed to poor understanding of the neural mechanisms underlying the pathophysiology of chronic pain. Neuro-modulatory and cognitive interventions such as mindfulness-based cognitive therapy have shown to modulate abnormalities of pain anticipation (Brown and Jones 2010) by enhancing top-down cortical control mechanisms. Approaches to therapy that enhance these top-down control mechanisms may therefore be more promising than pharmacological treatments.

Osteoarthritic pain relief resulting from the administration of the opioid-like agent tramadol is paired with an increased measure of cortical oscillatory alpha power (7-14Hz), as measured by electroencephalography (EEG) (Freye and Levy 2006). Evidence of the amplified presence of the cortical alpha rhythm in acute pain relief is abundant (Bromm 1986, Bromm, Ganzel et al. 1986, Chang, Arendt-Nielsen et al. 2001, Chang, Arendt-Nielsen et al. 2001, Kakigi, Nakata et al. 2005, Huber, Bartling et al. 2006). Alpha rhythm neurofeedback training has thus been attempted for the amelioration of chronic pain with promising results (Kayiran, Dursun et al. 2010, Jensen, Gertz et al. 2013). However, neurofeedback involves numerous training sessions over a prolonged period of time, requiring long-term commitment and motivation from patients. No work has thus far been completed investigating the immediate effect of temporarily increasing alpha power, through an external stimulus, on the perception of acute and chronic pain in osteoarthritic patients.

Converging evidence suggests patients suffering from chronic pain may have central nervous system abnormalities, influencing pathophysiology and resulting in enhanced processing of pain (Mease, Hanna et al. 2011, Schmidt-Wilcke and Clauw 2011, Jones, Huneke et al. 2012, Brown, El-Deredy et al. 2014).

In the present study, the main aim was to investigate the effect of alpha entrainment on the perception of, and electrophysiological response to, an acute noxious stimulus in OA patients. Albeit incomplete, experimental pain offers a relevant model of brain activity during osteoarthritic pain (Kulkarni, Bentley et al. 2007). Results of the present study may provide an insight to the mechanisms underlying persistent, or recurrent osteoarthritic pain. To investigate the analgesic effect of increased alpha power across the frequency range, a flickering visual stimulus was presented at 8Hz, 10Hz and 12Hz. Visual entrainment at 7-14Hz, through rhythmic photic stimulation, allows an almost immediate increase in alpha band power (Timmermann, Lubar et al. 1999, de Graaf, Gross et al. 2013). The aim of the study was to determine the effect of alpha entrainment on the perception of a moderately painful stimulus, whilst monitoring participants' osteoarthritic pain. The participants' pain was assessed by means of verbal pain ratings and electrophysiological responses. As patients suffering from osteoarthritis tend to be older adults, it was hypothesised that the largest amelioration in pain scores would be observed following the 8Hz visual stimulation, as the peak alpha frequency deviates lower from the typical 10Hz with age (Dustman, Shearer et al. 1993, Klimesch 1999).

3. Materials and Methods

3.1.1. Ethics statement

The protocol of this study was ethically approved by the NRES Committee North West – Liverpool Central (reference number 13/NW/0007). All study participants had to provide written informed consent according to the International Conference on Harmonisation Good Clinical Practice guidelines, prior to participating in the study.

3.1.2. Study Participants

Twenty-one self-reported right-handed volunteers with the diagnosis of painful Osteoarthritis (OA) (9 Males, 12 female, mean age 54.9 years ±9.62 SD, with a range of 38-72 years) were recruited. Although OA was the primary diagnosis of all participants, 5 were additionally diagnosed with Fibromyalgia (FM). All OA and FM patients fulfilled the American College of Rheumatology criteria for the diagnosis of OA (Altman et al., 1986; Arnett et al., 1988) and FM (Wolfe et al., 2011). All participants had to be aged 18 or over, and were excluded from the study if their medical record showed, or they verbally informed us of a morbid psychiatric illness (e.g. major depression, schizophrenia, bipolar disorder), neurological disorder, ischemic heart disease, uncontrolled high blood pressure, cardiovascular disease, chronic skin disease (e.g. eczema, psoriasis) hypertension not controlled by medication, or a history, or family history of epilepsy. Sub-clinical levels of anxiety and depression are not uncommon in OA and FM populations. Patients in the category were identified by pre-experimental questionnaires but were not excluded from the study. All participants were receiving their usual medication, analgesic (most frequently paracetamol (non-opioid analgesic), Meloxicam (NSAID COX-2), Celecoxib (NSAID COX-2) and Naproxen (NSAID)) or other, at the time of the study. A record was made of all medication participants were taking at the beginning of the visit. Three participants were excluded prior to the start of the experiment perceiving the laser stimulus as painful only at a laser voltage deemed unsafe, and potentially harmful to the skin. Participants were unaware of the aims and objectives of the study.

3.1.3. Pre-experimental questionnaires

Questionnaires were completed by participants at the beginning of the visit, after signing the consent form. All participants were assessed by a trained research nurse, who carried out a tender point examination, scored by use of the Manuel Tender Point Survey (methodology published by Okifuji et al., 1997). All participants completed the Western Ontario and McMaster Universities Arthritis Index (WOMAC), Health Assessment Questionnaire Disability Index (HAQ-DI) and the

Fibromyalgia Impact Questionnaire (FIQ) to assess the patients' condition according to standardised measures. Although the HAD-DI is designed for the assessment of Rheumatoid Arthritis and the FIQ is tailored to FM patients, all participants were asked to complete these questionnaires for consistency. Participants were additionally asked to complete the Participant Health Questionnaire-9, the Pain Catastrophizing Questionnaire, the State and Trait Anxiety Inventory and the Karolinska Sleepiness scale in order to assess depressive symptoms, pain related catastrophic thinking, anxiety and sleepiness.

An assessment of the participants' current osteoarthritic pain, and a measure of their osteoarthritic pain over the last two weeks was acquired through a clinical pain 0-10 visual analogue scale (VAS) scale, with 0 marked as 'no pain' and 10 as 'very severe'.

3.1.4. Experimental Procedure

A CO_2 heat laser was used to induce an acutely painful stimulus to the dorsal surface of the right forearm, through the activation of nociceptors located in the skin (Meyer et al., 1976). The laser stimulus occurred every 10 seconds, with a 150ms duration and a 15mm stimulated surface diameter. To avoid sensitization, habituation or skin damage by portions of the forearm being excessively stimulated, the laser was relocated at random over a 3 x 5cm area after each stimulus. Participants were obliged to wear safety spectacles every time the laser was in use.

Prior to the start of the experiment, a psychophysics procedure was initiated using a 0-10 numeric ratings scale. Level 4 on the scale marked the pain threshold, level 7 indicated a moderately painful level, and level 10 represented the maximum pain the participant could tolerate. A ramping procedure was conducted a minimum of three times, to determine the average laser energy needed to attain a level-7 (moderately painful) rating, as executed previously (Brown et al., 2008)

The experimental procedure was initiated with the rating of 30 individual laser pulses at the level-7 (moderately painful) intensity. A current rating of clinical pain,

sleepiness (KSS) and anxiety (STAI- state) was then recorded as a baseline measure. Participants were asked to fasten a pair of LED goggles, and received 10 minutes of four randomised visual 'on/off light' flashing stimulations: 8Hz flashing, 10Hz flashing, 12Hz flashing or 1Hz (control) flashing. Each stimulation session was followed by the rating of 30 level-7 laser pulses, the KSS, the STAI-state and current clinical pain on the VAS.

Experimental Procedure

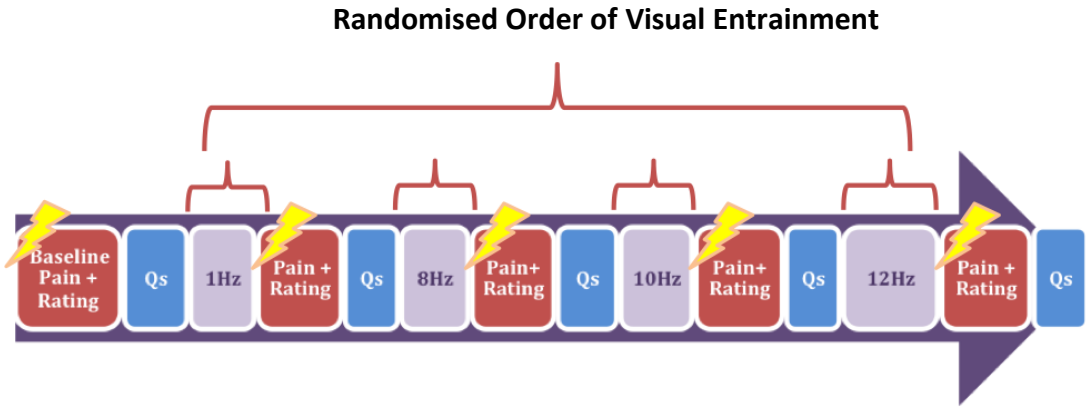


Figure 7.1 Experimental Procedure. All study participants were subjected to 30 pulses of their 'level-7' pain (Baseline Ratings). Participants were then asked to fill out a set of baseline questionnaires (Sleep Questionnaire, PCS, PHQ-9, PASS, POMS, STAI, KSS). Participants were presented with four visual stimulation sessions (using flashing LED goggle) at 8Hz, 10Hz, 12Hz and 1Hz (control), of 10 minute duration, in a randomised order. Participants were asked to rate 30 pulses of 'level-7' pain following each visual stimulation session. Following each pain rating session, volunteers were asked to complete the KSS, STAI-state and POMS. **Qs:** Behavioural Questionnaires

3.1.5. Acquisition of EEG Data

Electrophysiological recordings were completed using electroencephalography (EEG) with 64 Ag/AgCl fixed surface electrodes positioned according to the extended standard 10-20 system (BrainAmp, Brain Products GmBH, Germany). EEG activity was recorded during the visual stimulation, and during the pain ratings sessions. Vertical and horizontal electro-oculograms were measured by a set of four electrodes in order to identify off line blink and eye-movement artefacts. The right

mastoid electrode acted as a reference for all other electrodes, and the AFz electrode was used as a ground. A sampling rate of 500Hz was used. Band-pass filters were set to DC- 100Hz and a 50Hz notch filter was applied to reduce electrical interference. EEG activity was recorded using BrainVision Recorder 1.10 (Brain Products GmBH, Germany).

3.1.6. Pre-processing of EEG data

Electrophysiological data were analysed using BrainVision Analyzer 2.0. Data from the pain session were down-sampled to 125Hz. After applying a 50Hz notch filter, data were segmented into epochs 1000ms prior to the painful stimulus and 1500ms after, to isolate the laser-evoked potential (LEP; resulting from stimulus presented at time 0). Ocular artefactual components were removed after performing independent components analysis (ICA), with 25 components and an Infomax (Gradient) Restricted Biased algorithm. 0 to 6 components (median of 4) were removed per analysis. Linear trends were removed from the segment with a DC detrend applied to the first 500ms and the last 100 of the epoch. The 500ms directly preceding the stimulus were used for baseline correction of the LEP. After referencing the data to a common electrode, segments containing LEPs were averaged for each condition across all subjects.

The N2 and P2 peaks of the LEP were selected for analysis. For each subject and condition, the N2 and P2 peak latencies were measured as the most negative point between 200ms-300ms, and the most positive point between 350-500ms after the stimulus, respectively. Electrical activity in the nine central electrodes was pooled (CPz, Cz, Fcz, CP1, CP2, C1, C2, FC2, FC1), and an average 20ms window of peak N2 and P2 amplitudes were extracted for statistical analysis.

3.1.7. Spectral Analysis

Data recorded during the 10 minute visual stimulation was referenced to the common average and a low cut off filter at 0.05Hz (12 dB/oct), a high cut off filter at 35 Hz (48 dB/oct) were implemented. A range of 1-7 (median 4) components were removed from the data following a 25 components ICA. The remaining data

was segmented into 1-second epochs and a fast-fourier transformation with a 10% Hanning window was applied. Power in the stimulated frequency bands 8Hz ±1Hz, 10Hz ± 1Hz, 12Hz ± 1Hz, was compared to activity in the control bands. The average power was calculated as frequency density (expressed in log units ($10*\log10(\mu V^2/Hz)$).

3.1.8. Source Localisation

The stimulation conditions that resulted in the largest decrease in P2 amplitude, N2 amplitude and increase in alpha power were selected for source localisation. Cortical sources were estimated using low-resolution electromagnetic tomography (LORETA), using the LORETA-KEY software (Lantz, Michel et al. 1997, Pascual-Marqui, Esslen et al. 2002). A spherical head model registered to the Talairach anatomical brain atlas was used (Talairach, Tournoux et al. 1988). Sources of alpha activity and LEP peaks were estimated to a $7mm^3$ resolution (2394 voxels) in grey matter volume (Mazziotta, Toga et al. 2001). With each voxel represented a single potential activity zone, the spatially smoothest source was calculated.

3.1.9. Statistical Analysis

All statistical analysis was performed in SPSS (v. 20). A *p* value of less than 0.05 was considered statistically significant. Using a mixed linear model we were able to determine significant differences in pain ratings in the three visual stimulation conditions, compared to control. Main effects in the model included baseline pain ratings as covariates, and condition and order as factors. To establish if there were differences in pain ratings between the visual stimulation conditions the model was individually refitted with a Bonferroni correction, with each condition as a reference catagory. An identical model was applied separately to the clinical pain ratings, the STAI-state and the KSS scores. The baseline questionnaires completed at the beginning of the visit were correlated to change in pain ratings, and change in N2 and P2 peaks from baseline.

Changes in N2 and P2 peaks from the control condition were also assessed by applying the mixed linear model independently to the positive and negative peaks, taking baseline peaks as covariates and session order and condition as main effect factors.

To assess the change in alpha power from control in each condition, the 64 scalp electrodes were first divided into 9 scalp regions: Left Anterior (AF7, F9, F7, F5, F3) Central Anterior (Fp1, Fpz, Fp2, AF3, AFz, AF4, F1, Fz, F2), Right Anterior (AF8, F4, F6, F8, F10), Left Middle (FT7, FC5, FC3, T7, C5, C3, TP7, CP5, CP3), Central Middle (FC1, FCz, FC2, C1, Cz, C2, CP1, CPz, CP2), Right Middle (FC3, FC6, FT10, C4, C6, T8, CP4, CP6, TP8), Left Posterior (P7, P5, P3, PO7), Central Posterior (P1, Pz, P2, PO3, POz, PO4, O1, Oz, O2) and Right Posterior (P4, P6, P8, PO8). Activity in each of the scalp regions was pooled across electrodes. A repeated measures ANOVA was completed for each condition, comparing the activity of the stimulated frequency band, to the activity of the same frequency band in the control condition. For each condition the ANOVA was run with factors Left/Central/Right x Anterior/Middle/Posterior x Control/Condition. Main effects and interactions were following up with paired two-tailed t-tests.

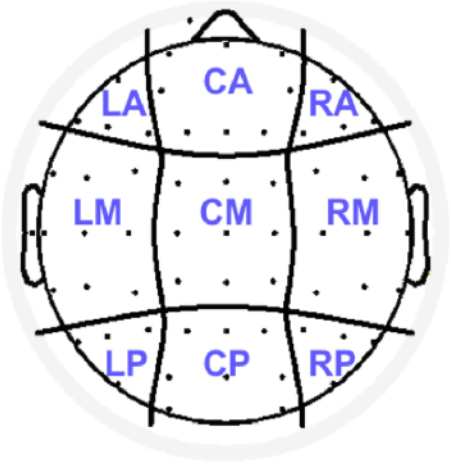


Figure 7.2 Map of Scalp Electrode Regions. Schematic representation of the division of electrodes into the 9 scalp regions: Left Anterior (AF7, F9, F7, F5, F3) Central Anterior (Fp1, Fpz, Fp2, AF3, AFz, AF4, F1, Fz, F2), Right Anterior (AF8, F4, F6, F8, F10), Left Middle (FT7, FC5, FC3, T7, C5, C3, TP7, CP5, CP3), Central Middle (FC1, FCz, FC2, C1, Cz, C2, CP1, CPz, CP2), Right Middle (FC3, FC6, FT10, C4, C6, T8, CP4, CP6, TP8), Left Posterior (P7, P5, P3, PO7), Central Posterior (P1, Pz, P2, PO3, POz, PO4, O1, Oz, O2) and Right Posterior (P4, P6, P8, PO8).

The statistical analysis on the sources identified by LORETA was completed using a voxel-wise binary t-test using non-parametric randomisation to compare the selected condition to the control condition separately for P2 amplitude, N2 amplitude and alpha power. To identify significant areas, LORETA produced a threshold (*t*) of activation for p<0.05.

4. Results

4.1.1. Behavioural Results

The numeric pain ratings of the moderately painful 'level-7' laser stimulus following the 8Hz, 10Hz and 12Hz visual stimulation, all reduced significantly from the stimulus ratings following the 1Hz visual control ($t(17) = -2.66$, $p<0.05$; $t(17) = -2.44$, $p<0.05$; $t(17) = -2.25$, $p<0.05$, respectively). Decreases in pain ratings from ratings in the control condition were -0.9097 (SE 0.342) following 8Hz, -0.8359 (SE 0.342) following 10Hz and -0.769063 (SE .342) points following 12Hz visual stimulation. Although pain ratings following 8Hz entrainment appeared to decrease more than following the other conditions, this difference was not significant.

Table Showing Changes in Acute Laser Pain Ratings from Control							
Parameter n = 18	**Change in Pain**	**Std. Error**	**df**	**t**	**Sig.**	**95% Confidence Interval**	
						Lower Bound	**Upper Bound**
12Hz Alpha	-0.77	0.342	17	-2.249	.028	-1.45	-0.08
10Hz Alpha	-0.84	0.342	17	-2.444	.018	-1.52	-0.15
8Hz Alpha	-0.91	0.342	17	-2.660	.010	-1.59	-0.22

Table 7.1 Numeric Pain Ratings. Changes in pain ratings of level-7, moderately painful laser pulses from control following the 8Hz, 10Hz, 12Hz visual alpha stimulation.

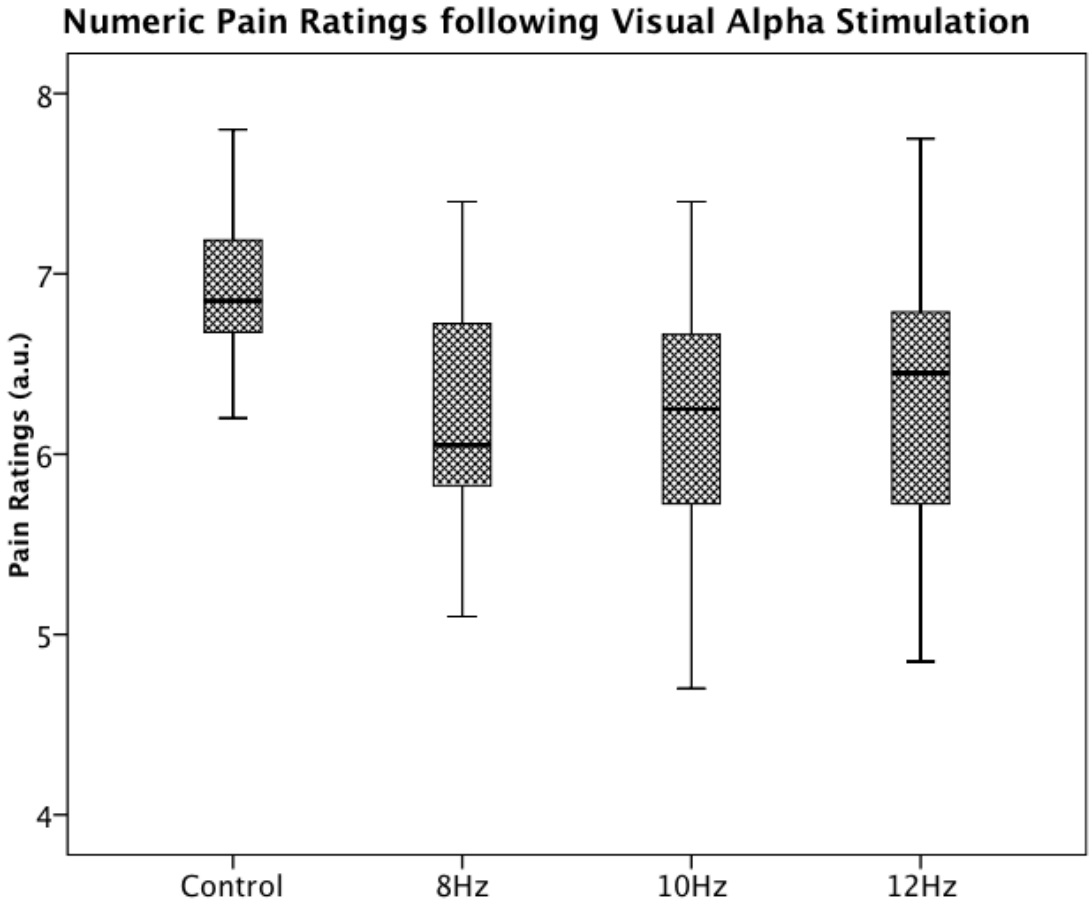


Figure 7.3 Pain Ratings following Alpha Stimulation. Averaged pain ratings on a 0-10 numeric ratings scale at baseline and following 1Hz control, 8Hz, 10Hz and 12Hz visual stimulation. Pain ratings are averaged across all subjects. Pain ratings following 8Hz, 10Hz and 12Hz stimulation are significantly lower than following 1Hz control stimulation ($p<0.05$)

4.1.2. Clinical Pain Ratings

Participants were asked to rate their osteoarthritic pain on a VAS following each entrainment session. A mixed liner modal was applied to determine changes in clinical pain rating from control, following each alpha stimulation condition. No significant decreases in clinical pain ratings were found following visual alpha stimulation.

Mixed Linear Model Results – Clinical Pain							
Parameter n = 18	**Change in Pain**	**Std. Error**	**df**	**t**	**Sig.**	**95% Confidence Interval**	
						Lower Bound	**Upper Bound**
12Hz	.394	.72	17	.548	.585	-1.04	1.83
10Hz	.088	.72	17	.123	.903	-1.35	1.52
8Hz	.194	.72	17	.270	.788	-1.24	1.63

Table 7.2 Clinical Pain. Changes in clinical pain ratings on the VAS from control rating following 8Hz, 10Hz and 12Hz visual stimulation

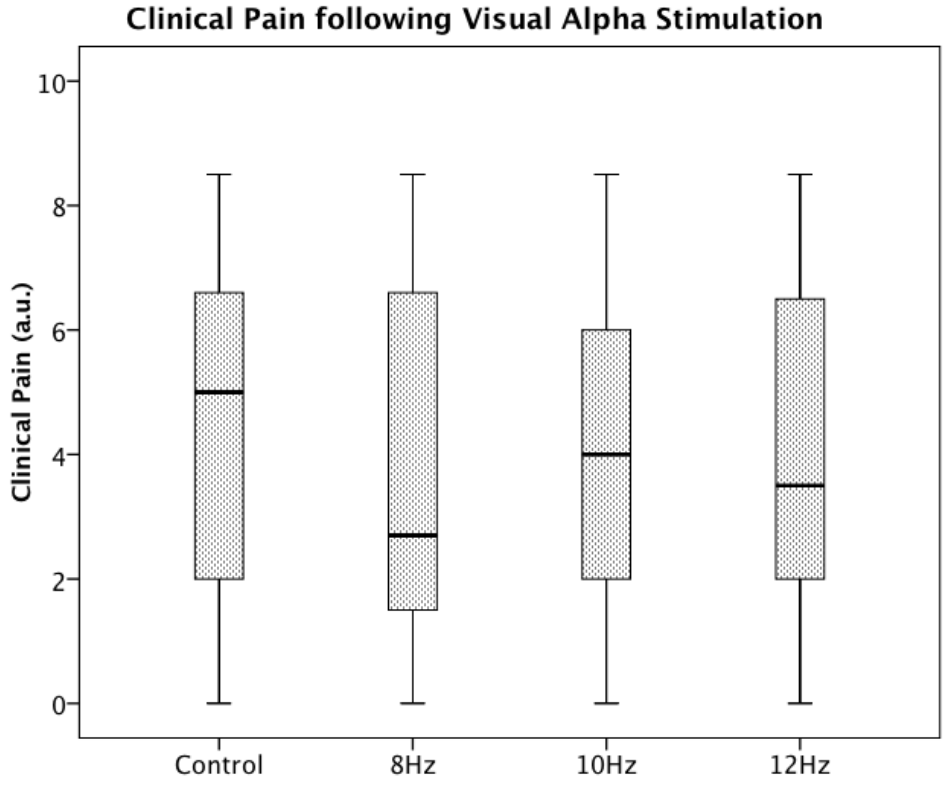


Figure 7.4 Changes in Clinical Pain. Following 8Hz, 10Hz and 12Hz visual stimulation, participants were asked to rate their clinical pain. Participants' clinical pain did not differ significantly from the control and from the baseline ratings.

4.1.3. Anxiety and Sleepiness

The STAI-state and the KSS were given to the volunteers following each of the visual stimulation conditions to assess the participants' current state anxiety and sleepiness. The mixed linear model revealed that the STAI-state and KSS scores did not differ significantly from control, or between conditions.

4.1.4. Electrophysiological Results

Following the 8Hz, 10Hz and 12Hz alpha entrainment, there was no change in the negative N2 peak amplitude. The P2 peak reduced significantly from control following the 12Hz entrainment ($t(17) = 2.14$, $p<0.05$), but not following the 8Hz or 10Hz entrainment conditions. The P2 peak significantly reduced in amplitude by an average of 1.15 mV (SE 0.54; CI 0.078 to 2.217), from control whereas following the 8Hz and 10Hz stimulation the reduction was 0.620 mV and 0.909 mV respectively (not significant).

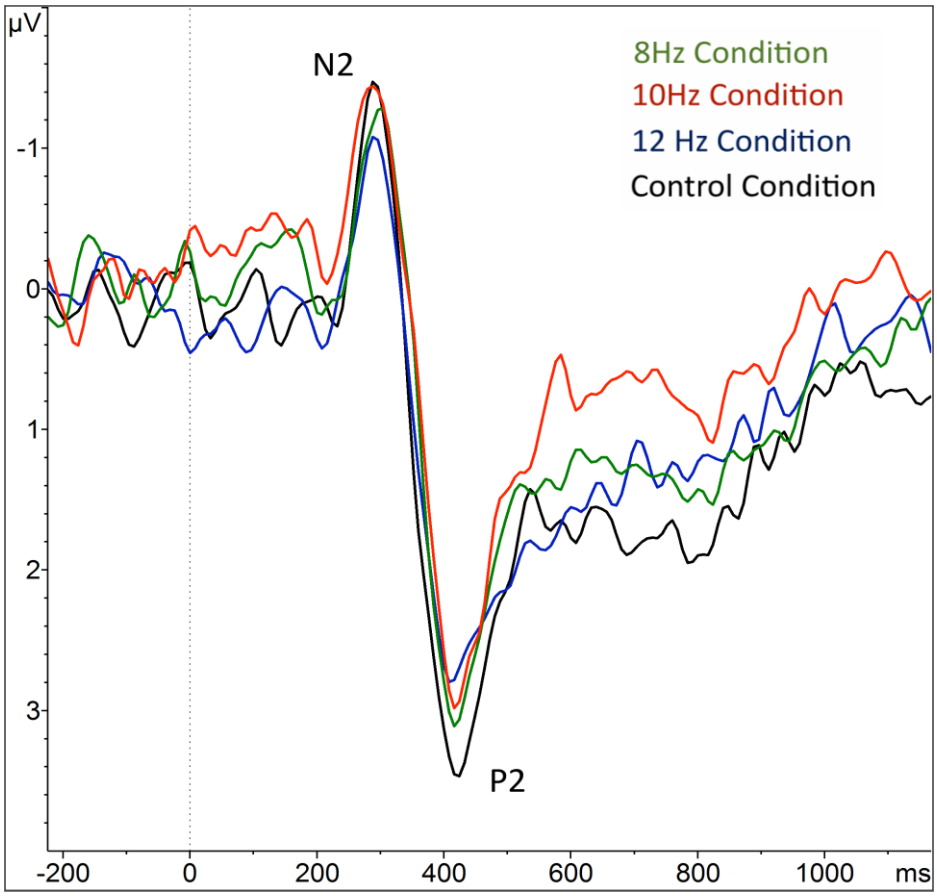


Figure 7.5 Laser-Evoked Potentials in the Central Middle Electrodes. The central 9 electrodes (FC1, FCz, FC2, C1, Cz, C2, CP1, CPz, CP2) were pooled to calculate the laser-evoked potential following the 8Hz stimulation (green), the 10Hz stimulation (red), the 12Hz stimulation (blue) and the control condition (black). The P2 peak following the 12Hz stimulation was significantly smaller than the P2 peak in the 1Hz control visual stimulation.

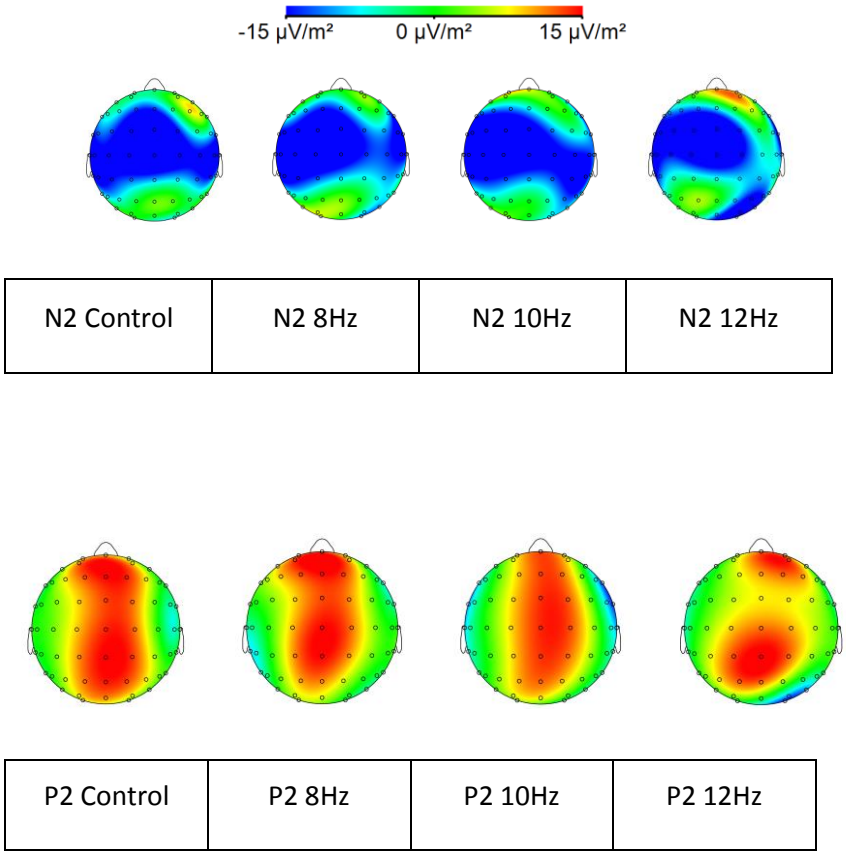


Figure 7.6 Topographies of LEPs. Topographical maps of the negative N2 peaks (top) and positive P2 peaks (bottom) following visual stimulation at 1Hz (Control), 8Hz, 10Hz and 12Hz.

4.1.5. Alpha Activity

To calculate the change in alpha activity, a repeated-measures ANOVA was run assessing interactions between the 9 scalp regions from left to right, and anterior to posterior for each condition compared to control. No significant effects or interactions were observed. Following the ANOVA with paired t-tests, comparing the power of the frequency stimulated to the matching frequency band in the control over the nine scalp regions, confirmed that no significant changes in alpha power were present. This suggested the visual stimulus did not result in significant entrainment.

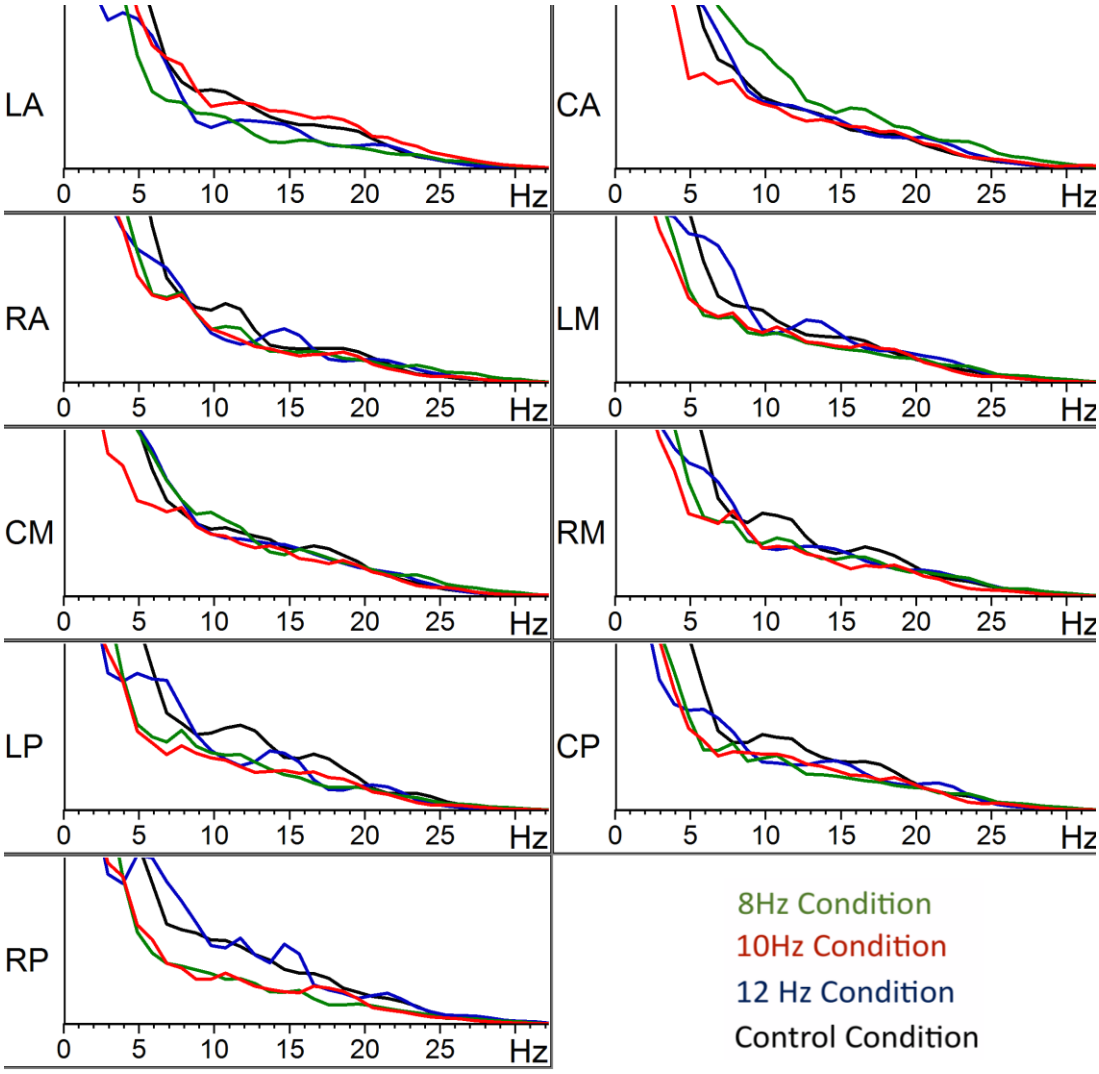
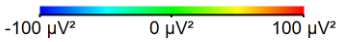
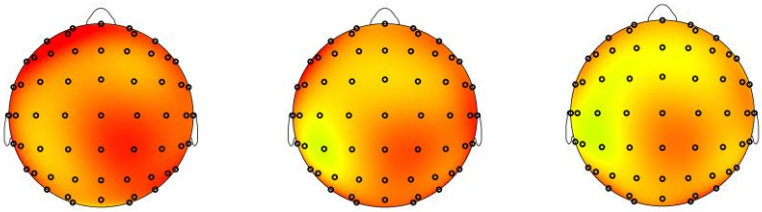
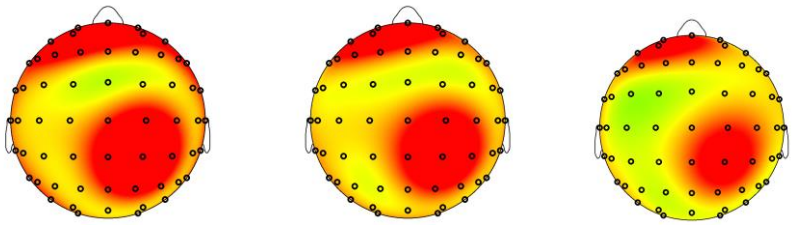


Figure 7.7 Alpha Power across Scalp Regions. The average alpha power was calculated for each of the 9 scalp regions. Depicted here are the Left Anterior, LA (AF7, F9, F7, F5, F3) Central Anterior, CA (Fp1, Fpz, Fp2, AF3, AFz, AF4, F1, Fz, F2), Right Anterior, RA (AF8, F4, F6, F8, F10), Left Middle, LM (FT7, FC5, FC3, T7, C5, C3, TP7, CP5, CP3), Central Middle, CM (FC1, FCz, FC2, C1, Cz, C2, CP1, CPz, CP2), Right Middle, RM (FC3, FC6, FT10, C4, C6, T8, CP4, CP6, TP8), Left Posterior, LP (P7, P5, P3, PO7), Central Posterior, CP (P1, Pz, P2, PO3, POz, PO4, O1, Oz, O2) and Right Posterior, RP (P4, P6, P8, PO8) region. Alpha activity during 1Hz stimulation is shown in black, 8Hz in green, 10Hz in red and 12Hz in blue.

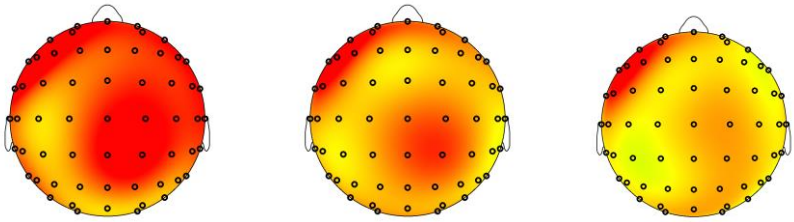




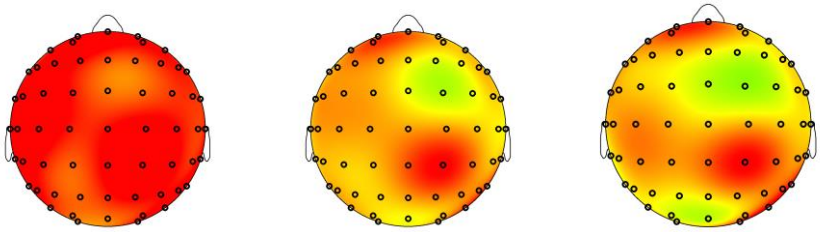
8Hz Band at 1Hz Stimulation	10Hz Band at 1Hz Stimulation	12Hz Band at 1Hz Stimulation



8Hz Band at 8Hz Stimulation	10Hz Band at 8Hz Stimulation	12Hz Band at 8Hz Stimulation



8Hz Band at 10Hz Stimulation	10Hz Band at 10Hz Stimulation	12Hz Band at 10Hz Stimulation



8Hz Band at 12Hz Stimulation	10Hz Band at 12Hz Stimulation	12Hz Band at 12Hz Stimulation

Figure 7.8 Alpha Band Topographies. Topographies of alpha bands 8Hz (+/- 1Hz), 10Hz (+/- 1Hz), 12Hz (+/- 1Hz) following 1Hz control, 8Hz, 10Hz and 12Hz visual alpha stimulation.

4.1.6. LORETA

The P2 peak amplitude following the 12Hz stimulation was selected for LORETA analysis, as this is where the largest and only significant reduction in P2 was observed. Source localisation of the electrical activity leading to the difference in P2 amplitude was identified bilaterally, in the Insular Cortex ($p<0.05$). Details of the LORETA results can be found in Figure 9. No significant areas were identified for the contrasts made for the N2 and 12Hz alpha activity.

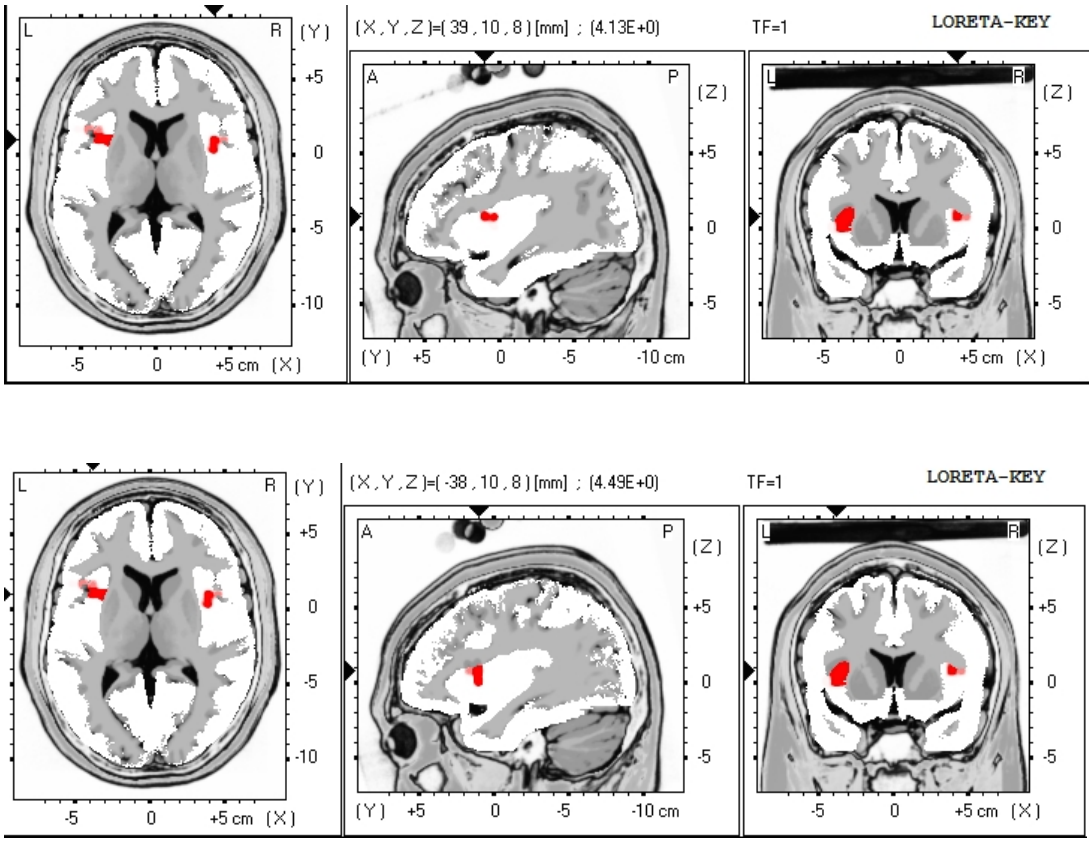


Figure 7.9 LORETA Results of P2 Peaks. LORETA results comparing P2 amplitude following 12Hz stimulation and P2 amplitude following the 1Hz control stimulation. Significant sources identified are the right and left insula.

Brodmann Area	Talairach Co-ordinates	Area
13	X= 39 , Y= 10 , Z= 8	Right Insula
13	X= -38 , Y= 10 , Z= 8	Left Insula

Table 7.3 LORETA Results of P2 Peaks. Cortical sources identified as having differences in current density when comparing the P2 peaks amplitude in the 12Hz and the control condition, quoted in Talairach coordinates. For all results, t($p<0.05$) = 2.46 for single voxels. Positive X coordinates signify right hemisphere activation.

5. Discussion

The present study was designed to investigate the effect of a flashing visual stimulus across the alpha range on acute laser pain ratings, in patients with osteoarthritis. The study revealed that following 8Hz, 10Hz and 12Hz visual stimulation, all participants rated the moderately painful heat-laser stimulus significantly lower than following the 1Hz control stimulation. A significant reduction could additionally be observed in the laser-evoked potential amplitude following the 12Hz stimulation. However, we could not demonstrate a change in alpha power following visual stimulation at any frequency. In sum, exposure to alpha stimulation, even without discernible entrainment, significantly reduces the behavioural and electrophysiological pain response.

Effects on Pain Ratings

In line with our hypothesis, pain ratings following the 8Hz visual stimulation averaged a lower overall score than following the 10Hz and 12Hz stimulation. Individual alpha frequency, known to decrease with age (Klimesch 1999), may thus play a fundamental role in alpha analgesia. Although the lower frequencies of the alpha band appear more successful candidates for targeted entrainment than 10-12Hz, differences in average pain ratings between the conditions were not significant. Conclusions from the observed trend should hence be made with caution.

Effects of P2 Laser-Evoked Potentials

The P2 peaks of the laser-evoked potentials displayed a reduction in amplitude following all three visual alpha stimulation frequencies. However, the only significant reduction in peak amplitude was observed in the 12Hz condition. It was unexpected for only one condition to be displaying a significant reduction, as LEPs have been identified to correlate with pain ratings (Carmon, Mor et al. 1976, Bromm and Scharein 1983, Truini, Rossi et al. 2004). Considering the largest reduction in pain ratings can be observed in the 8Hz condition, this is where we expected to see a significant decrease in P2 peak amplitude. A possible explanation for this may be overall low amplitude LEPs, and high variability in the LEP amplitude of the sample population at baseline.

P2 peaks following the 8Hz and 10Hz stimulation displayed a non-significant decrease in amplitude, but on a similar order of magnitude to the 12Hz decrease, suggesting that greater variability in the EEG data may have limited the statistical significance of the decrease at 8Hz and 10Hz. This was confirmed upon comparing the standard deviation of the 12Hz condition (SD 2.04 and variance of 4.2) to the 8Hz (SD 2.6 and variance of 6.9) and 10Hz (SD f 2.8 and variance of 7.6).

Furthermore, as LEP amplitude correlates negatively with older age (Truini, Galeotti et al. 2005), baseline and control amplitudes were already low in our osteoarthritic patient population, and may therefore be difficult to reduce further, despite reductions in pain perception. Additionally, as the patient population sample displayed a large age range, the amplitude presumed to correlate with age may have resulted in variable electrophysiological responses at baseline.

Further variance was introduced by the naturally larger electrophysiological irregularity found in older adults. The latter mentioned factors may deem it difficult to establish whether variance and sample size have influenced the accuracy of the electrophysiological results. To assess the validity of the present findings, a larger population sample, with more consistent LEP amplitudes may be required.

On the other hand, the relationship between pain perception and LEP amplitude is known to break down under some conditions. Indeed, the P2 peak amplitude has been linked to stimulus salience, rather than pain perception per se (Mouraux and Iannetti 2009). The population selected for the present study all suffered from persistent osteoarthritic pain. As a result, a heat-laser noxious stimulus may not be perceived as salient in patients relative to healthy, pain-free volunteers. It is possible that smaller reductions in LEP amplitude, compared to the reductions in pain perception, may be attributed to a lack of novelty of the painful sensation. Whether the smaller reduction in P2 amplitude in the 8Hz and 10Hz condition is due to lack of salience and constant pain state or, because changes in EEG activity are harder to detect in an older population, cannot be determined from the present results.

The Effect of Alpha Power

Following all three visual alpha stimulation sessions, no significant increase in alpha power was observed in any of the nine scalp regions, yet analgesic effects ensued. It may be that visual alpha stimulation alone, without frequency entrainment, could be sufficient to reduce the perception of experimental pain. However, although it appears to imply no entrainment occurred, sample heterogeneity and the resulting data variability might have simply been too great to observe statistically significant changes in alpha power with the sample size used in this study.

When inspecting the data visually it is immediately apparent that no clear alpha peak is displayed in any of the conditions, including baseline and control. This low baseline alpha activity may be explained by the inverse relationship between alpha power and persistent pain (Trifiletti 1984, Nir, Sinai et al. 2012, Huneke, Brown et al. 2013, Jensen, Sherlin et al. 2013, Peng, Hu et al. 2014). As the participants in this study all suffered from chronic osteoarthritic pain, it is possible that this is the reason for their lack of alpha peak in the 7-14Hz frequency band. Additionally, voltage, abundance and persistence of the alpha rhythm decreases rapidly above the age of 60 (Roubicek 1977). The combination of these two factors may have resulted in initially low alpha activity. As baseline alpha activity is known to

influence the ability to entrain frequencies in the 8-14Hz range (Rosenfeld, Reinhart et al. 1997), entrainment might not have occurred in subjects lacking an alpha peak, or with low initial alpha power. Nevertheless, visual stimulation across the alpha range significantly reduced the perception of acute pain. Whether this is due to a mechanism not detected by our recordings or whether alpha stimulation alone is enough to reduce pain ratings, requires further investigation.

Along with decreases in power and frequency, with age, alpha activity shows decreasing coherence (Klimesch et al., 1999). The decline in alpha coherence is stronger than in any other frequency band. Enhanced alpha desynchronisation with age can in part be attributed to a reduction in cortical connectivity (Vysata, Kukal et al. 2014). This decrease in coherence with age may influence the approximation of EEG power, or changes in such activity, as expected during alpha entrainment, due to phase cancellation. It could therefore be possible that visual stimulation has affected the alpha waves in this experiment, but due to phase cancellation, these changes are not apparent. Nevertheless, these undetected increases may still have an analgesic influence of the perception of pain.

With increasing age and decreasing alpha band prominence, oscillatory activity in the 8-14Hz frequency range becomes less reactive to external stimuli (Breslau, Starr et al. 1989). Early studies using photic driving observed that visually entrained alpha is less present in older than in younger participants (Verdeaux, Verdeaux et al. 1961). This lack of ability for alpha activity to mimic an external driving stimulus may explain the lack of entrainment following the visual stimulus.

With alpha power being lower and less reactive to external stimuli, perhaps a brief exposure to alpha stimuli will not have the full effect that is seen in younger healthy volunteers. Neurofeedback training, where participants practice over a prolonged period of time to increase their baseline alpha activity, may hence be a more viable treatment for osteoarthritic pain. Significant reductions in chronic pain in fibromyalgia patients and following spinal cord injury have been observed following

completed alpha neurofeedback training sessions (Kayiran, Dursun et al. 2010, Jensen, Sherlin et al. 2013).

Although alpha activity is maximal in the occipital lobe in the young healthy population (Herrmann 2001, de Graaf, Gross et al. 2013), with increasing age maximum alpha activity shifts from a posterior to a fronto-central or mid-parietal location (Breslau, Starr et al. 1989). As a result, targeting the occipital cortex by means of visual entrainment may not be the most effective method in an elderly population. For the present population sample, alpha entrainment through auditory binaural beats or tactile vibrations may target cortical locations more relevant for the present population sample.

Effect on Clinical Pain

This study was not primarily designed to assess effects of alpha-stimulation on clinical pain. Therefore, it should not be completely surprising that no significant changes in clinical pain ratings were observed following alpha stimulation. Although this might suggest that alpha stimulation is not suitable for the modulation of clinical pain, the majority of participants in the present study had low amounts of osteoarthritic pain prior to the start of the experiment. As a result, a reduction in clinical pain was difficult to quantify. In order to assess the influence of visual alpha stimulation on osteoarthritic pain, a moderate amount of clinical pain would have to be present at the start of the study.

Patients participating in the present study were asked to continue taking their prescribed medication for their osteoarthritic pain. These pharmacological analgesics may have contributed to the low baseline clinical pain ratings by reducing participants' resting state alpha power. Although participants' alpha power might be lower than in healthy young adults, it may well still be higher than when not regularly taking their medication. The already pharmacologically increased alpha power might hence be a contributing factor to the lack of statistically significant alpha entrainment. As there may be a limit to amount of cortical alpha power present, it could be postulated that is it difficult to further

entrain their already (relatively) high alpha power. Without patients taking their usual analgesic medication, we may have been able to observe a greater or even significant entrainment effect. However, to test this hypothesis a group of patients would need to complete the study twice, with and without medication.

Localisation of P2 Sources

In the current study, the bilateral insula displayed a significantly higher activation of the P2 peak amplitude in the control, compared to the 12Hz condition. Activation of the insular cortex has previously been shown to positively correlate with subjective pain ratings (Prichep, John et al. 2011). Brodmann area 13 specifically identifies the posterior portion of the insula, whose bilateral activation is known to be involved in the generation of LEPs (Garcia-Larrea, Frot et al. 2003), and positively correlate with pain intensity (Coghill, Sang et al. 1999). This is substantiated by my data, where pain ratings and P2 peaks amplitudes were significantly lower than control following the 12Hz stimulation. The insula has been shown to be involved in the modulation of pain expectancy (Sawamoto, Honda et al. 2000). As the P2 peak of the LEP waveform is also influenced by expectation (Brown, Seymour et al. 2008) and increased alpha power has been correlated with expectation of analgesia during experimental placebo (Huneke et al., 2013), alpha stimulation induced changes in expectancy may be the underlying link in this relationship.

The insula cortices play a functional role not only during the perception of acute pain stimuli, but furthermore at rest, in chronic pain patients (Cook, Lange et al. 2004, Napadow, LaCount et al. 2010, Kim, Chang et al. 2011). Previous work proclaims the insula receives input from thalamic nuclei (Coghill, Sang et al. 1999) and cortical alpha power is predominantly generated in thalamo-cortical feedback loops (Steriade, Gloor et al. 1990, Lopes da Silva 1991). Results from the present study demonstrated a reduction in the perception of acute pain, paired with reduced insula activity, following alpha stimulation. It could thus be hypothesized that increases in the thalamic alpha activity influences activity in the insula, which in turn regulates pain intensity coding. Accordingly, in a patient population with a

higher baseline clinical pain, alpha stimulation may be able to reduce osteoarthritic pain, by reducing activation of the insula.

Conclusion

Patients suffering from OA have a lower basal alpha activity due to their persistent pain, making it difficult to entrain frequencies in the 7-14Hz frequency band. Nevertheless, exposure to a visual alpha stimulus at 8Hz, 10Hz and 12Hz was enough to significantly reduce the perception and electrophysiological response to a moderately painful stimulus. However, we could not determine whether or not this reduction was dependent on alpha entrainment due to a lack of clear effects of visual stimulation on alpha power, which we suggest may have been due to low baseline alpha activity in chronic pain patients and/or a lack of coherency, but this requires further investigation.

CHAPTER 8

General Discussion

1. Introduction to Chapter 8

The main aim of this thesis was to develop an analgesic alpha entrainment intervention for acute and clinical pain. Prior to attempting to modulate pain, pilot work assessing the ability to increase alpha frequency power, through an external oscillatory stimulus, was completed. Upon establishing the capability of neural entrainment, a selection of stimulation modalities and frequencies were assessed to determine the most effective analgesic intervention for acute experimental pain, prior to progressing to the more complex, chronic pain. This final chapter is divided into four parts and will open by providing a summary of how the results presented in experimental Chapters 3 to 7 of this thesis individually address the hypotheses stated in Chapter 1. A critical review of the behavioural and electrophysiological results of this thesis will be presented in the broader context of pain research, and found the proposition of an ultimate neuro-modulatory analgesic model. The clinical implications of the final neuro-modulatory model for therapeutic research and pain management will be examined. Finally, limitations of the experimental work will be highlighted and discussed, prior to summarising future experimental work and directions based on the novel findings of this PhD thesis.

2. Addressing Hypotheses:

> ***Hypothesis 1*****:** *Alpha power can be significantly increased from spontaneous baseline activity following frequency entrainment in healthy volunteers*

Neural entrainment of frequencies across the alpha band was investigated in Chapter 3, where an external visual rhythmically alternating checkerboard stimulus was presented individually from 7Hz to 14Hz. In support of Hypothesis 1, Chapter 3 revealed that visual stimulation at 10 Hz and at 11 Hz resulted in a significant increase in alpha power. Although power increases following the 10Hz stimulus were widespread throughout the cortex, a larger power increase from baseline was observed in the posterior regions compared to the anterior scalp regions. This augmented entrainment may be due to the high resonance of the visual cortex

(Herrmann et al., 2011), and furthermore, this was the cortex targeted by the checkerboard stimulus. Alpha power appeared to increase more uniformly following the 11Hz stimulus, where no scalp regions increased significantly more than others. The difference in entrainment patterns of the 11Hz and 10Hz stimulation could be attributed to 11Hz not resonating as well as 10Hz in the visual cortex, resulting in increases in alpha power being more evenly distributed over all nine scalp electrode regions. Following both the 10Hz and the 11Hz stimulation, power increases were not limited to the targeted visual cortex; instead, surrounding cortices fell into lock step with the oscillations of the stimulus. This suggests, as hypothesised, the alpha power increase was not due to rhythmically evoked-potentials, but due to a widespread frequency entrainment.

Although according to the results in Chapter 3 no entrainment occurred following the 7Hz-9Hz and 12Hz-14Hz stimulations, the analysis may not provide an entirely accurate representation of the oscillatory changes elicited. The entrainment analysis was limited by selectively comparing the stimulated frequency band, to the same band in the control condition. Increases in the central alpha band (around 10Hz) following frequency stimulation of the peripheral frequencies (7-9 and 12-14) could not be detected in the analysis. Although non-frequency-matched increases in alpha power may not be relevant in frequency specific entrainment studies, as analgesic effects occur along the alpha frequency range, non-specific power increases in the alpha band range may still be of use in analgesic neuro-modulation.

Nevertheless, in experimental Chapters 5 and 6 (auditory and visual entrainment respectively), we can observe significant increases in alpha power following the 8Hz and 12Hz stimulations, as well as following the 10Hz stimulation. It should however be noted that a significant increase the stimulated frequency band does not equate to maximal entrainment at the stimulation frequency. Rather than generating a shift in peak alpha frequency, power increases following 8Hz and 12Hz stimulation in Chapters 5 and 6 may have been higher in the central frequencies, similar to the results in Chapter 3. The visual ‘on/off’ stimulus developed for Chapter 6 appears to produce significant results not because it creates a more frequency-specific

increase than the checkerboard stimulus in Chapter 3, but because it elicits a larger increase of the entire alpha band. That is, all frequencies of the bell-shaped alpha band display increases of similar proportions, resulting in peak alpha frequency remaining around 10Hz (central to the frequency range), after 8Hz and 12Hz entrainment.

In brief, Chapters 3, 5 and 6 confirm the hypothesis that alpha power can be significantly increased from spontaneous baseline activity. However, increases in alpha power may not necessarily be matched to the frequency of the stimulus, but instead, be central to the alpha range, around the 10Hz mark.

> ***Hypothesis 2*****:** *Auditory and visual alpha entrainment can significantly reduce behavioural ratings of acute pain in healthy volunteers*
> *2.1) The largest decrease in behavioural pain ratings will be apparent following the 10Hz entrainment in healthy volunteers*

To investigate hypothesis 2, an on/off flashing light stimulus and binaural beat stimulation was implemented at 8Hz 10Hz and 12Hz. In both the auditory and visual groups, all three entrainment frequencies resulted in pain ratings significantly lower than control. As hypothesized, the largest reduction in pain ratings was found after the 10Hz entrainment in both the visual and auditory groups. However, only in the visual group was the reduction after the 10Hz stimulation significantly larger than following the 8Hz and 12Hz stimulation. Although, in both conditions, there was no significant difference in analgesic effects of the 8Hz and 12Hz stimulus, pain ratings following the 8Hz stimulus were numerically lower than following the 12Hz stimulation.

In the introductory Chapter 1, the concept of alpha analgesia and current neuro-modulatory interventions are introduced. The literature reveals mixed results for the frequencies across the alpha range. Nir et al. separately investigated the association of tonic pain with the lower alpha-1 frequencies (8Hz-10Hz) and higher alpha-2 (10Hz-12Hz) frequencies, seeing only alpha-1 correlate negatively with

behavioural pain ratings (Nir et al., 2012). Nevertheless, numerous mindfulness meditation and neurofeedback studies aiming to increase power across the alpha frequency spectrum have recorded negative correlations with pain perception (Brown et al., 2008; Jensen et al., 2013).

The results fit in with the current literature as they show an analgesic effect across the alpha range (8Hz – 12Hz) using both visual and auditory stimulation. Additionally, larger effects were shown in the lower frequencies (8Hz-10Hz), although this difference was not significant in the auditory group, which Nir et al. identified to have the strongest correlation with pain. The lower alpha frequencies are thought to be representative of active information processing and are most dominant during anticipation of and attention to external stimuli (Moont et al., 2010). Higher band alpha-2 activity is hypothesised to be representative of inhibitory processes allowing task unrelated information to be filtered out and important signals to be refined. Alpha-2 activity has continuously been recorded as being more prominent during sensorimotor or cognitive processing related to external stimuli (Franciotti et al., 2009). In support of this, in Chapter 5, auditory entrainment was not significant following the 8Hz binaural beat stimulation. Yet, Chapter 4 reveals a reduction in behavioural pain ratings following the 8Hz auditory entrainment effectively equal to that observed after the auditory 10Hz and 12Hz stimulations, neither of which revealed significant entrainment in the EEG analysis in Chapter 5. Therefore, a higher increase in power after 10Hz and 12Hz auditory stimulation is needed to produce an equivalent analgesic effect as following 8Hz auditory stimulation. It should however be noted that entrainment was not significant following the 12Hz visual stimulation in Chapter 6, and might hence provide an alternative explanation for why alpha analgesia was significantly less following the 12Hz visual stimulation, compared to following the 10Hz stimulation in the visual group.

Although we were not able to compare the analgesic effects of the auditory and visual studies directly due a difference in paradigms, a numerically larger decrease could be observed following the visual entrainment (with the exception of the 12Hz

condition). It could be postulated that the increased effectiveness of the visual stimulation could be attributed to the largest size of the stimulated visual cortex, allowing a more powerful propagation of alpha power across the entire cortex. However, further investigation comparing the analgesic effects of auditory and visual entrainment is required.

> ***Hypothesis 3*****:** *Auditory entrainment can significantly increase alpha power and significantly reduce pain-evoked potentials in healthy volunteers*

In Chapter 5, hypothesis 3 was tested by measuring the increase in alpha power and decrease in N2 - P2 peak amplitudes of the laser-evoked potentials, following 10 minutes of auditory entrainment at 8Hz, 10Hz and 12Hz.

A significant increase in alpha power from control was found following all three binaural beat frequency stimulations. Posterior alpha power was higher at baseline and following all three frequency stimulations, increasing proportionally to other topographical regions following entrainment. Following the 10Hz auditory entrainment, a topographic left anterior condition effect was observed. The topographic results displaying greater entrainment in the left anterior scalp electrodes were reflected in the source analysis results. Differences in activity of both the P2 amplitudes and 10Hz alpha entrainment were found in Brodmann's Area 10, also located left anteriorly. In Chapter 6, visual entrainment produced a posterior entrainment effect. Although this is justified by the high resonance of the primary visual cortex, this discrepancy in topographic increases may be attributed to the nature of the stimulus. Binaural beat entrainment may modulate frontal sources of alpha power, whereas visual entrainment may predominantly influence occipital sources.

Surprisingly, P2 sources estimated by LORETA did not include structure in the pain matrix, defined in Chapter 1. However, as LORETA solely identifies structures with significantly different activation between the control and the 10Hz condition, it could be postulated that the high variability in the sources of participants' alpha

power hindered a significant difference in pain matrix structures to be detected. The extent of influences for pain-associated structures is therefore difficult to determine. Changes in activation of frontal sources may be reflecting alterations in ongoing brain states that co-occur with the electrophysiological pain response. Rather than revealing the sources contributing the changes in P2 amplitude, they may be revealing sources associated with psychological modulators of pain.

Different patterns of effects could be observed on N2 and P2 peak amplitudes following the entrainment sessions. All P2 peaks revealed a significant reduction compared to control, with the peaks after the 10Hz and 12Hz entrainment additionally being significantly smaller than following the 8Hz entrainment. The N2 peak showed a significant reduction after the 10Hz entrainment, but not following 8Hz and 12Hz. Following the visual entrainment in Chapter 6, solely changes in P2 and not in N2 can be observed. It's possible that auditory entrainment modulates pain differently than visual entrainment, as represented by the different aspects of pain characterized by the N2 and P2 peaks, although this can't be firmly established with the current data as there are other confounding variables (e.g. visual and auditory experiments taking place in different, and relatively small, groups of subjects) that may also explain the differences. It may be that the visual entrainment study simply lacked statistical power to detect a difference in the N2 peak.

The N2 peak is believed to be influenced more by somatosensory-specific activity (i.e. activity resulting from both nociceptive and non-nociceptive somatosensory stimuli) and activity elicited from selectively attending to the spatial location of pain (Bentley, Watson et al. 2004, Mouraux and Iannetti 2009). The P2 component is influenced more by activity elicited by stimuli of other sensory modalities, such as the cognitive and affective components of pain (e.g. attention)(Siedenberg and Treede 1996, Zaslansky, Sprecher et al. 1996, Bentley, Watson et al. 2004, Brown, Seymour et al. 2008). Taking the current results at face value, auditory entrainment may modulate both the somatosensory and the cognitive aspects of pain, whereas visual entrainment may predominantly influence the cognitive and affective

components of pain. However, this remains a hypothesis to be tested in future work involving either intra-subject comparisons between auditory and visual entrainment or larger sample sizes to confirm a lack of N2 modulation by visual entrainment.

> ***Hypothesis 4*****:** *Visual alpha entrainment can significantly increase alpha power and significantly reduce laser-evoked potentials in healthy volunteers*
> 4.1) The largest reduction in pain will be observed following 10Hz

Hypothesis 4 is addressed in Chapter 6 where flashing LED goggles are used as a visual stimulus to entrain alpha at 8Hz, 10Hz and 12Hz. Following the 8Hz and 10Hz stimulation, significantly higher alpha power was recorded across the majority of electrodes, confirming entrainment was successful. However, after the 12Hz stimulation, no significant effect of condition was observed, suggesting no entrainment occurred in the selected frequency band. A significant main anterior-to-posterior effect, with higher posterior alpha power was additionally found across both control and entrainment conditions. As there was no interaction between condition and anterior-to-posterior alpha power following any of the frequency stimulations, it appears that alpha power was consistently higher posteriorly, and increases in alpha power in the 8Hz and 10Hz conditions occurred globally, across multiple scalp regions.

In Chapter 6, a significant reduction in the P2 peak of the LEP waveform could only be observed following the 10Hz visual entrainment, and not following the 8Hz and 12Hz. This is in line with my hypothesis that 10Hz would result in the largest reduction in electrophysiological pain responses. I hypothesised the significant reduction at 10Hz could be attributed to the targeted cortex having the highest resonance at 10Hz. The lack of N2 change in this experiment suggests pain was mainly modulated by cognitive factors.

The source localization showed that increases in alpha power were associated with a change in activation of pain matrix structures. When comparing the source

localization of the change in P2 peaks to the change in alpha power, an overlap of structures, namely, the precuneus and the posterior cingulate, suggest an interaction between alpha and pain processing in these regions. The precuneus is known to be involved in shifting attention to different spatial locations (Wenderoth, Debaere et al. 2005). Changes in source activity between the 10Hz entrainment and control condition might imply that changes in attention (as a results of alpha entrainment), reduces sensitivity to the spatial location of the painful stimulus, making it harder to distinguish the sensory characteristic of the stimulus, such as its intensity (reflected in the P2 peak amplitude).

Activity in the posterior cingulate has been associated with changes in pain intensity (Tolle, Kaufmann et al. 1999). My results display greater activity in the posterior cingulate in the high P2 amplitude, high pain control condition. Higher P2 posterior cingulate source activation may reflect a more intense perception of the noxious heat laser stimulus in the control condition. Furthermore, changes in posterior cingulate activity were observed following the 10Hz alpha entrainment. It could hence be suggested that alpha entrainment mediates changes in pain intensity (reflected in P2 amplitude) by modulating the activity in the posterior cingulate.

> ***Hypothesis 5*****:** *Visual entrainment can significantly reduce electrophysiological and behavioural responses to experimental pain and can significantly reduce clinical pain in osteoarthritic patients*

Chapter 7 was designed to investigate the effect of a flashing visual stimulus across the alpha range on acute laser pain ratings, in patients with osteoarthritis. The study revealed that following 8Hz, 10Hz and 12Hz visual stimulation, all participants rated the moderately painful heat-laser stimulus significantly lower than following control. Pain ratings following the 8Hz visual stimulation averaged a lower overall numeric score than following the 10Hz and 12Hz stimulation, however, this difference was not significant. Although no significant decrease in clinical pain

ratings were recorded, patients reported very low baseline chronic pain ratings, making any analgesic effects challenging to detect. Future work could aim to recruit patients with greater levels of chronic pain at rest to overcome this limitation.

A significant reduction could additionally be observed in the laser-evoked potential P2 peak amplitude following the 12Hz stimulation. P2 peaks following the 8Hz and 10Hz stimulation displayed a non-significant decrease in amplitude, suggesting that the large variability in the data may have influenced the results, again suggesting that larger sample sizes may be needed in future work. It was unexpected for the only significant reductions in P2 peak amplitude to be observed following the 12Hz stimulation, as LEPs have been identified to correlate with pain ratings (Carmon, Mor et al. 1976, Bromm and Scharein 1983, Truini, Rossi et al. 2004). Considering the largest reduction in pain ratings can be observed in the 8Hz condition, this is where I expected to see a significant decrease in P2 peak amplitudes.

Source localisation of the electrical activity leading to the decrease in P2 amplitude observed following the 12Hz entrainment was identified bilaterally, in the insular cortex. In contrast to Chapter 6, far fewer sources displayed a change in activity contributing to P2 peak changes between control and entrainment. Recordings of EEG rhythms were far less pronounced in the older volunteers recruited in Chapter 7, compared to the healthy volunteers in Chapter 6. It could thus be postulated that had electrophysiological recordings been stronger, a higher overlap of sources may have been observed between the patients in Chapter 7 and healthy volunteers in Chapter 6. If, on the other hand, a completely different set of sources displayed a change in P2 peak activity in patients, discrepancies in the way a healthy brain and an osteoarthritic brain processes experimental pain stimuli may have been highlighted. Additionally, ameliorated recordings of EEG activity may have been able to detect significant alpha entrainment and sources of that entrainment, enlightening us on whether changes in alpha power are elicited by different or similar sources as in healthy volunteers.

Nevertheless, as EEG recordings struggled to record the weaker neural signals of

the older population of Chapter 7, we can only conclude that exposure to alpha stimulation, without discernible entrainment, is enough to significantly reduce the behavioural and electrophysiological pain response.

3. Clinical Implications of the Results

As pharmacological treatments alone are rarely sufficient for a substantial reduction in pain, neuro-modulatory interventions are becoming more widely accepted as a possible supplementary treatment option. The benefits of neurofeedback training have been mixed, with weak results often attributed to lack of commitment and difficulty of the training (Jenson et al., 2013). However, short-term results of neurofeedback training are promising as patients embrace the idea of self-management and self-efficacy (Jensen et al., 2014). Neural entrainment maintains the positive features of neurofeedback training and eliminates the difficulty and concentration required for neurofeedback neuro-modulation. Although Chapter 7 reveals no significant changes in clinical pain ratings, this result is not discouraging of the use of entrainment in the clinic. As aforementioned, the patients recruited in Chapter 7 were not experiencing sufficient pain at the start of the study to reveal a significant reduction in clinical pain. Furthermore, the study was primarily designed to modulate the patients' acute pain, and not their clinical pain. Visual alpha entrainment was successful at reducing the study targeted acute pain, underlining the potential of alpha analgesia in the chronic pain population.

Chapters 4 to 6 reveal analgesic alpha entrainment is easy to achieve, alleviates pain, and does not produce any unexpected negative side effects, nor did it have undesirable emotional effects such as exacerbating sleepiness, anxiety and negative moods. Entrainment could thus be employed alongside pharmacological treatments, as neurofeedback training has been, to assess the positive influence on chronic pain. If entrainment proves effective at managing clinical pain, it could be used in the home in addition to analgesic drugs to alleviate pain. Entrainment equipment is relatively inexpensive to construct and easy to use. Visual stimulation requires the manufacturing of LED goggles. However, listening to binaural beat

alpha entrainment tracks could be easily prescribed by a general practitioner, and listened to at home, or away from home, whenever in pain.

Furthermore, if alpha entrainment proves not to be as effective as neurofeedback training or mindfulness meditation, neural entrainment may be used as a diagnostic tool, evaluating participants' responsiveness to alpha analgesia prior to committing to extensive training. This would allow the segregation of alpha analgesia responders, and non-responders, allowing neurofeedback or mindfulness meditation to be personalised, and hence more effective.

From the work completed in this thesis, we can conclude with confidence that alpha entrainment has robust analgesic effects on acute pain. Entrainment could therefore be used as rapid acute pain relief, at home or in the clinic. Alpha entrainment could be used in the waiting rooms of A&E for minor injuries such as broken limbs, while they wait to be seen by the doctor. Furthermore, entrainment may be useful for dental pain and could be prescribed following a tooth extraction when pain becomes intolerable.

4. Limitations and Future Directions

Although the results obtained from this thesis are promising, the project contained several limitations. Firstly, from the work completed we are unable to conclude with certainty how long the increased power effect of the alpha stimulus lasted. It is therefore not possible to tell whether the alpha entrainment observed during the stimulation period is representative of the alpha power during or after the pain stimulus. Further research investigating the relationship of the longevity of increased alpha power and the frequency stimulated needs to be completed. Furthermore, Chapter 7 indicates that we may be able to modulate pain without alpha entrainment. It would hence be advantageous to further our knowledge of the necessity of prolonged entrained alpha power, or whether solely the stimulation of alpha is required to achieve analgesia. It could be postulated that the analgesic effects of alpha entrainment outlast the entrainment, and although alpha

power is no longer high, pain perception is still reduced. However, it may not be possible to distinguish the isolated effects of alpha stimulation and alpha entrainment, as rhythmic stimulation may not be feasible without subsequent entrainment.

The results of this thesis make it apparent that alpha power is a robust modulator of acute pain. The laser stimulus used elicited a pure activation of nociceptors, without mechanoreceptor activation. Although this stimulus is ideal for investigating the thesis hypotheses, from my recordings it is not possible to determine whether the modulatory effects of alpha entrainment are pain specific, or coupled with reductions in perception of other sensory inputs. As aforementioned in Chapters 5 and 6, increases in alpha power may be influencing the saliency of the stimulus, as opposed to directly manipulating pain processes. Further research into the effects of alpha entrainment on inputs from other sensory modalities, including light touch, sound or even taste, requires to be completed. The completion of such an assessment is necessary prior to introducing alpha entrainment into the clinic as an analgesic tool, as reductions in sensitivity to other environmental inputs may not be beneficial for the patient.

Throughout this thesis, I have primarily focussed on experimental laser pain. In order to strengthen my results different types of pain should be considered in future studies prior to clinical trials. Moreover, not only different types of pain, but potentially different oscillatory rhythms; both the theta rhythm in mindfulness meditation, and the gamma rhythm in experimental pain studies have shown a strong correlation with pain (Schultz et al., 2011; Aftanas et al., 2001). Theta and gamma entrainment may therefore be a valuable investigation, either directly as an intervention for acute and chronic pain, or to test the efficacy on patients prior to submitting them to longer-term neurofeedback training or mindfulness meditation.

In this thesis, due to time constraints, I have not been able to investigate the effect of alpha binaural beat entrainment on chronic pain. As mentioned previously, although the analgesic effects of visual entrainment appeared more robust in

healthy participants, the neural connectivity in older patients' cortices may be altered, allowing binaural beat stimulation to be the preferential modality. Although listening to binaural beats is more pleasant than observing a flashing light, binaural beats do require binaural hearing. As auditory sensitivity declines with age, this may be a key disadvantage of binaural beat stimulation in the older population. Additionally, tactile neural entrainment may be a further promising candidate for the alleviation of pain. Tactile entrainment is believed to have a relatively weak effect, compared to auditory or visual entrainment (Collura and Siever 2009). However, tactile entrainment through a vibrating stimulus placed on the hand, or dorsal surface of the arm, will primarily entrain the sensorimotor cortex. The somatosensory cortex is heavily interlinked within the pain matrix, and hence, may allow tactile entrainment to have a stronger analgesic effect than auditory or visual alpha entrainment.

The experiment completed in Chapter 7 was not optimised to investigate relief of chronic pain. The recorded baseline clinical pain ratings were varied, and predominantly too low to record a significant decrease. To thus more accurately determine the effect of alpha entrainment on chronic pain; further study with experimentally induced clinical pain, through pressure or uncomfortable movement, is necessary. If alpha entrainment proves successful at reducing clinical pain, a prolonged study comparing a group regularly entraining alpha, to one relying on pharmaceutical interventions, is required in order to evaluate the efficacy of alpha entrainment as a therapy.

In the experimental Chapters 4 to 7, no significant correlations between the pre-experimental questionnaires and the change in pain ratings could be observed. Additionally, anxiety, negative moods and sleepiness scores did not display significant differences between the three conditions. This implies that visual and auditory alpha entrainment alone could be enough to reduce pain ratings. Alpha entrainment may affect cortical processes independently of the measures considered in this study. Alternately, the questionnaires selected may have been unsuitable for detecting alpha manipulated behavioural changes. My

electrophysiological results suggest the change in pain may have been modulated by alpha entrainment influencing behavioural measures not considered in the study. As previously mentioned, anxiety, sleepiness and negative moods may still be affected by alpha and in turn, modulate pain. However, similar to the clinical pain ratings, initial values were too low to record a significant reduction in questionnaire scores. To further investigate the relationship between alpha power, emotional factors and pain modulation, a naturally anxious population, or a sample of high pain catastrophizers could be selected for further study.

Finally, upon repeating the paradigm used in Chapters 4 to 7, rhythmic patterns other than the entrainment stimulus should be avoided to diminish confounding entrainment factors. Specifically, presenting a pain stimulus every 10 seconds exposes the volunteer to a rhythmic stimulus of 0.1 Hz. In its place, a randomly selected time interval, ranging from 1-10 seconds in between pain stimulations should be employed. In addition to withdrawing rhythmic influences, randomising the timing of pain reduces pain expectancy and increases stimulus saliency. Additionally, comparing effects on predictable to unpredictable laser stimuli would test whether a change in expectancy modulates pain perception and laser-evoked potentials.

5. Final Conclusions

Mindfulness meditation and neurofeedback training from the alleviation of chronic pain are already indirectly relying on analgesic alpha neuro-modulation. Neural entrainment enables a direct increase in alpha power, known to be negatively correlated with pain perception. The work in this thesis provides solid evidence for neuro-modulatory alpha entrainment having a robust analgesic effect on both the perception of, and the electrophysiological response to, acute pain.

Auditory entrainment resulted in an analgesic effect in healthy volunteers in which frontal sources appeared to play a central role in modulating acute pain. In both healthy volunteers and patients with osteoarthritis, visual entrainment significantly

reduced electrophysiological and behavioural pain ratings. These changes appeared to be influenced by altered activity in the pain matrix, particularly the bilateral insula in osteoarthritic patients. Healthy volunteers revealed a more widespread pain matrix involvement with the precuneus and the posterior cingulate revealing changes in activation during both visual alpha entrainment and during the reduction of electrophysiological pain responses.

The work presented in this thesis provides a physiological foundation for further investigation and development of neuro-modulatory alpha entrainment treatments for acute and chronic pain. The behavioural and electrophysiological measures implemented in this thesis may furthermore aid the understanding of alternative neuro-modulatory treatments and predicting therapeutic outcomes. With the work presented in this thesis, I hope to contribute to the development of pain management, and provide a more targeted analgesic treatment with the aim to better address the unmet clinical needs of patients.

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