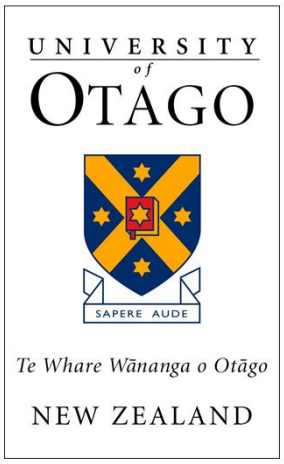


Factors Associated with Orthodontic Pain

DClinDent Thesis

Victoria Beck

Supervisors

Professor Mauro Farella

Professor Jules Kieser

Professor Murray Thomson

Associate Professor Nicholas Chandler

Table of Contents

Acknowledgements

This study was supported by grants from the Foundation for Orthodontic Research and Education, NZAO (FORENZAO) Charitable Trust and Fuller Scholarship, Sir John Walsh Research Institute, Faculty of Dentistry, University of Otago.

I would like to sincerely thank:

My research supervisors Professor Mauro Farella, Professor Murray Thomson, Associate Professor Nicholas Chandler and Professor Jules Kieser - thank you for sharing your passion for research and immense knowledge with me. You have helped make this project far more enjoyable than I had anticipated! Also to Tony Merriman and his team for their help with my genetic testing.

My clinical supervisors: Professor Mauro Farella, Dr Winifred Harding, Dr Chris Robertson, Dr Suzan Stacknik, Dr Peter Gilbert and visiting lecturer Dr Shari Daniels – thank you for your patience, guidance and generous donation of your time, as well as for sharing your enthusiasm for orthodontics with us.

Orthodontic clinic staff – thank you for always being ready for a chat and a laugh, I really appreciate how hard you ladies work for us!

My classmates Jen and Joe, quite possibly the shortest class ever to graduate - we made it!! It has been so much fun, I'm really lucky to have such supportive classmates as you.

Josh – your constant support has been incredible throughout this journey.

Amber and Meng – I couldn't have asked for more from you guys, you are amazing!

To my dog Archie, the best running buddy ever – sorry for ignoring you so much while I worked on this thesis, I promise there will be a lot more play-time next year!

Mum and Dad – thank you for all your love, support and encouragement. You have always been my number one supporters, I couldn't have come this far without you guys behind me. You have made me the person I am today and I love you so much.

Shaun – thank you for being my rock. Your love and support mean everything to me and I can't wait to begin our life together.

Abstract

Up to 95% of orthodontic patients report pain during orthodontic treatment, with up to 10% of patients interrupting their treatment due to the pain experienced. Pain is highly subjective: there is a range of pain response among individuals undergoing orthodontic treatment, with some patients feeling high levels of pain and others just mild discomfort. The reasons for this variability are largely unknown. **Objective:** To investigate factors that may be associated with orthodontic pain experience. **Methods:** First, 107 participants were screened for pain response over 48 hours following placement of orthodontic elastomeric separators. Second, the highest (n=10) and lowest (n=10) pain responders were identified and data collected on age, ethnicity, sex, self-rated oral health, anxiety, mood, dental anxiety and fear, catastrophising, general sensitivity (cold) and tooth sensitivity. They also provided a saliva sample for Catechol-O-Methyltransferase (COMT) gene sequencing. **Results:** Statistically significant differences between high and low pain responders were identified with the Pain Catastrophising Scale (PCS), Dental Anxiety Scale (DAS) and Cold Pressor Tests. Multivariate analysis was carried out using a generalised linear model. The empty model showed that 39.3% of pain response type (high or low) is explained by the magnification subcategory of the PCS; once all other variables were controlled for, the adjusted model explained 80% of the variance in the magnification subscale of the PCS. Of the three single nucleotide polymorphisms of the COMT gene analysed, only rs6269 showed an association with pain responders' haplotypes (albeit marginal). **Conclusions:** Pain catastrophising, dental anxiety and cold sensitivity appear to modify orthodontic pain experience. A few simple screening questions may help to identify patients at risk prior to commencing orthodontic treatment, so that patient-specific management strategies can minimise orthodontic discomfort.

Table of Abbreviations

Abbreviation	Definition
ANOVA	Analysis of Variance
ANS	Autonomic Nervous System
ATP	Adenosine Triphosphate
CNS	Central Nervous System
COMT	Catechol-O-Methyltransferase
COX	Cyclooxygenase
DAS	Dental Anxiety Scale
DNA	Deoxyribonucleic Acid
EPT	Electrical Pulp Tester
LD	Linkage Disequilibrium
met	Methionine
NS	Nociceptive Specific
PANAS	Positive and Negative Affect Schedule
PCS	Pain Catastrophising Scale
PDL	Periodontal Ligament
RCT	Randomised Control Trial
RT-PCR	Real-Time Polymerase Chain Reaction
SNP	Single Nucleotide Polymorphism
STAI	Spielberger's State–Trait Anxiety Inventory
TMD	Temporomandibular Dysfunction
val	Valine
VAS	Visual Analogue Scale
WDR	Wide Dynamic Range

Literature Review

Pain is an important factor in clinical orthodontic practice, so this section will initially encompass the basics of pain pathways and systems in the human body. It will begin with neural pathways of pain (focusing on the trigeminal system); secondly, discussing the autonomic nervous system (again with particular focus on the trigeminal afferent pathway); thirdly, background information will be presented on neurophysiology of pain; nociception; pain modulation; and finally psychological factors that may affect pain modulation. Lastly, this section will cover orthodontic pain and factors that may affect this, including genetics and anxiety and means of testing such factors.

Pain

Managing orofacial pain is a concern to clinicians worldwide. Pain is a highly personal experience, with the degree of pain and suffering reported not always related to the amount of tissue injury (Okeson and Bell, 2005). Pain cannot be sensed in a detached manner; it comes in combination with sensations such as dislike, anxiety, fear and urgency (Muller and Calvo, 2001). Acute symptomatic pain serves a biological function to warn the individual that something is wrong in the area manifesting the pain, whereas chronic pathologic pain may serve no clear biological function, but causes stress (emotional, physical and social) to the sufferer (Sessle, 1987). The ability of clinicians to treat pain lies in the knowledge and understanding of the various mechanisms and behavioural characteristics of pain and its manifestations (Okeson and Bell, 2005).

Pain is more of an experience than just a sensation that registers the nature of the stimulus, which can be described in terms of quality (such as itchy or sharp), intensity (such as dull or severe), location (where on the body they feel pain and whether it is superficial or deep) and duration (such as seconds or days as well as the frequency of pain). It also has *cognitive* (the individual's ability to comprehend and evaluate significance of experience), *emotional* (the feelings that are generated), and *motivational* (the drive to terminate the pain) dimensions (Okeson and Bell, 2005).

Definitions

Before proceeding, it is appropriate to define some key terms.

Nociceptors are free nerve endings; in the face and mouth, these are the peripheral receptors that respond to noxious orofacial stimuli (Sessle, 1987). *Nociception* refers to the noxious stimulus originating from the sensory receptor. This information is carried to the central nervous system (CNS) by the primary afferent neuron (Okeson and Bell, 2005). *Pain* is defined as the unpleasant sensation perceived in the cortex, usually as a result of incoming nociceptive input. However, the presence or absence of nociceptive input does not always relate closely to pain (Okeson and Bell, 2005), because the CNS can alter or modulate nociceptive input before it reaches the cortex for recognition. This modulation input can either increase or decrease the perception of pain.

There are three functional components of the brain system (Liebgott, 2001). First, there is the spinal cord and medulla (or basic reptilian brain), which provide protective reflex activity against challenges. Second, there are the limbic structures (or mammalian brain) which are wrapped around the upper portion of the medulla and spinal cord and provide the individual with instinctive drives and emotions (such as when pain is felt, the individual will instinctively direct behaviour towards activities to reduce pain and stimulate pleasure if possible). Finally, the cortex (or human brain) provides the individual with the ability to reason and think (so the individual can apply meaning and consequence to the situation). Sensations in the cortex can be influenced by attention, anxiety and fear (Liebgott, 2001).

Neural Pathway of Pain

The *nervous system* (Figure 1) can be divided into the central nervous system (which co-ordinates peripheral and autonomic systems) and peripheral nervous systems (which carries information from musculoskeletal and cutaneous system). The peripheral nervous system is then further divided into the somatic and autonomic systems. The latter co-ordinates the activities of visceral structures such as blood flow and breathing (Okeson and Bell, 2005).

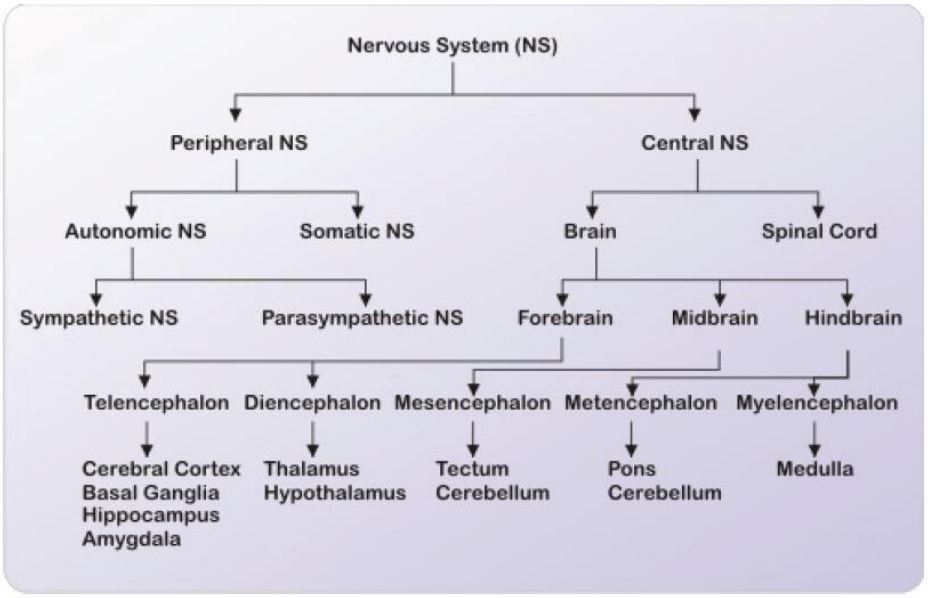


Figure 1 The Nervous System[i]

The neural pathway for pain consists of four processes; these are transduction, transmission, modulation and perception (Kelly et al., 2001). Transduction is where noxious stimuli causes electrical stimulation in specific nerve endings, the noxious input is then transmitted via the neural system to the CNS for processing. The neural system which processes the noxious input consists of three parts; initially the peripheral sensory nerve primary afferent neuron, which carries the nociceptive input via the dorsal root, to the dorsal horn in the spinal cord. All cell bodies of primary neurons are located in the dorsal root ganglion; followed by the second order neuron, which can involve more than one neuron and carries the input across the spinal cord, and via the anterolateral spinothalamic pathway, to the thalamus; the final part of the system involves neural interactions between the thalamus, cortex and limbic system. This is where modulation occurs, as the cortex and brainstem can control the pain-transmitting neurons (to enhance or reduce the arriving input). Finally, perception is achieved when nociceptive input reaches the cortex and a complex interaction of neurons between the higher centres of the brain occurs and pain behaviours begin (Okeson and Bell, 2005).

[i] Adapted from http://www.news-medical.net/health/What-is-the-Nervous-System.aspx on 8/4/13

Neural Structures

Nerves convey electrical and chemical impulses and are comprised of bundles of nerve fibres (each surrounded by its own connective tissue sheath) which are bound together by another connective tissue sheath (Okeson and Bell, 2005). Nerve fibres are comprised of nerve cells (neurons) which have a cell body and a process (axons or dendrites). Cell bodies which are located outside the CNS are grouped together in ganglia, and the dendritic processes conduct impulses towards the cell body. Axons are the central impulse-conducting core of the nerve fibre; they are essentially an extension of the neuronal cytoplasm (Okeson and Bell, 2005).

Neurons can have any number of axons (and are termed unipolar, bipolar; or multipolar depending on the number of axons present). Peripheral neurons are unipolar. The axon then divides into two; the peripheral part extends to terminate at a sensory receptor, and the central branch passes through the roots of the nerve to terminate in the grey matter of the CNS.

Depending on their location, neurons are either *afferent* (conduct impulses towards the CNS) or *efferent* (conduct nerve impulses peripherally, away from the CNS). Nerve impulses are transmitted from neuron to neuron through synaptic junctions (where two neuronal processes are in close proximity). Afferent synapses are located in the grey substance of the CNS.

Nociceptors

Nociceptors are each responsible for what is known as a *receptive field*. They are polymodal, meaning that they can respond to many different stimuli (such as heat or cold). Two types of nociceptors have been identified. The first is the *C-fibre*, which is sensitive to mechanical, chemical and heat stimuli (Dalili, 2009) and conveys a burning or dull aching pain sensation. C-fibres have a receptive field of 10cm, a diameter of 0.5-1μm and are unmyelinated, making them slower at conducting impulses. C-fibres are the most common nociceptor found in the dental pulp (Sessle, 1987); the other type of nociceptor is the *A-delta (A-δ) fibre,* which elicits a pricking, sharp pain and is sensitive to mechanical and heat stimuli (Dalili, 2009). *A-δ* -fibres are myelinated, so that they have faster signal transduction (Racich, 2005).

Brainstem and Brain

Input is carried to higher centres for interpretation via second-order neurons. These higher centres can be divided into four regions according to their location (from inferior to superior). The *brainstem* is made up of the medulla oblongata, the pons and the midbrain. The *cerebellum* has an outer part which comprises grey matter, and an inner part of white matter (Liebgott, 2001). The *diencephalon* consists mainly of the thalamus and hypothalamus. The *cerebrum* is made up of the cerebral cortex, basal ganglia and limbic structures (Okeson and Bell, 2005).

Trigeminal System

Orofacial input does not enter the spinal cord via the spinal nerves, but via the fifth cranial (trigeminal) nerve (Racich, 2005). The trigeminal spinal tract also receives input from other cranial nerves (the glossopharyngeal (IX) and vagus (X)) and the upper cervical nerves (Liebgott, 2001).

The afferent trigeminal nerve cell bodies are in the large Gasserian (trigeminal) ganglion; they enter directly into the brainstem at the pons to synapse in the trigeminal spinal tract nucleus (Sessle, 1987), which is structurally similar to the dorsal horn of the spinal cord.

The brainstem trigeminal nucleus consists of a main sensory trigeminal nucleus which is located rostrally and receives periodontal and some pulpal afferents. The spinal tract of the trigeminal nucleus is located caudally and is divided into three parts: the *subnucleus oralis* (a significant area in oral pain mechanisms); the *subnucleus interpolaris;* and the *subnucleus caudalis* (which is implicated in trigeminal nociceptive mechanisms; Miles et al., 2004; Racich, 2005; Sessle, 1987). Evidence points to facial nociceptive afferents projecting to the subnucleus caudalis; however, the other subnuclei also play important roles in nociception (Okeson and Bell, 2005). The trigeminal brainstem complex also has a motor nucleus, which interprets impulses that demand motor responses, such as the muscles of mastication (Racich, 2005).

Second-order trigeminal neurons project to the thalamus from synaptic junctions with primary afferents in the subnucleus caudalis (Miles et al., 2004) and at the dorsal horn. These inter-neurons represent three types of transmission cells. *Wide dynamic range*

neurons receive input from cutaneous and deeper orofacial structures, and respond to both noxious and non-noxious (such as tactile) stimuli. *Nociceptive specific* neurons respond only to input from small-diameter nociceptive fibres (A-δ and/or C-fibres) that are activated by high-intensity orofacial stimulus applied to a localised receptive field (Miles et al., 2004). *Low-threshold mechano-sensitive* neurons, which are usually non-nociceptive, respond to light tactile stimuli and are excited by strong electrical stimulation of the dental pulp (Sessle, 1987).

The Autonomic Nervous System

The autonomic nervous system (ANS) is made up of the *parasympathetic* (cranio-sacral) portion and the *sympathetic* (thoraco-lumbar) portion. They receive afferent input that is usually below the level of consciousness (Ranson, 1918). Efferent portions of these nerves make up the autonomic nervous system, which is again divided into sympathetic and parasympathetic components. The ANS plays important roles in the control of phenomena such as arterial blood pressure, digestion, sweating and body temperature, with most functions occurring continuously and below the conscious level. The ANS can respond quickly when stimulated, to help adaptation to environmental challenges.

Sympathetic Nervous System

Sympathetic nerves originate in the spine between T1 and L2, travelling through the sympathetic chain and on to tissues and organs. The sympathetic chain is a chain of ganglia which lie either side of the spinal column. The sympathetic pathway consists of two neurons, with the body of the preganglionic neuron lying in the intermediolateral horn of the spinal cord; the postganglionic neuron transmits the impulse to the target organ or tissue. Some sympathetic nerves pass all the way through the sympathetic chain without synapsing, to end at the adrenal medulla. Here, modified neuronal cells are stimulated to secrete adrenaline and noradrenaline into the bloodstream (Okeson and Bell, 2005).

Some of the postganglionic fibres pass back from the sympathetic chain onto the spinal nerves. Theses fibres are C-fibres which extend around the body in the skeletal nerves to control sweat glands, blood vessels, and so on. Approximately 8% of the fibres in skeletal nerves are sympathetic fibres (Okeson and Bell, 2005); this may have significance for muscle pain, because pains of muscular origin are a frequent cause of

discomfort in the head and neck (Miles et al., 2004). Thus, the possibility of orofacial pain being muscular (rather than dental) in origin must always be taken into account.

Parasympathetic Nervous System

The parasympathetic system is made up of fibres from cranial nerves III, VII, IX, and X, as well as fibres from the second and third sacral spinal nerves. Most parasympathetic fibres (approximately 75%) are in the vagus nerve (X), which innervates the thoracic and abdominal regions (Okeson and Bell, 2005). In the orofacial region, parasympathetic fibres travel with cranial nerve III to pupillary sphincters and ciliary muscles, cranial nerve VII to the lacrimal, nasal and submandibular glands, and cranial nerve XI to the parotid gland (Liebgott, 2001).

The parasympathetic nervous system is also made up of two types of neuron; however, the pre-ganglionic fibre travels all the way to the target organ, where it synapses with the post-ganglionic neuron in the wall of the organ.

The ANS maintains proper function of the body by having both the sympathetic and parasympathetic systems constantly active at a low level (Jänig and McLachlan, 1992; Okeson and Bell, 2005). This also allows for rapid adaptation to environmental challenges (the *fight or flight* reaction).

Trigeminal Afferent (parasympathetic sensory) Pathway

The main afferent nerve for orofacial somatic sensation is the trigeminal nerve (V), which divides into three sensory branches (Liebgott, 2001) and has a sensory field from vertically in front of the ears and across the top of the head, as shown in Figure 2[ii]. The ophthalmic division (V_1) supplies the parietal and frontal areas as well as the upper eyelid and nasal bridge. The maxillary division (V_2) supplies the anterior portion of the temple, malar and maxillary areas, as well as the lower eyelid, alar of nose and upper lip (Liebgott, 2001).

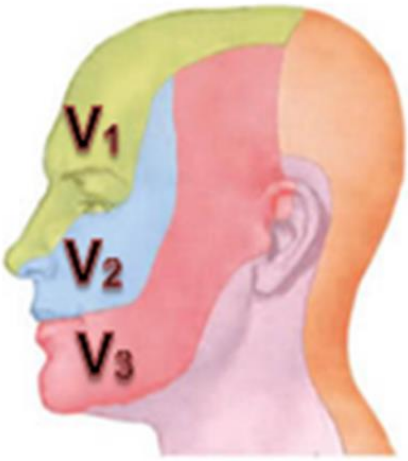


Figure 2 Sensory fields of the Trigeminal nerve branches [ii]

Intraorally, V_2 supplies the palate, maxillary process and associated teeth, periodontium and gingiva and some of the posterior buccal mucosa. The mandibular division (V_3) supplies the posterior temple, tragus, preauricular area, masseter, lower lip, external auditory canal and mandibular area (excluding the angle of the mandible). Intraorally, V_3 supplies the anterior two-thirds of the tongue, mandibular teeth, the periodontium and overlying gingiva and mucosa of the floor of the mouth (Liebgott, 2001).

The Neurophysiology of Pain

The neurophysiology of pain encompasses how neural impulses are transferred from one neuron to another from peripheral receptors to the CNS and back out again to receptor organs for appropriate action.

[ii] Adapted from: http://www.minclinic.ru/pns/pns_eng/trigeminus_eng.html on 17/09/11.

Nerve Action Potential

Impulses received from the dendrites are carried down the axon as an action potential. This comprises a change in the charge on the cell surface, which opens the sodium *ion channels* on its surface, leading to depolarisation, which is followed by a return to its resting state and repolarisation. This process continues down the sensory pathway until it reaches the CNS.

The signal is transmitted between neurons via junctions called *synapses* (often where there is contact with dendritic processes), and impulses are carried across to cause an action potential and subsequent depolarisation of the next neuron. There are chemical and electrical synapses, but the CNS predominantly comprises chemical synapses (Okeson and Bell, 2005). As the afferent input ascends to higher centres, it can be modulated by the brainstem or cortex (as discussed in "Neural Pathways of Pain" section). This is known as the *gate control theory*, where the signal can be blocked, transmitted, or modified at different central regions (Melzack, 2001).

Ion Channels

Synaptic membranes contain many ion channels, of which there are three main types. The first type, v*oltage-gated ion channels*, are the basic component of the membrane's ability to rapidly depolarise, allowing positively and negatively charged ions to pass in and out of the cell, via sodium, potassium and calcium channels (Armstrong and Hille, 1998). The second type of ion channels are *G-protein-linked ion channels*, which have receptors which are activated by mediators (e.g. bradykinin and prostaglandins) which stimulate intracellular messengers, leading to slow opening of the ion channels (Hepler and Gilman, 1992). Third, there are *ligand-gated ion channels*, where ligands (for example ATP) act as mediators and cause the opening of channels (Hucho and Weise, 2001).

Neurotransmitters

Neurotransmitters are the chemical mediators released by the pre-synaptic neuron into the synaptic cleft; they activate ion channels. The two types of neurotransmitters are small, fast-acting neurotransmitters and larger, slow-acting neuropeptides.

These small, rapid-acting molecules lead to acute responses of the nervous system. Examples include: acetylcholine, noradrenaline, glutamate, dopamine and serotonin. Acetylcholine, noradrenaline and glutamate usually have an excitatory effect on the

post-synaptic neuron, yet dopamine normally has an inhibitory effect. Centrally, serotonin potentiates endorphin analgesia and peripherally it is a neurovascular algogenic (pain-causing) agent (Okeson and Bell, 2005).

The large, slower-acting neuropeptides are manufactured in ribosomes and transported to the synapse for release. Examples of these include substance P, endorphins and bradykinin. Substance P is a polypeptide that is released at the primary terminals of nociceptive *A-δ* and C-fibre afferents (including in dental pulp nerve fibres) and acts as a transport substance, exciting *wide dynamic range* (WDR) and *nociceptive specific* (NS) neurons (Sessle, 1987) in the dorsal horn. Substance P has a rapid and short-lived action as a strong vasodilator, promoting oedema and the release of histamine—which is also an excitatory neurotransmitter that promotes vasodilation and oedema—from mast-cells (Besson, 1999). Endorphins are polypeptides that bind to morphine receptors and cause a dulling of pain, thus acting as "endogenous opiates" (Amir et al., 1980; Sessle, 1987; Zubieta et al., 2003). Bradykinin is released during inflammation and ischaemia, and is an algogenic agent that excites all receptors and sensitises high-threshold receptors so that they respond to otherwise sub-threshold stimuli (hyperalgesia). It requires the presence of prostaglandins to act (Chapman and Dickenson, 1992; Mizumura et al., 2009). There is a lot of interaction between neurotransmitters in pain modulation; for example, endorphin is potentiated by serotonin, which is only released in the presence of dopamine (Okeson and Bell, 2005).

Following the release of a neurotransmitter into the synapse, it is eliminated via various means. These include *diffusion*, where the neurotransmitter diffuses out of the synaptic cleft; *enzymatic destruction*, by enzymes that are either already present or are released; or *reuptake*, which can include active transportation back into the presynaptic terminal for re-use e.g. noradrenaline in the sympathetic nervous system (Okeson and Bell, 2005).

Neurochemistry of Nociception

Peripheral nociceptors can be activated by thermal, chemical and mechanical stimulation. Once stimulation has been removed, the most likely reason for continued nociceptive input is neurochemical substances accumulating near the nociceptor. Sources of these neurochemicals can include the damaged cell (via leakage of

intracellular substances), plasma extravasation, lymphocyte migration or the nociceptor itself, for example, by releasing substance P.

Histamine and potassium are known to be released from damaged tissues, and they can activate or sensitise the nociceptor. Bradykinin is known to induce the thermal sensitisation of polymodal nociceptors (Kumazawa et al., 1991).

In regions of tissue damage, metabolites of arachidonic acid are synthesised. These are inflammatory mediators. *Prostaglandins* are one type of inflammatory mediator. They are not algogenic substances, but help to sensitise nociceptors, effectively reducing pain thresholds (Besson, 1999). Prostaglandin E_2 is metabolised from arachidonic acid via the enzyme cyclooxygenase (COX), which has two isomers: COX_1 (which produces prostaglandins that aid normal physiologic functioning) and COX_2 (present in most tissues, it produces prostaglandins involved in inflammation). Thus, if COX_2 is inhibited, the inflammatory reaction is reduced, reducing pain (Besson, 1999). The presence of prostaglandins is required for bradykinin to act; this in turn stimulates the release of prostaglandins, each effectively potentiating the other. Another important metabolic part of the arachidonic pathway is the lipoxygenase pathway, which produces *leukotrienes* (Lewis et al., 1990). Leukotriene B_4 causes hyperalgesia which is not inhibited by COX inhibitors.

Neuronal Sensitisation

Following the release of excitatory neurotransmitters into the synaptic cleft, excitation of the post-synaptic neuron and carriage of the impulse down the axon, excitatory neurochemicals may remain in the synaptic cleft. This will lead to the neuron being depolarised more easily when neurotransmitters are next released, via a lowering of the excitatory threshold. This leads to greater sensitivity of adjacent primary afferent neurons so that even light mechanical stimulation may cause depolarisation (Besson, 1999; Miles et al., 2004; Racich, 2005). This process is known as neuronal sensitisation.

Pain Modulation by Psychological Factors

Pain experienced can be influenced positively and negatively by the psychological state of the individual (Sessle, 1987). It can be increased by such psychological excitatory

factors as the attention paid to the injury (Okeson and Bell, 2005), the individual expecting pain, usually based on past experiences (Arntz et al., 1990; Bergius et al., 2000), anxiety and fear as a result of previous pain experiences (Bergius et al., 2000; Klages et al., 2004; Klages et al., 2006), and depression (Keefe et al., 2001). Conversely, some inhibitory factors can include increasing patients' expectations that a drug will produce powerful analgesia or a "placebo effect" (Miles et al., 2004), attention being directed away from the injury (distraction), and confidence and assurance (Loggia et al., 2008).

Psychological factors which are associated with poor adjustment to pain include anxiety (Litt, 1996; Sergl et al., 1998), pain catastrophising, and pain-related anxiety or fear of pain. Pain catastrophising relates to the individual's tendency to focus on pain, along with an inability to deal with it (Keefe et al., 2001). Previous studies have shown that a high level of dental anxiety mirrors a strong influence of fear of both specific and general painful objects and situations (Litt, 1996).

State-Dependent Sensory Processing

The way in which the CNS receives, modulates and interprets input can be complicated further by the state of the dorsal horn when the nociceptive input arrives. Mechanical, chemical, or thermal threats to tissue integrity cause nociceptive neurons to increase their discharge rate, and there are four states described (Woolf and Doubell, 1994). First, there is the *basal state* (Carr and Goudas, 1999), where processing of the nociceptive input follows the normal pattern; for example, low-intensity stimuli activates only low-threshold primary afferent neurons and is interpreted as innocuous (touch, warmth, vibration). Reaction to noxious stimuli elicits reflex and behavioural avoidance responses. Second, there is the *suppressed state*, where stress-induced analgesia reflects the bilateral descending inhibition of neural activity from the brainstem to spinal level and the analgesic and anti-inflammatory effects, such as β-endorphin (Carr and Goudas, 1999). In this situation, a high-intensity nociceptive stimulus will fail to evoke the sensation of pain. This is also known as *hyposensibility* and can help individuals during fight-or-flight to reduce pain sensation from injuries. Third, there is the *sensitised state,* where the excitability of cells in the spinal tract nucleus is markedly increased with the sensitised nociceptors having an increased rate

of discharge, a lowered stimulus threshold, a supranormal increase in discharge rate with each increase in stimulus strength, or a combination of these changes (Carr and Goudas, 1999). At the site of injury, inflammatory mediators (such as prostaglandins), neurotransmitters, and growth factors surround sensitised nociceptors so that a low-intensity input will elicit pain (allodynia) and noxious input will be exaggerated (secondary hyperalgesia). This may also play a part in survival, because it may help an individual to protect an injured body part from further trauma during healing (Carr and Goudas, 1999). Finally there is the *reorganised state.* In this mode, there is a structural reorganisation of synaptic circuitry in the spinal tract nucleus, where axons may have atrophied or died as a result of injury to the nervous system. This alters the way in which input is processed, and it may result in neuropathic pain long after the injury heals (Woolf and Doubell, 1994). The first three modes represent normal, healthy functioning, and transitions between these modes occur in response to different types, intensities and qualities of input (Okeson and Bell, 2005).

Orthodontic Pain

Literature shows that up to 95% of orthodontic patients report pain at some stage during their treatment (Bergius et al., 2008), beginning with initial discomfort when orthodontic force is applied, but which disappears immediately. The second response appears much later, with peak intensity on day one or two and lasts a few days (Jones and Chan, 1992). This was demonstrated in a Swedish study, where 87% of patients reported pain on the first evening after elastic separators were placed between their teeth, with pain reaching a maximum score after 24 hours, as rated on a visual analogue scale (Bergius et al., 2002). The pain generally begins to subside after 48 hours and decreases to pre-application levels within seven days (Krishnan, 2007); however, up to 10% of patients will discontinue their treatment due to the pain experienced (Patel, 1989).

Orthodontic forces are designed to move teeth. Pain is thought to be caused by a range of orthodontic procedures, such as separator placement, archwire placement and activations (Krishnan, 2007). It has been reported that orthodontic procedures will reduce the proprioceptive and discriminating abilities of patients for up to four days, which results in lowering of the pain threshold and disruption of normal mechanisms associated with proprioception input from nerve endings in the periodontal ligament (PDL; Soltis et al., 1971). The forces applied to teeth also cause compression of the PDL, leading to pressure, ischaemia, inflammation and oedema in the PDL space (Krishnan, 2007). Periodontal nerve endings consist of low-threshold mechano-receptors and nociceptors. Pain is initiated via compression and stretch of the low-threshold mechano-receptors, whereas nociceptors are activated by tissue injury or heavy forces (Fujiyoshi et al., 2000). Orthodontic tooth movement may initiate mechanically-induced inflammatory responses in the periodontium (Yamashiro et al., 1998), stimulating the release of neuropeptides such as prostaglandin and substance P from peripheral nerves (Krishnan, 2007). The level of prostaglandin in the periodontium peaks at approximately 24 hours and gradually subsides over the next few days, to reach base level again by 7-14 days (Yamashiro et al., 1998).

Fujiyoshi et al. (2000) described two different responses during experimental tooth movement. The initial response, within the first two hours of force application, occurs at the ipsilateral medullary dorsal horn and then quickly disappears. This reaction may be

due to compression of the PDL (Bergius et al., 2000). The second response appears around four hours after the application of orthodontic force; it occurs at the trigeminal subnucleus oralis and lasts for up to a few days. This response has been called hyperalgesia of the periodontal ligament and is due to a greater sensitivity of nerve fibres to noxious stimuli, such as prostaglandins, histamines and substance P (Besson, 1999; Bergius et al., 2000; Miles et al., 2004). In a cat model, substance P appeared in the dental pulp within three hours, but later in the PDL (24 hours to 14 days), mainly at sites of compression (Nicolay et al., 1990).

It has been suggested that, due to the greater tenderness to pressure of the teeth involved, pain may also be due in part to a mild pulpitis reaction (Bergius et al., 2000) caused by periodontal inflammatory mediators spreading and diffusing into the pulp (Dalili, 2009). Pain can also be due to soft tissue injury from fixed orthodontic appliances (Scheurer et al., 1996).

The trigeminal nerve supplies sensory innervation to the teeth, with unmyelinated (C-) fibres located in the pulp, providing dull, poorly-localised, lingering pain, whereas myelinated (mostly A-δ) fibres are located in the dentine or pulpal periphery and provide brief, localised, sharp pain (Chaudhary et al., 2001).

There appears to be a variable response among individuals undergoing orthodontic treatment (Ngan et al., 1989; Krishnan, 2007; Bergius et al., 2008), with some patients feeling high levels of pain and others just mild discomfort, despite similar sex, race and age.

In a small study of 24 patients over the initial 16 days following placement of fixed appliances, there was no correlation between the total discomfort experienced and the severity of dental crowding. There was also no difference in the amount of crowding when patients were compared by pain response type (none, mild moderate or severe pain; Jones and Richmond, 1985). The same authors also conducted a randomised control trial of pain and discomfort during orthodontic treatment of two initial aligning wires (Japanese nickel titanium 0.014’ diameter and multi-stranded stainless steel 0.015’ diameter). They again concluded that the severity of initial dental crowding had no influence on the pain experienced and also found that there was no difference in the prevalence, intensity and duration of pain between the two initial archwires (Jones and

Chan, 1992). Comparing a 2x4 appliance, single arch and two arch appliances, no statistically significant differences have been found in reported pain frequency, general intensity of pain, tooth pain, discomfort when biting and chewing, and analgesic consumption. Overall, upon placement of the archwire, more pain has been reported from anterior than posterior teeth; this may be due to levelling of the arches, because the anterior teeth are more involved and have smaller root surfaces (Scheurer et al., 1996); however, following placement of separating elastics, more discomfort from posterior teeth was reported (Ngan et al., 1989).

Factors Influencing Orthodontic Pain

There have been few studies of pain experience during orthodontic treatment, fewer on possible modifying factors which may predispose individuals to experience more pain during treatment, and no studies into the possibility of COMT gene influence. There are some factors that have been identified as influencing orofacial pain, which some believe may also influence orthodontic pain.

Age

There appear to be conflicting findings with regard to age differences in orthodontic pain experience. This may be due to different treatment approaches; for example, a patient in the mixed dentition may not receive the same appliance as older patients (Bergius et al., 2000; Krishnan, 2007). Ngan (1989) found no statistically significant difference in relation to treatment with fixed appliances between adolescents and adults. However, Scheurer (1996) reported the most pain in the 13-16 age group, while other orthodontic studies reported that the older the patient, the greater the pain reported (Jones and Chan, 1992; Bergius et al., 2008), the greater the pain sensitivity (Jones, 1984), and the lower the pain tolerance (Walsh et al., 1989).

Sex

Orofacial and dental pain does tend to show statistically significant differences between sexes (Krishnan, 2007; Svensson et al., 2011), with women reported as having lower pain thresholds and reporting more pain (Woodrow et al., 1972; Walsh et al., 1989). It has been proposed that sex-dependent differences in pain sensitivity may be due to evolutionary pressure for women to be more aware of environmental threats against their offspring (Diatchenko et al., 2007). Sex differences have also been reported in the orthodontic literature (Scheurer et al., 1996; Bergius et al., 2002; Krishnan, 2007);

however, this is controversial, because there seem to be very few statistically significant associations reported between sex and orthodontic pain (Ngan et al., 1989; Jones and Chan, 1992).

Ethnicity

The term ethnicity distinguishes between groups of people based on behaviour and culture as well as physical characteristics; it includes what is usually categorised as *race* (for example Asian), but also refers to characteristics that are of social, psychological, cultural, and political nature (Edwards et al., 2001).

Some dimensions of pain are universal but others are learned. Some cultures encourage stoical attitudes and behaviour (Bergius et al., 2000). In some other cultures, individuals are encouraged to openly express their responses (Krishnan, 2007) and receive sympathy and attention for this behaviour (Bergius et al., 2000).

Social factors, such as differences in socioeconomic status, can influence access to health care, so that some ethnic groups do not receive adequate pain relief due to inadequate medical treatment (Edwards et al., 2001). Under-treatment of pain in Hispanic patients (relative to non-Hispanic Caucasian patients) has been previously reported to be at high levels in Emergency Department settings of American hospitals (Todd et al., 2000). Long term under-treatment of pain has been shown to produce increased psychological strain and reduced coping levels, as well as high levels of sympathetic nervous system activation. This may reduce an individuals' ability to cope with acute or persistent pain (Edwards et al., 2001).

Anxiety and Fear

Dental anxiety or previous negative dental experiences can increase the risk of reporting pain (Bergius et al., 2008; Mobilio et al., 2011) and psychological factors, such as anxiety (De Jongh et al., 1994; Newton and Buck, 2000; Fuentes et al., 2009; Armfield, 2010b) and dental fear (Vassend, 1993; Armfield, 2010a; b) have been associated with more pain during dental procedures.

It has been shown that dental anxiety lowers the pain threshold and can lead to the perception of normally non-painful stimuli as painful, and that a high level of dental anxiety mirrors a strong influence of fear of both specific and general painful objects and situations (Vassend, 1993; Litt, 1996). When anxiety sensitivity is combined with

high dental fear, pain perception is increased (Klages et al., 2006), because fearful patients have also been shown to expect and experience more pain in dental situations than less fearful patients (Klages et al., 2004; Klages et al., 2006). There are also genetic influences on anxiety disorders (discussed below).

An efficient, reliable and sensitive means to measure anxiety as a general aspect of personality (trait) and anxiety as a response to a specific situation (state) is *Spielberger's State–Trait Anxiety Inventory* (STAI; Appendix IV). This 40-item Likert-type frequency scale (responses are scored along a range) questionnaire is divided into two 20 question parts, with state anxiety questions assessing the transitory emotional state of the individual, which may be characterised by feelings of tension, apprehension and increased autonomic nervous system activity. The trait anxiety questions measure relatively stable individual differences in anxiety-proneness (Spielberger et al., 1970; Newton and Buck, 2000).

Dental anxiety is commonly measured using the *Corah Dental Anxiety Scale* (DAS; Appendix IV), which is a four-item measure in which participants are asked about dentally related situations (Newton and Buck, 2000). It has been shown to have both high reliability and test-retest stability when assessing dental anxiety (Corah, 1969; Corah et al., 1978).

An American study of pain after placement of orthodontic appliances used a group of 129 patients who were randomly divided into three groups and all told to "expect some pain and discomfort" (Bartlett et al., 2005). They also received a standardised set of oral and written instructions about general orthodontic care. The first group also received a structured telephone call demonstrating care and reassurance; the second group received an attention-only telephone call thanking them for participating in the study; and the third group, the control, received no telephone call. The findings showed that a telephone call from a health-care provider within 24 hours of orthodontic appliance placement significantly reduced patients' self-reported pain and anxiety, illustrating that the content of the telephone call was not important, yet it led to a decrease in anxiety and therefore a reduction in pain.

Mood

The *Positive and Negative Affect Schedule* (PANAS; Appendix IV) measures the two primary dimensions of mood (positive and negative affect) in a reliable, valid and efficient way (Watson et al., 1988).

Self-rated Oral Health

Locker's Global Item (Appendix IV) is a self-rating of patients' oral health and is scored on a Likert-type frequency scale (Locker, 1988; Locker et al., 2004). A higher score indicates worse oral-health-related quality of life. Locker (2004) stated that, when discussing oral health, the focus is not on the oral cavity itself but on the individual and the way in which oral diseases, disorders and conditions, whether confined to the oral cavity or linked to other medical conditions, threaten health, wellbeing and the quality of life. One described measure of quality of life focuses on physical functioning, psychological wellbeing and pain and discomfort, showing how tightly psychological wellbeing is linked to pain and discomfort through an individuals' health status (Stewart and King, 1994).

Catastrophising

Catastrophising is defined as "an individual's tendency to focus on and exaggerate the threat value of painful stimuli and negatively evaluate one's own ability to deal with pain" (Rosenstiel and Keefe, 1983). It is a multifactorial concept, comprising rumination ("I keep thinking about how much it hurts"), magnification ("I wonder whether something serious may happen"), and helplessness ("It's awful and I feel it overwhelms me"; Sullivan and Neish, 1998). The *Pain Catastrophising Scale* (PCS; Appendix IV) evaluates any exaggerated negative orientation toward noxious stimuli (such as helplessness and inability to cope effectively with pain), identifying individuals who may be susceptible to heightened distress responses. It has been shown to be a reliable and valid measure of catastrophising (Sullivan et al., 1995).

There is an apparent relationship between catastrophising (Sullivan and Neish, 1998; Keefe et al., 2001; Sullivan et al., 2006), heightened pain behaviour (Sullivan and Neish, 1998) and also more intense pain (Sullivan et al., 1995). Catastrophisers were shown to report more negative pain-related statements during a cold pressor test (discussed later) than non-catastrophisers (Sullivan et al., 1995). There have been no studies of the relationship of catastrophising to orthodontic pain experience, but it has been shown

that there is an association between catastrophic thinking and dental anxiety (De Jongh et al., 1994). A Canadian study of catastrophising, anxiety and pain during dental hygiene treatment found that an excessive focus on pain sensations played an important role in the experience of pain, and that the heightened pain experience of catastrophisers may lead them to become dentally anxious (Sullivan and Neish, 1998). The study found that the rumination subscale of the PCS contributed significant unique variance to the prediction of pain.

Both dispositional and situational studies have shown an effect of sex on pain catastrophising (Sullivan et al., 1995; Sullivan et al., 2000), with differences observed in the rumination and helplessness subscales, but not the magnification subscale. An American study into catastrophising as a mediator of sex differences in pain showed that women tend to report higher levels of catastrophising, along with more recent painful experiences, lower pain thresholds and tolerances; yet, after controlling for catastrophising, the sex disparity was somewhat diminished (Edwards et al., 2004). This association was shown again in a Canadian study, but, after PCS scores were statistically controlled for, sex was no longer associated with pain (Sullivan et al., 2000); thus, catastrophising may act a as a confounder, explaining some of the sex disparities in pain experiences. To date, no study has reported higher catastrophising levels in men.

Pain Sensitivity

Individual variation can potentially occur at any stage in pain processing, ranging from the peripheral nociceptor, through pain-regulating mechanisms in the brainstem and spinal cord, to the psychological and cognitive processes involved in interpreting and experiencing pain (Nielsen et al., 2009).

The Catechol-O-Methyltransferase (COMT) gene

Human cells have 46 chromosomes, located in the nucleus of the cell. These provide the information required for a functioning individual. Chromosomes consist of strands of deoxyribonucleic acid (DNA), which can be further divided into subunits known as *nucleotides*. Genes are specific series of nucleotides within a strand of DNA (Kardos and Kieser, 2000).

An allele is one of a number of alternative forms of the same gene for a character producing different effects. Sometimes, different alleles can result in different

observable or *phenotypic* traits, such as different pigmentation. However, many variations at the genetic level result in little or no observable variation. *Haplotypes* are the particular combinations of alleles observed in a population. When a new mutation arises, it happens on a specific chromosomal haplotype (Gabriel et al., 2002).

Individual sensitivity to pain is believed to be genetically determined by many genes which influence the pain perception pathway. Despite the small effects of individual genes, interactions between genes and environment play a big part in pain modulation. One important gene in this process is the COMT gene. The role of the COMT gene has been well established for individual susceptibility to orofacial pain (Diatchenko et al., 2005) and temporomandibular dysfunction (TMD; Slade et al., 2008), but there have been no studies into its possible influence on orthodontic pain.

Located on the long arm of chromosome 22, the COMT enzyme regulates levels of catecholamines and encephalins (Andersen and Skorpen, 2009). It acts as a key modulator in dopaminergic and adrenergic/non-adrenergic neurotransmission, which is characterised by heightened sensitivity to noxious stimuli and affects approximately 15% of the American adult population (Slade et al., 2008). Substitution of valine (*val*) by methionine (*met*) at codon 158 ($val^{158}met$) causes a difference in thermostability, leading to a three- to four-fold reduction of activity in the COMT enzyme. This substitution is also known as single nucleotide polymorphism (SNP) rs4680. Two other synonymous haplotypes of COMT (rs4633 and rs4818), combined with the $val^{158}met$ SNPs, can lead to a 30-fold difference in enzymatic activity related to pain sensitivity (low enzymatic activity leads to high pain sensitivity - to be discussed later; Diatchenko et al., 2007). Protein regulation is controlled by interaction between three alleles in these haplotypes. A further SNP (rs6269) has been implicated, along with the previous three SNPs, in contributing to a higher risk of developing TMD (Slade et al., 2008).

Three rs4680 ($val^{158}met$) genotypes have been identified to be associated with pain sensitivity phenotypes: high pain sensitivity, average pain sensitivity and low pain sensitivity (Andersen and Skorpen, 2009). In a recent study, these were shown to encompass 96% of examined genotypes (Diatchenko et al., 2005). The alleles are co-dominant, so that the *val/val* genotype has the highest COMT activity and the *met/met* genotype has the lowest COMT enzyme activity, and lower thermostability due to a

difference in COMT molecular structure (Lotta et al., 1995). Heterozygous individuals are intermediate (Zubieta et al., 2003). In the high pain sensitivity *met/met* genotype, a variation has been associated with low enzymatic activity of COMT (leading to high pain sensitivity due to more neurotransmitters being present to bind to excitatory receptors following noxious stimuli), as well as diminished activation of μ-opioid system (reduced analgesia) (Slade et al., 2008). Both homozygous genotypes are common, with the *met/met* allele frequency reported to be around 42% in US Caucasians (Enoch et al., 2003).

Within the CNS, there are several opiate receptors (μ, δ and κ) involved in pain and its control (Sessle, 1987). One of the functions of the COMT enzyme is metabolising catecholamines (such as dopamine, epinephrine, and norepinephrine) which are secondarily involved in the modulation of pain, via the endogenous opioid pathway. The level of COMT activity by different genotypes influences the functions regulated by neurotransmitters, such as the μ-opioid pathway, which is activated in response to prolonged (not acute) stressors and pain, and acts to reduce pain and stress responses. Polymorphisms in COMT have been shown to be associated with resistance to the effects of analgesics. Polymorphisms can result in lower morphine efficacy (Kolesnikov et al., 2011). The COMT haplotype has recently been shown to be associated with the efficacy of the β-adrenergic antagonist propranolol for pain reduction in patients with TMD (Tchivileva et al., 2010). Four COMT SNPs have been identified (rs6269, rs4633; rs4818; rs4680) that may contribute to a haplotype characterised by differences in COMT metabolic enzyme activity (Diatchenko et al., 2006) that are inversely correlated with alterations in pain perception (Young et al., 2012).

The application of orthodontic force may be seen as a prolonged pain challenge. Even moderate levels of pain, as they become sustained, can become a significant physical and emotional stressor, activating the μ-opioid neurotransmitter responses and subsequent compensatory changes in μ-opioid receptor binding (Zubieta et al., 2003). This pathway generally only becomes activated with prolonged, non-acute pain, so that individuals with the *met/met* genotype (high pain sensitivity) undergoing orthodontic treatment will most likely experience greater discomfort or pain during treatment (Slade et al., 2008).

Recently, Michelotti and colleagues (Michelotti et al., 2013) investigated the role of the COMT enzyme as a risk factor for chronic TMD pain. A statistically significant difference in SNPs genotype frequencies between 50 patients affected by chronic TMD pain and the 132 controls was found for the polymorphisms rs165656 (P =0.001). They also investigated a new SNP rs4646310, which was shown with logistic regression analysis to confer an increased risk of chronic TMD pain of 5.3. There was no statistically significant difference between TMD patients and controls for rs4680 or rs6269 SNPs.

Linkage disequilibrium (LD) refers to correlations among neighbouring alleles, reflecting 'haplotypes' descended from single, ancestral chromosomes (Reich et al., 2001). Because disease mutations are inherited by individuals who share a common, distant ancestor, they will also share a region of the ancestor's haplotype from where the disease mutation originated. Markers within this shared haplotype are associated with the disease, and each other, and are in "linkage disequilibrium" (Abecasis and Cookson, 2000). Linkage disequilibrium mapping presents a powerful analysis for the mapping and detection of genes with a modest effect on risk (Li et al., 2000). LD between several COMT SNPs associated with orofacial pain is shown in Figure 3.

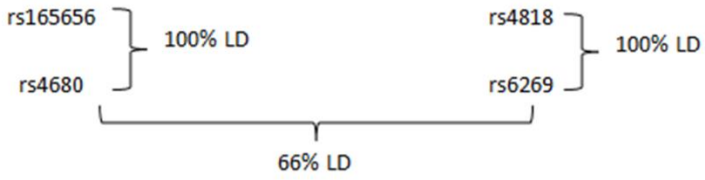


Figure 3 Linkage disequilibrium of COMT SNPs associated with orofacial pain [iii]

It has been shown that, during the follicular phase of women's menstrual cycles (days 1-13), oestradiol levels are at their lowest, and have the least effects on COMT transcription (oestrogen can inhibit COMT gene transcription; Enoch et al., 2003). COMT plays a significant role in metabolisation of basal ganglia dopamine in humans,

[iii] Information was generated by the Merriman lab using publically-available genotype data from the 1000 Genomes project and Haploview software.

and the amygdala has been implicated in the μ-opioid receptor-dependent, stress-, and fear-induced analgesia and emotional regulation (Zubieta et al., 2003). The three COMT haplotypes may account for approximately 11% of variation in pain perception; this is quite considerable (Diatchenko et al., 2005). A different SNP of COMT (rs740603) showed an association with maximum post-operative pain following dental extraction (Kim et al., 2006).

As an aside, on a small sample of 29 healthy participants, Zubieta et al. (2003) found that in response to a sustained pain challenge (internal emotional state was measured using the PANAS and pain was measured using the McGill Pain Questionnaire), there were higher negative affect scores in individuals with the lowest (*met/met*) COMT activity, followed by heterozygotes and *val/val* homozygotes. Enoch et al. (2003) found no association between the rs4680 genotype and the Spielberger *State* anxiety scores in 401 US participants.

Stein et al. (2005) genotyped 497 undergraduate students for three common COMT polymorphisms (including rs4680) that combine to define a haplotype that is associated with reduced COMT activity in the human brain. Individuals with the chromosome 22q11 micro-deletion in the COMT gene have a high frequency of mood disorders, including anxiety (Enoch et al., 2003). Anxiety-related traits (such as high neuroticism) were associated with rs4680 and rs737865 (located in the first intron) polymorphisms. The rs4680 polymorphisms have been associated with a variety of personality traits, such as harm avoidance and neuroticism (Enoch et al., 2003).

There is sexual dimorphism in anxiety disorders, with generalised anxiety disorders being more common in women (Runeson and Rich, 1994). Studies on mice have shown that COMT-deficient female mice (but not males) display greater anxiety. In humans, women have been shown to have significantly lower COMT activity than men (this could be due to the fact that oestrogen can inhibit COMT gene transcription), which may lead to an increased vulnerability to anxiety disorders when COMT gene expression is below a minimum threshold (Enoch et al., 2003). In a study of 58 patients undergoing arthroscopic shoulder surgery, high pain catastrophising and low COMT activity were associated with higher pre-operative ratings and a greater risk of experiencing persistent pain following surgery (George et al., 2008b).

Genetic phenotyping shows great promise in the future of orthodontic treatment, because clinicians may better predict which of their patients may have greater pain experience, as well as being able to provide a more individualised approach to their analgesic regime, especially with opioids.

Measurements of Pain

Orthodontic pain has been simulated in previous clinical studies by placement of elastic separators, mesial and distal to first molar teeth, to allow assessment of pain perception as well as creating a simple homogenous research model, because this procedure is often the start of orthodontic treatment (Ngan et al., 1989; Bergius et al., 2002).

One way to assess potential clinical pain is by assessment of experimental pain thresholds and tolerance. Pain tolerance is defined as the highest level of pain stimulus at which the participant *requests* cessation of stimulus; the pain threshold is that stimulus at which the participant first *recognises* pain or discomfort (Woodrow et al., 1972). Gelfand (1964) found that pain tolerance has no strict association with pain threshold and that pain tolerance is more closely related to clinical pain than the pain threshold is.

Pain is a subjective experience, and the self-report approach must therefore be used for its assessment. The *Visual Analogue Scale* (VAS) is a widely used non-verbal measure for orthodontic pain (Ngan et al., 1989; Jones and Chan, 1992; Scheurer et al., 1996), where patients describe their pain by placing a mark on a 100mm horizontal line that has anchors labelled “no pain” and “unbearable pain” (Duncan et al., 1989). The VAS has been validated as a measure of pain many times, proving more accurate than other pain scales (such as a five-point verbal rating scale or fixed-interval rating scale; Aitken, 1969). The reliability of the VAS for pain measures has been confirmed by Revill and colleagues (Revill et al., 1976) and it has been shown to be well suited to within-individual repeated measurements (Aitken, 1969) for those aged over five years (Bergius et al., 2008).

Elicitation of Pain

Methods of assessing experimental pain levels include electrical pulp testing (EPT) and the cold pressor test. Electrical pulp testing can be used to deliver an electric current to a tooth that is sufficient to overcome enamel and dentine resistance to stimulate the small, fast-conducting myelinated A-fibres at the pulp-dentine junction (there is not

enough current to stimulate the C-fibres; Bender et al., 1989). As the intensity of the stimulus increases, more sensory nerves are activated, and this results in a progressive increase in the sensory response (Lin and Chandler, 2008). In a Japanese clinical trial, EPT was found to be 99% accurate in 396 teeth with vital pulps (Kitamura et al., 1983). It was concluded from studies with a *Macaca mulatta* monkey, following five hours of continuous EPT stimulation, that prolonged electric pulp testing caused no histological damage to the dental pulp (McDaniel et al., 1973).

The Cold Pressor Test is an experimental technique for inducing an emotional/motivational pain experience (Walsh et al., 1989). It is a measure of pain tolerance which involves participants placing their hand and lower forearm in cold water, inducing a slowly mounting pain (via C-fibres) that dissipates quickly on withdrawal of the limb from the water (von Baeyer et al., 2005). It has been reported that preoperative cold pain tolerance, using the cold pressor test, can be used to predict post-operative pain after wisdom tooth extraction (Mobilio et al., 2011), laparoscopic cholecystectomies, even after controlling for neuroticism (Bisgaard et al., 2001). Collectively, these findings suggest that experimental pain responses are associated with clinical pain reports (namely, that greater pain sensitivity is associated with greater clinical pain; Edwards et al., 2005).

Current Study

Background

The International Association of Pain defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such" (Merskey and Bogduk, 1994). Pain is highly subjective and is influenced by a mixture of physical, cognitive, emotional and genetic factors (Andersen and Skorpen, 2009).

There appears to be a variable response among those undergoing orthodontic treatment (Ngan et al., 1989; Bergius et al., 2008; Krishnan, 2007), with some patients feeling high levels of pain and others just mild discomfort. There have been few investigations into why some patients experience more pain during treatment than others, despite similarities in malocclusion, sex, race and age.

The literature has identified modifying factors for orthodontic pain, including anxiety (Bergius et al., 2008), dental fear (Klages et al., 2006), psychological state (Loggia et al., 2008), patients' attitudes towards treatment (Sergl et al., 1998), age (Svensson et al., 2011) and ethnicity (Bergius et al., 2000; Krishnan, 2007). The literature on sex differences in response to orthodontic pain is inconclusive (Ngan et al., 1989; Scheurer et al., 1996; Bergius et al., 2000), and there also appears to be no association between the severity of crowding (Jones and Richmond, 1985) or type of archwire used and pain levels reported (Jones and Chan, 1992)Studies of the genetic predictors of orofacial pain and TMD have pinpointed the Catechol-O-Methyltransferase (COMT) gene as a major influence (Diatchenko et al., 2005; Slade et al., 2007, 2008). However, there have been no studies on the COMT gene's influence in orthodontic pain.

Elastomeric separators will be used in this study to introduce orthodontic force. As stated earlier, this allows assessment of pain perceived as well as creating a homogenous research model.

Being able to identify individuals who may have a high pain response to orthodontic treatment would help the clinician to provide the patient with a more comfortable experience, by utilising patient-specific management strategies to help minimise orthodontic discomfort.

Aims and Objectives

The aim of this study was to investigate possible modifying factors of pain experienced following application of orthodontic force.

The objectives of this study were:

1. To determine, in a convenience sample, the variability of pain responses following orthodontic force application with elastomeric separators and to identify high and low pain responders.
2. To compare high and low pain responders for differences by self-rated oral health, anxiety (both as a general aspects of personality and in response to a specific situation), mood, dental anxiety and fear, catastrophising, genetics, general sensitivity (cold) and tooth sensitivity.

Hypothesis

The study hypothesis was that the reported amount of orthodontic pain is associated with other pain thresholds, specific psychological and emotional factors (such as dental anxiety), sex and specific SNPs (rs4680, rs4646310 and rs6269) of the COMT gene.

Materials and Methods

Data and Collection

Data collection, in regard to the use and disclosure of personal information, was in compliance with The Privacy Act 1993 and the Health Information Privacy Code 1994. Participants were informed of the purpose for which we were collecting the information and the uses we proposed to make of it. They were allowed access to (and the right to correct) any personal information gathered.

Māori consultation was sought and Ethical Approval gained (Appendix I).

Data were kept in secure storage within the Orthodontic Clinic and any personal information held about the participants (such as contact details) was destroyed after it had been transcribed. After the completion of the study, all personal identifying information was removed and the remaining data will be stored indefinitely. The principal researcher had access to personal information. Supervising Faculty staff had access only via liaison with the principal researcher. Participants had access to the data in its raw format, and the research findings were made available to them upon completion of the project, if requested.

Sample

A convenience sample of 107 students (mean age = 24.1 years; 49.5% female, 50.5% male) in the Faculty of Dentistry (University of Otago) participated in Phase One of the study. Potential participants were invited to participate by the principal researcher through personal contact, advertising posters, online noticeboards and by dropping flyers into the pigeonholes of all undergraduate and postgraduate students at the Faculty of Dentistry.

Inclusion criteria included: age 18 years or older; good general and oral health; contact with adjacent teeth on mesial and distal surfaces of mandibular first molar teeth (tested by dental floss resistance) and a willingness to participate in the study (signalled by signing a written consent form).

Exclusion criteria included: diagnosed depressive illness; a history of Raynaud's phenomenon or any chronic pain syndrome; use of neurologically-acting medication or medication that can potentially affect pain sensitivity (such as anti-depressants); spacing in the mandibular dentition; active caries or periodontal disease; large restorations; and previous trauma or root canal treatment of the maxillary central incisors.

As an incentive for taking part in Phase One research, all participants were offered a movie voucher and went into a draw for one of two iPod Touch devices; for Phase Two, each participant received a $50 gift voucher.

Participants were provided with an explanation of the research goals and procedures and the investigator was available to participants after the project, should any stress, harm, or related concerns have arisen.

Experimental Procedure

This study consisted of two phases. During the first phase, participants were screened for pain response to the placement of an orthodontic elastomeric separating ring ("Sep-A-Ring Separators", maximum stretch 18 mm, TP Orthodontics Inc, Indiana, USA) between their teeth over 48 hours (Figure 4).

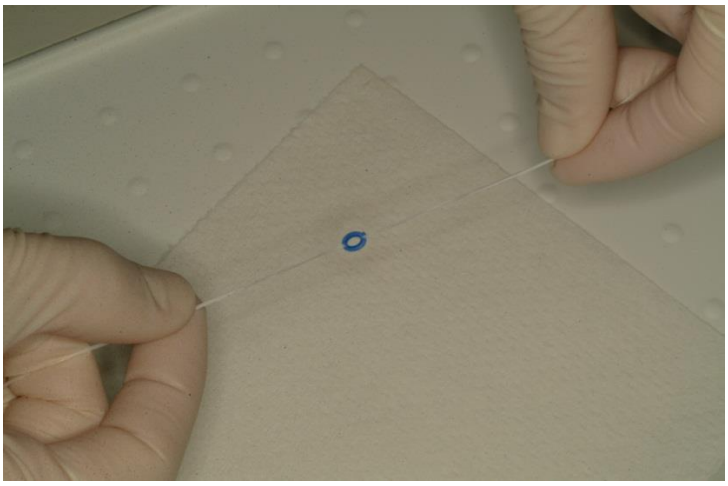


Figure 4 Orthodontic elastomeric separator

This was used to identify high and low pain responders, who were then invited to enter phase two testing. Phase two consisted of cold pressor testing, electric pulp testing, a self-rating on oral health, testing of anxiety levels and dental fear as well as testing genetic variations in order to investigate how the two groups differed.

Phase One

Following the placement (Figure 5) of two orthodontic separating rings – one mesial and one distal to the mandibular right first molar tooth (Figure 6) – participants were asked to complete six visual analogue scales on pain levels over the next 48 hours (McCormack et al., 1988; Appendix III).

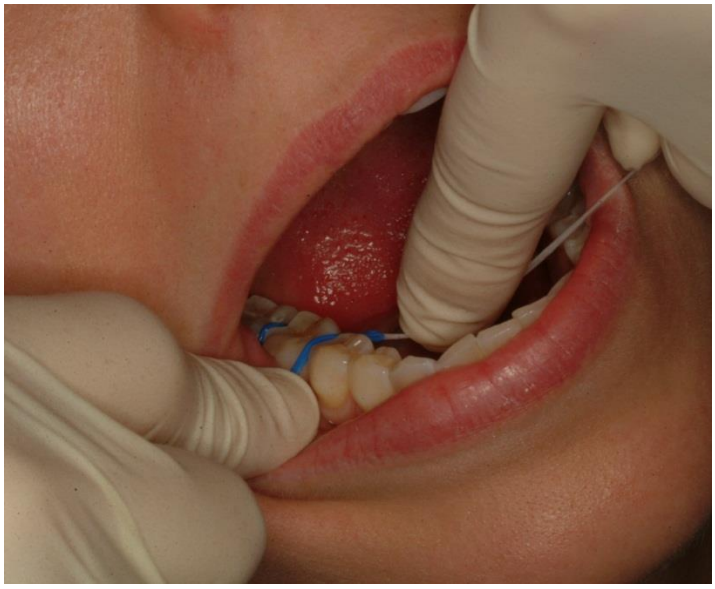


Figure 5 Insertion of elastomeric separators

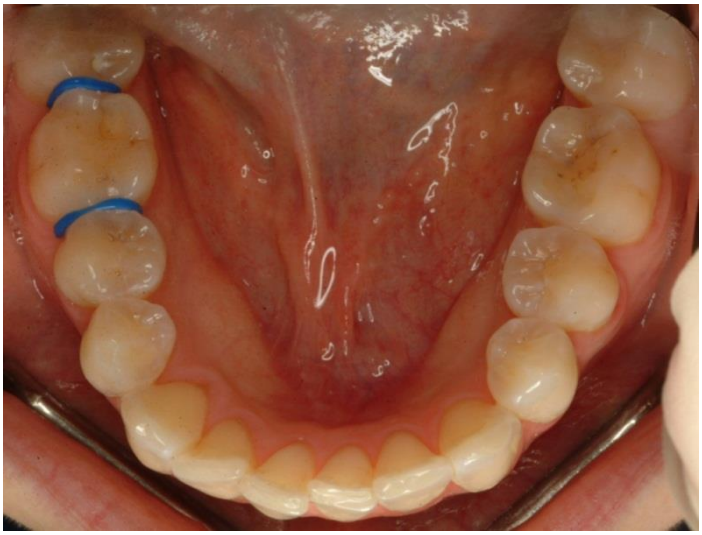


Figure 6 Elastomeric separators in situ

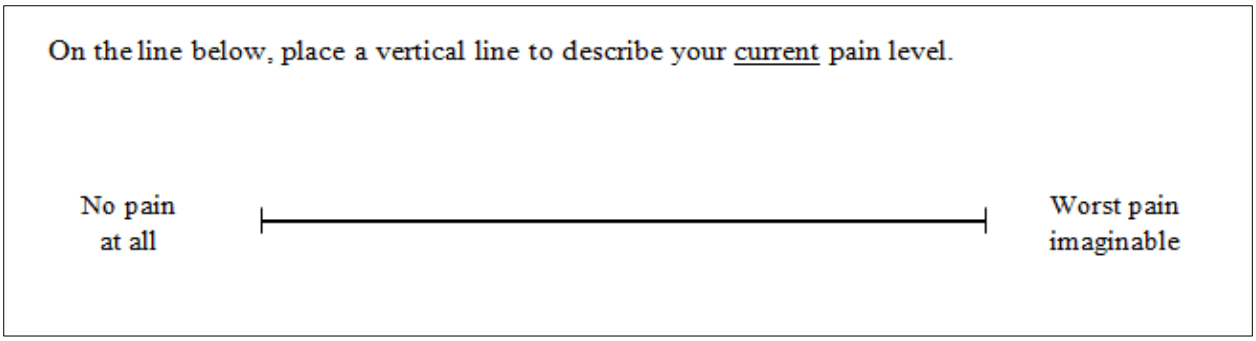


Figure 7 Visual Analogue Scale

The VAS (Figure 7) consisted of a 10 cm horizontal line on a sheet of paper with labels at either end. The participant placed a mark on the line wherever their level of current pain or highest intensity of pain since the last report lay. These reports took only seconds to complete, with the first one being completed under supervision in the clinic as soon as the separating rings were placed. The next one was four hours after insertion of the elastic separating rings, followed by one that evening before retiring, another report first thing the following morning, and another in the evening prior to retiring. The last report was made the following day (making six reports, in total, over 48 hours). The first VAS report related to the current pain level; subsequent reports included a second VAS on the maximum pain intensity felt over the time since the previous report as well. After the final VAS report was completed, participants returned to have the separating rings removed.

Based on these scores, 10 participants above the 90th percentile (peak VAS score from Phase One 8.00 cm or higher) and 10 participants below the 10th percentile (score 0.55 cm or lower), were selected and defined as “high” or “low” pain responders (respectively) and invited to participate further.

Phase Two

Phase Two commenced shortly after completion of Phase One.

(1) Emotion, Dental Fear and Anxiety Questionnaire (Appendix IV)

Participants were asked to complete a 15 minute questionnaire prior to testing. The questionnaire comprised: the Corah Dental Anxiety Scale (DAS; Corah, 1969); Locker's Global Item (Locker, 1988); the Positive and Negative Affect Schedule (PANAS; Watson et al., 1988); the Pain Catastrophising Scale (Sullivan et al., 1995); and Spielberger's State–Trait Anxiety Inventory (STAI; Spielberger et al., 1970).

(2) Cold Pressor Test

This test evaluates pain sensitivity in experimental conditions. It is based on von Baeyer's methodology (von Baeyer et al., 2005) and elicits an emotional/motivational pain experience from the immersion of a limb in a 21.5 litre tank of cold water which has been insulated to ensure constancy of temperature (Walsh et al., 1989).

In preparation for testing, participants immersed their non-dominant hand into a water tank at 36 °C (+/- 1°C) for two minutes. They were then seated comfortably next to the tank (Figure 8) and then placed their non-dominant hand into the tank of continuously circulating water (10°C +/- 1°C), flowing from a tap above the tank.

Figure 8 Cold pressor test apparatus

Hands were immersed unclenched and palm-up, to a depth of 5 cm above the wrist into the tank (arm not secured), for three minutes (Figure 9). After each minute, without removing their arm from the tank, participants were asked to mark on a VAS the pain/discomfort level currently experienced (Appendix VI). The last VAS was completed prior to removing the hand from the tank. If at any time the pain was intolerable, participants could remove the arm from the tank and complete the VAS.

Figure 9 Cold pressor test

Appropriate resuscitation equipment was readily available during this testing, but it was not required.

(3) Electric Pulp Testing

The dental pain threshold and tolerance were tested using an electrical pulp tester (EPT) of the type used routinely in general dental practice (Figure 10).

Figure 10 Electric pulp tester

Participants were seated comfortably in the dental chair. Their maxillary central incisors were isolated using cotton rolls, dried with gauze (no air-blast), and then Mylar strips were placed mesially and distally, to isolate them from the adjacent teeth.

The Kerr Vitality Scanner 2006 Electric Pulp Tester (SybronEndo, Anaheim, CA, USA) was used following manufacturer's instructions. In common with most contemporary EPT instruments, the instrument's scale runs from 0 to 80. The stimulus rate was fixed at level "4" throughout Phase Two testing. "Close Up Active Gel" 0.21% NaF toothpaste (Unilever, N.V, Rotterdam) was used as a contact medium on the probe tip.

The probe tip was placed on the incisal third of the enamel (to ensure no greater sensation due to exposed dentine on the incisal edge) as recommended by Bender et al. (1989) and, once positioned by the clinician, the participant was asked to grasp the handle of the probe (Figure 11).

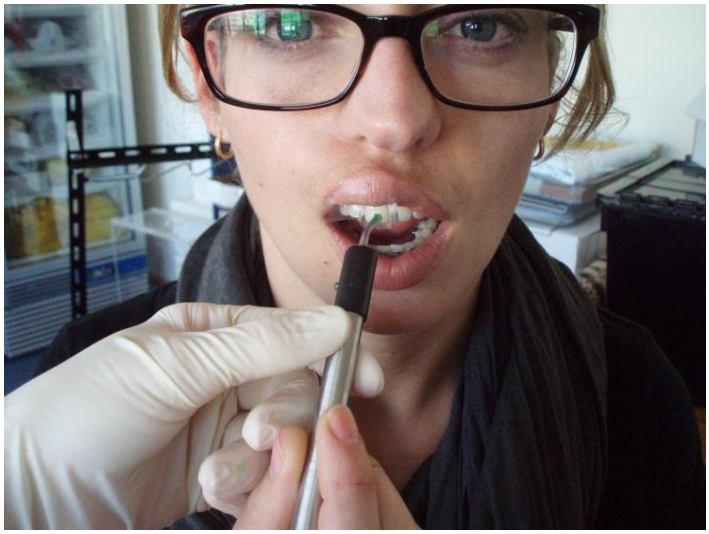


Figure 11 Electric pulp tester tolerance test

Three initial sensation readings were made on the maxillary left central incisor. These initial readings recorded the detection threshold of the central incisor. The participant was asked to let go of the probe handle as soon as an abnormal sensation (such as heat or tingling) was felt, and then to immediately report the pain experienced on a VAS (Appendix VII); the reading at this point was recorded (Appendix V). This was repeated two more times, with the tooth dried with gauze and a rest period of at least one minute between readings, in order to minimise nerve sensitisation arising from repeated stimulation (Bender et al., 1989; Lin et al., 2007).

Three further assessments were taken on the maxillary right central incisor. This second round of testing involved the participant holding the probe until discomfort was unbearable, at which point the participant released the probe handle. Immediately, the pain experienced was recorded on a VAS (Appendix VII). The reading at this point was recorded as the maximum tolerance (Appendix V). Following the first measurement, the tooth was re-dried and a recovery period of at least one minute was allowed (Lin et al., 2007), and the procedure was repeated twice. This procedure has been shown to cause no long-term pain or injury to the tooth (McDaniel et al., 1973).

(4) Saliva Test

Due to financial constraints, only three single nucleotide polymorphisms (SNPs) could be selected for analysis. Due to linkage disequilibrium (LD) values (discussed earlier) of 100% between SNPs rs165656 and rs4680, as well as for rs4818 and rs6269, it was only

necessary to analyse one SNP from each of these pairs, as well as analysing the novel rs464630, for which the LD was unknown.

Participants were asked to provide a saliva sample according to a standard protocol (Oragene-500 Kit, DNA Genotek, Ottawa, Canada). Samples were kept at room temperature until all samples had been collected, at which time they were taken to be processed at Associate Professor Tony Merriman's laboratory, Department of Biochemistry, University of Otago. Genomic DNA was extracted from the saliva samples according to manufacturer's instructions. Three SNPs (namely rs4680, rs6269 and rs4646310) from the COMT gene were genotyped using TaqMan assays (Diatchenko et al., 2005; Michelotti et al., 2013). TaqMan probes are hydrolysis probes that are designed to increase the specificity of real-time polymerase chain reaction (RT-PCR) assays.

Samples were destroyed under standard conditions at the laboratory, unless the participants had requested that their sample either be disposed of with appropriate karakia (Māori prayers or incantations) or returned to them at the conclusion of the study.

Duration of Testing

Phase One involved ten minutes in the Orthodontic Clinic and then five minutes over the following 48 hours.

Phase Two took 30-40 minutes to complete a questionnaire and participate in the testing, which on average took 20 minutes.

Data Analysis

Statistical analysis used conventional descriptive statistics, using SPSS Software (version 21, IBM, NY, USA). Scale scores were computed using the recommended method for each scale. Comparison of means was performed using Student's t-test (if the data were normally distributed) or the Mann-Whitney U test where data was not normally distributed. Comparisons of proportions used the Chi-square test. Repeated measurement analysis of variance and multivariate modelling were used to control confounding, and, in particular, to control for the influence of personality.

Results

Sample

The sample consisted of 107 undergraduate and postgraduate students within the Dental Faculty at the University of Otago (Table 1). There was an almost equal number of male and female participants (n = 54 and n = 53 respectively), with over 80% of the sample being of either Asian or European descent. Males and females did not differ with respect to ethnicity or mean age.

Table 1. Phase One Participants - Summary Data

Variable	Sex			Total
	Females (n = 53)	Males (n = 54)	*P* Value	(n = 107)
Ethnicity (%)				
European	28 (52.8)	19 (35.2)	0.823[a]	47 (43.9)
Asian	19 (35.8)	24 (44.4)		43 (40.2)
Other	6 (11.3)	11 (20.4)		17 (15.9)
Age (SD)	24.2 (4.2)	23.9 (4.1)	0.699[b]	24.1 (4.2)

[a] Fisher's Exact Test

[b] Unpaired Student's t-test

Phase One

Mean VAS peak pain over the 48-hour time interval was 3.1 cm (sd, 2.7), peaking during the night on the second evening. Generally, pain levels had elevated by the first evening and stayed elevated until the second morning (Figure 12).

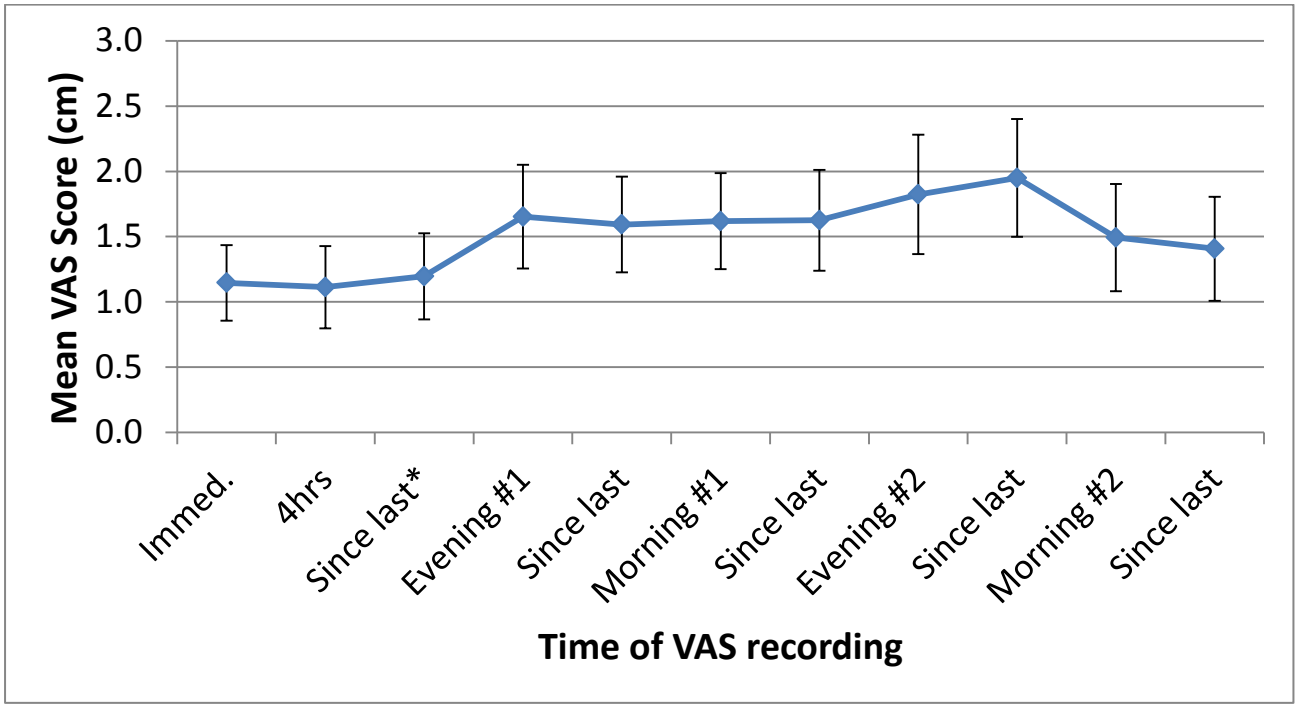


Figure 12 Phase One mean VAS pain over the duration of experiment

Error bar = 95% confidence interval
*_Since Last_ refers to the VAS report regarding maximum pain experienced between their previous pain report and the current report

Repeated measurements analysis of variance (ANOVA) showed that the pain scores over time were not significantly associated with sex (Fisher's Exact Test (F) = 0.121; *P* = 0.729), and that there was no significant interaction between time and sex (F = 1.780; *P* = 0.074). Pain response varied significantly over time following orthodontic force application (F = 3.026; *P* = 0.002).

The mean maximum VAS pain score over the 48-hour period did not differ between males and females (t = 0.730 and *P* = 0.467).

Phase Two

Phase Two involved the highest and lowest 10th percentile (n = 10 in each group) from Phase One. Again, there were nearly equal proportions of male and female participants in each group (high pain n = 4 and n = 6 respectively, and low pain group n = 5 in each group), and over 80% of the sample was of either Asian or European descent (Table 2).

Table 2. Phase Two Participants - Summary Data

Variable	Group			Both combined (n = 20)
	High Pain (n = 10)	Low Pain (n = 10)	*P* Value	
Age (SD)	22.4 (2.3)	24.6 (4.1)	0.164[a]	23.5 (3.4)
Sex (%)				
Male	4 (40.0)	5 (50.0)	1.000[b]	9 (45.0)
Female	6 (60.0)	5 (50.0)		11 (55.0)
Ethnicity (%)				
European	3 (30.0)	5 (50.0)	0.727[b]	8 (40.0)
Asian	5 (50.0)	4 (40.0)		9 (45.0)
Other	2 (20.0)	1 (10.0)		3 (15.0)

[a] Independent samples t-test (equal variance not assumed)
[b] Fisher's Exact Test

The phase one VAS pain scores differed substantially between the groups. Individually, each high pain responder had a peak VAS pain score of ≥8.0 cm for at least one time point over the 48-hour observation period, whereas, low pain responders had a mean peak pain of ≤0.6 cm, during the whole 48-hour period.

As a group, due to peak pain occurring at different time points, the high pain responders had a peak VAS pain score of 7.4 cm (sd, 2.3) on evening two; by contrast, the low pain responders had a peak VAS pain score of 0.2 cm (sd, 0.2) on morning one (Figure 13).

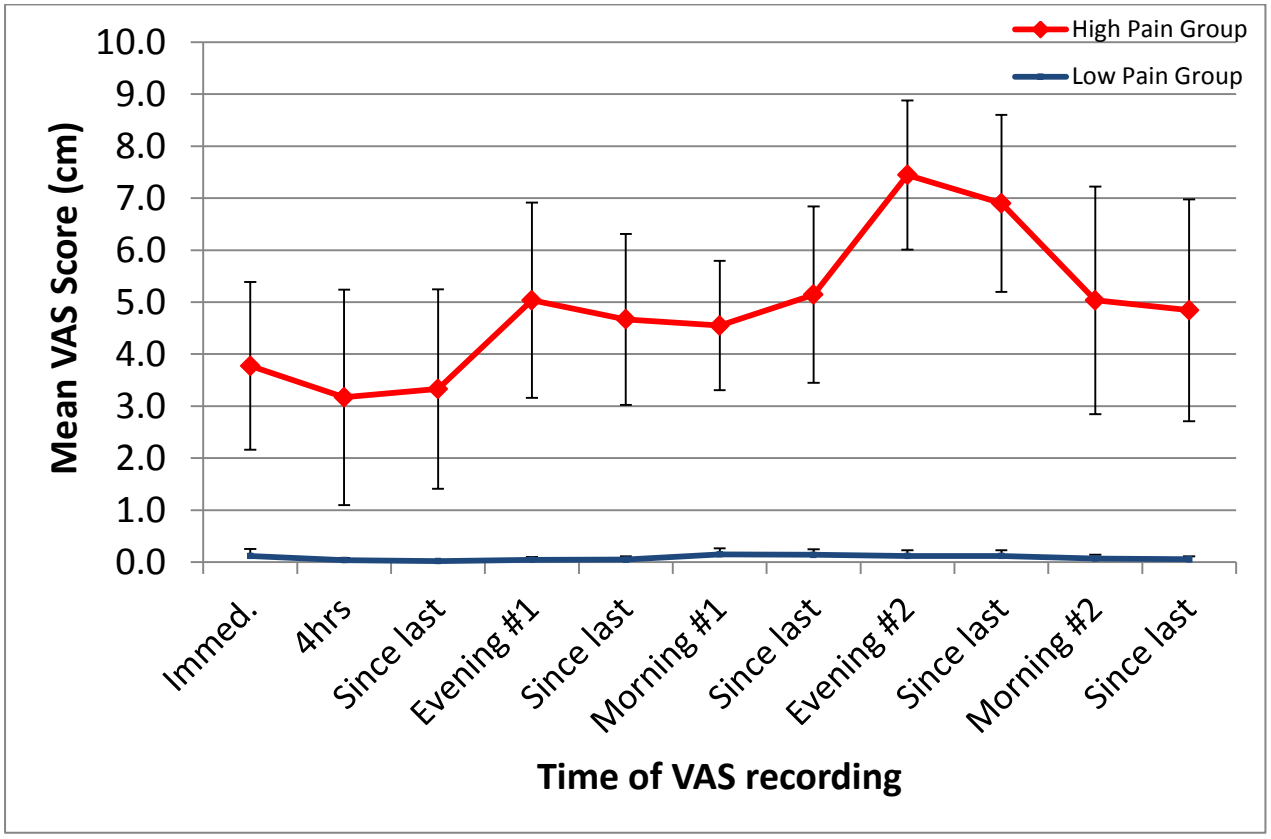


Figure 13 Highest and lowest pain responders

* Error bar = 95% confidence interval (for convenience, upper bar only shown for Low Pain Group)

The Pain Catastrophising Scale (PCS) showed a statistically significant difference ($P < 0.05$) between high and low pain responders across all three subcategories. The Corah Dental Anxiety Scale (DAS) also showed a statistically significant difference ($P < 0.05$) between the high and low pain responders (Table 3).

Table 3. Mean PCS and DAS scores, by group

	Group			Both combined (n = 20)
	High Pain (n = 10)	Low Pain (n = 10)	*P* Value[a]	
Catastrophising				
Rumination (SD)	9.1 (5.2)	3.5 (3.2)	0.019	6.3 (5.1)
Magnification (SD)	4.6 (1.8)	1.5 (2.0)	0.003	3.1 (2.4)
Helplessness (SD)	7.8 (5.8)	2.6 (2.7)	0.023	5.2 (5.1)
Mean DAS Score (SD)	9.4 (3.3)	6.5 (2.6)	0.043	8.0 (3.3)

[a] Mann-Whitney U Test

The two groups' responses to Locker's global item were identical (Table 4).

Table 4. Locker's global item responses, by group

Self Rating	Group			Both combined (n = 20)
	High Pain (n = 10)	Low Pain (n = 10)	*P* Value[a]	
Excellent (%)	2 (20.0)	2 (20.0)	1.000	4 (20.0)
Very Good (%)	6 (60.0)	6 (60.0)		12 (60.0)
Good (%)	2 (20.0)	2 (20.0)		4 (20.0)
Fair-Poor (%)	0 (0.0)	0 (0.0)		0 (0.0)

[a] Fisher's Exact Test

Neither the Positive and Negative Affect Schedule (PANAS) nor the Spielberger's State-Trait Anxiety Inventory (STAI) showed any statistically significant difference between the high and low pain responders (Table 5).

Table 5. Mean PANAS and STAI scores, by group

	Group			Both combined (n = 20)
	High Pain (n = 10)	Low Pain (n = 10)	*P* Value[a]	
Affectivity Schedule				
Positive (SD)	33.4 (8.0)	34.3 (4.9)	0.796	33.9 (6.5)
Negative (SD)	16.8 (6.1)	19.2 (7.0)	0.353	18.0 (6.5)
STAI				
A-Trait (SD)	29.1 (8.7)	26.6 (3.8)	0.579	27.9 (6.6)
A-State (SD)	40.6 (12.4)	35.6 (9.2)	0.353	38.1 (10.9)

[a] Mann-Whitney U Test

The Cold Pressor Test showed a statistically significant difference between high and low pain responders for minutes one and two, but there was no statistically significant difference at three minutes (Table 6).

Table 6. Mean Cold Pressor Test VAS scores, by group

Time Elapsed	Group			Both combined (n = 20)
	High Pain (n = 10)	Low Pain (n = 10)	*P* Value[a]	
Minute One (SD)	7.2 (2.7)	3.7 (2.8)	0.009	5.5 (3.2)
Minute Two (SD)	7.5 (2.9)	4.5 (3.1)	0.029	6.0 (3.3)
Minute Three (SD)	6.4 (2.9)	4.1 (3.1)	0.075	5.2 (3.2)

[a] Mann-Whitney U Test

Detection threshold and tolerance output measurements for EPT were compared between high and low pain responders, as were the VAS scores for pain experienced. Neither output reading value nor VAS scores for detection threshold or tolerance were significantly different between high and low pain responders.

Detection threshold and tolerance output values between high pain responders and low pain responders were tested using repeated measurement ANOVA. The ANOVA results suggested that there was a significant effect of "test number" on detection threshold (F = 5.5; *P* = 0.008), with the mean detection threshold value (Table 7) becoming progressively lower with subsequent tests. The effect of "test number" was not significant (F = 1.3; *P* = 0.28) for tolerance thresholds (Table 8).

VAS scores for pain were also tested for detection threshold and tolerance differences between high and low pain responders using repeated measurement ANOVA. Detection threshold VAS pain (Table 9) was not influenced by test number (F = 0.38; *P* = 0.69); however, the VAS pain score for tolerance (Table 10) was statistically significantly higher with subsequent tests (F = 6.4; *P* = 0.004).

Table 7. Mean Electric Pulp Tester Threshold Output Values (arbitrary units), by group

Test number	Group			Both combined (n = 20)
	High Pain (n = 10)	Low Pain (n = 10)	*P* Value[a]	
Test One (SD)	19.1 (5.2)	20.4 (7.2)	0.280	19.8 (6.1)
Test Two (SD)	14.7 (7.2)	19.7 (7.3)	0.075	17.2 (7.5)
Test Three (SD)	15.5 (8.3)	18.5 (6.3)	0.218	17.0 (7.3)
Mean (SD)	16.4 (6.9)	19.5 (6.9)	0.143	18.0 (6.6)

[a] Mann-Whitney U Test

Table 8. Mean Electric Pulp Tester Tolerance Output Values (arbitrary units), by group

Test	Group			Both combined (n = 20)
	High Pain (n = 10)	Low Pain (n = 10)	*P* Value[a]	
Test One (SD)	26.4 (6.9)	35.7 (16.7)	0.075	31.1 (13.3)
Test Two (SD)	25.7 (5.6)	33.6 (17.9)	0.247	29.7 (13.5)
Test Three (SD)	26.3 (7.2)	35.0 (16.6)	0.123	30.7 (13.2)
Mean (SD)	26.1 (6.6)	34.8 (17.1)	0.105	30.5 (13.2)

[a] Mann-Whitney U Test

Table 9. Mean Electric Pulp Tester Threshold VAS pain scores, by group

Test	Group			Both combined (n = 20)
	High Pain (n = 10)	Low Pain (n = 10)	*P* Value[a]	
Test One (SD)	3.0 (2.3)	1.6 (1.9)	0.190	2.3 (2.2)
Test Two (SD)	2.9 (2.6)	1.5 (2.3)	0.218	2.2 (2.5)
Test Three (SD)	3.0 (2.8)	1.7 (2.4)	0.190	2.3 (2.6)
Mean (SD)	3.0 (2.6)	1.6 (2.2)	0.190	2.3 (2.4)

[a] Mann-Whitney U Test

Table 10. Mean Electric Pulp Tester Tolerance VAS pain scores, by group

Test	Group			Both combined (n = 20)
	High Pain (n = 10)	Low Pain (n = 10)	*P* Value[a]	
Test One (SD)	7.2 (2.0)	5.6 (2.4)	0.123	6.4 (2.3)
Test Two (SD)	7.5 (1.5)	5.9 (2.5)	0.143	6.7 (2.1)
Test Three (SD)	8.0 (1.4)	5.9 (2.7)	0.105	7.0 (2.4)
Mean (SD)	7.6 (1.6)	5.8 (2.5)	0.123	6.7 (2.3)

[a] Mann-Whitney U Test

Owing to the low number of participants in Phase Two, logistic regression could not be used to compare low and high pain responders. Therefore, analysis was carried out using pain responder type as the main independent variable, in order to investigate the significant differences described above (PCS, DAS, Cold Pressor One Minute; Table 11).

The empty model shows that 39.3% of pain response type (high or low) was explained by the magnification subcategory of the PCS, with high pain responders having a score in this subcategory which was approximately 3.1 units higher; after controlling for other factors, it was still 1.8 units higher than low pain responders.

Once all other variables were controlled for, the adjusted model accounted for 80% of the variance in the magnification subscale of the PCS ($P = 0.011$).

Table 11. Multivariate analysis, using statistically significant variables from Phase Two to compare pain responder types (Regression coefficient pertains to high pain response type).

Scale	Empty Model			Full Model[a]		
	Unadjusted difference (95% CI)	*P* Value	Variance explained (%)	Adjusted difference (95% CI)	*P* Value	Variance explained (%)
PCS						
Rumination	5.6 (1.6, 9.6)	0.009	28.2	-0.5 (-3.8, 2.7)	0.728	82.9
Magnification	3.1 (1.3, 4.9)	0.002	39.3	1.8 (0.5, 3.2)	0.011	79.9
Helplessness	5.2 (-0.4, 5.6)	0.019	22.8	-2.1 (-5.0, 0.8)	0.144	84.6
DAS	2.9 (0.1, 5.7)	0.044	16.3	1.8 (-1.3, 4.8)	0.239	59.7
Cold pressor test minute 1	3.5 (1.0, 6.1)	0.010	27.9	3.0 (-0.2, 6.2)	0.064	47.1

[a] Independent variable = pain responder type

Three SNPs of the COMT gene were analysed from saliva samples of both high and low pain responders (Table 12). None of the three SNPs showed a statistically significant difference between high and low pain responders.

Table 12. Genotype frequencies of COMT SNPs, by group

SNP	Genotype	Group		
		High Pain (%)	Low Pain (%)	*P* Value[a]
rs4680	G/G	2 (20)	2 (20)	
	G/A	5 (50)	7 (70)	0.820
	A/A	3 (30)	1 (10)	
rs6269	A/A	6 (60)	3 (30)	
	A/G	3 (30)	7 (70)	0.179
	G/G	1 (10)	0	
rs4646310	G/G	9 (90)	6 (60)	
	G/A	1 (10)	3 (30)	0.303
	A/A	0	1 (10)	

[a] Fisher's exact test

Discussion

The findings of this study suggest that there are three characteristics that modify the pain experienced following application of orthodontic force, namely: pain catastrophising, dental anxiety, and cold sensitivity.

The factors investigated in this study included: anxiety, both as a general aspect of personality and in response to a specific situation; the two primary dimensions of mood (positive and negative affect); dental anxiety and fear; self-rating of oral health; catastrophising; three SNPs of the COMT gene; general sensitivity (cold) and tooth sensitivity (EPT). Only three factors proved to be related to pain experienced following application of orthodontic force; these were pain catastrophising, dental anxiety and cold sensitivity. The strongest of the three orthodontic pain modifying factors was the magnification subscale of the PCS, as shown by using a predictive multivariate model for orthodontic pain sensitivity, which accounted for 39.3% of the variance in the magnification subscale of the PCS ($P = 0.011$).

There were some weaknesses in this study: a lack of power due to low participant numbers; a possibly biased sample; timing of administration of questionnaires and pain reporting protocol. The most important weakness was Type II error, due to low participant numbers. This was especially highlighted in the COMT analyses. Participation was entirely voluntary, with 107 volunteers for Phase One, leading to just 20 participants in Phase Two. Dental students have a very busy timetable; thus, a self-selecting group of students was involved in this study. This compromises the study's external validity.

It is unlikely that a truly representative sample of participants could ever be recruited to studies like this, and so multiple studies of different populations are required to determine whether the findings can be a true representation of orthodontic patients. This lack of power meant that no strong conclusions could be drawn from the EPT or

COMT SNP analysis[iv]. Electrical pulp testing is based on stimulation of sensory nerves and, as with the cold pressor testing, requires and relies on subjective assessment from the patient (Lin and Chandler, 2008). EPT can produce a false-positive response in teeth with pulpal necrosis, a condition which was avoided as much as possible via exclusion criteria. However, one study has demonstrated an overlap of the thresholds of pulpal and periodontal nerves, thus providing a "false" response via periodontal nerve stimulation – a scenario which was unaccounted for in this study (Närhi et al. 1979).

With this study being based within a dental faculty, the external validity of the findings may be further compromised. This is particularly evident with Locker's global item (self-rated oral health), where all participants rated their oral health as "good" or better. Participants would have higher dental awareness as well as easier access to oral health care. However, dental anxiety still featured as a statistically significant orthodontic pain predictor, which indicates that despite the study being based within a dental institution, there was still good representation of dentally anxious people.

Some internal bias may have been encountered in Phase One due to participants allowing previous VAS scores to influence their current report. Efforts were made to reduce this, by placing each VAS time report on a new page (Appendix I). Previous studies have telephoned the participants at each time interval to collect a verbal VAS (Bergius et al., 2008), or participants have mailed back VAS forms on completion (Scheurer et al., 1996; Bergius et al., 2002). A novel way to prevent bias could be to create a "smart phone" application which would prompt a VAS report at the required time intervals, whereby the data would be sent straight back to the server and the investigators.

Another factor to consider is that the STAI questionnaire was administered at the start of Phase Two; thus, the *State* section which pertains to how they feel at a particular time

[iv] We are collaborating with a dental school in Naples, Italy, to increase the power of this study.

(such as the "intensity induced by stressful experimental procedures") was not temporally related to the application of orthodontic separators (Spielberger et al., 1970). The situation is similar for the PCS which ideally is assessed in two different ways, either as a reaction measured during or immediately after exposure to a noxious stimulation (situational or state assessment; Leung, 2012). Ideally, the PCS could have been administered immediately after separator removal, so that participants could reflect on this experience when answering their questionnaire. However, catastrophising, assessed while individuals are in a pain-free state, can predict pain ratings made in response to aversive stimulation, as shown by PCS scores obtained one week (Sullivan and Neish, 1999) or up to eight weeks (Sullivan et al., 1995) before a painful procedure. The findings from this study can still be an indication of catastrophising and anxiety when there is the threat or anticipation of pain including dental pain, since all Phase Two participants were aware that following completion of their questionnaires, they would be taking part in EPT and cold pressor tests.

Pain has the two dimensions of *algosity* and *unpleasantness*. Algosity refers to the quality of an unpleasant somatic sensation that allows it to be identified as pain (Fields, 1999), whereas unpleasantness is a dimension of pain that allows a somatic sensation to be identified as noxious, yet it may not necessarily be associated with noxious stimuli (Mobilio et al., 2011). An example is an itch, where unpleasantness is tightly coupled with stimulus intensity (Fields, 1999). Both of these dimensions can be tested using a VAS (with different end-point labels), which would allow a better understanding of the quality of the pain that participants were experiencing. In the current study, there was only one VAS used throughout the study, and it was noted during Phase Two testing that some participants had asked whether they were reporting on *discomfort* (unpleasantness) or *pain.* Having separate VAS reports of those aspects would help participants to clarify what they were experiencing. It is important to remember when scrutinising the pain literature that the severity of pain is different for different experimental modalities of testing; thus, modifying factors may not be comparable between all studies.

The menstrual phase of female participants was not recorded in this study. A review of pain perception across the menstrual cycle (Riley et al., 1999) concluded that, for cold pressor pain, higher thresholds were apparent in the follicular phase than in other phases; however, these were regarded as only trivial to mild, in line with the conclusions of a more recent systematic review which showed inconsistent menstrual cycle effects in regard to cold pressor pain (Fillingim et al., 2009). Because of such inconclusive results, menstrual phase was not seen to be an influence, as there were no sex differences in pain response during this study.

The participants in Phase One were evenly split between males (50.5%) and females (49.5%). When the influence of sex on the Phase One VAS scores was investigated, both as the maximum pain score and throughout the 48-hour observation period, it was not a significant predictor of pain. This was also true upon investigation of the interaction between time and sex with regard to pain level. This is consistent with the findings of other orthodontic studies using orthodontic separators (Ngan et al., 1989), including those of Jones and Chan (1992) who did a randomised control trial (RCT) with fixed orthodontic appliances. Both studies found no sex influence on pain reported by VAS, yet this is the opposite of some other studies looking at sex differences in pain perception, most of which were not orthodontic studies (Svensson et al., 2011). However, Scheurer and colleagues (1996) found a significantly higher reporting of pain from females following placement of orthodontic appliances and Bergius and co-workers (2002) found that, following orthodontic separator placement, females experienced significantly higher levels of pain on days three, five and seven post-insertion.

The time of the VAS pain recording had a significant influence on pain levels reported, which, of course, is to be expected, because it is well known that orthodontic pain generally follows a pattern of increasing after four hours of force application, usually rising to a peak after approximately 24 hours, before beginning to subside after 48 hours (Krishnan, 2007). This temporal pattern has been shown in previous studies (Ngan et al., 1989; Bergius et al., 2002) following placement of orthodontic separators

on patients. Jones and Chan (1992) also showed this in a RCT following placement of fixed orthodontic appliances.

The age or ethnicity of participants did not significantly influence pain response type in either Phase One or Phase Two. Since the sample for this study consisted of students, there was a limited spread of ages; the literature has mixed reports on the influence of age on orthodontic pain, with some reports stating no difference (Ngan et al., 1989; Bergius et al., 2008) and others concluding that the older the patient (over 16 years), the worse the pain (Jones and Chan, 1992). Further studies have concluded that the greatest pain reported was in the 13-16 age group (Scheurer et al., 1996).

There is no literature on ethnic differences in orthodontic pain. Literature on pain that compares similar ethnic groups to those in this study has found similarities in reported pain responses, but it has been pointed out that groups can be quite different with regard to factors (such as social and cultural) which influence the responses (Lipton and Marbach, 1984). By contrast, Woodrow and colleagues (1972) suggested that Caucasians can tolerate more pain than Asians. This was also found in a small study using the cold pressor test, where Asians reported significantly more pain and distress (in response to cold pressor pain) than Caucasian participants (Knox et al., 1977). In the present study, there were no ethnic differences, possibly because of low statistical power.

As stated earlier, the PCS evaluates any exaggerated negative orientation toward noxious stimuli (such as helplessness and inability to cope effectively with pain), identifying individuals who may be susceptible to heightened distress responses. In this study, there was a statistically significant difference between high and low pain responders for all three subscales (rumination, magnification and helplessness), meaning that catastrophising appears to be a strong predictor of orthodontic pain. When Sullivan and colleagues (1995) developed the PCS, it was also compared with cold pressor pain VAS scores, and they too found that catastrophisers reported significantly

more pain than non-catastrophisers. Although research has demonstrated a strong relationship between catastrophising and pain experience, the precise mechanism remains unclear (Sullivan et al., 1995). As discussed later, it has been suggested that pain catastrophising might be associated with suppression of the dopamine or endogenous opioid pain-control systems (Benedetti et al., 2010). It has also been suggested that pain catastrophising might interfere with pain modulation.

Multivariate analysis using all the predicting factors that we found to be statistically significant (cold pressor test, the three subcategories of PCS and DAS), with pain responder type as the independent variable, showed that 80% of the variance in the magnification subscale of the PCS was explained after other factors were controlled for, making magnification the strongest predictor for orthodontic pain. Magnification describes a person's likelihood to exaggerate the threat value or seriousness of the pain sensations (such as "I wonder whether something serious may happen").

The current study findings underline the importance of cortical processing on pain perception following the application of orthodontic force, because cerebral cortical areas receiving afferent nociceptive information can be readily modulated by expectation-induced information. Positive expectations (of lower pain) can produce a greater reduction in perceived pain than the effects of a clinically analgesic dose of morphine, and expectations of a negative outcome (greater pain) can result in the amplification of pain (Koyama et al., 2005). Several regions in the cortex and the hippocampus have been found to be activated during the anticipation of pain (Koyama et al., 2005). As discussed earlier, the cerebral cortex is an area where pain modulation can occur; thus, influencing pain signal transmission (Okeson and Bell, 2005), for example, via suppression of opioid or dopamine pathways (Benedetti et al., 2010). The way in which the pain signal is received and modulated (either by enhancement or reduction) can be further influenced by the state of the dorsal horn when the input arrives, such as in the sensitised state, as discussed earlier, which will lead to an exaggerated noxious input (Carr and Goudas, 1999).

It has been found that following dental hygiene treatment, the rumination subscale of the PCS contributed significant unique variance to the prediction of pain, but the magnification subscale had no significant contribution (Sullivan and Neish, 1998). No dental literature highlights the magnification subscale of the PCS as being a modifying factor for pain experience, but there has been a tentative conclusion drawn in a sample of patients with whiplash at a specialist pain clinic. One year after the injury, the magnification subscale was the best predictor of their pain and disability (Sullivan et al., 2002).

A study of dental students investigated how to reduce pain experienced by catastrophisers during dental hygiene treatment. It showed that catastrophisers who were given the opportunity to disclose their dental worries and concerns before the dental procedure had lower ratings of pain and emotional distress (Sullivan and Neish, 1999).

Dental anxiety may also be a modifying factor for orthodontic pain as there was a significant difference in DAS scores between high and low pain responder groups, meaning that high pain responders seem to be more dentally anxious. A similar conclusion was made by Bergius and colleagues (2008) who investigated the characteristics of patients who experienced prolonged pain (pain still at day seven) following orthodontic separator placement. They found the DAS scores were significantly higher in the group who experienced prolonged pain. The DAS was also found to be a strong predictor of pain intensity associated with routine dental procedures (Tickle et al., 2012). Catastrophising can be a significant confounder on the influence of dental anxiety. De Jongh and colleagues (1994) concurred with previous research on student populations, finding that dental anxiety was significantly related to catastrophising.

Orthodontic pain experience may also be influenced by genetic differences in the COMT enzyme. The COMT SNPs investigated in this study were rs4680, rs6269 and the

recently identified “novel” SNP rs4646310 (Michelotti et al., 2013). While no SNPs were shown to differ between high and low pain responders, a factor heightened due to the low power of this study, the rs6269 SNP difference approached statistical significance, pointing at a marginal relationship of an A/A haplotype for high pain responders, and A/G haplotype for low pain responders ($P = 0.179$). Doubling the sample size gave a P Value of 0.035, and doubling again gave $P < 0.0001$.

Evidence for the COMT SNP rs6269 being a significant contributor to orofacial pain sensitivity can be found in the study by Diatchenko’s group (2005), where it was determined that rs6269 accounted for 6% of the variation in pain sensitivity ($P < 0.01$) and that homozygous genotypes were associated with significant differences in mean pain sensitivity, whereas rs4680 showed a marginal association with pain sensitivity ($P = 0.18$). Diatchenko’s group also reported that, following multivariate analysis, all possible combinations of four SNPs in the coding region (including rs4680 and rs6269 plus two other SNPs), accounted for 10.6% of the variation in the summary measure of pain sensitivity. This was also found by Lee and colleagues (2011), who studied pain following mandibular third molar tooth extraction in a sample that did not differ in psychological factors (including STAI and PCS measures). It was found that pain VAS scores one week post-operatively showed statistically significant differences between rs6269 high- and low-pain sensitivity genotypes (GG versus GA, respectively). Interestingly, there was again no statistically significant difference in pain VAS scores between rs4680 genotypes.

There appears to be a strong link between genetic and psychological factors. Patients with high PCS scores and low COMT activity (high pain sensitivity) with shoulder pain had significantly higher preoperative pain ratings (32.9% of variance explained) in comparison to those patients with high PCS and high COMT activity, showing that pain catastrophising and COMT diplotype interaction was a significant predictor of post-operative pain (George et al., 2008b). George and colleagues had earlier shown with an induced shoulder pain experiment and a hierarchical regression model that the interaction between pain catastrophising and COMT diplotype was the strongest unique

predictor of 72-hour pain ratings (George et al., 2008a). Enoch and colleagues (2003) had previously concluded that the COMT rs4680 SNP played an important role in the pathogenesis of anxiety in women. This was also shown by Slade's group (2007) in a prospective study of TMD of asymptomatic females, where it was found that psychological factors (including STAI scores) and COMT haplotypes (including rs6269 and rs4680) increased the risk of clinical pain.

The cold pressor test was used in the current study to investigate whether the pain experienced from hand immersion in cold water predicts the pain experienced following application of orthodontic force. For the cold pressor test, a technique validated for use with children was selected (von Baeyer et al., 2005), because this would ensure that the maximum number of participants would complete the task, providing the maximum amount of data to analyse, given that half of our participants were pain-sensitive. It is worth noting that one of the high pain responder participants did remove his/her arm from the tank shortly after two minutes, as they felt the pain was unbearable. The cold pressor test showed a statistically significant difference between high and low pain responders for minutes one and two of immersion, but there was no significant difference at three minutes. This is probably due to the fact that after two minutes of hand immersion in 10°C water, peripheral vasoconstriction occurs to reduce heat loss (cold adaptation) in both groups (Greene et al., 1965; Walsh et al., 1989).

Multivariate analysis, using pain responder type as the independent variable, showed borderline statistical significance (P = 0.064) once all other variables were controlled for, and accounting for 47% of the variance in the cold pressor one minute test VAS score. Preoperative pain sensitivity testing on patients prior to third molar tooth removal has shown that the cold pressor test can be used to identify patients at risk of developing greater pain after this type of surgery (Mobilio et al., 2011). A prospective study on patients undergoing a laparoscopic cholecystectomy has also identified that pre-operative greater sensitivity to cold pressor pain is an independent risk factor for early postoperative pain (Bisgaard et al., 2001). These findings support those of the

current study, whereby cold pressor VAS pain scores were associated with pain experienced following the application of orthodontic force (P = 0.009 after one minute of immersion). However, the influence of the cold pressor test on pain following application of orthodontic force is small compared to that of the magnification subscale of the PCS.

The EPT was used to compare both Threshold Detection and Tolerance levels between the high and low pain responder groups. For both sets of data, there was no significant difference between the groups, meaning that EPT pain VAS and output values are not related to pain experienced following application of orthodontic force. The ANOVA results showed a statistically significant difference within each responder group (high- or low-pain response) with subsequent tests for tolerance VAS scores being higher, as well as a progressive lowering of the detection threshold during the three tests (P = 0.008). This may be described as intra-group conditioning (due to primary hyperalgesia or peripheral sensitisation of the nociceptors at the test site) accounting for the greater pain sensitivity of the central incisors (Besson, 1999; Miles et al., 2004). This result contradicts that found in some EPT studies, which have described nerve accommodation after repeated nerve stimulation, and participants feeling less pain or tolerating higher output readings before releasing the probe tip (Mumford, 1965; Bender et al., 1989).

The PANAS measures the two primary dimensions of mood (positive and negative affect). It did not show a statistically significant difference between high and low pain responder groups. A similar Swedish orthodontic study (Bergius et al., 2008) - which tested personality factors (temperament, self-concepts and self-esteem) using the EAS survey for adults (Buss and Plomin, 1984) and the "I think I am" self-report questionnaire (Ouvinen-Birgerstam, 1984) to try and characterise the patients who felt prolonged pain following orthodontic separators - also found that the two groups did not differ according to these psychological dimensions.

The STAI was used as a measure of anxiety as a general aspect of personality (trait) and anxiety as a response to a specific situation (state). Neither measure showed any statistically significant differences between the high and low pain responder groups. As discussed earlier, the "state" questionnaire was not specifically in relation to the orthodontic separators; however, the STAI was administered just prior to commencing the experimental procedures of Phase Two, for which the threat of pain had been explained. The general indication shown is that dental anxiety can be a predictor of orthodontic pain, but there is no influence from a generally anxious personality.

Clinical Implications

These findings show the importance of cortical processing for pain perception, demonstrating that the pain perceived following orthodontic force application can be influenced to a greater extent by psychological factors rather than from factors related to the occlusion, dental anatomy or PDL. Dental anxiety and pain catastrophising were found to significantly influence the pain reported over 48 hours following placement of orthodontic elastomeric separators, with the magnification subscale of the PCS explaining the most variation between pain responder types (high or low). Cold sensitivity was also found to significantly modify the pain experienced; however, its influence was smaller than that of the modification subscale of the PCS.

Clinicians may consider adding PCS and DAS questions into their new patient forms, because pain catastrophising and dental anxiety appear to modify orthodontic pain experience. The PCS involves 13 multiple choice questions (the magnification subscale makes up three of these), and the DAS comprises four. They are quick to complete and may provide information so that patient-specific management strategies can be employed. These might include: ensuring they are given an opportunity to discuss their worries and concerns prior to treatment; phoning patients within 24 hours of orthodontic treatment, since this has been shown to reduce patients' self-reported pain and anxiety (Bartlett et al., 2005); or even ensuring that analgesic recommendations are discussed. Clinicians would then be doing as much as possible to minimise orthodontic discomfort.

Conclusion

Pain is a very subjective experience which may be influenced by genetic, psychological and environmental factors, among others. Early and accurate identification of patients who are more sensitive to orthodontic pain is currently not possible because valid prognostic factors have not been discovered.

This study set out to determine whether high and low responders to pain caused by orthodontic force application differed in regards to age, ethnicity, sex, self-rated oral health, anxiety (both as a general aspects of personality and in response to a specific situation), mood, dental anxiety and fear, catastrophising, genetics, general sensitivity (cold) and tooth sensitivity. It identified three significant characteristics that may modify the pain experience following application of orthodontic force: dental anxiety, pain catastrophising and cold sensitivity. Age, sex, ethnicity, anxiety, mood, and tooth sensitivity were not modifiers.

These findings highlight the importance of cortical processing for pain perception, as the pain signal can be either enhanced, or reduced, thus influencing the pain experienced.

Based on our preliminary findings, it seems that a few screening questions may help to identify patients at risk of a high pain response prior to commencing orthodontic treatment, so that patient-specific management strategies can be employed to minimise orthodontic discomfort.

Future directions for this research could look at sampling the general population, and exploring the exciting field of genetic prediction, especially with respect to the COMT gene, which is established as a modifier of individual susceptibility to TMD and orofacial pain.

References

Abecasis GR, Cookson WOC (2000). GOLD—Graphical Overview of Linkage Disequilibrium. *Bioinformatics* 16(2)*:*182-183.

Aitken RC (1969). Measurement of feelings using visual analogue scales. *Proc R Soc Med* 62(10)*:*989-993.

Amir S, Brown ZW, Amit Z (1980). The role of endorphins in stress: Evidence and speculations. *Neurosci Biobehav R* 4(1)*:*77-86.

Andersen S, Skorpen F (2009). Variation in the COMT gene: implications for pain perception and pain treatment. *Pharmacogenomics* 10(4)*:*669-684.

Armfield JM (2010a). How do we measure dental fear and what are we measuring anyway? *Oral Health Prev Dent* 8(2)*:*107-115.

Armfield JM (2010b). Towards a better understanding of dental anxiety and fear: cognitions vs. experiences. *Eur J Oral Sci* 118(3)*:*259-264.

Armstrong CM, Hille B (1998). Voltage-gated ion channels and electrical excitability. *Neuron* 20(3)*:*371-380.

Arntz A, van Eck M, Heijmans M (1990). Predictions of dental pain: the fear of any expected evil, is worse than the evil itself. *Behav Res Ther* 28(1)*:*29-41.

Bartlett BW, Firestone AR, Vig KW, Beck FM, Marucha PT (2005). The influence of a structured telephone call on orthodontic pain and anxiety. *Am J Orthod Dentofacial Orthop* 128(4)*:*435-441.

Bender IB, Landau MA, Fonsecca S, Trowbridge HO (1989). The optimum placement-site of the electrode in electric pulp testing of the 12 anterior teeth. *J Am Dent Assoc* 118(3)*:*305-310.

Benedetti F, Carlino E, Pollo A (2010). How placebos change the patient's brain. *Neuropsychopharmacol* 36(1)*:*339-354.

Bergius M, Kiliaridis S, Berggren U (2000). Pain in orthodontics. A review and discussion of the literature. *J Orofac Orthop* 61(2)*:*125-137.

Bergius M, Berggren U, Kiliaridis S (2002). Experience of pain during an orthodontic procedure. *Eur J Oral Sci* 110(2)*:*92-98.

Bergius M, Broberg AG, Hakeberg M, Berggren U (2008). Prediction of prolonged pain experiences during orthodontic treatment. *Am J Orthod Dentofacial Orthop* 133(3)*:*331-338.

Besson JM (1999). The neurobiology of pain. *Lancet* 353(9164)*:*1610-1615.

Bisgaard T, Klarskov B, Rosenberg J, Kehlet H (2001). Characteristics and prediction of early pain after laparoscopic cholecystectomy. *Pain* 90(3)*:*261-269.

Buss AH, Plomin R (1984). Temperament: Early developing personality traits. Hillsdale, New Jersey: L. Erlbaum Associates

Carr DB, Goudas LC (1999). Acute pain. *Lancet* 353(9169)*:*2051-2058.

Chapman V, Dickenson AH (1992). The spinal and peripheral roles of bradykinin and prostaglandins in nociceptive processing in the rat. *Eur J Pharmacol* 219(3–4)*:*427-433.

Chaudhary P, Martenson ME, Baumann TK (2001). Vanilloid receptor expression and capsaicin excitation of rat dental primary afferent neurons. *J Dent Res* 80(6)*:*1518-1523.

Corah NL (1969). Development of a dental anxiety scale. *J Dent Res* 48(4)*:*596.

Corah NL, Gale EN, Illig SJ (1978). Assessment of a dental anxiety scale. *J Am Dent Assoc* 97(5)*:*816-819.

Dalili F (2009). Pain Perception at Different Stages of Orthodontic Treatment. *Doctoral Thesis, University of Kuopio, Finland*.

De Jongh A, Muris P, ter Horst G, Van Zuuren FJ, De Wit CA (1994). Cognitive correlates of dental anxiety. *J Dent Res* 73(2)*:*561-566.

Diatchenko L, Slade GD, Nackley AG, Bhalang K, Sigurdsson A, Belfer I *et al.* (2005). Genetic basis for individual variations in pain perception and the development of a chronic pain condition. *Hum Mol Genet* 14(1)*:*135-143.

Diatchenko L, Nackley AG, Slade GD, Bhalang K, Belfer I, Max MB *et al.* (2006). Catechol-O-methyltransferase gene polymorphisms are associated with multiple pain-evoking stimuli. *Pain* 125(3)*:*216-224.

Diatchenko L, Nackley AG, Tchivileva IE, Shabalina SA, Maixner W (2007). Genetic architecture of human pain perception. *Trends Genet* 23(12)*:*605-613.

Duncan GH, Bushnell MC, Lavigne GJ (1989). Comparison of verbal and visual analogue scales for measuring the intensity and unpleasantness of experimental pain. *Pain* 37(3)*:*295-303.

Edwards CL, Fillingim RB, Keefe F (2001). Race, ethnicity and pain. *Pain* 94(2)*:*133-137.

Edwards RR, Haythornthwaite JA, Sullivan MJ, Fillingim RB (2004). Catastrophizing as a mediator of sex differences in pain: differential effects for daily pain versus laboratory-induced pain. *Pain* 111(3)*:*335-341.

Edwards RR, Sarlani E, Wesselmann U, Fillingim RB (2005). Quantitative assessment of experimental pain perception: multiple domains of clinical relevance. *Pain* 114(3)*:*315-319.

Enoch MA, Xu K, Ferro E, Harris CR, Goldman D (2003). Genetic origins of anxiety in women: a role for a functional catechol-O-methyltransferase polymorphism. *Psychiatr Genet* 13(1)*:*33-41.

Fields HL (1999). Pain: an unpleasant topic. *Pain* Suppl 6:S61-69.

Fillingim RB, King CD, Ribeiro-Dasilva MC, Rahim-Williams B, Riley JL, 3rd (2009). Sex, gender, and pain: a review of recent clinical and experimental findings. *J Pain* 10(5)*:*447-485.

Fuentes D, Gorenstein C, Hu LW (2009). Dental anxiety and trait anxiety: an investigation of their relationship. *Br Dent J* 206(8)*:*E17.

Fujiyoshi Y, Yamashiro T, Deguchi T, Sugimoto T, Takano-Yamamoto T (2000). The difference in temporal distribution of c-Fos immunoreactive neurons between the medullary dorsal horn and the trigeminal subnucleus oralis in the rat following experimental tooth movement. *Neurosci Lett* 283(3)*:*205-208.

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B *et al.* (2002). The structure of haplotype blocks in the human genome. *Science* 296(5576)*:*2225-2229.

Gelfand S (1964). The Relationship of Experimental Pain Tolerance to Pain Threshold. *Can J Psychol* 18(36-42).

George SZ, Dover GC, Wallace MR, Sack BK, Herbstman DM, Aydog E *et al.* (2008a). Biopsychosocial influence on exercise-induced delayed onset muscle soreness at the shoulder: pain catastrophizing and catechol-o-methyltransferase (COMT) diplotype predict pain ratings. *Clin J Pain* 24(9)*:*793-801.

George SZ, Wallace MR, Wright TW, Moser MW, Greenfield WH, 3rd, Sack BK *et al.* (2008b). Evidence for a biopsychosocial influence on shoulder pain: pain catastrophizing and catechol-O-methyltransferase (COMT) diplotype predict clinical pain ratings. *Pain* 136(1-2)*:*53-61.

Greene MA, Boltax AJ, Lustig GA, Rogow E (1965). Circulatory dynamics during the cold pressor test. *Am J Cardiol* 16(54-60).

Hepler JR, Gilman AG (1992). G proteins. *Trends Biochem Sci* 17(10)*:*383-387.

Hucho F, Weise C (2001). Ligand-Gated Ion Channels. *Angew Chem Int Edit* 40(17)*:*3100-3116.

Jänig W, McLachlan EM (1992). Characteristics of function-specific pathways in the sympathetic nervous system. *Trends Neurosci* 15(12)*:*475-481.

Jones M, Chan C (1992). The pain and discomfort experienced during orthodontic treatment - A randomized controlled clinical trial of 2 initial aligning arch wires. *Am J Orthod Dentofacial Orthop* 102(4)*:*373-381.

Jones ML (1984). An investigation into the initial discomfort caused by placement of an archwire. *Eur J Orthod* 6(1)*:*48-54.

Jones ML, Richmond S (1985). Initial tooth movement: force application and pain-a relationship? *Am J Orthod* 88(2)*:*111-116.

Jones M, Chan C (1992). The pain and discomfort experienced during orthodontic treatment - A randomized controlled clinical-trial of 2 initial aligning arch wires. *Am J Orthod* 102(4)*:*373-381.

Kardos T, Kieser J (2000). Clinical Oral Biology. Second ed. Dunedin: Otago University Print (pages 1-2).

Keefe FJ, Lumley M, Anderson T, Lynch T, Studts JL, Carson KL (2001). Pain and emotion: new research directions. *J Clin Psychol* 57(4)*:*587-607.

Kelly DJ, Ahmad M, Brull SJ (2001). Preemptive analgesia I: physiological pathways and pharmacological modalities. *Can J Anaesth* 48(10)*:*1000-1010.

Kim H, Lee H, Rowan J, Brahim J, Dionne RA (2006). Genetic polymorphisms in monoamine neurotransmitter systems show only weak association with acute post-surgical pain in humans. *Mol Pain* 2:24 (online).

Kitamura T, Takahashi T, Horiuchi H (1983). Electrical characteristics and clinical application of a new automatic pulp tester. *Quintessence Int* 1:45-53.

Klages U, Ulusoy O, Kianifard S, Wehrbein H (2004). Dental trait anxiety and pain sensitivity as predictors of expected and experienced pain in stressful dental procedures. *Eur J Oral Sci* 112(6)*:*477-483.

Klages U, Kianifard S, Ulusoy O, Wehrbein H (2006). Anxiety sensitivity as predictor of pain in patients undergoing restorative dental procedures. *Community Dent Oral Epidemiol* 34(2)*:*139-145.

Knox VJ, Shum K, McLaughlin DM (1977). Response to cold pressor pain and to acupuncture analgesia in oriental and occidental subjects. *Pain* 4*:*49-57.

Kolesnikov Y, Gabovits B, Levin A, Voiko E, Veske A (2011). Combined catechol-O-methyltransferase and mu-opioid receptor gene polymorphisms affect morphine postoperative analgesia and central side effects. *Anesth Analg* 112(2)*:*448-453.

Koyama T, McHaffie JG, Laurienti PJ, Coghill RC (2005). The subjective experience of pain: where expectations become reality. *P Natl Acad Sci USA* 102(36)*:*12950-12955.

Krishnan V (2007). Orthodontic pain: from causes to management-a review. *Eur J Orthod* 29(2)*:*170-179.

Kumazawa T, Mizumura K, Minagawa M, Tsujii Y (1991). Sensitizing effects of bradykinin on the heat responses of the visceral nociceptor. *J Neurophysiol* 66(6)*:*1819-1824.

Lee PJ, Delaney P, Keogh J, Sleeman D, Shorten GD (2011). Catecholamine-o-methyltransferase polymorphisms are associated with postoperative pain intensity. *Clin J Pain* 27(2)*:*93-101.

Leung L (2012). Pain catastrophizing: an updated review. *Indian J Psychol Med* 34(3)*:*204-217.

Lewis RA, Austen KF, Soberman RJ (1990). Leukotrienes and other products of the 5-lipoxygenase pathway. Biochemistry and relation to pathobiology in human diseases. *New Eng J Med* 323(10)*:*645-655.

Li T, Ball D, Zhao J, Murray RM, Liu X, Sham PC *et al.* (2000). Family-based linkage disequilibrium mapping using SNP marker haplotypes: application to a potential locus for schizophrenia at chromosome 22q11. *Mol Psychiatry* 5(4)*:*452.

Liebgott B (2001). The anatomical basis of dentistry. 2nd ed. St. Louis: Mosby (pages 268-271, 275, 404-409).

Lin J, Chandler N, Purton D, Monteith B (2007). Appropriate electrode placement site for electric pulp testing first molar teeth. *J Endod* 33(11)*:*1296-1298.

Lin J, Chandler NP (2008). Electric pulp testing: a review. *Int Endod J* 41(5)*:*365-374.

Lipton JA, Marbach JJ (1984). Ethnicity and the pain experience. *Soc Sci Med* 19(12)*:*1279-1298.

Litt MD (1996). A model of pain and anxiety associated with acute stressors: distress in dental procedures. *Behav Res Ther* 34(5-6)*:*459-476.

Locker D (1988). Measuring oral health: a conceptual framework. *Community Dent Health* 5(1)*:*3-18.

Locker D, Jokovic A, Clarke M (2004). Assessing the responsiveness of measures of oral health-related quality of life. *Community Dent Oral Epidemiol* 32(1)*:*10-18.

Loggia ML, Schweinhardt P, Villemure C, Bushnell MC (2008). Effects of psychological state on pain perception in the dental environment. *J Can Dent Assoc* 74(7)*:*651-656.

Lotta T, Vidgren J, Tilgmann C, Ulmanen I, Melen K, Julkunen I *et al.* (1995). Kinetics of human soluble and membrane-bound catechol O-methyltransferase: a revised

mechanism and description of the thermolabile variant of the enzyme. *Biochemistry-US* 34(13)*:*4202-4210.

McCormack HM, Horne DJ, Sheather S (1988). Clinical applications of visual analogue scales: a critical review. *Psychol Med* 18(4)*:*1007-1019.

McDaniel KF, Rowe NH, Charbeneau GT (1973). Tissue response to an electric pulp tester. *J Prosthet Dent* 29(1)*:*84-87.

Melzack R (2001). Pain and the neuromatrix in the brain. *J Dent Educ* 65(12)*:*1378-1382.

Merskey H, Bogduk N (1994). Classification of chronic pain: descriptions of chronic pain syndromes and definitions of pain terms: Seattle: IASP press (pages 209-214).

Michelotti A, Liguori R, Toriello M, D'Anto V, Vitale D, Castaldo G *et al.* (2013). Catechol-O-Methyltransferase (COMT) gene polymorphisms as risk factor in temporomandibular disorders patients from southern Italy. *Clin J Pain* (online article): 1-5.

Miles TS, Nauntofte B, Svensson P (2004). Clinical oral physiology. Copenhagen: Quintessence (pages 93, 101, 103, 104, 114, 116-118).

Mizumura K, Sugiura T, Katanosaka K, Banik RK, Kozaki Y (2009). Excitation and sensitization of nociceptors by bradykinin: what do we know? *Ex Brain Res* 196(1)*:*53-65.

Mobilio N, Gremigni P, Pramstraller M, Vecchiatini R, Calura G, Catapano S (2011). Explaining pain after lower third molar extraction by preoperative pain assessment. *J Oral Maxillofac Surg* 69(11)*:*2731-2738.

Muller E, Calvo M (2001). Pain and Dental Implantology: Sensory Quantification and Affective Aspects.: Part I: At the Private Dental Office. *Implant Dent* 10(1)*:*14-22.

Mumford JM (1965). Pain perception threshold and adaptation of normal human teeth. *Arch Oral Biol* 10(6)*:*957-968.

Närhi MVO (1985). The characteristics of intradental sensory units and their responses to stimulation. *J Dent Res* 64, 564–71.

Nielsen CS, Staud R, Price DD (2009). Individual differences in pain sensitivity: measurement, causation, and consequences. *J Pain*10(3)*:*231-237.

Newton JT, Buck DJ (2000). Anxiety and pain measures in dentistry: a guide to their quality and application. *J Am Dent Assoc* 131(10)*:*1449-1457.

Ngan P, Kess B, Wilson S (1989). Perception of Discomfort by Patients Undergoing Orthodontic Treatment. *Am J Orthod Dentofacial Orthop* 96(1)*:*47-53.

Nicolay OF, Davidovitch Z, Shanfeld JL, Alley K (1990). Substance P immunoreactivity in periodontal tissues during orthodontic tooth movement. *Bone Miner* 11(1)*:*19-29.

Okeson JP, Bell WE (2005). Bell's orofacial pains : the clinical management of orofacial pain. 6th ed. Chicago: Quintessence Pub. Co (pages 9-11, 13-41, 45-60, 87-89, 96, 97).

Ouvinen-Birgerstam P (1984). Jag tycker jag är. En metod för studier av barns och ungdomars självuppfattning. Manual: Stockholm: Psykologiförlaget.

Patel V (1989). Non-completion of orthodontic treatment: a study of patient and parental factors contributing to discontinuation in the hospital service and specialist practice. *Thesis, University of Wales, Heath Park, UK*.

Racich MJ (2005). Orofacial pain and occlusion: Is there a link? An overview of current concepts and the clinical implications. *J Prosthet Dent* 93(2)*:*189-196.

Ranson SW (1918). An introduction to a series of studies on the sympathetic nervous system. *J Comp Neurol* 29(4)*:*305-312.

Reich DE, Cargill M, Bolk S, Ireland J, Sabeti PC, Richter DJ *et al.* (2001). Linkage disequilibrium in the human genome. *Nature* 411(6834)*:*199-204.

Revill SI, Robinson JO, Rosen M, Hogg MI (1976). The reliability of a linear analogue for evaluating pain. *Anaesthesia* 31(9)*:*1191-1198.

Riley JL, 3rd, Robinson ME, Wise EA, Price DD (1999). A meta-analytic review of pain perception across the menstrual cycle. *Pain* 81(3)*:*225-235.

Rosenstiel AK, Keefe FJ (1983). The use of coping strategies in chronic low back pain patients: relationship to patient characteristics and current adjustment. *Pain* 17(1)*:*33-44.

Runeson BS, Rich CL (1994). Diagnostic and statistical manual of mental disorders, 3rd ed. (DSM-III), adaptive functioning in young Swedish suicides. *Ann Clin Psychiatry* 6(3)*:*181-183.

Scheurer PA, Firestone AR, Burgin WB (1996). Perception of pain as a result of orthodontic treatment with fixed appliances. *Eur J Orthod* 18(4)*:*349-357.

Sergl HG, Klages U, Zentner A (1998). Pain and discomfort during orthodontic treatment: causative factors and effects on compliance. *Am J Orthod Dentofacial Orthop* 114(6)*:*684-691.

Sessle BJ (1987). The neurobiology of facial and dental pain: present knowledge, future directions. *J Dent Res* 66(5)*:*962-981.

Slade GD, Diatchenko L, Bhalang K, Sigurdsson A, Fillingim RB, Belfer I *et al.* (2007). Influence of psychological factors on risk of temporomandibular disorders. *J Dent Res* 86(11)*:*1120-1125.

Slade GD, Diatchenko L, Ohrbach R, Maixner W (2008). Orthodontic treatment, genetic factors and risk of temporomandibular disorder. *Semin Orthod* 14(2)*:*146-156.

Soltis JE, Nakfoor PR, Bowman DC (1971). Changes in ability of patients to differentiate intensity of forces applied to maxillary central incisors during orthodontic treatment. *J Dent Res* 50(3)*:*590-596.

Spielberger CD, Gorsuch RL, Lushene RE (1970). STAI Manual for the Stait-Trait Anxiety Inventory ("Self-Evaluation Questionnaire"). Palo Alto: Consulting Psychologists Press, Inc.

Stein MB, Fallin MD, Schork NJ, Gelernter J (2005). COMT polymorphisms and anxiety-related personality traits. *Neuropsychopharmacol* 30(11)*:*2092-2102.

Stewart AL, King AC (1994). Conceptualizing and measuring quality of life in older populations. In: Aging and quality of life. New York, USA. Springer Publishing Co xvii (pages 27-54).

Sullivan MJ, Neish N (1999). The effects of disclosure on pain during dental hygiene treatment: the moderating role of catastrophizing. *Pain* 79(2-3)*:*155-163.

Sullivan MJ, Stanish W, Sullivan ME, Tripp D (2002). Differential predictors of pain and disability in patients with whiplash injuries. *Pain Res Manag* 7(2)*:*68-74.

Sullivan MJL, Bishop SR, Pivik J (1995). The Pain Catastrophizing Scale: Development and validation. *Psychol Assessment* 7(4)*:*524-532.

Sullivan MJL, Neish NR (1998). Catastrophizing, anxiety and pain during dental hygiene treatment. *Community Dent Oral Epidemiol* 26(5)*:*344-349.

Sullivan MJL, Tripp DA, Santor D (2000). Gender differences in pain and pain behavior: The role of catastrophizing. *Cognitive Ther Res* 24(1)*:*121-134.

Sullivan MJL, Martel MO, Tripp D, Savard A, Crombez G (2006). The relation between catastrophizing and the communication of pain experience. *Pain* 122(3)*:*282-288.

Svensson P, Baad-Hansen L, Pigg M, List T, Eliav E, Ettlin D *et al.* (2011). Guidelines and recommendations for assessment of somatosensory function in oro-facial pain conditions - a taskforce report. *J Oral Rehabil* 38(5)*:*366-394.

Tchivileva IE, Lim PF, Smith SB, Slade GD, Diatchenko L, McLean SA *et al.* (2010). Effect of catechol-O-methyltransferase polymorphism on response to propranolol therapy in chronic musculoskeletal pain: a randomized, double-blind, placebo-controlled, crossover pilot study. *Pharmacogenet Genomics* 20(4)*:*239-248.

Tickle M, Milsom K, Crawford FI, Aggarwal VR (2012). Predictors of pain associated with routine procedures performed in general dental practice. *Community Dent Oral Epidemiol* 40(4)*:*343-350.

Todd KH, Deaton C, D'Adamo AP, Goe L (2000). Ethnicity and analgesic practice. *Ann Emerg Med* 35(1)*:*11-16.

Vassend O (1993). Anxiety, pain and discomfort associated with dental treatment. *Behav Res Ther* 31(7)*:*659-666.

von Baeyer CL, Piira T, Chambers CT, Trapanotto M, Zeltzer LK (2005). Guidelines for the cold pressor task as an experimental pain stimulus for use with children. *Pain* 6(4)*:*218-227.

Walsh NE, Schoenfeld L, Ramamurthy S, Hoffman J (1989). Normative model for cold pressor test. *Am J Phys Med Rehabil* 68(1)*:*6-11.

Watson D, Clark LA, Tellegen A (1988). Development and validation of brief measures of positive and negative affect: the PANAS scales. *J Pers Soc Psychol* 54(6)*:*1063-1070.

Woodrow KM, Friedman GD, Siegelaub AB, Collen MF (1972). Pain tolerance: differences according to age, sex and race. *Psychosom Med* 34(6)*:*548-556.

Woolf CJ, Doubell TP (1994). The pathophysiology of chronic pain—increased sensitivity to low threshold Aβ-fibre inputs. *Curr Opin Neurobiol* 4(4)*:*525-534.

Yamashiro T, Satoh K, Nakagawa K, Moriyama H, Yagi T, Takada K (1998). Expression of Fos in the rat forebrain following experimental tooth movement. *J Dent Res* 77(11)*:*1920-1925.

Young EE, Lariviere WR, Belfer I (2012). Genetic basis of pain variability: recent advances. *J Med Genet* 49(1)*:*1-9.

Zubieta JK, Heitzeg MM, Smith YR, Bueller JA, Xu K, Xu Y *et al.* (2003). COMT val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor. *Science* 299(5610)*:*1240-1243.

Appendix I

11/172

Academic Services
Manager, Academic Committees, Mr Gary Witte

12 August 2011

Professor J Kieser
Sir John Walsh Research Institute
Department of Oral Diagnostic and Surgical Sciences
Faculty of Dentistry

Dear Professor Kieser

I am again writing to you concerning your proposal entitled "**Pain predictors during orthodontic treatment**", Ethics Committee reference number **11/172**.

Thank you for sending to me an amended copy of the application addressing the Committee's concerns.

On the basis of this response, I am pleased to confirm that the proposal now has full ethical approval to proceed.

Approval is for up to three years. If this project has not been completed within three years from the date of this letter, re-approval must be requested. If the nature, consent, location, procedures or personnel of your approved application change, please advise me in writing.

Yours sincerely,

Gary Witte

Mr Gary Witte
Manager, Academic Committees
Tel: 479 8256
Email: gary.witte@otago.ac.nz

c.c. Sir John Walsh Research Institute

NGĀI TAHU RESEARCH CONSULTATION COMMITTEE
TE KOMITI RAKAHAU KI KĀI TAHU

31/05/2011 - 28
Tuesday, 31 May 2011

Dr Beck
Orthodontics
Dunedin

Tēnā koe Dr Beck

Title: Predicting factors of pain during orthodontic treatment.

The Ngāi Tahu Research Consultation Committee (The Committee) met on Tuesday, 31 May 2011 to discuss your research proposition.

By way of introduction, this response from the Committee is provided as part of the Memorandum of Understanding between Te Rūnanga o Ngāi Tahu and the University. In the statement of principles of the memorandum, it states "Ngāi Tahu acknowledges that the consultation process outlined in this policy provides no power of veto by Ngāi Tahu to research undertaken at the University of Otago". As such, this response is not "approval" or "mandate" for the research, rather it is a mandated response from a Ngāi Tahu appointed committee. This process is part of a number of requirements for researchers to undertake and does not cover other issues relating to ethics, including methodology; they are separate requirements with other committees, for example the Human Ethics Committee, etc.

Within the context of the Policy for Research Consultation with Māori, the Committee base consultation on that defined by Justice McGechan:

"Consultation does not mean negotiation or agreement. It means: setting out a proposal not fully decided upon; adequately informing a party about relevant information upon which the proposal is based; listening to what the others have to say with an open mind (in that there is room to be persuaded against the proposal); undertaking that task in a genuine and not cosmetic manner. Reaching a decision that may or may not alter the original proposal."

The Committee considers the research to be of importance to Māori health.

As this study involves human participants, the Committee strongly encourage that ethnicity data be collected as part of the research project. That is the questions on self-identified ethnicity and descent, these questions are contained in the 2006 census.

The Ministry of Health website http://www.Māorihealth.govt.nz/moh.nsf/indexma/publications contains a list of Māori health publications. The Committee recommends you review the Māori health publications on this website, e.g. Unequal Impact II: Māori and Non-Māori Cancer Statistics by Deprivation and Rural-Urban Status 2002-2006 and Tatau Kahukura: Māori Health Chart Book 2010. Another Publication, Hauora: Māori Standards of Health IV (200-2005), has its own website,

http://www.hauora.Māori.nz/. These publications provide information on a range of Māori health issues and will assist in ensuring your research has an appropriate Māori health focus.

The Committee suggests dissemination of the findings to relevant Māori health organisations, for example the National Māori Organisation for Dental Health, Oranga Niho and to Associate Professor John Broughton, who is involved in Māori Dental Health, University of Otago.

We wish you every success in your research and the Committee also requests a copy of the research findings.

This letter of suggestion, recommendation and advice is current for an 18 month period from Tuesday, 31 May 2011 to 01 December 2012.

The recommendations and suggestions above are provided on your proposal submitted through the consultation website process. These recommendations and suggestions do not necessarily relate to ethical issues with the research, including methodology. Other committees may also provide feedback in these areas.

Nāhaku noa, nā

pp NTRCC

Mark Brunton
Kaiwhakahaere Rangahau Māori
Facilitator Research Māori
Research Division
Te Whare Wānanga o Otāgo
Ph: +64 3 479 8738
email: mark.brunton@otago.ac.nz
Web: www.otago.ac.nz

Appendix II

Budget

Materials and consumables

Cheek Swab Collection Kit - 25 @ $29.30 per person = $732.50

Red Stamp 25 Litre Chilly Bin - 3 @ $24.99 = $74.97

Aqua One (Sydney, Australia) ST-3 Electronic Thermometer $13

DSE Digital LCD Stopwatch $14

Gift Vouchers for Phase Two participants 20 @ $50 = $1000

iPod Touch 2 @ $259.99 = $519.98

Technical Services

Genetic testing-

Set-up Cost (TaqMan probes) - 3 @ $614 per COMT SNP = $1842

Genetic testing of COMT variations - $100

Total

$4296

$2855 Funded by the Foundation for Orthodontic Research and Education, NZAO (FORENZAO) Charitable Trust

$1441 Funded by a Fuller Scholarship, Sir John Walsh Research Institute

ID Number				

Appendix III

Factors Associated with Orthodontic Pain

Phase One

Sex (*please circle*)

Male Female

Date of Birth

_____

Ethnicity (*please circle)*

NZ European

Māori

Pacific Islander

European

Asian

Other (*please specify*) _____

The clinician will go through this section with you:

- Anxiety disorders or depressive illness Yes/No
- Raynaud's phenomenon Yes/No
- Chronic pain syndromes Yes/No
- Use of neurologically-acting medication or medication Yes/No
- Spacing between mandibular teeth Yes/No
- Active decay or gum disease Yes/No
- Large fillings, previous trauma or root canal treatment on upper central incisors Yes/No

Following insertion of the separating rings, you are requested to complete the Visual Analogue Scales (VAS) on the following pages.

The first one will be completed under supervision, immediately following insertion. After this, you are required to complete a VAS at the following times:

- Four hours after insertion
- Evening One before retiring
- Morning One shortly after rising
- Evening Two before retiring
- Morning Two shortly after rising

You are required to bring these forms back with you on Day Two, when you return to have the separating rings removed.

Immediately post-insertion

On the line below, place a vertical line to describe your current pain level.

No pain at all — Worst pain imaginable

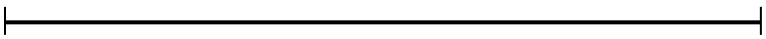
Four hours post-insertion

On the line below, place a vertical line to describe your current pain level.

No pain at all — Worst pain imaginable

On the line below, place a vertical line to describe the maximum pain level experienced since the last questionnaire.

No pain at all — Worst pain imaginable



Evening One

On the line below, place a vertical line to describe your current pain level.

No pain at all — Worst pain imaginable

On the line below, place a vertical line to describe the maximum pain level experienced since the last questionnaire.

No pain at all — Worst pain imaginable

Morning One

On the line below, place a vertical line to describe your current pain level.

No pain at all — Worst pain imaginable

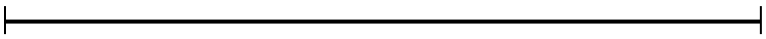
On the line below, place a vertical line to describe the maximum pain level experienced since the last questionnaire.

No pain at all — Worst pain imaginable

Evening Two

On the line below, place a vertical line to describe your current pain level.

No pain at all — Worst pain imaginable



On the line below, place a vertical line to describe the maximum pain level experienced since the last questionnaire.

No pain at all — Worst pain imaginable

Morning Two

On the line below, place a vertical line to describe your current pain level.

No pain at all — Worst pain imaginable

On the line below, place a vertical line to describe the maximum pain level experienced since the last questionnaire.

No pain at all — Worst pain imaginable

Thank you for your participation.

Please return to the Orthodontic clinic this morning at the scheduled time to have the separating rings removed.

ID Number				

Appendix IV

Factors Associated with Orthodontic Pain

Phase Two

Please complete every question in this questionnaire.

The Positive And Negative Affectivity Schedule

This scale consists of a number of words that describe different feelings and emotions. Read each item and then mark the appropriate answer in the space next to that word. Indicate <u>to what extent you generally feel this way</u>, that is, how you feel on average.

Use the following scale to record your answers.

1	2	3	4	5
Very slightly or not at all	A little	Moderately	Quite a bit	Extremely

____ interested	____ irritable
____ distressed	____ alert
____ excited	____ ashamed
____ upset	____ inspired
____ strong	____ nervous
____ guilty	____ determined
____ scared	____ attentive
____ hostile	____ jittery
____ enthusiastic	____ active
____ proud	____ afraid

Corah Dental Anxiety Scale

This next section asks more about how you feel when you go to the dentist. For each question, please tick the box of the answer which comes closest to how you feel.

If you had to go to the dentist tomorrow, how would you feel about it?

- I would look forward to it as a reasonably enjoyable experience ☐
- I wouldn't care one way or the other ☐
- I would be a little uneasy about it ☐
- I would be afraid that it would be unpleasant and painful ☐
- I would be very frightened of what the dentist might do ☐

When you are waiting in the dentist's surgery for your turn in the chair, how do you feel?

- Relaxed ☐
- A little uneasy ☐
- Tense ☐
- Anxious ☐
- So anxious that I sometimes break out in a sweat or almost feel physically sick ☐

When you are waiting in the dentist's chair while they get their drill ready to begin working on your teeth, how do you feel?

- Relaxed ☐
- A little uneasy ☐
- Tense ☐
- Anxious ☐
- So anxious that I sometimes break out in a sweat or almost feel physically sick ☐

You are waiting in the dentist's chair to have your teeth cleaned. While you are waiting and the dentist is getting out the instruments which they will use to scrape your teeth around the gums, how do you feel?

- Relaxed ☐
- A little uneasy ☐
- Tense ☐
- Anxious ☐
- So anxious that I sometimes break out in a sweat or almost feel physically sick ☐

Locker's Global Item

How would you describe the health of your teeth and mouth?

Excellent (1) Very good (2) Good (3) Fair (4) Poor (5)

The State-Trait Anxiety Inventory:

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the most appropriate number to the right of the statement to indicate how you *feel* right now, that is, *at this moment*. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

	Not at all	Somewhat	Moderately so	Very much so
1. I feel calm	1	2	3	4
2. I feel secure	1	2	3	4
3. I am tense	1	2	3	4
4. I am regretful	1	2	3	4
5. I feel at ease	1	2	3	4
6. I feel upset	1	2	3	4
7. I am presently worrying over possible misfortunes	1	2	3	4
8. I feel rested	1	2	3	4
9. I feel anxious	1	2	3	4
10. I feel comfortable	1	2	3	4
11. I feel self-confident	1	2	3	4
12. I feel nervous	1	2	3	4
13. I am jittery	1	2	3	4
14. I feel “high strung”	1	2	3	4
15. I am relaxed	1	2	3	4
16. I feel content	1	2	3	4
17. I am worried	1	2	3	4
18. I feel over-excited and “rattled”	1	2	3	4
19. I feel joyful	1	2	3	4
20. I feel pleasant	1	2	3	4

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the most appropriate number to the right of the statement to indicate how you *generally* feel. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you generally feel.

	Not at all	Somewhat	Moderately so	Very much so
21. I feel pleasant	1	2	3	4
22. I tire quickly	1	2	3	4
23. I feel like crying	1	2	3	4
24. I wish I could be as happy as others seems to be	1	2	3	4
25. I am losing out on things because I can't make up my mind soon enough	1	2	3	4
26. I feel rested	1	2	3	4
27. I am "calm, cool, and collected"	1	2	3	4
28. I feel that difficulties are piling up on me so that I cannot overcome them	1	2	3	4
29. I worry too much over something that really doesn't matter	1	2	3	4
30. I am happy	1	2	3	4
31. I am inclined to take things hard	1	2	3	4
32. I lack self-confidence	1	2	3	4
33. I feel secure	1	2	3	4
34. I try to avoid facing a crisis or difficulty	1	2	3	4
35. I feel blue	1	2	3	4
36. I am content	1	2	3	4
37. Some unimportant thought runs through my mind and bothers me	1	2	3	4
38. I take disappointments so keenly that I can't put them out of my mind	1	2	3	4
39. I am a steady person	1	2	3	4
40. I get in a state of tension or turmoil as I think over my recent concerns and interests	1	2	3	4

Pain Catastrophising Scale

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the most appropriate number to the right of the statement to indicate how you feel when you have pain. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your feelings best

"When I have pain…"

	Not at all	To a slight degree	To a moderate degree	To a great degree	All the time
1. I worry all the time about whether the pain will end	0	1	2	3	4
2. I feel I can't go on	0	1	2	3	4
3. It's terrible and I think it's never going to get any better	0	1	2	3	4
4. It's awful and I feel that it overwhelms me	0	1	2	3	4
5. I feel I can't stand it anymore	0	1	2	3	4
6. I become afraid that the pain may get worse	0	1	2	3	4
7. I think of other painful experiences	0	1	2	3	4
8. I anxiously want the pain to go away	0	1	2	3	4
9. I can't seem to keep it out of my mind	0	1	2	3	4
10. I keep thinking about how much it hurts	0	1	2	3	4
11. I keep thinking about how badly I want the pain to stop	0	1	2	3	4
12. There is nothing I can do to reduce the intensity of the pain	0	1	2	3	4
13. I wonder whether something serious may happen	0	1	2	3	4

ID Number				

Appendix V

Factors Associated with Orthodontic Pain

Phase Two

Cold Pressor Test (non-dominant hand, 5cm from ulnar process)

Warm water temperature at start _____°C

Cold Water:

Temperature at start _____°C

Temperature at 1 minute _____°C

Temperature at 2 minutes _____°C

Temperature at conclusion _____°C

Time in cold (if not full 3 minutes) _____:_____ (mins:secs)

Electric Pulp Testing

Output Reading at Initial Sensation 1 **(tooth 21)** _____

Output Reading at Initial Sensation 2 **(tooth 21)** _____

Output Reading at Initial Sensation 3 **(tooth 21)** _____

Output Reading at Maximum Tolerance 1 **(tooth 11)** _____

Output Reading at Maximum Tolerance 2 **(tooth 11)** _____

Output Reading at Maximum Tolerance 3 **(tooth 11)** _____

Hair Colour _____

Saliva Test

*No bubbles & must be completed within 30mins

ID Number				

Appendix VI

Cold Pressor Test

1 Minute

On the line below, place a vertical line to describe the maximum pain level experienced.

No pain at all — Worst pain imaginable

2 Minutes

On the line below, place a vertical line to describe the maximum pain level experienced.

No pain at all — Worst pain imaginable

3 Minutes

On the line below, place a vertical line to describe the maximum pain level experienced.

No pain at all — Worst pain imaginable

ID Number				

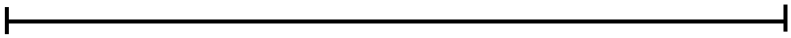
Appendix VII

Electric Pulp Testing

Threshold 1

On the line below, place a vertical line to describe the maximum pain level experienced.

No pain at all — Worst pain imaginable



Threshold 2

On the line below, place a vertical line to describe the maximum pain level experienced.

No pain at all — Worst pain imaginable

Threshold 3

On the line below, place a vertical line to describe the maximum pain level experienced.

No pain at all — Worst pain imaginable

Maximum Tolerance 1

On the line below, place a vertical line to describe the maximum pain level experienced.

No pain at all — Worst pain imaginable

Maximum Tolerance 2

On the line below, place a vertical line to describe the maximum pain level experienced.

No pain at all — Worst pain imaginable

Maximum Tolerance 3

On the line below, place a vertical line to describe the maximum pain level experienced.

No pain at all — Worst pain imaginable