EXPLORING INDIVIDUAL VARIATION IN ORAL PERCEPTION

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ABSTRACT

Diet plays a pivotal role in preventing, managing, and reducing the risk of weight gain, diabetes and heart disease. Increasing pressure is directed towards food manufacturers to offer healthier options. The challenge is to develop products which are both nutritious and accepted by the consumer. Oral sensitivity, and therefore product perception, varies greatly amongst individuals, and likely affects food choice. Taste phenotype and genotype are frequently proposed as markers for overall oral sensitivity. This thesis performs fundamental research to further current understanding of the impact of taste phenotype and genotype on the response to oral stimuli.

The effect of 6-n-propylthiouracil (PROP) taster status (PTS), fungiform papillae density, TAS2R38 and gustin rs2274333 genotype on the perceived intensity of prototypical tastants and metallic stimuli is explored. Experiments were first conducted to develop oral stimuli for use in the subsequent fMRI studies, which interestingly identified that some metallic stimuli may have a gustatory component. Perceptually, few or no differences were identified across taste phenotypes or genotypes. Interestingly, functional Magnetic Resonance Imaging (fMRI) identified variation in cortical processing that was associated with PTS. PROP intensity ratings were found to correlate with cortical activation in the anterior insula, an area of the brain thought to be the primary gustatory cortex, in response to sweet and metallic stimuli, but not for sour, salt, bitter or umami stimuli. These limited differences observed may have been due to the occurrence of a concentration effect, where the increased gustatory sensitivity frequently associated with PROP tasters compared to PROP non-tasters was lost when administering strong supra-threshold stimuli used in the current study. These findings are of interest to food manufacturers and health professionals

as they could indicate that taste phenotype and genotype has less impact on product perception, and therefore food choice, than previously proposed.

Thermal taster status (TTS) refers to a new taste phenotype in which individuals perceive phantom tastes when the tongue is thermally stimulated, whilst thermal non-tasters (TnTs) only perceive temperature. In this thesis, variation in the phantom tastes reported by thermal tasters (TTs) are explored, and for the first time the temporal phantom taste response is measured. Different categories of temporal taste responses are identified, and interestingly it is shown that phantom tastes are perceived at variable temperature ranges across both TTs and taste qualities. Importantly, the onset of sweet taste was found to occur as the temperature increased between 22-35°C, supporting the hypothesis that the TRPM5 may be involved in sweet phantom taste responses. This is the first study to assess the brain's response when thermally stimulating the tongue of TTs to elicit a phantom taste response. Interestingly, when using fMRI it is shown that at the time when TTs perceive a phantom taste, cortical activation is induced in the anterior insula, which is thought to be the primary gustatory cortex. This indicates that thermal stimulation may activate temperature sensitive gustatory nerve fibres in TTs, and supports the hypothesis of cross wiring between gustatory and trigeminal nerves. When comparing the cortical response to thermal stimulation of the tongue across TTs and TnTs, greater activation is observed in oral somatosensory areas of the brain in TTs compared to TnTs. These findings show cortical processing differs across thermal taste phenotype, and supports evidence that thermal taster status may be a marker for oral sensitivity.

This original research provides a valuable contribution towards understanding the effect of taste phenotype and genotype on perception of prototypical taste,

metallic, and thermal stimuli. The novel multidisciplinary approach of utilising sensory evaluation and fMRI techniques has provided valuable insights into the impact of taste phenotype on gustatory responses, and has suggested possible mechanisms that may be involved in thermal taste phenotype.

PREFACE

Individual variation in sensory perception is of interest to food manufacturers and health professionals, as it influences dietary behaviours and health outcomes. Taste phenotype and genotype have been proposed as markers for overall oral sensitivity. Whilst 6-n-propylthiouracil (PROP) taster status (PTS) and fungiform papillae density (FPD) phenotypes have been extensively researched, the more recently discovered thermal taster status phenotype requires significant investigation, along with the TAS2R38 and gustin genotypes. Evidence of their impact on oral sensitivity remains contradictory, and little is known of the mechanisms which drive individual variation in perception.

The research in this thesis adopts a novel approach to explore variation in the perceptual response to oral stimuli across taste phenotype and genotype using sensory evaluation techniques, and functional Magnetic Resonance Imaging (fMRI) measures of the brain's response. This work was performed as part of a collaboration between the Sensory Science Centre (SSC), and Sir Peter Mansfield Imaging Centre (SPMIC) at the University of Nottingham. The primary aim was to explore individual variation in gustatory perception across PTS, and measure the temperature related responses across thermal taste phenotype. Thermal tasters (TTs) are those individuals who perceive a 'phantom' taste sensation when the tongue is thermally stimulated despite there being no chemical taste in the mouth, whilst thermal non-tasters (TnTs) only perceive temperature. The mechanism behind this unusual phenotype is unknown.

Chapter 1 provides a general introduction to sensory perception, from the peripheral level to the central nervous system. It also introduces taste

phenotypes and genotypes, and the basic principles of fMRI to measure brain activation. **Chapter 2** details the sensory panel work completed when developing gustatory stimuli, and a range of experiments used to inform the experimental design of the subsequent fMRI experiment detailed in Chapter 4. Whether perceived 'metallic' sensations have a gustatory component is still under debate. In **Chapter 3**, the oronasal contributions to metallic perception are explored. Chapters 4 and 5 explore individual variation in oral sensitivity. In **Chapter 4**, sensory evaluation experiments measuring individual variation in taste perception across PTS, FPD, and TAS2R38 and gustin genotypes, as well as variation in cortical processing associated with PTS are described. **Chapter 5** is divided into two parts; part one uses sensory evaluation techniques to measure variation in the phantom tastes perceived by 36 TTs, part two measures variation in the cortical response to temperature across 12 TTs and 12 TnTs. Conclusions drawn from this body of work are detailed in **Chapter 6**, alongside the implications of these findings, and recommendations for future work.

1 GENERAL INTRODUCTION

Changes in the global food system have moved towards producing increasingly processed, energy dense, affordable, and readily accessible products. This has contributed to the rise in obesity and co-morbidities, including cardiovascular disease, cancer, and type 2 diabetes mellitus over the past 40 years (Swinburn et al., 2011). In 2014, 39% of adults were overweight, and 13% obese (World Health Organisation (WHO), 2016b). In 2015, 31% of all deaths were attributed to cardiovascular disease, making it the global number one cause of morbidity (WHO, 2017). Colorectal cancer was the third most commonly diagnosed cancer in 2012, with 1,361 new cases reported globally (World Cancer Research Fund, 2017). In 2014, 8.5% of adults worldwide were estimated to have diabetes, compared to 4.7% in 1980 (WHO, 2016a). Diet plays a pivotal role in preventing, managing, or reducing the risk of developing non-communicable diseases (Gandy, 2014). However, implementing behaviour change to improve dietary intake is challenging, and the effect size often small (Michie et al., 2009). Amongst other approaches, increasing pressure is directed towards food manufacturers to adapt product formulations to offer healthier options (Swinburn et al., 2011). The challenge is to develop products that are both nutritious and accepted by the consumer (Food and Drink Federation, 2016).

Multiple factors interplay in food choice, including biological (e.g. gender, age, genetic factors), sociological (e.g. culture, tradition, social status), psychological (e.g. personality traits), economic (e.g. price, availability) factors, and importantly the sensory properties of food and beverage (Koster, 2009). Oral sensitivity, and therefore product perception, varies greatly amongst individuals, and is likely affecting food choice. Better understanding of the impact of

individual variation on fundamental sensory perception of oral stimuli will aid in understanding the wider implications on food choice. This may provide valuable insights into the diversity of consumer demands, and identify the need to target consumer segments associated with sensory perception.

This chapter introduces the predominant sensory systems involved in oral perception of foods and beverages, discusses the impact of taste phenotype and genotype on sensory perception, and details the basic principles of functional Magnetic Resonance Imaging (fMRI).

1.1 PERIPHERAL SENSORY PROCESSING

Interaction with the external environment is perceived via the sensory systems broadly categorised as gustation, olfaction, vision, audition and somatosensation (Mader, 2010). A stimulus activates a receptor, initiating a nerve impulse to relay information to the brain where it is interpreted (Cohen and Taylor, 2005). Oral receptors are activated when a food or beverage is consumed, resulting in conscious perception of flavour. Traditionally flavour has been defined as the “complex combination of the olfactory, gustatory and trigeminal sensations perceived during tasting” (ISO-5492, 2017), where a trigeminal sensation refers to the sensations of ‘touch’, temperature and pain. Although still an area of debate (Small, 2012), it is now more widely accepted that every sensory system contributes to the overall flavour perceived (Kemp et al., 2009) at the peripheral (Delwiche, 2004) and/or central (Verhagen and Engelen, 2006) level. For example, the colour of wine influences the flavour profile reported (Morrot et al., 2001), and delivering auditory cues can manipulate how a food is perceived (Spence, 2015). However, sensations

arising from the mouth are defined as the core contributions to flavour perception (Small, 2012), and will be discussed individually.

1.1.1 Peripheral gustatory processing

There are currently five widely recognised and accepted tastes, with clearly identified receptor mechanisms; sweet, sour, salty, bitter and umami (Chandrashekar et al., 2006). Gustation acts as a nutrient-toxin detection system aiding food choices that are appropriate to bodily requirements; sweet indicates carbohydrates as a source of energy, umami reflects amino acids present in protein, salt informs intake of sodium and other salts relating to electrolyte homeostasis and mineral content, whilst the aversive taste of bitter frequently indicates potentially harmful poisons, and sourness signifies food spoilage and unripe fruit (Bachmanov and Beauchamp, 2007). However, exposure to food and learned behaviour can override innate responses (Chaudhari and Roper, 2010).

Chemical compounds termed tastants stimulate the gustatory system (Bachmanov and Beauchamp, 2007). Tastants dissolved in saliva come into contact with papillae primarily on the tongue, and also the soft palate and throat. There are four types of papillae: fungiform, foliate, circumvallate and filiform (Nuessle et al., 2015). Fungiform papillae located on the anterior tongue tip, foliate papillae on the lateral edges, and circumvallate papillae at the posterior of the tongue contain taste buds that are involved in gustation (**Fig 1.1**). Taste buds comprise 150-300 cells, including 50-150 taste receptor cells (TRCs) (Gravina et al., 2013). Type I cells are supporting cells which may also be involved in salt detection, Type II cells contain sweet, bitter and umami taste receptors, and Type III cells are involved in the detection of sour taste

(Chaudhari and Roper, 2010). TRCs have chemically sensitive apical ends that protrude through the taste pore to expose microvilli to saliva (Chandrashekar *et al*., 2006). Taste qualities have different transduction mechanisms. Salt and sour tastants permeate the cell wall via gated ion channels, whilst sweet, umami and bitter tastants bind to G-protein coupled receptors (G-PCRs) on the cell wall (Smith and Margolskee, 2001).

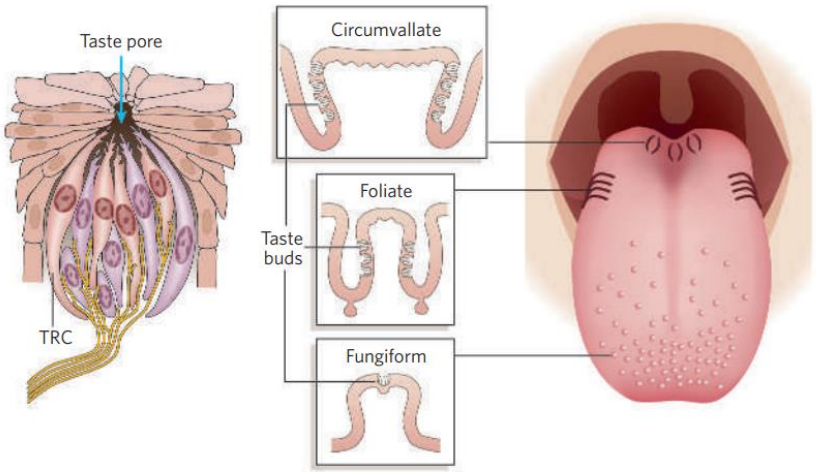

Figure 1.1: Type, location and anatomy of papillae that contain taste buds, and taste bud anatomy (source: Chandrashekar *et al*., 2006).

Two opposing theories regarding how taste is perceived have been proposed (Chandrashekar et al., 2006). The labelled line theory suggests TRCs are tuned to a specific taste quality by expressing only one type of receptor, and innervating nerves are individually tuned. Alternatively, an across-fibre model may occur, which can be broken down into two possible pathways. TRCs may express receptors for different taste qualities, and innervating nerves carry information for multiple tastes. Alternatively, TRCs express receptors for only one taste quality, and the innervating nerve carries information for multiple taste qualities. The facial, vagus, and glossopharangeal nerves are responsible for transmitting gustatory responses (**Fig 1.2)**. The chordia tympani branch of the facial nerve innervates the fungiform papillae on the anterior 2/3 of the tongue, the glossopharangeal nerve innervates the foliate and circumvallate papillae at

the posterior 1/3 of the tongue, and the vagus nerve innervates the larynx and epiglottis (Bromley, 2000). Sensory information is transmitted from activated TRCs to the brain via the cranial nerves, where the signals are encoded to give conscious taste perception.

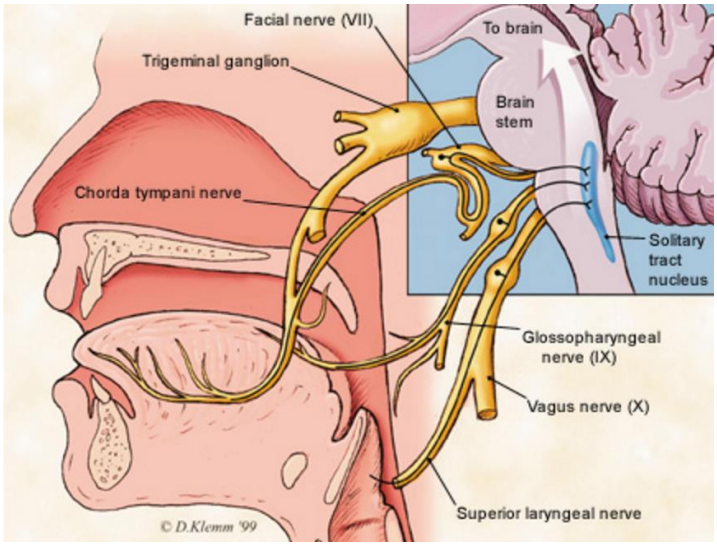

Figure 1.2: Cranial nerves involved in gustatory perception (source: Bromley, 2000).

1.1.1.1 Salt

Salts are made up of a negatively charged anion and a positively charged cation (Yang and Lawless, 2006). The prototypical salt stimulus is sodium chloride (NaCl), which is appetitive at low concentrations and aversive at high concentrations (Chandrashekar et al., 2010). NaCl is thought to activate TRCs when Na^+ enters the cell via gated ion channels on the microvilli or basolateral side of the cell, causing an inward current to the cell, and depolarisation (Smith and Margolskee, 2001). The epithelial amilioride-sensitive sodium channel (ENaC) mediates this process in rodents (Chandrashekar et al., 2010). However, this finding has not been replicated in humans, and its involvement in human gustation is unknown (Roper and Chaudhari, 2017). Salt remains one of the most poorly characterised taste qualities.

1.1.1.2 Sour

Sour perception arises due to hydrogen ions (H^+) in acids (Bear et al., 2016). H^+ are thought to influence TRCs by directly entering the cell via gated ion channels, blocking the K^+ channel, thus preventing K^+ exiting the cell, or binding to ion channels resulting in other positive ions entering the cell (Smith and Margolskee, 2001). This causes an accumulation of positive ions in the cell (intracellular acidification), cell depolarisation, and a nerve impulse sent to the brain (Smith and Margolskee, 2001). A number of candidate ion channels have been proposed, including hyperpolarisation-activated cyclic-nucleotide-gated channels, amilioride-sensitive cation channel 1, Na^+-H^+-exchanger isoform 1, epithelial amilioride-sensitive sodium channel, and polycystic kidney disease channel PKD1L2 + PKD3L1 (Bachmanov and Beauchamp, 2007). However, individual involvement remains unclear, and in some cases rodents lacking the proposed receptor remain responsive to sour stimuli (Roper and Chaudhari, 2017). Potassium leak conductance plasma membranes from the K_2P family are modulated by changes in intracellular acidification (Richter et al., 2003, Richter et al., 2004) and are a potential mechanism requiring further research (Chaudhari and Roper, 2010). As with salt perception, the mechanism behind sour taste detection is poorly understood.

1.1.1.3 Sweet

The perception of sweet, umami and bitter stimuli involves two families of GPCRs; T1Rs (T1R1, T1R2 and T1R3) are involved in sweet and umami detection, whilst T2Rs are responsive to bitter tastants (Bear et al., 2016). Twenty five genes have been found to encode for T2Rs, whereas only three have been identified to encode for T1Rs (Roper and Chaudhari, 2017).

Sweet stimuli, considered the most pleasant taste quality, include natural sugars (e.g. glucose) and artificial sweeteners (e.g. aspartame) (Chaudhari and Roper, 2010). The most studied sweet receptor is the GPCR heterodimer T1R2-T1R3 able to respond to natural sugars, artificial sweeteners, and sweet tasting amino acids (Chandrashekar et al., 2006). T1R3 knockout mice do not always loose total sensitivity to sweet stimuli, therefore T1R3-independent mechanisms, including the glucose transporter type 4 (GLUT4) and sodium/glucose cotransporter 1 (SGLT1), are proposed to be involved in sweet taste perception where transport of glucose into the cell causes elevated ATP levels, which in turn block K^+ channels, resulting in cell depolarisation (Roper and Chaudhari, 2017).

1.1.1.4 Umami

Umami is described as a 'meaty, savoury' sensation, indicating amino acids such as glutamate and aspartate which are found naturally in many foods, including meat, cheese and some vegetables (Chaudhari and Roper, 2010). A synergistic effect occurs with combinations of 5'-nucleotides (such as inosine 5' monophosphate) and glutamate, where the perceived intensity is greater than the sum of the individual components (Araujo et al., 2001). Umami is detected by the GCPR heterodimer T1R1-T1R3 (Bear et al., 2016). However, some researchers found T1R3 and T1R1 knockout mice are still responsive to umami stimuli, indicating other mechanisms are likely involved (Damak et al., 2003, Delay et al., 2006). The mGluR4 and mGluR1 glutamate receptors are possible mechanisms, as mice lacking these receptors have reduced nerve responses to umami stimuli (Roper and Chaudhari, 2017).

1.1.1.5 Bitter

Many bitter compounds signify toxic substances, although some nutritionally beneficial foods, such as phytochemicals in cruciferous vegetables, are also bitter (Chaudhari and Roper, 2010). A range of compounds with varying chemical structures activate T2Rs. T2Rs may be tuned to single or multiple tastants. For example, T2R3 responded to only one of 104 compounds tested, whereas T2R14 responded to 33 of the compounds (Meyerhof et al., 2010). Additionally bitter tastants may activate a single T2R (e.g. Phenylthiocarbamide), or multiple T2Rs (e.g. quinine activates nine receptors) (Meyerhof et al., 2010).

1.1.1.6 Signalling downstream of sweet, umami and bitter GPCRs

Despite the diversity in GPCRs responding to sweet, bitter and umami stimuli, and different transduction pathways being proposed in the past (Smith and Margolskee, 2001), a recent review indicates that the intracellular signalling pathway is consistent across taste qualities (Roper and Chaudhari, 2017) (**Fig 1.3**). GPCRs are coupled with a G-protein comprising of beta ($G\beta_3$), gamma ($G\gamma_{13}$), and alpha ($G\alpha_{gus}$, $G\alpha_{14}$ and $G\alpha_i$, also known as gustducin) sub units. Alpha subunits are thought to control the release of beta and gamma dimers when the GPCR is activated, stimulating the enzyme phospholipase C, which increases the production of an intracellular messenger inositol triphosphate (IP_3), mobilising Ca^2, resulting in the opening of transient receptor potential cation channel subfamily M member 5 (TRPM5), causing cell depolarisation. Cells express T1Rs and T2Rs in a non-overlapping pattern, explaining the differentiation between taste qualities (Bear et al., 2016). It is possible that some bitter and sweet compounds do not bind to a receptor, but permeate the cell

membrane to enter the cell (Bachmanov and Beauchamp, 2007), which requires further exploration.

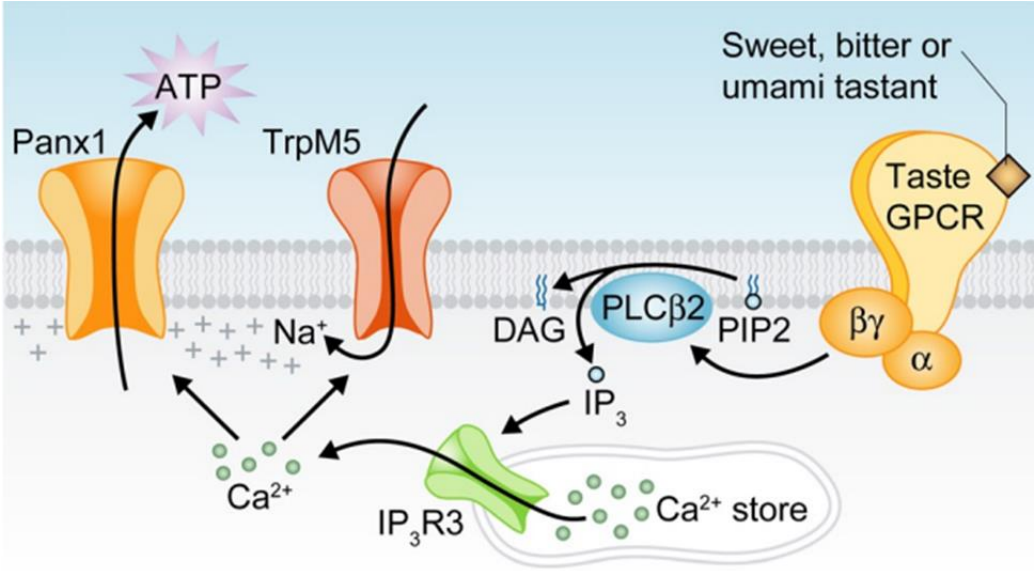

Figure 1.3: Sweet, umami or bitter tastants binding to GPCRs, activating the phosphoinositide pathway, causing a chemical cascade and cell depolarisation (Source: Chaudhari and Roper, 2010).

Although there are currently only five confirmed tastes, metallic (Bartoshuk, 1978), fatty acids (Mattes, 2011), kokumi (Ueda et al., 1990, Ohsu et al., 2010), calcium (Tordoff et al., 2012) and even water (Bachmanov and Beauchamp, 2007) have also been proposed as additional taste qualities. The definition of what constitutes a taste is still under debate, although Mattes (2011) proposed criteria should include that they; a) provide an adaptive advantage, b) be a defined class of effective stimuli, c) exhibit a unique transduction mechanism to convert the chemical signal to an electric signal, d) have a distinct pathway to project the electrical signal from the peripheral to central nervous system, e) be distinguishable from other taste qualities, f) evoke a functional physiological and/or behavioural response.

1.1.1.7 Metallic

Metallic perception has been proposed as a taste quality in the past (Bartoshuk, 1978). A range of oral stimuli, including divalent salt solutions (Lawless et al., 2004), electrical stimulation of the tongue, and solid metal (Lawless et al., 2005) elicit a metallic sensation. Occluding the nose to block retronasal aroma delivery reduces or eliminates metallic perception after oral exposure to divalent salts (Lawless et al., 2004), indicating volatiles released from the oral cavity during contact with the divalent salts are involved in the response (Omur-Ozbek et al., 2012). Interestingly, occluding the nose does not influence the metallic sensation reported after electrical current or solid metal stimuli on the tongue, indicating multiple mechanisms may be involved in the metallic sensations perceived (Lawless et al., 2005). Whether metallic constitutes a taste quality remains a controversial topic, and requires further investigation.

1.1.2 Peripheral olfactory processing

The olfactory system functions closely with gustation to determine the flavour perceived when consuming food (Small, 2012). Aroma provides valuable information about food prior to consumption, indicating, for example, food spoilage (Hayes et al., 2002) or the ripeness of fruit (Espino-Diaz et al., 2016). Aroma is perceived when volatile chemical stimuli, termed odorants, are dispersed in the air and activate receptors in the nasal cavity (Dietrich, 2009). Humans are able to detect 10,000-100,000 odours, using around 350 types of receptors (Buck, 2004). Odorants enter the nasal cavity via two different pathways, the orthonasal and retronasal routes (Rozin, 1982). Odorants from the external environment enter the nasal cavity via the nostrils (orthonasal olfaction), whilst those released from food and beverage enter the nasal cavity

via the nasophanrynx during exhalation when masticating or swallowing (retronasal olfaction) (**Fig 1.4**).

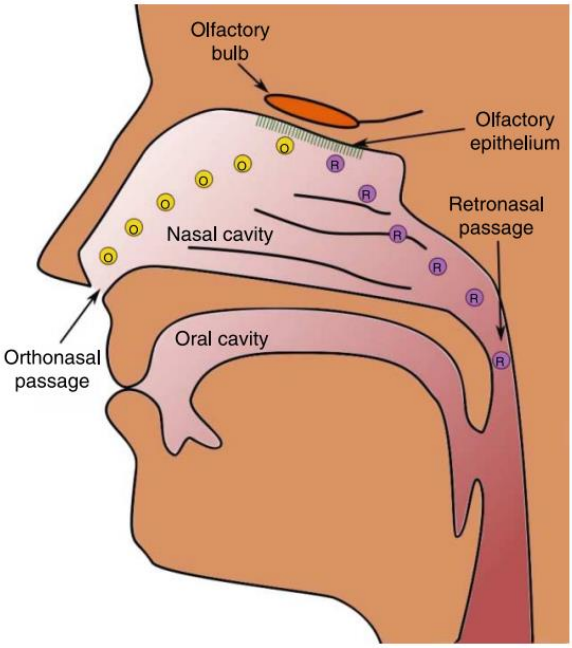

Figure 1.4: Odourants enter the nasal cavity via the orthonasal (O) and retronasal (R) pathways (source: Dietrich, 2009).

The nasal cavity contains olfactory epithelium comprising of olfactory receptor neurons (ORNs) and supporting cells (**Fig 1.5**). Small clusters of ORNs penetrate the cribriform plate, and collectively constitute the cranial nerve I (olfactory nerve). ORNs contain only one type of odour receptor from the GPCR superfamily, and therefore only interact with certain types of molecules (Buck, 2004). ORNs expressing a particular receptor type converge at the same glomerulus in the olfactory bulb (Bear et al., 2016). Odorants dissolved in mucous that coats the olfactory epithelium come into contact with cilia on the ORNs, bind to GPCRs, and activate the transduction process (Bear et al., 2016). Individual receptors may be broadly tuned to a range of chemical stimuli (Buck, 2004). Odours activate large populations of neurons, and the population coding of these responses permits the differentiation between the thousands of aromas that are perceived (Zarzo, 2007). The location of the activated neurons,

and their temporal response, may also influence olfactory perception (Bear et al., 2016). An intracellular chemical cascade occurs, resulting in cell depolarisation, and signal transduction in the brain.

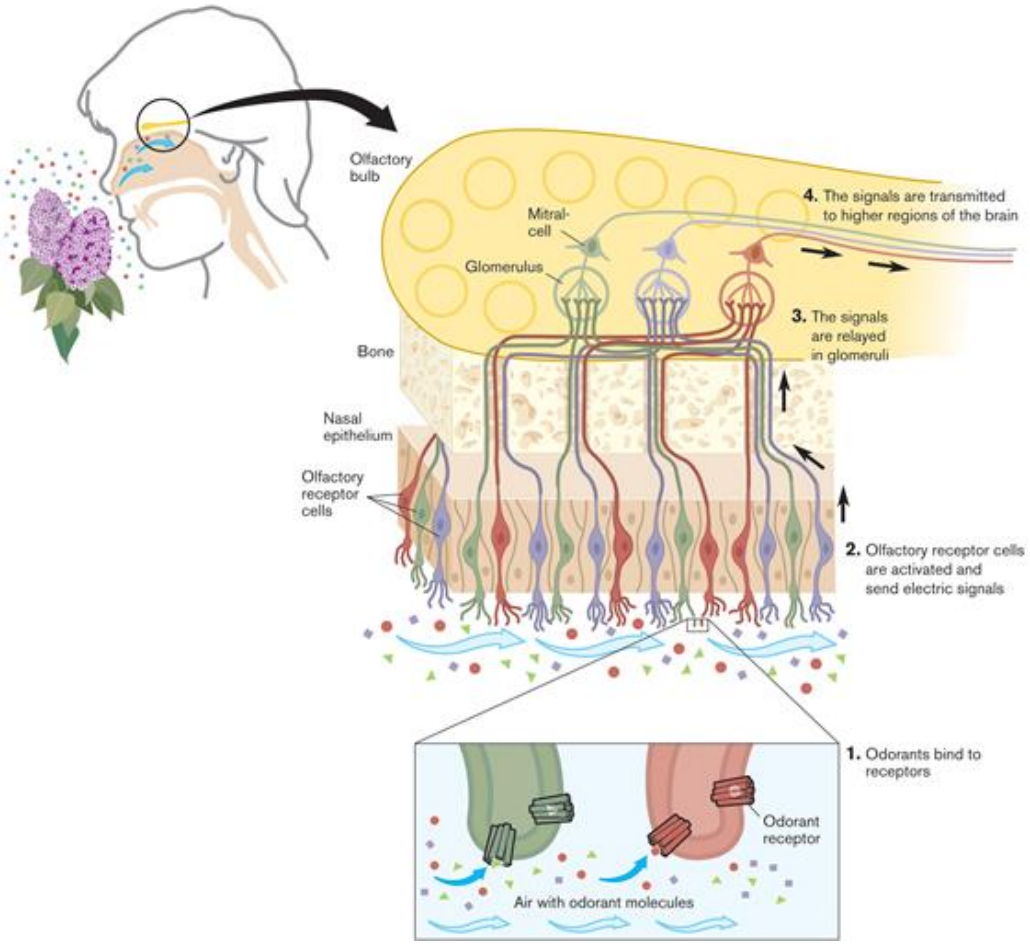

Figure 1.5: Organisation of the peripheral olfactory system (source: Buck and Axel, 2004).

1.1.3 Peripheral trigeminal processing

The somatosensory system differs from the other sensory systems as receptors are located across the body, as opposed to one localised area, and respond to different types of stimuli (Cohen and Taylor, 2005). This system is divided into three key areas, proprioception, interoception and extroception (Bear et al., 2016). Proprioception relates to body position and movement, interoception senses the internal body state - major organ systems - and is not always

consciously perceived, whilst extroception detects stimuli arising from outside of the body, for which touch is the primary sensation.

Oral somatosensory signals provide information about the touch, temperature, and nociception (pain) properties of food, relayed via the trigeminal nerves, therefore termed the trigeminal system (Bear et al., 2016). The range of sensations classified as 'touch' can be divided into somesthesis, kinesthesis and chemesthesis (Kemp et al., 2009). Somesthesis is the perception of tactile sensations and skin feel, for example detected in the lips and oral cavity (Meilgaard et al., 2007). Kinesthesis is perceived via nerve fibres in the muscles, tendons and joints, and relates to proprioception and the mechanical movement of muscles, for example during chewing (Meilgaard et al., 2007). Some chemical stimuli activate trigeminal nerves to elicit sensations such as heat, burning, stinging, cooling, and tingling, termed chemestheisis (Kemp et al., 2009). Chemesthesis is not only perceived in the oral cavity, but volatile compounds also stimulate receptors in the nasal cavity (Cometto-Muniz and Hernandez, 1990). Slow changes in body temperature between 31-36°C are not usually noticed, but innoxious (non-painful) warming and cooling sensations are perceived as temperature moves further away from this range (Ferrandiz-Huertas et al., 2014). A noxious (painful) sensation is perceived when temperature reaches <5°C or >45°C (Gardener and Johnson, 2013). Together, these sensations determine how attributes such as texture, mouthfeel, and chemical irritation are experienced when eating.

Trigeminal receptor types can generally be categorised into mechanoreceptors, thermoreceptors and nocioceptors (Mader, 2010). Mechanoreceptors respond to touch, pressure, vibration and proprioception (Cohen and Taylor, 2005). The simplest form are positioned deep in the tissue, have high thresholds, and are

slow in responding, whilst those located more superficially have low thresholds and are fast responding (Haggard and de Boer, 2014). The anterior tongue is more densely innervated than the posterior, and the middle more innervated than the lateral edges (Haggard and de Boer, 2014). Thermoreceptors are free nerve endings which respond to changes in temperature, with separate receptors for warm and cool stimuli (Gardener and Johnson, 2013). Thermoreceptors on the tongue are more responsive to temperature change than receptors on other areas of the oral cavity (Haggard and de Boer, 2014). Nociceptors are located on free nerve endings, and aim to detect potentially harmful chemical, mechanical, electrical or thermal stimuli (Mader, 2010). Two pathways transmit pain responses to the CNS, one for acute sharp pain, and the second for slow, chronic pain (Cohen and Taylor, 2005). The number and variety of receptors in the oral cavity make it one of the more densely innervated parts of the body (Haggard and de Boer, 2014). Gustatory and filiform papillae are highly innervated by trigeminal nerves, influencing sensitivity to stimuli (Witt and Reutter, 2015).

Trigeminal information from the anterior two thirds of the tongue is transmitted via the trigeminal nerve (cranial nerve V), which splits into three main branches that innervate the face, mouth and tongue on each side of the face (**Fig 1.6**) (Haggard and de Boer, 2014). The opothalamic (V1) and maxillary (V2) are responsible for sensory stumuli reception alone, whilst the mandibular nerve (V3) is responsible for sensory and motor function. V3 controls the motor movement of muscles relating to biting, chewing, mastication and swallowing, and is the main nerve innervating the oral cavity. The largest branch of the V3 is the lingual nerve which innervates the anterior two thirds of the tongue. The facial nerve (CN VII), glossopharangeal nerve (CN IX) and vagus nerve (CN X)

innervate the pharynx and nasal area. When trigeminal receptors are activated, the trigeminal nerves transmit signals to the brain.

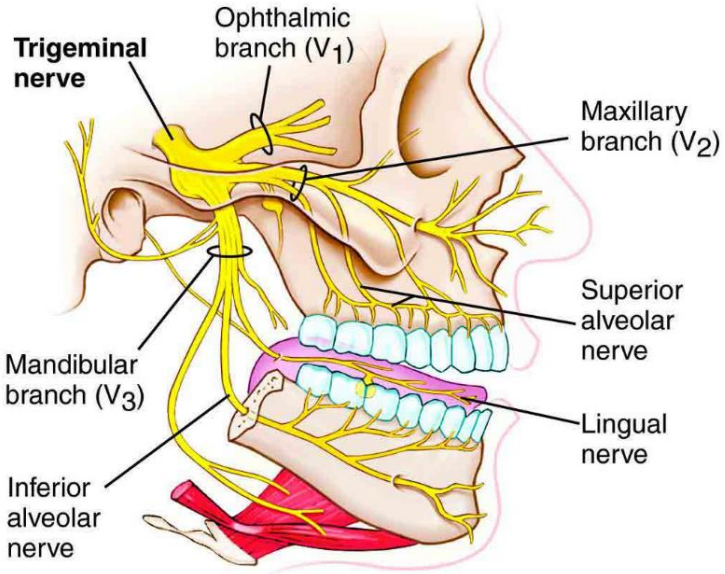

Figure 1.6: Branches of the trigeminal nerve innervating the face and oral cavity (Source: http://www.yourarticlelibrary.com/biology/human-beings/trigeminal-nerve-the-largest-of-all-cranial-nerves-1166-words/9555/).

1.2 VARIATION IN SENSORY PERCEPTION

Multiple factors influence oral sensitivity, including age (Methven et al., 2012), gender (da Silva et al., 2014) ethnicity (Guo and Reed, 2001), body weight (Proserpio et al., 2016), smoking status (Vennemann et al., 2008), health status (Mizuta, 2015) and taste disorders (Naik and Claussen, 2010), and taste phenotype and genotype (Yang, 2015). 6-n-propylthiouracil (PROP), thermal taste, and FPD phenotypes, and TAS2R38 and gustin genotypes will be discussed individually.

1.2.1 PROP taster status (PTS)

The scientist Arthur Fox first noticed individual variation in the ability to perceive the bitterness of phenylthiocarbamide (PTC) in 1931 (Blakeslee and Fox, 1932). He observed that some individuals were unable to detect the chemical (termed

'non-tasters'), whilst others perceived it to varying degrees of bitterness (termed 'tasters'). Due to concerns over the safety of using PTC, it was replaced with PROP, a compound of a similar chemical structure and bitterness. Using PROP, it was later determined that the 'taster' group could be further divided into two sub groups; 'PROP medium tasters' (PMTs) who perceive the bitterness of PROP at a similar intensity to a reference NaCl sample, and 'PROP supertasters' (PSTs) comprising of those individuals who perceived the intensity of PROP at a much higher intensity than the reference NaCl sample (Bartoshuk, 1993, Bartoshuk et al., 1994). The prevalence of phenotypes varies with ethnicity and between 0 and 67% of studied populations have been classified as PNTs (Guo and Reed, 2001). On average, a Caucasian population is thought to comprise of 70% tasters and 30% non-tasters (Blakeslee and Fox, 1932). A modest decline in sensitivity to PROP occurs with age, with children perceiving more bitterness than adults (Guo and Reed, 2001, Tepper et al., 2014). Although no gender differences in PROP taster status (PTS) are observed in children, at and beyond puberty a higher percentage of females are tasters (Guo and Reed, 2001, Monteleone et al., 2017) and supertasters (Bartoshuk et al., 1994). However, some studies have found no effect of gender (Chang et al., 2006, Von Atzingen and Silva, 2012). Interestingly a large study identified a decline in sensitivity to PROP in females with age, whilst no significant difference was observed in males (Monteleone et al., 2017).

PTS is frequently considered a marker for sensitivity to other oral stimuli. PSTs and/or PMTs are frequently observed to report taste, trigeminal (Bajec and Pickering, 2008) and aroma (Yang et al., 2014) stimuli more intensely than PNTs, as well as attributes within a range of products including yoghurt (Prescott et al., 2004), cheese (Hayes et al., 2010), grapefruit (Drewnowski et al., 1997c), soft drinks (Zhao and Tepper, 2007), and coffee (Masi et al., 2015).

Individuals can be categorised according to their hedonic response to sweet stimuli, where a positive correlation is identified between increasing sweet concentration and reported liking by 'sweet likers', whereas 'sweet dislikers' exhibit increasing dislike as the concentration increases (Looy and Weingarten, 1992). Interestingly, PNTs have more frequently been classified as sweet likers, and PSTs as sweet dislikers (Yeomans et al., 2007, Looy and Weingarten, 1992), whereas other researchers identified no difference across PTS groups (Drewnowski et al., 1997c). Due to the potential impact on calorie intake and weight status, the influence of PTS on the perception and consumption of fat has been widely researched. Fat stimuli exhibit complex sensory profiles that are thought to include a combination of trigeminal, aroma, and proposed taste components (Mattes, 2011). Some interesting findings include evidence that tasters perceive more creaminess from dairy fats (Hayes and Duffy, 2007), and are more able to discriminate between varying fat concentrations in salad dressings than non-tasters (Tepper and Nurse, 1997, Tepper and Nurse, 1998). Whilst examples of conflicting evidence found no difference across PTS groups for the ability to discriminate between fat concentrations in a range of products (Yackinous and Guinard, 2001) or the perceived creaminess or fat content of dairy products (Drewnowski et al., 1998). Two studies have explored variation in central processing of oral stimuli across PTS groups. Differences in cortical activation in areas associated with somatosensory and/or taste responses differed between groups in response to sweet-carbonated (Clark, 2011) and fat (Eldeghaidy et al., 2012) stimuli.

Observed differences in oral sensitivity across PTS groups are of interest due to the potential influence on food preference, which in turn affects dietary behaviour, nutrition, weight status, and health outcomes (Tepper, 2008). A review of this literature is beyond the scope of this thesis, although some

examples follow. One hypothesis is that increased bitter sensitivity exhibited by PTs reduces consumption of bitter eliciting products. For example, some evidence indicates that PTs have a lower preference (Drewnowski et al., 1999, Monteleone et al., 2017) and intake (Basson et al., 2005, Bell and Tepper, 2006, Duffy et al., 2010, Dinehart et al., 2006, Yackinous and Guinard, 2002) of cruciferous vegetables such as glucosinolates containing brussel sprouts and cabbage. However, other studies have found no significant differences in intake across groups (Shen et al., 2016, Baranowski et al., 2011). PTs sometimes report a lower preference for grapefruit (Drewnowski et al., 1997c), whilst others have identified no difference across PTS groups (Pasquet et al., 2002). In some cases PTs are observed to perceive more bitterness and irritation from alcohol, and consume less than non-tasters (Duffy et al., 2004a, Duffy et al., 2004b, Intranuovo and Powers, 1998, Lanier et al., 2005), whilst others report no differences across groups (Yackinous and Guinard, 2002, Pasquet et al., 2002). PROP sensitivity has been associated with coffee consumption, where PTs in some cases report lower preference (Drewnowski et al., 1999), a finding that has not been replicated by others (Yackinous and Guinard, 2002, Pasquet et al., 2002, Masi et al., 2015, Ly and Drewnowski, 2001). Although interestingly, a trend is observed for PNTs to consume more black coffee compared to PTs consuming more white coffee (Ly and Drewnowski, 2001). Another study reported PNTs to add significantly more sugar to black coffee than PSTs did (Masi et al., 2015), indicating a potential link with dietary sugar intake and associated health outcomes. For example some researchers report PNTs to have a higher BMI and weight compared to PTs (Tepper et al., 2008), with weight negatively correlating to PROP sensitivity (Tepper and Nurse, 1998), although others report no difference across groups (Yeomans et al., 2007, Yackinous and Guinard, 2002, Von Atzingen and Silva, 2012; Ly et al, 2001). A lower intake of bitter vegetables by PTs has been linked to a higher number of

colonic polyps, an indicator of colorectal cancer (Basson et al., 2005). The higher alcohol consumption reported by PNTs compared to PTS (Duffy et al, 2004a; Duffy et al, 2004b) may have negative health consequences, although other researchers have found no difference in alcohol consumption across PTS groups (O'Brien et al, 2010).

Although many interesting associations have been made between PTS, oral sensitivity, food behaviours, and health outcomes, the degree of conflicting results make it difficult to draw conclusions relating to the true impact of PTS, thus highlighting the need to better understand not only this taste phenotype, but also other influences and phenotypes.

1.2.2 TAS2R38 genotype

Variation in the ability to perceive PROP has a genetic component. TAS2R38 is one of the 25 bitter receptor genes, is found on chromosome 7, and expresses the TAS2R38 receptor that is responsible for detecting the N-C=S group of thiourea-containing compounds, including PTC and PROP (Bufe et al., 2005). Kim et al (2003) identified three single nucleotide polymorphisms (SNPs) on the TAS2R38 gene that result in amino acid substitutions at positions 49 (alanine49proline), 262 (valine262alanine) and 296 (isoleucine296valine), thought to alter the biochemical functioning and ability of the encoded bitter receptor to detect thiourea-containing compounds. This results in two main haplotypes; the dominant allele PAV, the recessive allele AVI (Kim et al., 2003, Boxer and Garneau, 2015), and the rare halotypes AAV, AVV, PAI, PVI, AAI, and PVV (Risso et al., 2016). PAV is associated with the PROP taster variant, and AVI with non-tasting (Kim et al., 2003, Prodi et al., 2004, Bufe et al., 2005, Duffy et al., 2010, Khataan et al., 2009, Calo et al., 2011, Melis et al., 2013b,

Barbarossa et al., 2015, Yang, 2015, Shen et al., 2016, Barajas-Ramirez et al., 2016, Sollai et al., 2017). Prevalence varies across ethnicities, where 46-69% of a population carry the PAV haplotype, only 35-49% have the AVI haplotype, and the unusual PVV haplotype was found exclusively in Europeans, whilst the AAI is predominantly found in Africans (Risso et al., 2016). No differences have been identified across TAS2R38 genotypes for gender (Tepper et al., 2008, Shen et al., 2016), BMI (Timpson et al., 2005, Tepper et al., 2008, Barajas-Ramirez et al., 2016), waist circumference (Tepper et al., 2008, Barajas-Ramirez et al., 2016) or coronary heart disease (Timpson et al., 2005).

Little (Yang, 2015) or no difference (Barajas-Ramirez et al., 2016, Smutzer et al., 2013, Duffy et al., 2010) is observed in sensitivity to aroma, taste or trigeminal stimuli at detection, recognition or supra-threshold intensities across TAS2R38 genotypes. However, PAV homozygotes perceive the bitterness of brassica vegetables (Sandell and Breslin, 2006, Shen et al., 2016) and rocket (Bell et al., 2017) more intensely than AVI homozygotes or heterozygotes. Interestingly, observed liking (Shen et al., 2016) and consumption (Duffy et al., 2010, Sacerdote et al., 2007) of vegetables is reported higher in AVI homozygotes, although this finding has not been replicated in other studies that identified no difference across TAS2R38 genotypes (Timpson et al., 2005, Barajas-Ramirez et al., 2016). Although no significant differences were observed in the perceived intensity of bitterness from alcohol, consumption was significantly higher in AVI homozygotes (Duffy et al., 2004a).

1.2.3 Gustin rs2274333 genotype

Associations have been found between the chemical composition and physical properties of salivary proteins and sensitivity to PROP. One example is the

variance in the composition of bPRP Ps-1 and II2 salivary proteins across groups, thought to influence PROP binding to the receptor (Melis et al., 2013a). A genetic polymorphism (rs2274327) on the gene for gustin (located at chromosome 1) was found to modulate salivary buffer capacity when maintaining oral homeostasis (Peres et al., 2010), and subsequent studies explored associations between gustin polymorphisms and gustatory functioning. The most widely researched salivary protein is the zinc dependant metalloprotein gustin (carbonic anhydrase VI, or CA6) (Henkin et al., 1975). Gustin is thought to act on taste bud stem cells to promote growth and development (Henkin et al., 1999a). The gustin gene polymorphism rs2274333 (A/G) results in substitution of the amino acid at position Serine90Glycine, which is believed to result in structural changes and reduced functionality of gustin (Padiglia et al., 2010). Some researchers have found an association between this genotype and PTS, where the AA genotype is more frequently carried by PTS, the GG genotype by PNTs (Padiglia et al., 2010, Calo et al., 2011, Melis et al., 2013b), whereas others have found no association (Feeney and Hayes, 2014, Bering et al., 2014, Yang, 2015, Barbarossa et al., 2015, Shen et al., 2016, Shen et al., 2017). When exploring the influence of gustin polymorphism on sensory perception, differences in sensitivity to pure tastant or aroma stimuli have not been identified across genotypes (Yang, 2015). When investigating more complex products, the perceived bitterness, liking, or consumption of brassica and non-brassica vegetables does not differ across gustin rs2274333 genotypes (Shen et al., 2016). Significant differences were observed across genotype for mouthfeel, and overall liking of ice cream, however, no differences between any specific SNP pair was identified (Shen et al., 2017).

1.2.4 Fungiform papillae density (FPD)

The TAS2R38 and gustin genotypes do not fully explain differences in oral sensitivity across PTS groups, and FPD is also thought to contribute to the variation. Fungiform papillae are innervated by both gustatory and trigeminal nerves, and house taste buds and trigeminal receptors (Whitehead et al., 1985). FPD is sometimes reported to be higher in females than males (Bartoshuk et al., 1994, Hayes et al., 2008, Duffy et al., 2010, Fischer et al., 2013), whilst other studies have found no difference across genders (Masi et al., 2015, Hayes and Duffy, 2007). FPD modestly declines with age, and is influenced by environmental factors such as smoking and heavy alcohol consumption (Fischer et al., 2013). In some instances FPD has been associated with food preference (e.g. Hayes et al., 2008, Bakke and Vickers, 2011), and a lower FPD has been associated with obesity (Proserpio et al., 2016). Higher FPD is frequently associated with increased sensitivity to:

- PROP (Bajec and Pickering, 2008, Tepper and Nurse, 1997, Yackinous and Guinard, 2001, Tepper and Nurse, 1998, Yackinous and Guinard, 2002, Essick et al., 2003, Miller and Reedy, 1990b, Yeomans et al., 2007, Hayes et al., 2008, Melis et al., 2013b, Sollai et al., 2017, Nachtsheim and Schlich, 2013, Hayes and Duffy, 2007, Hayes et al., 2010, Bartoshuk et al., 1994, Duffy and Bartoshuk, 2000, Duffy et al., 2004a, Duffy et al., 2004b, Duffy et al., 2010, Bakke and Vickers, 2008)
- Gustatory stimuli (Delwiche et al., 2001, Masi et al., 2015, Zhang et al., 2009, Hayes et al., 2008, Hayes et al., 2010, Tepper and Nurse, 1997, Zuniga et al., 1993, Miller and Reedy, 1990b, Proserpio et al., 2016).
- Trigeminal stimuli (Nachtsheim and Schlich, 2013, Prutkin et al., 2000, Duffy et al., 2004b, Essick et al., 2003).

1.2.5 Thermal taster status (TTS)

In 2000, Cruz and Green identified an unusual new taste phenotype (Cruz and Green, 2000). When thermally stimulating the tongue to temperatures ranging between 5-40°C with a temperature thermode, some individuals were found to perceive phantom taste sensations, despite there being no chemical stimulation present. Those perceiving phantom tastes were termed 'thermal tasters' (TTs), whilst those only perceiving temperature were termed 'thermal non-tasters' (TnTs) (Cruz and Green, 2000). A range of prototypical tastes (sweet, sour, salty, bitter, umami) and taste-related sensations (metallic, mint, spicy) are reported during thermal stimulation, and the perceived taste quality and intensity varies across TTs (Yang et al., 2014, Hort et al., 2016). The prevalence of TTs is reported to be from 20% (Bajec and Pickering, 2008) to 50% (Cruz and Green, 2000) of participants. No differences in gender (Bajec and Pickering, 2008, Yang, 2015), BMI, salivary flow rate, or FPD (Bajec and Pickering, 2008) is observed across groups. Thermal taster status is thought to be independent of PTS (Bajec and Pickering, 2008, Yang, 2015). Interestingly, TTs not only perceived phantom taste sensations, but they are also observed to perceive a range of taste and oral trigeminal stimuli (Bajec and Pickering, 2008, Yang, 2014, Green and George, 2004) more intensely than TnTs. Evidence for aroma is contradictory, as one study reported that TTs perceived olfactory stimuli more intensely than TnTs (Green and George, 2004), whilst a second study identified no significant difference across groups (Yang et al., 2014). It is possible that the sometimes heightened response observed in TTs is due to a multimodal interaction between taste and aroma at the central level. These differences in oronasal sensitivity are thought to affect the perception of food and beverage, which may impact on dietary behaviours and health outcomes. In some cases, questionnaires have been administered to explore differences across thermal taster status groups. No significant difference across phenotypes were identified

in food neophobia scores (Bajec and Pickering, 2010, Yang, 2015), whilst TTs had significantly higher food involvement scores than TnTs for some questions (Yang, 2015). Interestingly, TTs were observed to have lower preference ratings for a range of foods classified within the 'mushy food group'. Therefore, differing trigeminal sensitivity across phenotypes was hypothesised to explain the differences across groups (Bajec and Pickering, 2010). TTs rated certain attributes of wine (Pickering et al., 2010b) and beer (Pickering et al., 2010a) more intensely than TnTs, but this did not translate into a significant difference in preference across the groups. Two recent studies further explored the influence of thermal taster status on the perception of a wide range of complex food products, and found few significant differences in perceived attribute intensity or preference when comparing ratings between thermal taster status groups. TTs rated the perceived bitterness of cranberry juice to be more intense than TnTs, and had a higher preference for foods classified within the 'grainy' (Pickering et al., 2016) and 'creamy' (Pickering and Klodnicki, 2016) food groups, supporting previous findings indicating differences in trigeminal sensitivity may influence the observed differences between groups. Two recent fMRI studies have detailed differences in cortical activation between TTs and TnTs when delivering gustatory-trigeminal stimuli to the oral cavity (Yang et al., 2015, Hort et al., 2016).

1.2.6 Measuring variation in oral perception

A variety of different measurement scales can be used when recording the response to a stimuli, and the appropriate type is dependent on the test objectives. Accurately deciding upon oral sensitivity measures to detect differences across people is both challenging and subjective. Detection or recognition level thresholds were a popular measure to compare differences across people (Doty, 2015). However, these measures do not always give

sufficient detail on the range of sensory function, or indicate sensitivity to suprathreshold stimuli (Yang et al., 2014). Suprathreshold intensity ratings give a more accurate measure of overall taste sensitivity, and advances in measurement techniques have increased the popularity and use of suprathreshold testing in gustatory research (Doty, 2015). Individual perceptions differ, but direct comparisons across people are invalid when using most sensory scales. The oral labelled magnitude scale (LAM) was designed to identify and compare differences in oral sensitivity across assessors (Green et al., 1993). Based on the previously developed Borg scale (Borg, 1982), it comprises of a vertical line with unequal quasi-logarithmic spacing between adverb and adjective word descriptors; 'no sensation', 'barely detectable', 'weak', 'moderate', 'strong', 'very strong' and 'strongest imaginable oral sensation', placed at 0, 1.4, 6, 17, 35, 53 and 100% of the scale respectively. The upper limit of 'strongest imaginable oral sensation' and other word descriptors are assumed to mean the same thing across individuals. However, as understanding on variation in taste sensitivity developed, overall oral sensitivity was found to vary across people, making this assumption incorrect (Bartoshuk et al., 2002). For example, whilst a sour stimuli may be rated as 'strong' in intensity by a number of assessors, their perceptual experience of what constitutes 'strong' may differ. Using an upper anchor point that is unrelated to the modality being tested resolves this problem. The general labelled magnitude scale (gLMS) is not limited to oral sensations, and instead uses the upper anchor point of 'strongest imaginable sensation of any kind' (Bartoshuk et al., 2002). This scale is thought to make valid across group comparisons when exploring variation in oral sensitivity across PTS groups (Bartoshuk et al., 2004), and has since been used to identify differences across thermal taster status groups (Green et al., 2005).

1.3 CENTRAL SENSORY PROCESSING

1.3.1 Nervous system signal transduction

The nervous system has two major divisions, the peripheral nervous system (PNS) and central nervous system (CNS). The PNS comprises the spinal and cranial nerves conveying messages between the CNS and other areas of the body, and the CNS comprises the brain and spinal cord (Nolte, 2009). The nervous system contains nerve cells (neurons) and glial cells (Nicholls et al., 2001). Glial cells are not directly involved in information processing, but are in high abundance and support neuronal functioning. Neurons contain a cell body (soma), axon, and dendrites, and convey electro-chemical signals (action potentials). The soma contains the nucleus and organelles involved in cell functioning. Axons conduct action potentials to other neurons (efferent), and are typically covered with a myelin sheath to speed up signal transmission. Dendrites receive signals from sensory receptors or other neurons (afferent).

Intracellular and extracellular ion distributions determine the neurons membrane potential, at rest the inside is more negatively charged than the outside, (-65 mV). When the cell is not conducting an impulse the sodium-potassium pump maintains unequal distribution of ions by the transport of sodium (Na^+) and potassium (K^+) in/out of the cell. An appropriate stimulus opens Na^+ channels, resulting in an influx of Na^+ into the cell, thus depolarising to +40 mV, and an action potential to spread across the membrane. The cell repolarises to -65 mV by opening K^+ channels and allowing K^+ to exit the cell.

Action potentials are transferred between neurons by synapses occurring at specialised junctions on the neuron (Squire et al., 2013). Most are chemical

synapses, where the terminal of a presynaptic axon is located adjacent to the postsynaptic membrane of another neurons dendrite or soma, creating a small gap termed the synaptic cleft. The presynaptic neuron releases neurotransmitters that act on receptors on the postsynaptic nerve. Less frequently a more rapid electrical synapse occurs when an electrical signal is transferred directly between the membranes of pre synaptic and postsynaptic nerves (Bear et al., 2016).

1.3.2 Anatomy of the brain

The central nervous system can be divided into white matter made up of axons, where the lipid component of the myelination gives the white colour, and grey matter containing unmyelinated dendrites and somas. All functions of the body are controlled by the brain, including thoughts, emotions, motor movements, and responses to sensory stimuli (Bear et al., 2016). It comprises of four main areas; the cerebrum, cerebellum, brainstem, and diencephalon. The cerebrum is the largest part of the brain containing the cerebral cortex which contains a left and right hemisphere (Squire et al., 2013). The cerebral cortex is divided into four lobes, termed the frontal, occipital, parietal and temporal lobes (**Fig 1.7**). The anterior frontal lobe involves functions such as written and spoken language, and executive functions relating to personality, insight and foresight, whilst the posterior frontal lobe contains the motor cortex, which controls movement (Nolte, 2009). The parietal lobe contains the somatosensory cortex and processes sensations such as tactile and proprioceptive information, is involved in language comprehension, and directing attention (Nolte, 2009). The temporal lobe is involved in distinguishing sound, smell, and complex aspects of learning and memory, whilst functioning of the occipital lobe almost exclusively functions to process visual information (Nolte, 2009).

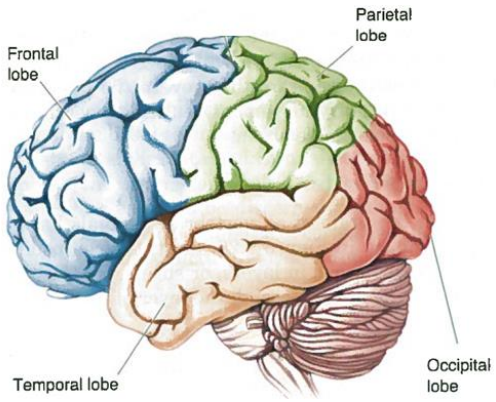

Figure 1.7: Location of the four lobes of the cerebrum (source: Bear et al., 2016).

The primary function of the cerebellum is to coordinate movements and balance (Nicholls et al., 2001). The brainstem is divided into midbrain, pons and medulla oblongata, and it relays neural messages from the cerebral cortex and cerebellum to the spinal cord and vice versa (Bear et al., 2016). Regulation of survival mechanisms such as breathing, sleep, arousal, and the control of food intake are controlled here (Squire et al., 2013). The diencephalon contains the thalamus and hypothalamus (Bear et al., 2016). Most sensory inputs are processed through the thalamus before being directed to their relevant cortical areas (Jones, 2001). The hypothalamus is involved in many processes, including regulating the autonomic nervous system, hormones, and regulating food and liquid intake (Rajmohan and Mohandas, 2007, Kullmann et al., 2014).

Processing of the senses occurs at different spatial locations in the cerebrum, spanning across the two hemispheres (**Fig 1.8**). The sensory cortex includes the somatosensory cortex located on the post central gyrus of the parietal lobe, the visual cortex on the occipital lobe, the auditory cortex on the temporal lobe, the primary olfactory cortex on the piriform area of the temporal lobe, and the primary gustatory cortex on the anterior insula. The limbic system includes the thalamus, hypothalamus, cingulate gyrus, amygdala, hippocampus, and basal ganglia (Rajmohan and Mohandas, 2007), and has been proposed as a system

that processes and regulates emotions, including those relating to eating behaviour (Rajmohan and Mohandas, 2007).

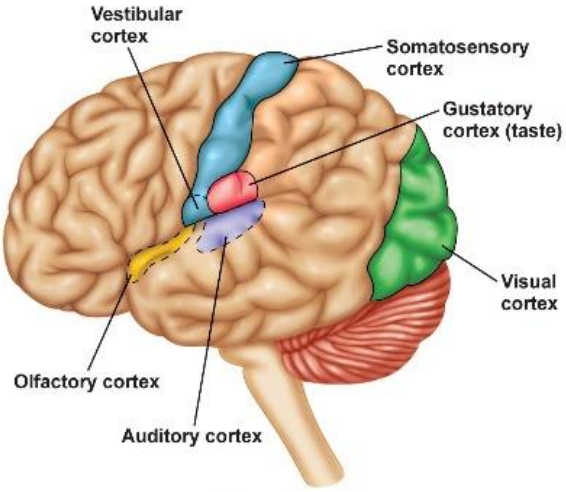

Figure 1.8: Location of the somatosensory, visual, auditory, olfactory and gustatory cortex (Source: https://fuzzyscience.wikispaces.com/Somatosensory+Cortex).

1.3.3 Central gustatory processing

A number of studies explore gustatory processing in the human brain, and have been the focus of various reviews and meta-analyses (Small et al., 1999, Faurion et al., 2005, Verhagen and Engelen, 2006, Kurth et al., 2010, Veldhuizen et al., 2011, Small, 2012, Rolls, 2016a, Yeung et al., 2017). A gustatory response is initiated when tastants dissolved in saliva activate taste receptors in the oral cavity, as discussed in Section 1.1.1. Taste signals are sent via the facial, glossapharangeal and vagus cranial nerves, and converge in the rostral nucleus of solitary tract (Verhagen and Engelen, 2006, Small, 2012, Rolls, 2016a). Projections are directed via the ventral-posterior-medial thalamus (VPM) to the anterior insula and/or adjoining frontal operculum (Small et al., 1999, Faurion et al., 2005, Verhagen and Engelen, 2006, Kurth et al., 2010, Veldhuizen et al., 2011, Small, 2012, Rolls, 2016a, Yeung et al., 2017). Further projections are frequently transmitted to the orbitofrontal cortex (OFC) (Small et al., 1999, Faurion et al., 2005, Verhagen and Engelen, 2006, Veldhuizen et al.,

2011, Small, 2012, Rolls, 2016a), mid insula (Kurth et al., 2010, Veldhuizen et al., 2011, Small, 2012, Rolls, 2016a, Yeung et al., 2017), thalamus, and post central gyrus (Veldhuizen et al., 2011, Yeung et al., 2017). In some cases activation is projected to the rolandic operculum, posterior insula, anterior cingulate cortex (ACC) (Veldhuizen et al., 2011, Rolls, 2016a), amygdala (Faurion et al., 2005, Small, 2012, Rolls, 2016a, Yeung et al., 2017), hippocampus and caudate (Yeung et al., 2017). Primary functions of the key areas in gustatory processing will be discussed individually.

As well as acting as a relay centre for gustatory inputs entering the CNS (Veldhuizen et al., 2011, Small, 2012, Yeung et al., 2017), the thalamus has also been implicated in being responsible for the attention to taste (Veldhuizen et al., 2007) and increased activation during hunger compared to satiety (Haase et al., 2009b), indicating involvement in eating behaviours modulated by reward.

The anterior insula (AI) and frontal operculum are thought to be the primary gustatory cortex (PGC), frequently associated with taste identification and intensity (Small et al., 1999, Faurion et al., 2005, Veldhuizen et al., 2011, Rolls, 2016a). However, involvement of the AI in the pleasant and aversive qualities of taste, and the mid insula in intensity perception has also been proposed (Small et al., 2003, Small, 2010). The AI is activated when attempting to detect taste in a tasteless solution, indicating involvement in attention to taste (Veldhuizen et al., 2007). The AI, mid insula, and thalamus are frequently activated alongside other brain regions, suggesting functional networks are involved with the integration between taste and other contexts, such as attentiveness and emotional response (Yeung et al., 2017).

The orbitofrontal cortex is thought to be the secondary gustatory cortex (SGC) (Small et al., 1999, Verhagen and Engelen, 2006,, Veldhuizen et al., 2011, Rolls, 2016a) involved in the hedonic response to taste, as activation is linearly correlated with pleasantness of taste (Grabenhorst and Rolls, 2008), and associated with changes (O'Doherty et al., 2001) and anticipation of pleasantness (de Araujo et al., 2003b). Activation is reduced when fed to satiety (Kringelbach et al., 2003, Haase et al, 2009b), and pleasant ratings decrease. OFC is likely involved in predicting taste quality and evaluation of stimuli, as it is activated when participants are uncertain about the taste quality being delivered (Veldhuizen et al., 2007). It is also an integration area with other sensory inputs (de Araujo et al., 2003, Rolls and Grabenhorst, 2008).

The amygdala is typically associated with the pleasant/aversive aspect of taste (O'Doherty et al., 2001, Zald et al., 2002), but also intensity perception (Small et al., 2003). Activation is higher when hungry relative to satiated (Haase et al., 2009b), dietary intake of artificial sweetener can influence sucrose related activation (Rudenga and Small, 2012), and a greater response is observed in the anticipation of taste reward compared to receiving the reward (O'Doherty et al., 2002). These findings indicate the response is modulated by both physiological satiety, and processing the expectation of food and reward. The ACC is also responsive to aversive and pleasant taste (de Araujo and Rolls, 2004, Grabenhorst and Rolls, 2008, Grabenhorst et al., 2008). It is often co-activated with the OFC, and thought to act together to control goal directed behaviours relating to pleasantness (Kringelbach, 2005).

The association with the hippocampus and caudate are less frequently discussed in taste literature compared to other brain regions. The hippocampus is activated in response to taste (Gautier et al., 1999) and is decreased when

satiated compared to hungry (Haase et al., 2009b), thus it is hypothesised to be involved in the motivation to eat, and termination of eating behaviour (Haase et al., 2009b). Taste also activates the caudate (Cerf-Ducastel et al., 2012), a response negatively correlated to BMI and waist circumference (O'Doherty et al., 2006). Interestingly both regions have been implicated in the response to food (Pelchat et al., 2004) and drug craving (Breiter et al., 1997), indicating involvement in food reward and eating behaviours.

1.3.4 Central olfactory processing

When olfactory receptors are activated, the signal is transmitted via the olfactory nerve to the olfactory bulb, and projected directly to the primary olfactory cortex (consisting of the piriform cortex, anterior cortical amygdaloid nucleus, periamygdaloid cortex, anterior olfactory nucleus and olfactory tubercle), involved in the perception and discrimination of odour (Marciani et al., 2010). This is unique, as all other sensory inputs pass through the thalamus before projecting to the cerebral cortex. From here projections are made to multiple brain regions, including the hypothalamus and amygdala of the limbic system, where the emotional response, learning and behaviour related to aroma is controlled (Marciani et al., 2010).

1.3.5 Central trigeminal processing

Figure 1.9 details the trigeminal nerve fibres which pass from the periphery to the trigeminal ganglion, onto the trigeminal nuclei in the brainstem, before being directed to the thalamus and onto the primary somatosensory cortex (SI), located behind the central sulcus in the lateral post central gyrus, and secondary somatosensory cortex (SII) in the parietal operculum (Haggard and de Boer, 2014). Further projections to areas associated with somatosensory processing

include the OFC, ACC, insula and the primary motor cortex (Marciani et al., 2010).

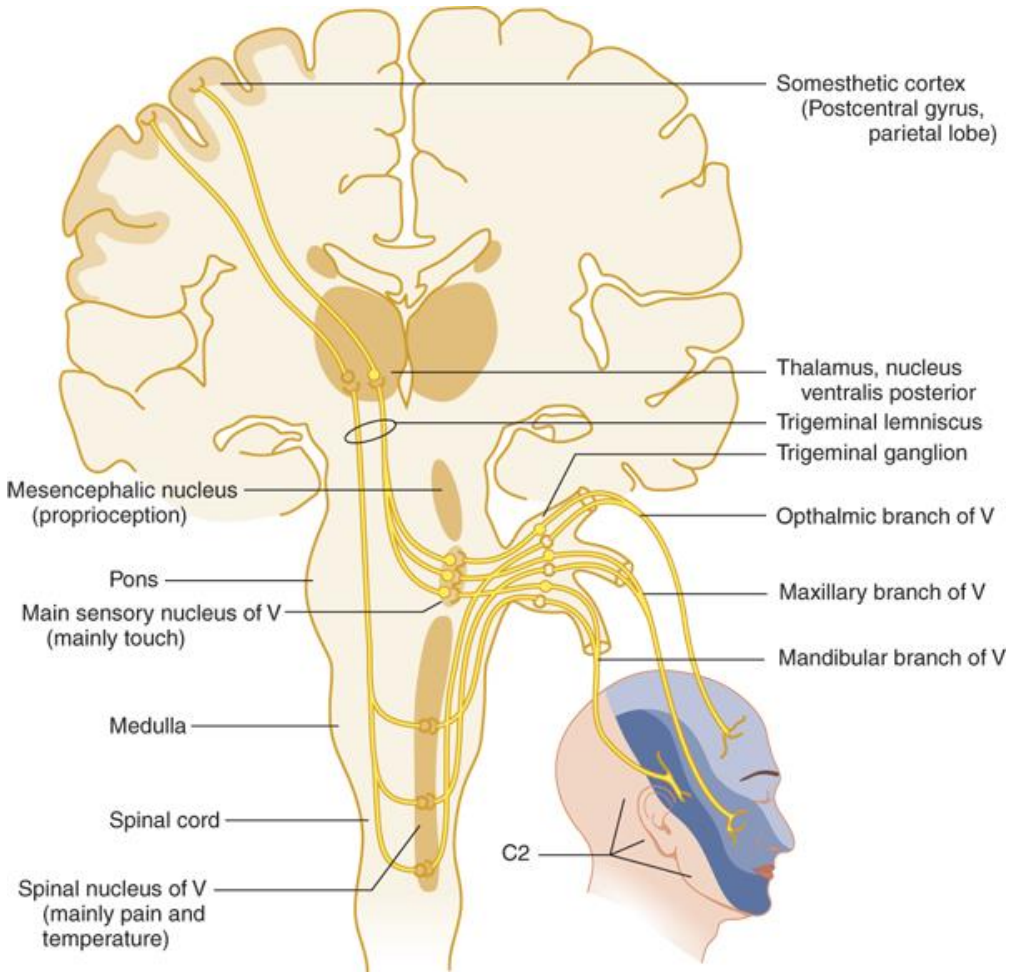

Figure 1.9: Trigeminal pathway from the peripheral trigeminal nerve to the primary somatosensory cortex (source: https://neurology.mhmedical.com/content.aspx?bookid=1043&sectionid=59094689&jumpsectionID=59096257).

A somatotopic map of the body pictorially represented by the sensory homunculus (**Fig 1.10**) has been identified in SI (Penfield and Boldrey, 1937). The size of the body part correlates with the density of sensory input from that area. The oral cavity, located at the posterior of SI (Tamura et al., 2008), can be subdivided into the lips, tongue (Nakahara et al., 2004) and teeth (Miyamoto et al., 2006).

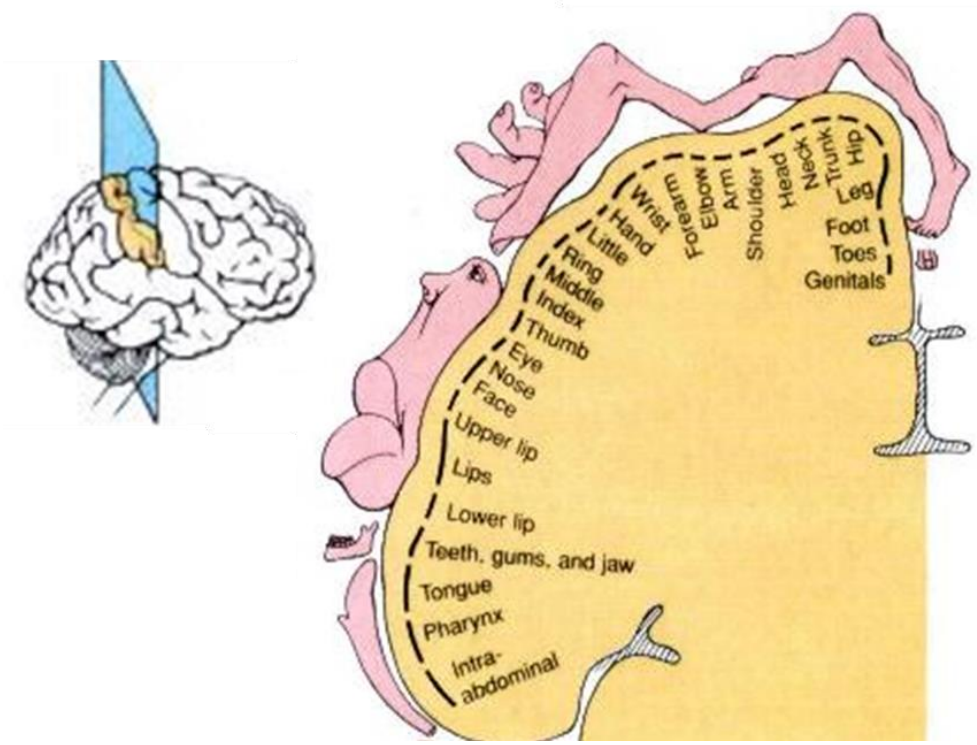

Figure 1.10: Sensory homunculus indicating a somatotopic map of the body represented in the primary somatosensory cortex (source: http://www.harmonicresolution.com/Sensory%20Homunculus.htm).

The cortical response to some oral trigeminal stimuli, including texture, temperature and pain, has been explored. The viscosity of carboxymethyl cellulose activates the mid and anterior insula, OFC, and perigenual cingulate cortex (de Araujo and Rolls, 2004). The viscosity of algenate also activates the anterior insula, but unlike carboxymethyl cellulose, activation is also observed in the rolandic operculum/parietal operculum and post central gyrus/somatosensory cortex, but not the OFC (Alonso et al., 2007). The OFC is typically considered an area where inputs from different sensory modalities converge (de Araujo et al., 2003, Rolls and Grabenhorst, 2008), therefore the complexity of a given stimulus may determine the extent of activation in this area. Understanding the cortical response to oral fat is of interest to determine what drives dietary consumption (Eldeghaidy et al., 2011). Iso-viscous, iso-sweet fat emulsions (Eldeghaidy et al., 2011) and fat emulsions (Eldeghaidy et al., 2012) are represented in the anterior insula, frontal operculum, ACC,

amygdala and SII, and are positively correlated with increasing fat concentration (Eldeghaidy et al., 2011). The pleasantness of oral fat is represented in the ACC and OFC, whilst high-fat stimuli activate the hypothalamus and amygdala significantly more compared to low-fat stimuli (Grabenhorst et al., 2010).

Limited evidence is currently available to identify the oral response to temperature in humans. Guest et al (2007) identified that the anterior insula, somatosensory cortex, OFC, ACC, and the ventral stratium were activated in response to thermal stimulation, whereas the OFC and pegenual cingulate cortex areas were thought to correlate to the pleasantness of the stimuli. Activation in response to oral pain (elicited by electrical stimulation) has consistently been observed in the thalamus, insula and cingulate cortices (Lin et al., 2014). However, these studies focused only on stimulating pain in the tooth pulp, relating to dental pain, and the findings may not translate to other oral stimuli. Compared to the vast number of studies conducted on the cortical response to taste and flavour, there is limited understanding of the complex oral trigeminal response.

1.4 NEUROIMAGING TECHNIQUES

Until relatively recently brain function has been poorly understood. The development of neuroimaging techniques since the early 1900's has played a pivotal role in progressing knowledge in this area, and bridging the gap between stimulus delivery and the perceived response. Neuroimaging identifies areas of brain activation associated with a particular stimulus, and maps areas related to mental functioning. A range of functional imaging techniques are available, including positron emission topography (PET), electroencephalography (EEG), magnetencephalography (MEG), and functional magnetic resonance imaging

(fMRI). The benefits and limitations of each technique should be considered when selecting an appropriate method to meet study objectives.

Discovered in the 1920's, EEG was the first brain imaging technique used. Electrical signals termed 'event related potentials' are associated with neuronal activity, and are detected by electrodes placed on the scalp (Puce and Hamalainen, 2017). MEG was discovered in the 1960's, magnetic fields generated by neuronal activity are measured by detectors positioned around the head (Cohen, 1968). EEG and MEG are non-invasive, achieve direct measures of neuronal activity, and have good temporal resolution, whilst a major limitation is the relatively poor spatial resolution achieved (Puce and Hamalainen, 2017). In contrast, PET and fMRI indirectly measure neuronal activity by measuring how much oxygen (PET and fMRI) or glucose (PET) are used during metabolism associated with neuronal activity (Huettel and Song, 2009). PET was discovered in the 1970's (Phelps et al., 1975). A radioactive labelled tracer is injected into the bloodstream, and changes in the brain relating to glucose metabolism or blood flow associated with neuronal activity can be identified. The drawbacks of PET include the invasive tracer, dangers associated with exposure to radiation, and the relatively low spatial and temporal resolution achieved (Bear et al., 2016). The use of PET has declined since the birth of fMRI, first discovered on the rodent brain in the 1980's (Ogawa et al., 1990), and soon after translated to the human brain (Bandettini et al., 1992, Kwong et al., 1992). Being non-invasive, and having no detrimental side effects, it has become the dominant technique in cognitive neuroscience, and by 2013 over 13,000 fMRI studies had been published (Passingham et al, 2013). fMRI indirectly measures neuronal activity using a method called Blood Oxygenation Level Dependent (BOLD) contrast which detects changes in the

haemodynamic response arising from changes in blood flow, blood volume and oxygen consumption associated with neuronal activation (Kornak et al., 2011). Acquired images have good spatial resolution, whilst a relatively poor temporal resolution is achieved as compared to EEG and MEG (Sturzbecher and de Araujo, 2012). When measuring brain activation associated with gustatory processing (Yeung et al., 2017) and other oral stimuli such as temperature (Guest et al., 2007), fMRI is a popular choice.

1.4.1 The basic principles of MRI

Magnetic resonance imaging (MRI) is used to obtain detailed anatomical images, whereas fMRI collects functional images of the brain to identify areas activated in response to a sensory and cognitive processes.

1.4.1.1 The MR scanner

The primary components of a magnetic resonance (MR) scanner include a superconducting electromagnet, three gradient coils, a radiofrequency (RF) coil, shimming coils, and the patient table, which is positioned inside the scanner bore during data acquisition (**Fig 1.11**).

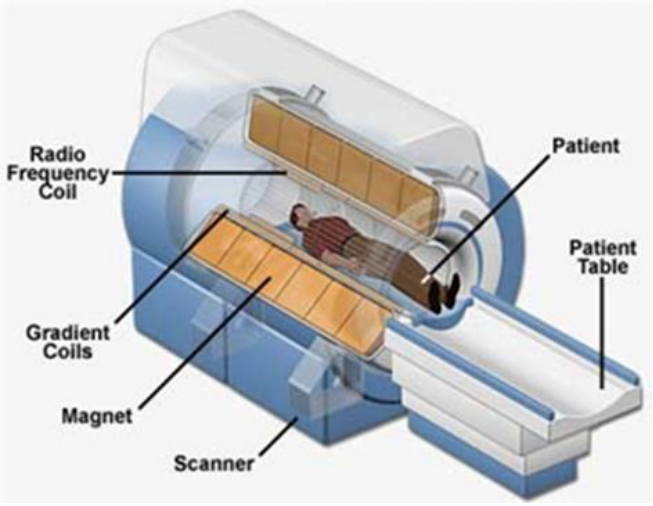

Figure 1.11: The key components of an MR scanner (source: https://www.medventura.com/healthaffairs/mri-scanner-buying-guide/).

The superconducting electromagnet creates a strong static magnetic field, most frequently of the order of 0.5, 1.5, 3 or 7 Tesla, and remains on at all times. Work in this thesis is collected on both the 3 and 7 Tesla scanner. The radio frequency (RF) coil sends and receives RF pulses of a specified electromagnetic energy during scanning, and the received signals form the basis of images produced. RF coils come in different shapes and sizes in order to be placed near the area being imaged to optimise signal detection. Three magnet field gradients are used to resolve the spatial location of the MR signal using slice selection, frequency encoding and phase encoding. Ideally, the static magnetic field would be homogeneous, and the magnetic field gradient perfectly linear, although in reality this is not usually the case. Therefore, shimming coils are used to control for field inhomogeneity across the system, and to control for additional field distortion created when placing an individual in the scanner. Unlike the static magnetic field, the shimming, gradient and RF magnetic fields are not on all the time, but turned on at specific time points termed the 'pulse sequences' to control the signal generated and image formation. The study objectives and desired image type determine the pulse sequence used.

1.4.1.2 MR sensitive nuclei

The characteristics of MR sensitive nuclei can be manipulated inside the scanner to generate the signal used to form images. However, only nuclei with mass, charge, spin and a magnetic moment are suitable for MR techniques. Atoms contain three types of particles; protons and neutrons which form the atomic nucleus, and electrons which orbit the nucleus. Nuclei with an odd mass number (the number of protons and neutrons) or atomic number (number of protons) cannot distribute electrical charge or mass evenly, and therefore possess spin which makes them sensitive to MR techniques. In contrast, those

with an even atomic number and mass do not possess spin, making them insensitive to MR methods. Important properties are angular momentum (J), a vector that describes the amount and direction of the angular motion of the nuclei, and the gyromagnetic ratio (γ) describing the ratio between the charge and mass of the nuclei. Together these characteristics give rise to a magnetic moment (μ). Only nuclei which contain both a magnetic moment and angular momentum are MR sensitive, and are said to possess nuclear magnetic resonance properties. Hydrogen nuclei (^{1}H proton) are MR sensitive, and most MRI methods use ^{1}H to generate images due to the high natural abundance of it, the body is made up of 70% water (H_2O), and its large gyromagnetic ratio (42.58 MHz/Tesla) resulting a large MR signal. But many molecules, such as ^{13}C, ^{19}F, ^{23}Na, ^{31}P, ^{39}K, ^{2}H, are also suitable for MRI.

1.4.1.3 Magnetisation of a spin system

An individual atomic nuclei is termed a ‘spin’. It is not possible to measure the magnetisation of an individual spin, instead the net magnetisation from all atomic nuclei in the sample is measured, termed a spin system or spins. In the absence of a strong magnetic field, spins are randomly distributed, causing the magnetic moments to cancel one another out (**Fig 1.12a**). When placed in the static magnetic field (B_0) of the MR scanner, spins align with B_0 in a parallel (low energy) or antiparallel (high energy) state, resulting in net magnetisation (M_0) parallel to B_0 (**Fig 1.12b**).

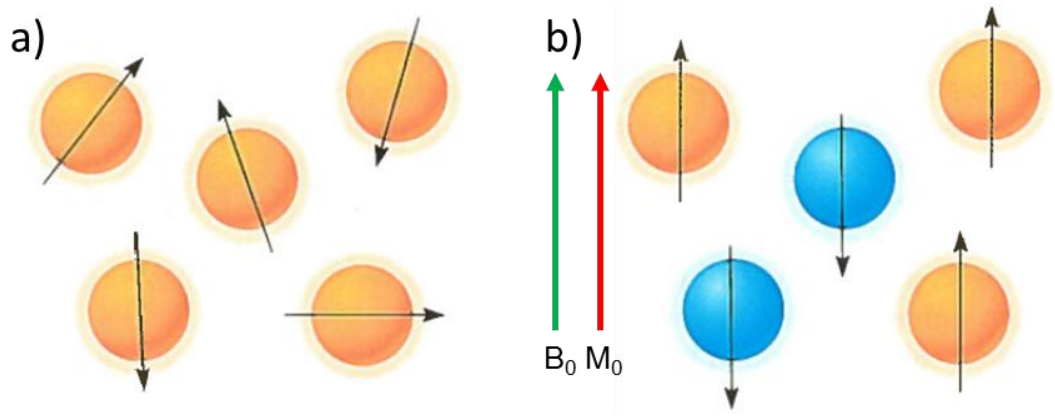

Figure 1.2: a) In the absence of a strong magnetic field spins randomly alligned b) In the static magnetic field (B_0) net magnetisation (M_0) is paralell to B_0 (adapted from: Huettel and Song, 2009).

Due to the magnetic moment and angular momentum, the nuclei spin on their own axis, as well as precessing about B_0 (**Fig 1.13a**). ^1H has a positive gyromagnetic ratio, causing it to precess in a clockwise direction. The precession frequency is dependent on the nuclei characteristics, and is calculated using the Larmor frequency (ω_L) determined by γ and B_0 ($\omega_L = \gamma B_o$). Spins precess at this precessional frequency, but are out of phase (or coherence) with one another (**Fig 1.13b**).

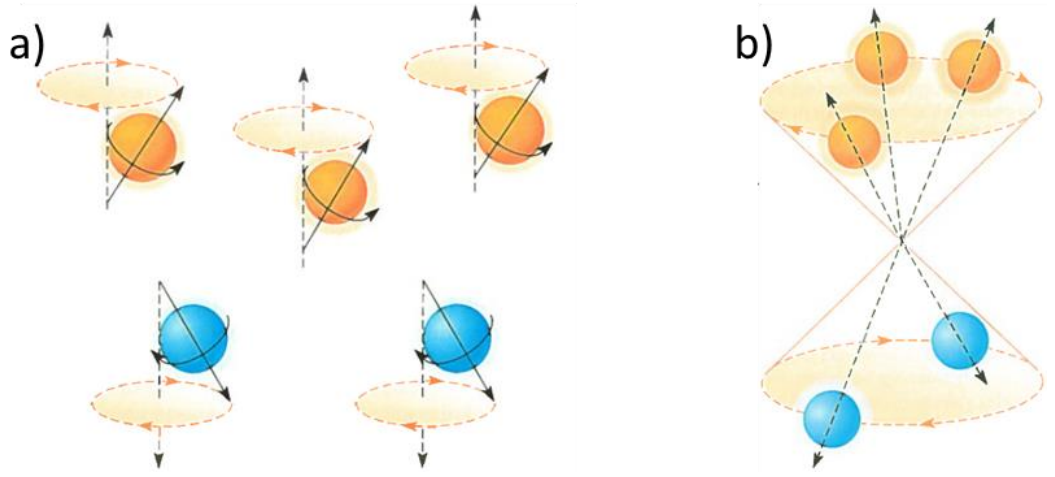

Figure 1.13: When placed in the static magnetic field, spins a) spin on their own axis, and precess about B_0 b) precess about B_0 at the same frequency but out of phase with one another (adapted from: Huettel and Song, 2009).

Temperature and static magnetic field strength determine the distribution of spins across spin states, as they are not equally distributed. If the temperature is held at 0°C all of the spins will take the low energy spin up state when placed in B_0, resulting in M_0 parallel with B_0. Temperature control is useful for non-biological samples, but for obvious reasons cannot be used when imaging humans. At temperatures suitable for imaging biological samples, more spins are in the low energy state, with M_0 parallel to B_0, termed longitudinal magnetisation. However, M_0 is relatively small and cannot be detected relative to the magnitude of main magnetic field, B_0.

1.4.1.4 Excitation of the spin system and MR signal generated

When an RF pulse of energy equal to the energy difference between the two states is applied, spins can transition from the low energy spin up state, to the high energy spin down state. The RF pulse delivers electromagnetic energy that oscillates at the resonant frequency (ω_L) of the targeted spins. This process is termed excitation, and has two effects. Firstly, spins in the low energy state parallel to B_0 absorb energy and flip into the high energy state antiparallel to B_0, secondly the spins precess in phase. Applying a 90° RF pulse delivers the precise amount of energy to equalise the number of spins in each state, thus cancelling out longitudinal magnetisation in the z plane parallel to B_0, and since the spins precess in phase, this results in a net magnetisation in the x, y plane perpendicular to B_0, termed the transverse magnetisation (**Fig 1.14**). Transverse magnetisation is not stationary, but oscillates at ω_L about B_0, and is the basis of the MR signal detected. The in-phase precession of spins in the x, y plane generates an electrical current oscillating at ω_L, which is detected by a RF receiver tuned to the same resonant frequency. Because the RF energy is

both delivered and received at ω_L, the same RF coil is used for both transmission and reception.

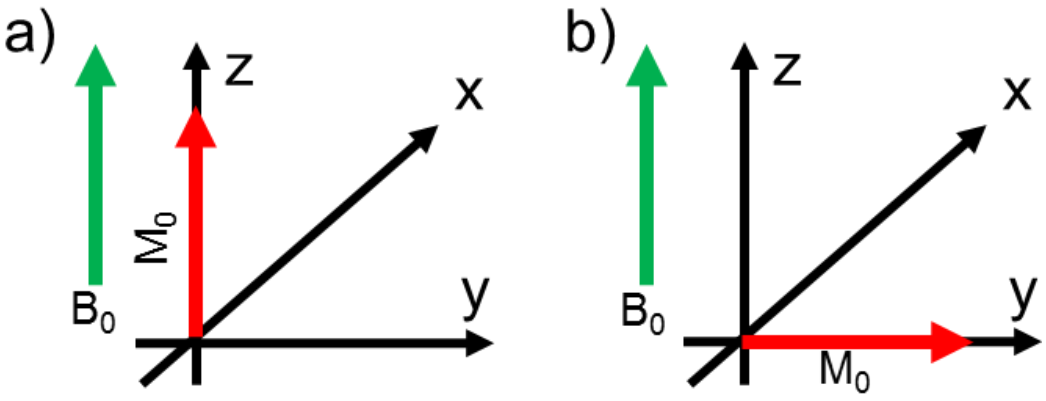

Figure 1.14: a) Longitudinal magnetisation in the z plane b) Transverse magnetisation knocked into the x, y plane after an RF excitation pulse is delivered.

1.4.1.5 Relaxation of the spin system

Following the RF pulses, the signal does not remain stable for long as the longitudinal magnetisation recovers, termed longitudinal relaxation, and transverse magnetisation decays, termed transverse relaxation. Longitudinal magnetisation recovers when spins that were in the spin down state return to their initial spin up state. The absorbed energy is released into the surrounding lattice, also termed spin-lattice relaxation, influencing the strength of signal detected. T_1 is the longitudinal relaxation time constant, typically in the order of seconds (**Fig 1.15a**). T_1 influences the timing of the excitation pulses in a MR pulse sequence, as spins must have recovered to equilibrium before another is delivered or the effects of incomplete recovery taken into account.

The strength of transverse magnetisation depends on the coherence of spins, and is strongest when they are precessing in phase at the same frequency. Spins interact with one another after excitation, resulting in out of phase precession and a decay in the transverse magnetisation, termed spin-spin

relaxation. Transverse relaxation is determined by the time constant T_2, usually shorter than T_1 and of the order of milliseconds (**Fig 1.15b**). In addition to the spin-spin interactions, field inhomogeneity influences spin precession and so dephasing. The time constant related to these combined factors is termed T_2*. The transverse relaxation time governs the time during which the MR signal can be detected.

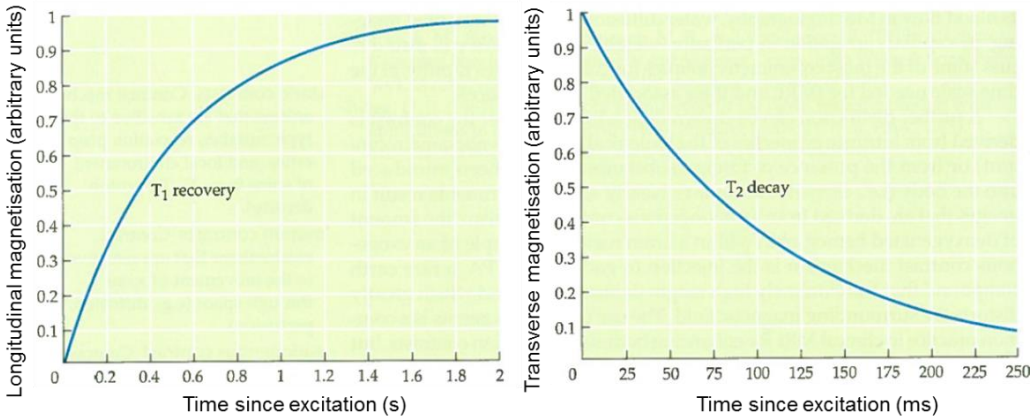

Figure 1.15: a) T_1 relaxation and the recovery of longitudinal magnetisation b) T_2 relaxation and the decay of transverse magnetisation (Source: Huettel and Song, 2009).

1.4.1.6 Image contrast

Tissue types exhibit different relaxation times thus causing variation in the signal strength generated, which defines the contrast in MR images. For example, a ^{1}H spin attached to a water molecule acts differently to a ^{1}H spin as part of a free fatty acid or carbohydrate molecule. Repetition time (TR) is how frequently the excitation RF pulse is applied, and echo time (TE) is the time after the excitation pulse at which the MR signal is detected. Different TR and TE are applied to define the time point of excitation signal detection, as defined by the tissue properties. This can result in a T_1 weighted or T_2 weighted image to determine the tissue contrast of interest. For example, a long TE and long TR gives a T_2-weighted image, frequently used to identify pathology as diseased

tissue frequently have a higher water content. Short TR and short TE gives a T_1-weighted image, typically used to produce detailed anatomical images.

1.4.1.7 Image formation

3D brain images are formed from acquiring and combining a number of 2D slices. Each slice is divided into voxels, and the signal corresponding to each voxel recorded. The spatial location of the signal across the sample must be isolated, and is achieved by applying field gradients to alter the magnetic field across the x, y and z planes. This causes the frequency and phase of spins to differ across the sample in a controlled way using slice selection, frequency and phase encoding. A sequence of alternating magnetic field gradients and RF pulses, known as a pulse sequence, is used to acquire signal across the sample. The slice selection gradient (e.g. z plane) alters the frequency of spins across the sample. Slice selection is achieved by exciting only those spins within the slice which are on-resonance with the RF pulse. The spatial location of the signal across voxels within the slice are isolated by applying magnetic gradients along the remaining planes (e.g. x and y planes). The phase and frequency encoding gradients are applied a number of times during image acquisition to manipulate the phase and frequency of the spins across the slice, further isolating the spins on resonance with the RF receiver, allowing the signal to be measured for each voxel. The signal is processed from digital to analogue, and held in k-space. A mathematical algorithm, termed a Fourier transform, converts k-space data into image data, this is termed image reconstruction.

The process described so far details the collection of highly detailed anatomical images that are time consuming to acquire as a number of RF pulses are applied to acquire a single image. In contrast, functional MRI (fMRI) measures

the brain functioning over time by acquiring large numbers of images in rapid succession.

1.4.2 The basic principles of functional MRI (fMRI)

fMRI measures brain function using BOLD contrast to detect changes in the cerebral haemodynamic response associated with neuronal activation. During an fMRI scan a series of BOLD images are collected to cover the whole brain, termed a brain volume. BOLD images are acquired in very quick succession, with a whole brain volume acquired every one-three seconds. In order to increase statistical power, hundreds of brain volumes are collected during delivery of multiple replicates of a stimulus. A specific pulse sequence termed 'echo-planar imaging' is used to acquire images at this speed, with the signal for all voxels within an image being acquired after just one excitation pulse. The resulting images generally have reasonably coarse spatial resolution, and are designed to identify changes in brain function related to a task or stimulus.

1.4.2.1 Blood Oxygen Level Dependent (BOLD) contrast

Haemoglobin is an iron-containing molecule which transports oxygen in the blood. One haemoglobin molecule can carry up to four oxygen molecules. Haemoglobin-carrying oxygen is termed oxyhaemoglobin, whilst those red blood cells without oxygen are termed deoxyhaemoglobin. Oxyhaemoglobin is diamagnetic, and has similar magnetic properties to the surrounding tissue. Deoxyhaemoglobin is paramagnetic, with differing magnetic properties to the surrounding tissue. The differences in magnetic susceptibility across blood oxygenation level influences T_2* properties, and determines the BOLD signal contrast. Deoxyhaemoglobin induces field inhomogeneity, increasing spin dephasing, resulting in a short T_2* decay time compared to oxyhaemoglobin,

which has a slower T_2* decay time. When a stimulus activates a receptor at the peripheral level, neurons transmit a signal to the brain, and a relatively localised area is activated. The metabolic demand of this activated area increases, requiring more oxygen and glucose from the blood. In order to meet these demands, a localised haemodynamic response occurs, involving blood vessel dilation, and an increase in cerebral blood volume (CBV) and cerebral blood flow (CBF) (**Fig 1.17**). The increase in blood flow overcompensates the oxygen required, resulting in activated areas of the brain containing increased oxyhaemoglobin levels. fMRI uses T2*-weighted imaging to highlight these increases in signal between resting and activated brain regions.

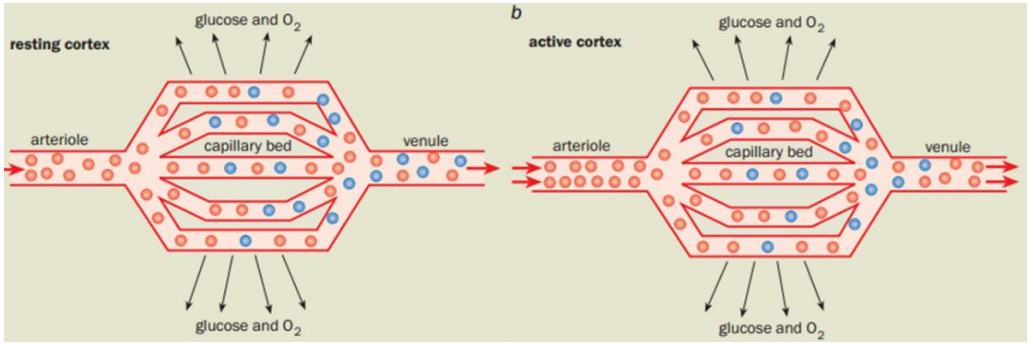

Figure 1.17: a) Haemodynamic response at rest b) change in haemodynamic response during cortical activation (Gowland et al., 2002).

1.4.2.2 Haemodynamic Response Function (HRF)

The BOLD signal time course associated with neuronal activity has three clearly defined features, and is termed the haemodynamic response function (HRF) (**Fig 1.8**). The initial dip occurs before blood flow to the activated area is sufficiently increased, and can last one-two seconds. The positive BOLD signal is the largest signal change and peaks after approximately six seconds, the time taken for blood flow and therefore blood oxygenation level to increase. Oxygenation supply exceeds demand, resulting in an increased blood oxygenation level. Activated areas with increased oxyhaemoglobin levels have slower spin dephasing, and a longer T2* decay time than areas not activated by

the stimulus. The final stage is the post-stimulus undershoot which is thought to occur due to stretching of venous vessels, increasing the amount of deoxygenated blood causing a reduction in the BOLD signal.

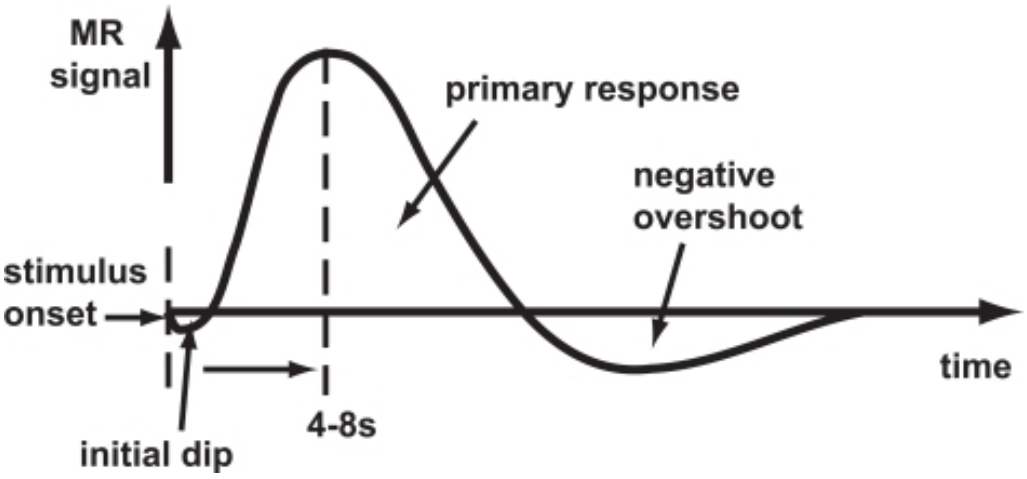

Figure 1.8: Schematic illustration of the typical BOLD haemodynamic response function (HRF), showing the initial dip, primary response, and negative overshoot – also termed the post-stimulus undershoot (source: Kornak et al., 2011).

1.4.2.3 fMRI data analysis

fMRI data is analysis is extensive and contains multiple stages which can be broken down into pre-processing and statistical analysis steps, as summarised in **Figure 1.19**. The final aim is to generate 'statistical parametric maps' (SPM's) identifying those areas of the brain significantly activated in response to a stimulus. The coarse resolution T_2* weighted BOLD images are designed to identify changes in brain function, but not provide detailed anatomical evaluation, therefore SPMs are subsequently overlaid onto high resolution T_1-weighted anatomical images.

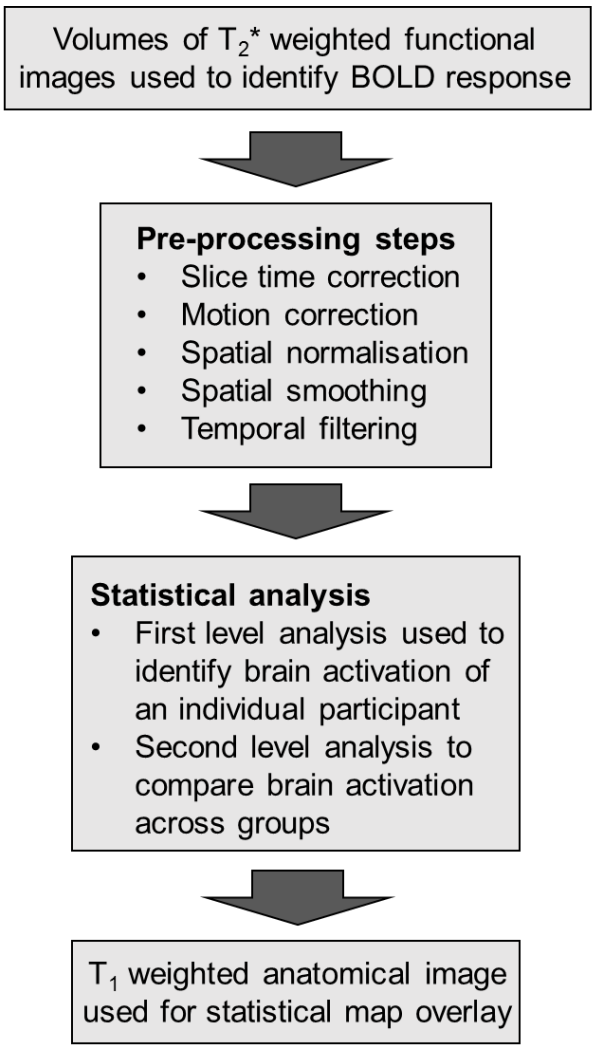

Figure 1.19: Summary of MR image processing undertaken when analysing fMRI data.

The purpose of fMRI pre-processing is twofold; firstly, to improve the signal (BOLD response) to noise (underlying variance from artefacts such as participant movement) ratio. Secondly, it aims to ensure that the data meets the assumptions required for statistical analysis. Pre-processing has a number of stages; slice time correction, motion correction, spatial normalisation, spatial smoothing, and temporal filtering, detailed in **Table 1.1**.

Table 1.1. Individual stages and descriptions of the pre-processing steps of fMRI data analysis (information sourced from Marciani et al., 2010).

Pre-processing step	Description
Slice time correction	Slices within a brain volume are typically collected over a period of two-three seconds, therefore each slice is acquired at a slightly different time. Slice time correction shifts the time series to a reference slice to account for this variance and the time series for each slice will appear as if it were acquired at the same time
Motion correction	Some degree of head movement during scanning is inevitable, and the likelihood increased with taste studies where participants are required to swallow stimuli. This can result in a change in voxel location during image acquisition. Data where movement in excess of one voxel has occurred should be eliminated. However, motion correction can realign images to a reference image to account for smaller movements. Typically rigid-body realignment is undertaken across 6 parameters (x, y, z, roll, pitch and yaw)
Spatial normalisation	When assessing brain activation across a group of individuals, the data must be combined across participants. The size and shape of each brain is variable, and must be standardised before comparisons can be made. Spatial normalisation transforms each brain into a standard template, one widely used template is the Montreal Neurological Institute template, as used in this thesis.
Spatial smoothing	Spatial smoothing is applied to account for the anatomical variation across participants. It convolves each voxel with a 3D Gausian kernel, typically two-three times the voxel size used. This reduces the resolution of the image, but can improve the signal to noise ratio and improve statistical power.
Temporal filtering	Temporal filter is applied to remove frequencies within the signal that are of no interest, such as physiological and scanner noise. As with spatial smoothing, this can improve the signal-to-noise ratio.

Statistical analysis identifies those brain areas activated in response to a stimulus. This can be broken down into first level analysis conducted to determine a statistical parametric map (SPM) on data from an individual subject, and second level or group analysis to make comparisons across groups. Second level analysis pools together individual SPMs to form a group map identifying overlapping areas significantly activated across the group.

A common approach to statistical analysis is the general linear model (GLM), where the expected BOLD response is linearly modelled by convolving the HRF with the stimulus waveform. The study variables, and covariates of no interest, such as the motion parameters, are included in the data matrix. Parametric statistical analysis is conducted to obtain a T-statistic or F-statistic determining if each voxel is significantly activated. The value is converted to represent Z values, and uncorrected probability scores. Due to the number of statistical tests conducted on each voxel, adjustments are made to control for false positives occurring due to multiple comparisons (Type I error). A colour scale is then used to represent those areas significantly activated, and this is superimposed over a detailed anatomical image or template. Second level analysis can be conducted on a number of individual SPMs (typically 15-20) to produce a group activation map.

1.5 THESIS AIMS AND OBJECTIVES

Individual variation in oral sensitivity is known to influence perception of oral stimuli, which in some cases has been found to affect food preference and food choice. Although evidence for variation in perceptual responses is growing, very few studies have measured variation in brain activation across taste phenotype and genotype. Therefore, the overall aim of this research is to bridge the gap between peripheral and central oral processing when exploring oral sensitivity to gustatory and trigeminal (temperature) stimuli across PROP, thermal taste, and FPD phenotypes, and TAS2R38 and Gustin genotypes. Specific hypotheses are detailed in the relevant chapters, individual objectives for each chapter follow:

- The focus of the data presented in Chapter 2 was the development of tastant stimuli to be delivered to participants during the fMRI sessions

detailed in Chapter 4, and to inform the experimental design of the fMRI session. This included collecting a range of perceptual responses to gustatory stimuli from both a sensory panel, and sensory assessors who were representative of the general population.

- Gustatory metallic sensations are complex in nature, and not well understood. The experiments detailed in Chapter 3 used sensory evaluation and headspace analysis to better understand how olfactory, trigeminal and gustatory aspects of divalent salts interact to elicit the overall metallic sensation perceived. Sensory assessors who were representative of the general population were used to collect the perceptual response to stimuli, and solid phase microextraction (SPME) and gas chromatography–mass spectrometry (GC–MS) were used to explore whether any volatiles were present in the sample headspace.
- Individual variation in taste sensitivity was explored in the experiments detailed in Chapter 4. PTS, TTS, FPD phenotype, and TAS2R38 and Gustin rs2274333 genotype were determined for 30 participants. The perceptual response to tastant stimuli was collected during sensory evaluation sessions, and the cortical response measured using fMRI. Differences across groups were compared.
- Chapter 5 explored temperature responses across TTs and TnTs. The perceptual phantom taste response was first measured across 36 TTs. The perceptual and cortical response to temperature was then measured in 12 TTs compared to 12 TnTs.
- The key findings of the thesis are discussed in Chapter 6, conclusions detailed, and recommendations for future work suggested.

2 DEVELOPMENT OF GUSTATORY STIMULI

2.1 INTRODUCTION

The focus of the experiments detailed in this chapter were to develop a set of tastant stimuli to be used to measure the perceptual and brain response to taste during sensory evaluation and functional Magnetic Resonance Imaging (fMRI) sessions, as detailed in Chapter 4.

2.1.1 Measuring taste response in an MR scanner

The MR scan environment introduces limitations to tastant delivery methods, as the participant is supine and head movement must be minimised (Marciani et al., 2006), limiting the volume of tastant delivered, and a large number of replicates of tastants are required for the stimulus effect on brain activation to reach statistical power (Cerf-Ducastel and Murphy, 2004). The method of stimulus delivery has been shown to influence the perceived intensity (Haase et al., 2009b, Meiselman, 1971). Therefore, a number of considerations must be made when developing appropriate stimuli and delivery protocols for taste experiments in the MR environment.

2.1.2 Considerations for taste experiments in an MR scanner

In order to compare perceptual and brain responses across both taste qualities and assessors in Chapter 4, it was important to develop a set of tastants of approximately the same intensity. To have sufficient power to map cortical responses, stimuli were also required, on average, to be perceived between moderate and strong intensity. This also ensured that taste qualities were easily differentiated. Variation in taste sensitivity is widely evidenced, therefore the challenge was to develop stimuli that were suprathreshold for those with low taste sensitivity, whilst also being palatable for more sensitive individuals. Due

to individual variation it was unlikely that stimuli would be rated as ‘equi-intense’ within each assessor without adjusting the sample concentration at an individual level. Therefore, an approach to identify tastants that were a ‘best fit’ between moderate and strong intensity was adopted when developing the set of stimuli.

Cleansing the palate before and after sample delivery aims to allow sensory receptors to recover between assessments, preventing adaptation (Kemp et al., 2009), and re-establishing the baseline environment to reduce or eliminate residuals effecting subsequent sample perception (Lucak and Delwiche, 2009). Inadequate palate recovery results in carryover and interaction effects between samples. Depending on the order of presentation, this can result in the following effects: enhancement (one sample increasing perceived intensity of the subsequent sample), suppression (one sample decreasing perceived intensity of the subsequent sample), or synergy (total perceived intensity of the sum of two compounds are greater than each individual part) (Kemp et al., 2009). Additionally, palate cleansers themselves exhibit sensory qualities which can influence sample perception (Vickers et al., 2008), and so an appropriate type should be selected for the stimuli being tested. Therefore, a range of palate cleansers may be used, including water, which is commonly used for a wide range of products, whilst samples with more lingering qualities may need something which targets its particular characteristics. For example, apple is effective for greasy foods such as chocolate, whilst the lingering that is experienced after drinking tea can be reduced using melon (Kemp et al, 2009). Again, the MR scan environment restricts palate cleanse options to those that can be delivered as a liquid, typically sprayed into the mouth. Those commonly used are water (Haase et al., 2009a) or tasteless ‘mock saliva’ (O'Doherty et al., 2001), whilst the carryover of more problematic samples such as lipids can be tackled using a lime wash in conjunction with a water wash (Eldeghaidy et

al., 2011). Water has been found to activate neurons in the insula and orbitofrontal taste cortices in primates (Yaxley et al., 1990; Rolls et al., 1990) and areas associated with taste processing in humans (de Araujo et al., 2003a). This is likely due to taste qualities such as bitter and metallic which are often associated with deionised water (Dalton et al., 2000). A tasteless 'mock saliva' solution containing the key ionic components of saliva has therefore become a popular palate cleanser and control stimulus in fMRI taste studies (Francis et al., 1999; O'Doherty et al., 2001). During sensory evaluation sessions, assessors can be permitted unlimited palate cleanser, or alternatively palate cleansing can be standardised in a way that allows enough time for adequate recovery before testing the subsequent sample. Restrictions in the MR scan environment prevent this flexibility as a large number of replicates must be delivered for the stimulus effect on the brain to meet statistical power (Cerf-Ducastel and Murphy, 2004), and they must be delivered in reasonably quick succession to prevent participants remaining in the scanner for prolonged periods of time. Therefore, when designing an fMRI experiment, careful consideration should be given to the type of palate cleanser used, the sample presentation order, and the minimum recovery time that is permitted between both replicates and taste qualities in order to minimise palate fatigue and interaction effects between samples.

2.1.3 Stimuli delivery methods

The way in which a tastant is delivered can affect the perceived intensity (Haase et al., 2009a, Meiselman, 1971). Sensory evaluation typically adopts sip and expectorate (or swallow), and dorsal flow techniques. Sipping stimulates the whole mouth and more closely replicates natural food and beverage consumption, whilst the dorsal flow method prevents stimulation of the whole

mouth, and limits saliva from interacting with the sample which affects receptor binding (Meiselman, 1971).

Here, sample delivery protocols are adapted to accommodate limitations posed by the MR scan environment where current methods typically involve spraying samples into the mouth using an automated pump system during scanning (Marciani et al., 2006). Haase et al (2009a) measured perceived intensity when syringing tastants onto the tongue to replicate the delivery method inside an MR scanner, and compared the reported intensity to values recorded in literature for both sip and spit, and dorsal flow delivery. Syringing samples onto the tongue yielded lower intensity ratings compared to the other delivery methods. However, this difference may have been influenced by the reduced sample volume (0.3 ml) that was delivered, compared to the 5-20 ml typically administered during sensory evaluation protocols. A greater volume of stimuli should also be delivered during fMRI in order to cover a wider range of oral receptors, and more closely replicate natural consumption behaviour *(*Marciani et al*.,* 2006*),* potentially reducing the difference in perceived intensity across delivery methods. Stimuli presentation order should be balanced and randomised to account for order effects (ISO-6658, 2005). Long breaks are typically given during sensory evaluation to allow the palate to recover and reduce psychological fatigue. In contrast, breaks given during fMRI testing are limited in order to reduce the duration that a participant is inside the scanner. This can cause restrictions in palate cleansing protocols and palate recovery as samples are delivered in a relatively quick succession, and should be considered when designing the sample delivery paradigm.

The position of the body when a stimulus is delivered can influence perception, and is of increasing interest due to the rise in neuroimaging research requiring

participants to be in a supine position during data collection. Body position has been found to influence auditory (Fukai et al., 2005) and visual (Marendaz et al., 1993; Mast et al., 2003) perception. In contrast, whilst taste and trigeminal thresholds were not altered by participants being supine compared to sitting (Vickers et al., 2001), the evidence for aroma is less consistent. Sensitivity to perithreshold aromas delivered orthonasally can be reduced when supine compared to seated (Lundstrom et al., 2006; Lundstrom et al., 2008), whilst body position has no significant effect on the ability to discriminate between flavours involving retronasal aroma delivery (Hort et al., 2008). Participants are able to replicate natural eating or drinking behaviour when tasting stimuli in a seated position during sensory evaluation protocols, whereas during fMRI experiments they are always supine. Possible influences of this on sample perception should therefore be considered.

2.1.4 Using a sensory panel to develop tastant stimuli

A sensory panel was used to quantify tastant intensities during stimuli development. Sensory panels are routinely used to evaluate samples during product development and quality control, as well as to categorise sample attributes and differences. A descriptive panel can be used to identify the characteristics of a sample to provide complex attribute profiles, whilst a difference panel is used to measure the overall or attribute differences between samples (Meilgaard et al., 2007). Panellists may be internal, external, or a combination of both, and the advantages and disadvantages of each should be evaluated in comparison to the panel aims when deciding which is used (Kemp et al., 2009). Validity of the results depends upon appropriately selected panellists meeting the study aims (Stone et al., 2012), where general requirements are willingness and motivation to develop sensory skills (Kemp et

al., 2009). The number of panellists required depends upon the panel aims and the types of statistical tests required. Selection typically involves pre-screening, which aims to determine basic inclusion criteria such as availability and health status, and one or more rounds of screening sessions where more thorough assessments are required. The types of tests selected, and the materials assessed, are guided by the panel aims, and should be the same or similar to those being assessed with the recruited panel. Tests can be divided into those aimed to determine sensory impairment, sensory acuity, and ability to describe and communicate sensory perceptions (ISO-8586, 2014). Screening can include tests to identify taste disorders such as dysgeusia (taste distortion), hypogeusia (reduced taste sensitivity) and ageusia (complete lack of ability to detect taste) (Naik and Claussen, 2010). Whilst prevalence of ageusia is thought to be low, five percent of the population are estimated to experience hypogeusia (Welge-Lussen et al., 2011), indicating the importance of measuring sensitivity during screening procedures. Panellists recruited onto a sensory panel are selected according to specific criteria, and will often have higher sensory acuity than that which is typical of the general population. Therefore, it was also necessary here to test the developed tastant samples on a wider range of individuals with varied taste sensitivities, and who were representative of the general population.

2.1.5 Aims

The overall aim of this study was to develop tastant stimuli to be delivered to participants during the fMRI sessions detailed in Chapter 4, and to inform the experimental design of the fMRI session. The study objectives for this chapter were to:

- Recruit and train a sensory panel that would be used to develop tastant stimuli, and participate in additional experiments that would inform stimuli delivery for fMRI studies.
- Use the sensory panel to develop tastant stimuli that were of approximately the same intensity between moderate and strong on the general labelled magnitude scale (gLMS). Due to individual variation in taste sensitivity it was hypothesised that perceived intensity would be variable. An approach to identify tastants that were a 'best fit' between moderate and strong intensity was therefore adopted.
- Measure perceived intensity of the developed tastant samples using 20 assessors who were representative of the general population. More variable tastant intensity ratings were expected here than with the sensory panel.
- Use the sensory panel to measure palate recovery time for each of the developed tastant stimuli, to guide fMRI delivery protocols. Taste persistence was expected to differ across tastants.
- Use the sensory panel to compare perceived tastant intensity across two sample delivery methods. It was hypothesised that perceived intensity may be lower when tastants were sprayed into the mouth when supine, compared to sipping stimuli when seated.

2.2 MATERIALS AND METHODS

An initial cohort of 40 volunteers including students and staff from the University of Nottingham were screened for recruitment onto a sensory panel, the minimum number of participants recommended to screen in order to obtain ten panellists (ISO-8586, 2014). An internal panel was recruited due to the need for panellists to attend for only a short duration (60 min) per session. The study had

ethics approval from the University of Nottingham Medical Ethics Committee (C13032014), and all participants gave informed consent to take part.

2.2.1 Tastant stimuli

Tastants (**Table 2.1)** were selected to represent the five prototypical tastes (sweet, sour, salt, bitter, umami) and the purported taste 'metallic' (Bartoshuk, 1978). Samples were prepared using deionised water, which was also used for all palate cleansing. Stimuli were delivered at a range of concentrations across experiments, as indicated in each section.

Table 2.1: Taste quality, chemical and source.

Taste	**Chemical**	**Source**
Sweet	D-glucose anhydrous (GLC)	Thermo Fischer Scientific, USA
Sour	Citric acid (CA)	Sigma Aldrich, UK
Salt	Sodium chloride (NaCl)	Sigma Aldrich, UK
Bitter	Quinine sulphate (QS)	Acros Chemicals, USA
Umami	Sodium L-glutamate monohydrate (MSG)	MERCK chemicals, Germany
Metallic	Iron sulphate heptahydrate II ($FeSO_4$)	Sigma Aldrich, UK
Water	Deionised water	University of Nottingham, UK

Prototypical tastants were prepared ≤ 24 hours in advance, stored at 5 ± 1°C, and adjusted to room temperature (22 ± 2 °C) before assessment. $FeSO_4$ samples were prepared fresh every three hours to prevent oxidation (Lim and Lawless, 2005b).

2.2.2 General methods

Unless otherwise stated, samples were presented in odour-free plastic cups (Fischer Scientific, UK) with removable lids. Participants were instructed not to wear strong smelling toiletries, or to consume anything but water for at least one

hour before testing commenced (ISO-8586, 2014). A standardised testing approach was adopted, as the way in which the assessor interacts with both the sample and test procedure adds a source of variation (Meilgaard et al., 2007). Specifically, participants were trained to hold samples and palate cleansers in the mouth and lift the tongue to the palate three times in order to coat the oral receptors before swallowing.

2.2.3 Sensory panel recruitment

2.2.3.1 Pre-screening

A recruitment email requiring respondents to complete a basic health questionnaire (appendix 1) and detail their availability was sent to staff and students at the University of Nottingham. Those individuals who reported food allergies to the stimuli being tested, were pregnant, a smoker, or had health conditions/taking medication which may affect their sensory perception were excluded. Of the 40 people who returned the paperwork, 32 met the requirements and agreed to attend the screening session.

2.2.3.2 Screening

Appropriate screening tests vary depending on the aims of the panel. Requirements here were the ability to detect the tastants, accurately rate using a sensory scale, and discriminate between samples. In order to make comparisons in performance across candidates, samples were presented in the same order across individuals.

2.2.3.3 Screening Session 1

A total of thirty candidates attended the first screening session (30 min) which involved two tests. The first test assessed ability to accurately rate on a sensory

scale as this would be important during development of the tastants. This was assessed using a series of ten geometric shapes with different percentages shaded. Participants were instructed to rate the proportion of each shape that was shaded on a horizontal continuous line scale, and those rating within 10-20% of the correct range across all responses passed (Meilgaard et al., 2007). It was important that panellists did not have a taste disorder that would prevent them from perceiving the tastants. Therefore, the second test measured taste acuity using the tastants identified in **Table 2.2**, and the identification of taste test method described by ISO standards (ISO-8586, 2014, ISO-3972, 2011).

Table 2.2: Tastant concentrations (ISO-3972, 2011) used for the identification of taste test. *Recognition threshold concentrations following Nakamura et al (2008) when chemicals differed from those suggested by ISO.

Taste	**ISO sample (g/L)**	**Sample used in current study (g/L)**
Sweet	Sucrose (5.76)	Glucose (17.7)*
Sour	Citric acid (0.28)	Citric acid (0.43)*
Salt	Sodium chloride (1.19)	Sodium chloride (1.19)
Bitter	Caffeine (0.2)	Quinine (0.00783)*
Umami	Monosodium glutamate (0.3)	Monosodium glutamate (0.29)
Metallic	Iron sulphate (0.0036)	Iron sulphate (0.0036)

Candidates were first familiarised with one replicate of known tastant samples (sweet, sour, salt, bitter, umami, metallic) (15 ml). Unknown tastant and water control samples were then presented with random three digit codes (two replicates). Information that each sample may contain one of the taste qualities introduced in the familiarisation task, or may be ‘tasteless’ (i.e. water) was delivered. Candidates were instructed to identify the taste perceived for each sample, or indicate ‘tasteless’ for water. A one minute palate cleansing break was given before moving onto the next sample. The number of correct

identifications required to pass this type of screening depends upon the panel aims, with 75-100% (Meilgaard et al., 2007) or 60-80% (Kemp et al., 2009) being recommended. A relatively low pass criteria of >65% was adopted here, as water was frequently identified as metallic or bitter. Although officially this reduced the percentage of correct identifications, these responses were considered 'correct' as deionised water is known to elicit these attributes (Dalton et al., 2000). Misidentification of the same tastant sample (not including the water control) twice resulted in exclusion, as this suggests aguesia to that taste quality.

2.2.3.4 Screening Session 2

The best sixteen candidates from Screening Session 1 were invited to attend a second screening session. This identified candidate dedication to joining the panel. This was important as poor panel attendance can be problematic with longer studies (Meilgaard et al., 2007), tellingly, four candidates dropped out of the study at this stage. This also enabled the assessment of the reproducibility of responses, and candidate ability to learn over time, as the identification of taste test was completed a second time. A ranking test (ISO-8587, 2006) was also introduced to determine candidate ability to discriminate between the relative intensity of tastant samples. NaCl was presented at four concentrations (1, 2, 5, 10 g/L), and candidates were asked to rank them from least to most intense. Successful individuals were required to rank the lowest and highest concentration samples in the correct order, and those with the highest performance also correctly ranked the concentrations between these (Meilgaard et al., 2007).

2.2.3.5 Data analysis of screening assessments

The percentage of correct responses was calculated for each candidate's response for the identification of taste testing during the first and second screening sessions, and the ranking of tastants during the second screening session.

2.2.4 Development of tastant stimuli using a sensory panel

2.2.4.1 Sensory panellists

The ten candidates achieving the highest overall scores in screening tests were recruited onto the sensory panel (eight female/two male). As recommended, correct responses in the identification of taste test results for the combined scores from the Screening Session 1 and 2 was >80% (ISO-8586, 2014).

2.2.4.2 Sensory panel sessions

Panel sessions (60 min each) were conducted at the Sensory Science Centre, University of Nottingham. Sessions were scheduled at the same time and day each week to minimise errors arising from irregular testing (Meilgaard et al., 2007). A minimum break of ten min was given during each session to reduce fatigue and promote palate recovery. Panellists were instructed not to attend if they felt ill as this would affect their sensory acuity (ISO-6658, 2005). Catch up sessions were completed for all missed assessments. An inconvenience allowance was provided at the end of each session. Data were collected using the computerised data acquisition system FIZZ (Biosystems, France).

2.2.4.3 Panel training and correct use of the gLMS

Three training sessions were conducted. The gLMS (see Section 1.2.6 for further details) was used to identify variation in taste perception across

assessors (Green et al., 1996). Training on the correct use of the gLMS is important as it explains the principal of rating across sensory modalities, and allows errors in scale use to be identified so that panellists can be re-trained if necessary (Hayes et al., 2013). A standard training exercise was completed (Bartoshuk et al., 2002) during which a blank gLMS was provided, and panellists were instructed to add their 'strongest imaginable sensation of any kind' at the top of the scale, before rating the perceived intensity of 15 remembered or imagined sensations anywhere on the scale (**Table 2.3**). This created a reference gLMS used by the assessor to guide sample intensity ratings. To ensure panellists understood how the gLMS is used, they were expected to rate 'whisper'<'conversation'<nearby jet plane take off (Bartoshuk et al., 2002).

Table 2.3: Remembered or imagined sensations list provided when training panellists on accurate use of the gLMS (Bartoshuk et al., 2002a).

	Remembered or imagined sensation
1	The brightness of a dimly lit restaurant
2	The brightness of a well-lit room
3	Staring at the sun
4	The loudness of a whisper
5	The loudness of a conversation
6	Hearing a nearby jet-plane take off
7	The warmth of freshly baked bread in your mouth
8	The coldness experienced sucking on an ice-cube
9	The smell of a rose
10	The strongest smell ever experienced
11	The sweetness of candyfloss
12	The bitterness of grapefruit
13	The strongest taste ever experienced
14	The strongest oral burn experienced
15	The strongest oral pain ever experienced

It has long been documented that training improves panel homogeneity, enhances sample discrimination, and produces reproducible and reliable ratings across repeat measures (Amerine et al., 1965). It provides an

opportunity to identify standardised testing protocols, and practice test methods by performing observations on dummy samples where the data is discarded (ASTM-E1499-16, 2009). Panel aims should determine the type of training required (ISO-8586, 2014). Here, ranking tests were used to develop panellist ability to discriminate between the intensity of tastant samples of differing concentrations. Easily distinguishable concentrations of randomly selected tastants were initially tested: citric acid (0.5, 0.8, 1.5, 2.0 g/L), NaCl (2, 3.5, 5, 7 g/L), quinine sulphate (0, 0.00853, 0.0256, 0.0427 g/L). As panellists gained experience and confidence in ranking the samples, the difference between sample intensities was reduced (identified from benchtop analysis), citric acid (0.6, 0.8, 1.3, 1.7), NaCl (3.1, 4.3, 5.5, 7 g/L), quinine sulphate (0, 0.007, 0.02, 0.033 g/L). Ranking tests were repeated with the adapted concentrations, which made discrimination between samples more challenging.

Once panellists were proficient in discriminating between tastant concentrations during the ranking tests, they were acclimatised to using the gLMS to rate stimuli intensity. Here, the tastant concentrations that were closest together (and therefore most difficult to discriminate) in the ranking tests were presented individually (one replicate of each) with a one minute palate recovery break between them. Panellists were instructed to rate the sample intensity on the gLMS, and were reminded to use their individual reference gLMS to guide intensity ratings. Panellists were expected to rate samples in the correct ranking order of sample concentration, and instructed to rate objectively without influence from the hedonic qualities of the sample.

2.2.4.4 Sensory panel performance

Monitoring and evaluating individual and group ability to consistently produce valid results is an important part of minimising both internal errors made by a panellist, and external errors made across the panel (Kermit and Lengard, 2005). The testing methods used in this study are particularly susceptible to sensitivity error (not discriminating across samples), reproducibility error (not reproducible across replicates), and non-discriminator error (rating all products similarly when others rate them differently) (Kermit and Lengard, 2005). A set of tools should be used to monitor performance, and re-training provided where appropriate. Here, panel performance was measured for precision and reliability. Consistency in rating across replicates was measured using the coefficient of variance (CoV) where ≤30% was an acceptable level (Kemp et al., 2009). Panellists ability to discriminate between samples of differing intensities and the water control was assessed using a two factor ANOVA (sample and assessor), and interaction plots were used to assess interactions between samples and assessors. Panellist motivation was enhanced by giving regular feedback on performance, and providing confectionary after sessions. The study objectives required repetitive testing of sample intensity, so different types of testing were introduced where possible (data not shown) to keep panellists stimulated. For example, some of the methodologies used during the experiments detailed in Chapter 3, 4 and 5 were pilot tested using the sensory panel.

2.2.4.5 Development of moderate-strong intensity tastant stimuli

The trained panel were used, over nine panel sessions, to develop a set of five prototypical tastes (sweet, sour, salt, bitter, umami) and purported taste 'metallic' stimuli that were on average perceived at a similar intensity, and rated

between moderate and strong on the gLMS. All data were collected in sensory booths designed to British standard (ISO-8589, 2014), and samples were presented with random three digit codes, in 5 ml volumes to closely mimic the 3 ml that would be delivered during the final fMRI scan sessions. During each panel session, a set of the six tastants were tested in replicate in a random balanced design. Panellists were presented with each sample individually, and instructed to rate the perceived intensity when it reached its maximum on a blank gLMS. They were always provided with their reference gLMS, and reminded to use it to guide intensity ratings. A one minute break was given between samples, where unlimited deionised water was provided for palate cleansing. The intensity ratings reported during each sensory panel session were subjected to statistical analysis to identify tastants which were rated at a significantly different intensity to those rated in the desired range (moderate-strong). Where necessary, tastant concentrations were adjusted (with the aim of bringing the perceived intensity to between moderate and strong) before the samples were again tested during the next panel session. Verbal reporting on palatability, and whether stimuli exhibited irritant qualities were also considered. This process was repeated until stimuli were rated at similar intensities between moderate and strong on the gLMS. Concentrations of the stimuli that were tested during the first panel session are indicated in **Table 2.4**, which were made at concentrations identified in literature to be 'strong' intensity. **Table 2.4** also indicates the range of concentrations that were tested over the nine panel sessions in order to develop the final set of tastant stimuli, which are also detailed in the table.

Table 2.4: Concentrations of tastant stimuli assessed during sensory sessions. Glucose (GLC), citric acid (CA), monosodium glutamate (MSG), sodium chloride (NaCl), quinine sulphate (QS) and ferrous sulphate ($FeSO_4$).

Tastant	Initial set of tastants assessed (g/L)	Range of concentrations assessed (g/L)	Final stimuli set (g/L)
GLC	180.16 (Moskowitz et al., 1976)	105-180.16	117.32
CA	7.92 (Mojet et al., 2005)	1.2-7.92	1.5
MSG	12.58 (Mojet et al., 2005)	11-20	20
NaCl	22.6 (Mojet et al., 2005)	10-22.6	10
QS	0.078 (Simons et al., 2002)	0.01-0.078	0.017
$FeSO_4$	0.834 (Lawless et al., 2004)	0.641-0.834	0.834

It was important to monitor panellists' ability to discriminate between samples of differing intensity, and to reduce rating by habituation (where stimuli that are subtly different are rated the same) (Kemp et al., 2009). Therefore, water and six 'low' intensity tastants were added to the experimental design and tested during each panel session (one replicate). The concentrations of these 'low' intensity samples varied, ranging from roughly recognition threshold, to around 'weak' intensity on the gLMS (**Table 2.5**).

Table 2.5: Range of concentrations used for 'low' intensity tastant stimuli. Glucose (GLC), citric acid (CA), monosodium glutamate (MSG), sodium chloride (NaCl), quinine sulphate (QS) and ferrous sulphate ($FeSO_4$).

Tastant	Concentration (g/L)
GLC	17.7-30
CA	0.34-0.43
MSG	0.29-0.8
NaCl	1.19-2
QS	0.00783-0.0086
$FeSO_4$	0.0036-0.019

2.2.4.6 Data analysis

Here, and for all subsequent gLMS intensity ratings, a value of 0 was converted to 0.5, and the data was $\log_{10}$ transformed before statistical analysis, as the data is not normally distributed. All statistical analyses were performed using SPSS, version 21 (SPSS IBM, USA) with an α-risk of 0.05.

A three factor ANOVA (sample, panellist, and replicate) with sample panellist interaction was performed on the tastant intensity ratings made by the sensory panel after each session. Tukey's Honest Significant Differences (HSD) post-hoc tests were used to determine where significant differences occurred. Tastant concentrations were adapted in response to the intensity ratings, and tastants made at the new concentrations were tested during the subsequent panel session. This analysis was conducted on each set of tastant concentrations rated by the panel, until the final set of stimuli perceived between moderate-strong intensity had been developed.

2.2.5 Pilot testing of taste stimuli intensity

The tastants developed in Section 2.2.4.5 were pilot tested on a random selection of sensory assessors (who were representative of the general population) to test perceived intensity across individuals with varied taste sensitivities. This was important as the participants who would be fMRI scanned would not be trained sensory panellists.

2.2.5.1 Participants

A recruitment email was sent to staff and students at the University of Nottingham. Study eligibility required assessors to be available to attend the scheduled session, have no known taste or smell abnormalities, and to pass a basic health questionnaire, as would be the case for fMRI participants

(Appendix 1). The first 20 people meeting these requirements were recruited, and food incentives were provided for participating.

2.2.5.2 Evaluation of tastant intensity

Participants were semi-trained during attendance of one sensory evaluation session (60 min), which included training on the use of the gLMS (as described in section 2.2.4.3) and familiarisation with known taste samples (15 ml) (as described in section 2.2.3.3). Practice intensity rating was performed on three concentrations of NaCl (2, 5, 7 g/L). One replicate of each of the unknown tastants (developed in Section 2.2.4.5) and a water control sample were presented (5 ml) with random three digit codes in a randomised balanced design. Participants were instructed to rate the perceived intensity once it had reached its maximum on a blank gLMS FIZZ form (Biosystems, France), and a one minute palate cleanse break was given between samples. Individual reference gLMS were used to guide intensity ratings.

2.2.5.3 Data analysis

Data from three assessors was removed due to poor use of the gLMS. A two factor ANOVA (sample and assessor) was performed to determine differences across samples or assessors. Tukey's HSD post-hoc tests were used to determine significant differences between samples.

2.2.6 Measuring palate recovery time for taste stimuli

In a further experiment, the recruited and trained sensory panel (n=10) were used to measure palate recovery time for each of the developed taste stimuli to inform sample presentation order during the fMRI scanning session. Tastants are delivered to participants in quicker succession, and over a larger number of

replicates during fMRI scanning compared to sensory evaluation, and so the risk of sample interaction effects (suppression/enhancement/synergy) is increased. It was therefore important to explore which tastants were the most persistent, as this would guide the delivery order when conducting the fMRI experiment. The developed tastant samples were tested in triplicate over three sensory panel sessions, where the presentation order was balanced and randomised across panellists and sessions.

Tastant stimuli and water for palate cleansing were presented in 5 ml volumes to reflect the 3 ml stimuli delivered during fMRI scanning (Marciani et al., 2006). Panellists were provided with a stopwatch which they started when sipping the sample from a cup, and 12 seconds later they were instructed to palate cleanse with 5 ml water as this is the typical delivery time during an fMRI scanning experiment to allow the haemodynamic response function to return to baseline (Hort et al., 2016). They recorded the time at which they perceived the palate to have returned to its normal state, and a timer indicated when they should move onto the next sample, 185 sec after the previous stimulus. If the palate had not returned to its normal state by this point they recorded >185 seconds.

2.2.6.1 Data analysis

To identify if palate recovery time differed across tastant qualities, a three factor ANOVA (sample, panellist, replicate) with sample panellist interaction was performed. Tukey's HSD post-hoc testing was conducted to determine where differences between samples occurred.

2.2.7 Comparison of sample delivery methods

The sensory panel performed one final experiment. For logistical reasons, panellists sipped samples from cups whilst in a seated position during stimuli

development. However, during fMRI testing, tastants are sprayed into the mouth whilst participants are supine (Marciani et al., 2006). The perceived intensity of tastant samples developed for subsequent fMRI testing were compared across delivery methods to determine how it influenced stimulus perception. Three panellists attended this additional session, conducted at the Sir Peter Mansfield Imaging Centre, where sample delivery used in the MR scanner was replicated in a training room. They were familiarised with their reference gLMS at the start of the session. Panellists lay supine to mimic the position inside the MR scanner, and wore prism glasses to view a presentation screen situated at their feet, which displayed the gLMS (Compusense, Five 5.4, Canada). An automated spray system using a custom made gustometer (Marciani et al., 2006) delivered tastant stimuli and water for palate cleansing (**Fig 2.1**). Samples were sprayed into the mouth through nozzles positioned between the lips, and delivery was controlled by presentation software (Neurobehavioral System, San Francisco, US). Practice intensity ratings across five replicates of water were conducted. The importance of stimuli covering the tongue (and hence oral receptors) was highlighted, and the nozzle position was adjusted by the assessor to meet this aim when necessary.

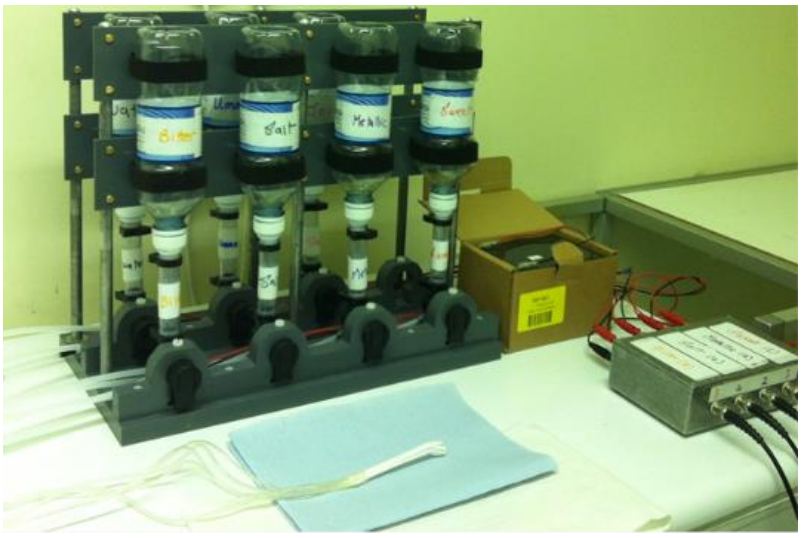

Figure 2.1: Gustometer and nozzles used for sample delivery during fMRI scanning (Marciani et al., 2006).

The sample delivery paradigm was designed to deliver tastants to participants during the fMRI experiment (Chapter 4). The gustometer flow rate was 1 ml/s, which allowed precise and reproducible delivery of 3 ml stimuli over three seconds. The cycle length for stimulus delivery was 15 seconds, which included a 12 second break between replicates (**Fig 2.2**).

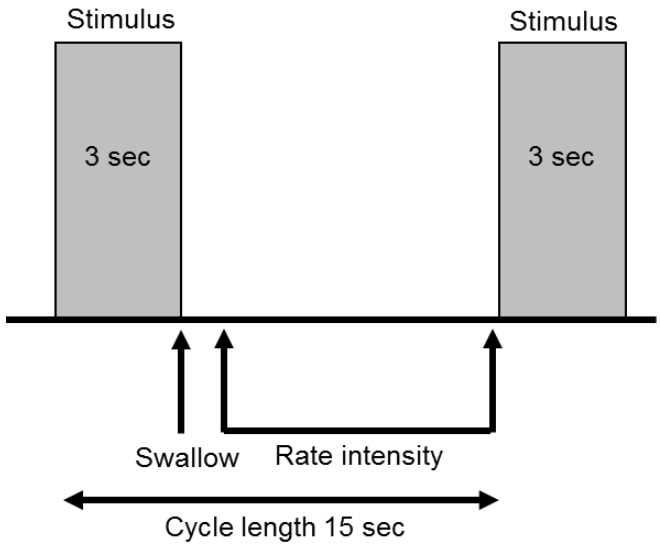

Figure 2.2: Cycle length of one replicate of stimulus.

A mouse positioned on a flat board on the participants stomach was used to rate stimulus intensity on a blank gLMS between replicates. **Figure 2.3** outlines the stimulus delivery paradigm used. Tastants were delivered across three 'blocks' each containing 'pairs' of two tastants. Ten replicates of water were delivered at the beginning of each tastant 'pair' block, as this was the control condition that would later be required to model the fMRI data in Chapter 4. Ten replicates of each tastant were delivered, followed by five replicates of water to cleanse the palate. Delivery of each tastant 'pair', including the associated water control and palate cleansers, lasted for 14 minutes. A three minute break was given between tastant pairs, during which panellists were able to remove the nozzles from the mouth.

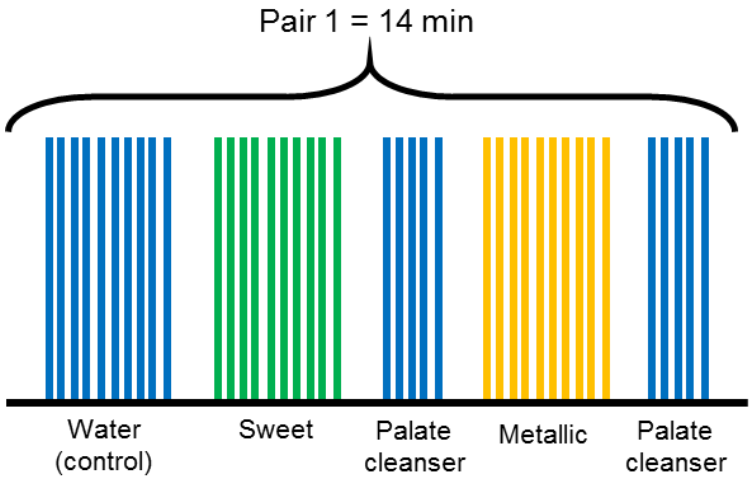

Figure 2.3: Schematic showing delivery of the water control sample (ten replicates), a 'pair' of tastants (ten replicates), and water palate cleanser (five replicates).

2.2.7.1 Data analysis

The mean perceived intensity when sipping samples when seated (two replicates) and spraying samples when supine (ten replicates) were calculated. Adaptation may occur over the ten replicates (Halpern and Meiselman, 1980), which would lower the mean rating for the ten replicates delivered when spraying into the mouth. Therefore, further comparisons were made by calculating the mean of the first two replicates sprayed, as well as the maximum intensity (*Imax*) reached across both delivery methods.

A two factor ANOVA (panellist and sample delivery condition) was conducted on the gLMS intensity ratings for tastants to compare the mean ratings across the two replicates delivered by sipping (when seated) compared to the mean of both the first two replicates and the ten replicates when tastants were sprayed into the mouth (when supine). A two factor ANOVA (panellist and sample delivery condition) was conducted on the gLMS *Imax* ratings to determine if the stimuli delivery method (sip/seated compared to spray/supine) influenced the perceived *Imax*.

2.3 RESULTS

2.3.1 Screening assessments

Screening results (**Table 2.6**) show the percentage of correct responses ranged from 25-100% in the identification of taste test, and 50-100% in the ranking test. Candidates in bold were recruited onto the panel (n=10).

Table 2.6: Screening test results for 30 candidates attending session one (S1), and 12 candidates attending session two (S2). The percentage of correct responses for individual tests, and the mean of S1 and S2.

		Identification of taste test (S1)	**Identification of taste test (S2)**	**Identification of taste test (mean S1 & S2)**	**Ranking test (S2)**
Candidate	1	41.6			
	2	**83.3**	**92.8**	**88**	**100**
	3	**83.3**	**92.8**	**88.1**	**100**
	4	**93**	**92.8**	**92.9**	**100**
	5	75			
	6	**75**	**92.8**	**83.9**	**100**
	7	75			
	8	25			
	9	**100**	**71.4**	**85.7**	**100**
	10	**83.3**	**92.8**	**88.05**	**50**
	11	58.3			
	12	66.7			
	13	**75**	**85.7**	**80.4**	**100**
	14	33.3			
	15	58.3			
	16	33.3			
	17	58.3			
	18	**75**	**85.7**	**80.4**	**100**
	19	50			
	20	41.6			
	21	75			
	22	41.6			
	23	41.6			
	24	75	57.1	66.05	100
	25	**83.3**	**85.7**	**84.5**	**100**
	26	**75**	**100**	**87.5**	**100**
	27	50			
	28	50			
	29	50			
	30	66.7	78.6	72.65	50

2.3.2 Development of moderate-strong intensity tastant stimuli

Although the panellists assessed a number of different tastant concentrations, for brevity, the following results only detail ratings for the initial tastants tested (identified as 'strong' in previous literature) (**Fig 2.4**), and the final set of tastant samples developed (**Fig 2.5**). Intensity ratings for each iteration of the adapted concentrations tested, between the initial and final set, are not shown.

Significant differences across samples ($p<0.001$) and panellists ($p<0.001$), but not replicates ($p=0.292$), were identified for the intensity ratings of tastants at the concentrations identified to be strong in previous literature, and a significant sample panellist interaction ($p<0.001$) occurred. Interaction plots identified crossover between panellist ratings. Tukey post-hoc testing identified no significant differences between sour, salt and bitter stimuli, which were significantly more intense than sweet, umami and metallic. Mean intensity ratings were rated between moderate and very strong on the gLMS for all samples. Some of the panellists verbally reported finding the concentrations of sour, salt and bitter unpalatable.

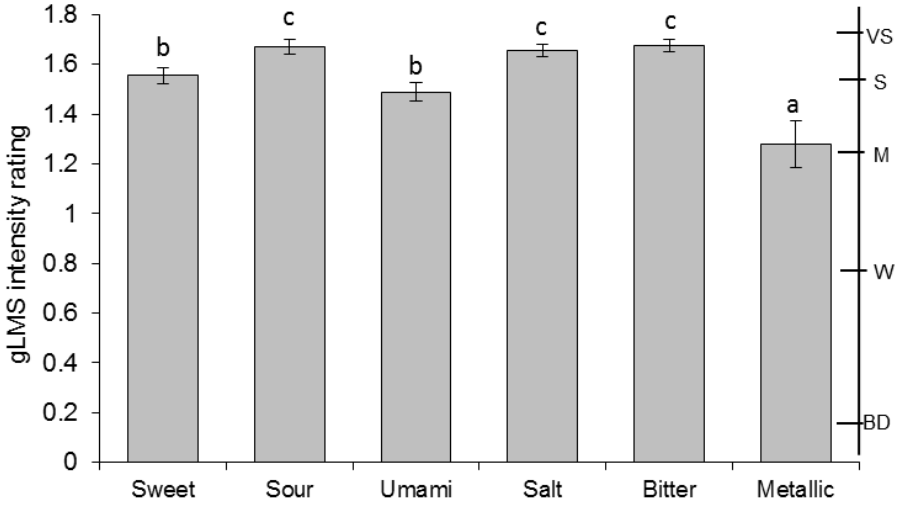

Figure 2.4: Mean intensity ratings reported by the sensory panel for tastants identified as 'strong' in previous literature. Bars represent mean ± S.E. Different letters above the bars ^{abc} indicates a significant difference between samples at $p<0.05$. BD - barely detectable, W - weak, M - moderate, S - strong, VS - very strong.

Intensity ratings for the developed tastants are shown in **Figure 2.5**, where a significant difference across samples ($p<0.028$) and panellists ($p<0.001$), but not replicates ($p=0.292$) was observed, and a significant sample panellist interaction ($p=0.01$) occurred due to cross-over in panellists' intensity ratings. Tukey post-hoc testing showed there was no significant difference across samples, except for metallic which was rated lower in intensity. All samples were rated between moderate and strong on the gLMS.

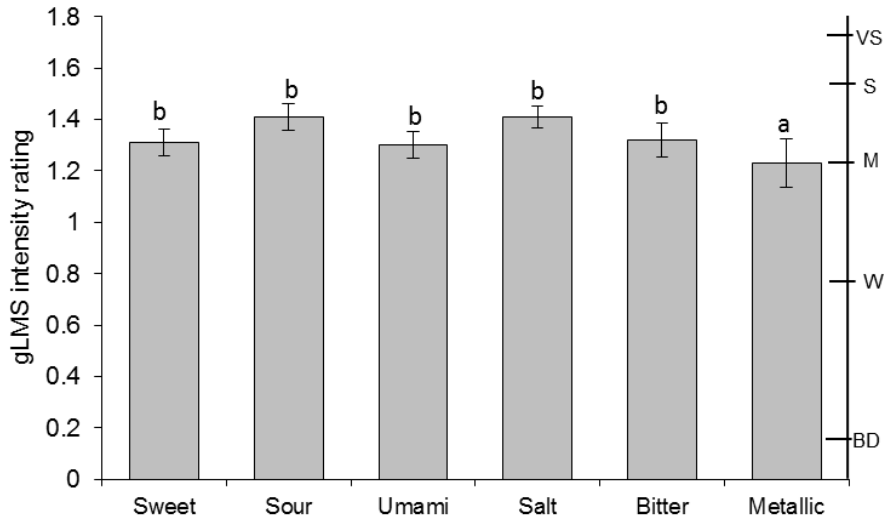

Figure 2.5: Mean intensity ratings for the final set of tastants tested with the sensory panel. Bars represent mean ± S.E. Different letters above the bars [ab] indicates a significant difference between samples at $p<0.05$. BD - barely detectable, W - weak, M - moderate, S - strong, VS - very strong.

2.3.3 Pilot testing of taste stimuli intensity

When the perceived intensity of tastants was tested on a group of 20 randomly selected semi-trained assessors, significant differences were identified across samples ($p<0.001$) and assessors ($p<0.001$). Tukey post-hoc testing identified that water was significantly less intense than all other samples, bitter was significantly more intense than umami and metallic, and metallic was significantly less intense than sour, salt and bitter. Mean intensity ratings for all tastants, excluding metallic, were between moderate and very strong.

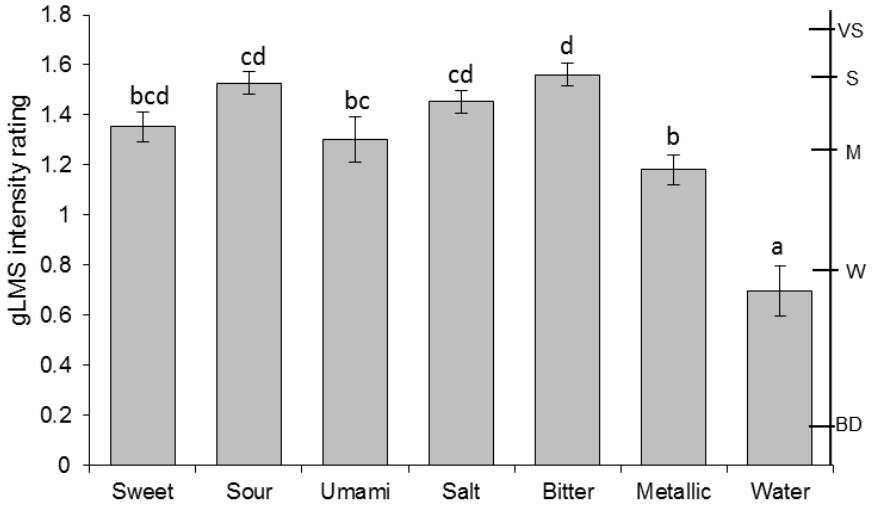

Figure 2.9: Mean sample intensity ratings reported by semi-trained assessors. Bars represent mean ± S.E. Different letters above the bars [abcd] indicate significant difference between samples at $p<0.05$. BD - barely detectable, W - weak, M - moderate, S - strong, VS - very strong.

2.3.4 Measuring palate recovery time for taste stimuli

Mean palate recovery time across samples was 44-121 sec (**Fig 2.6**). The longest palate recovery was reported for metallic (121 sec), followed by umami (106 sec) and then bitter (73 sec). Panellists reported that the palate had not recovered after 185 sec on 33% of responses for metallic, 27% of responses for umami, and 7% of responses for bitter. An ANOVA identified a significant difference in palate recovery times between samples ($p<0.001$) and panellists ($p<0.001$), but not between replicates ($p=0.6$), and a sample panellist interaction ($p<0.001$) occurred, as can be expected when measuring variation in taste sensitivity. Metallic and umami had significantly longer palate recovery times than all other samples, and bitter significantly longer than sour, sweet and salt.

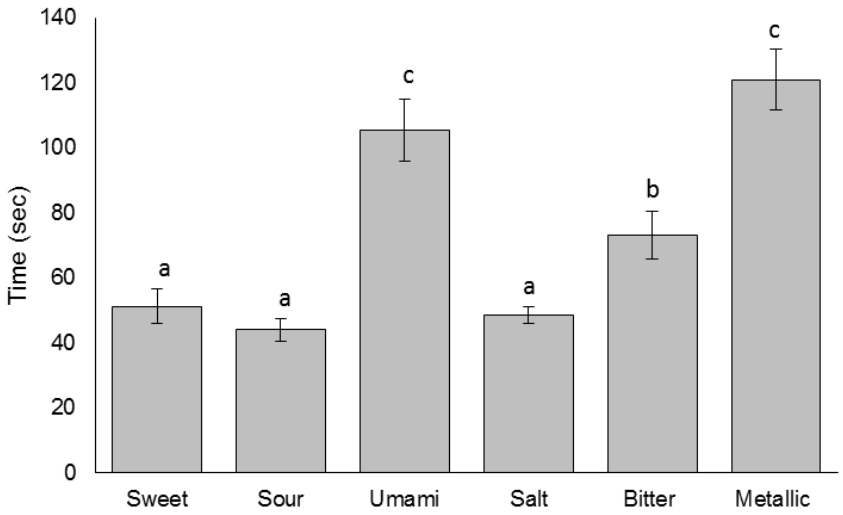

Figure 2.6: Mean palate recovery time for samples. Bars represent mean ± S.E. Different letters above the bars [abc] indicate significant difference between samples at $p<0.05$.

2.3.5 Comparison of sample delivery methods

Figure 2.7 shows a trend for the mean intensity to be higher when sipping samples (in a seated position) compared to when spraying tastants into the mouth (in a supine position). However, no significant differences were identified between panellists ($p=0.09$), or mean intensity ratings across delivery methods ($p=0.57$) when comparing the mean gLMS rating over two replicates of sipping the sample (when seated) with the mean of two or ten replicates when stimuli were sprayed into the mouth (when supine). *Imax* ratings were similar across delivery methods, and no significant differences across delivery method ($p=1.73$) or panellists ($p=0.138$) were identified. Importantly, the mean intensity remained between weak and very strong for all panellists and tastants across both delivery methods.

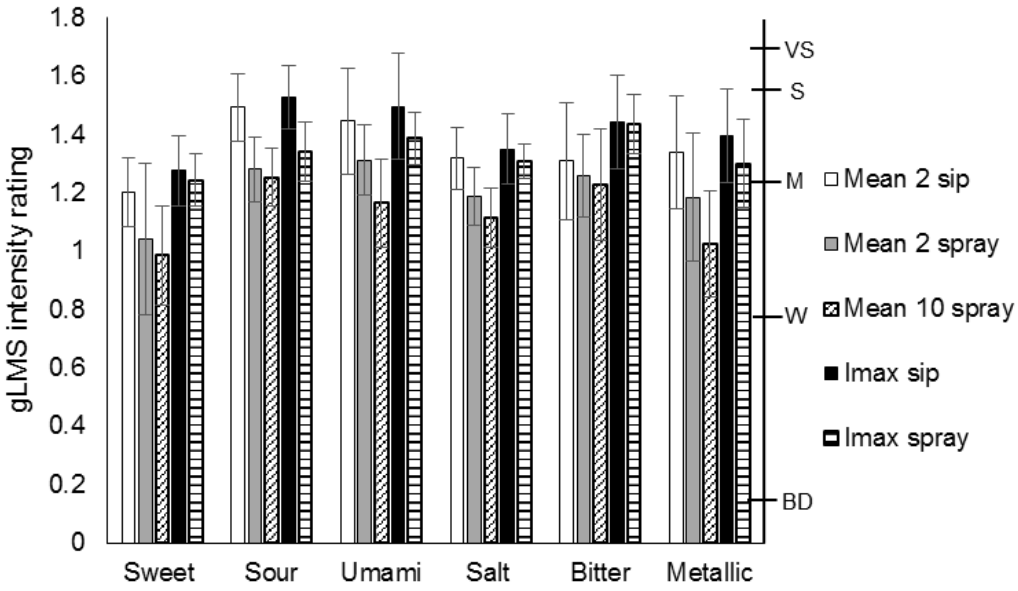

Figure 2.7: Mean intensity ratings across delivery methods when sipping or spraying tastants into the mouth. Bars represent mean ± S.E. Word descriptors: BD - barely detectable, W - weak, M - moderate, S - strong, VS - very strong.

2.4 DISCUSSION

2.4.1 Development of moderate-strong intensity tastant stimuli

Table 2.6 shows 31% of candidates passed the screening, which is similar to the estimated 10-30% pass rate (Kemp et al., 2009). A higher percentage of females passed due to a greater response to the recruitment email, and potentially higher taste acuity (Weiffenbach et al., 1982, da Silva et al., 2014).

Initial tastant concentrations, identified as 'strong' in previous literature, were rated between moderate and very strong (**Fig 2.4**) by the sensory panel, and on occasion sour, salt and bitter were verbally reported to be unpalatable. The variability in perceived intensity across studies may be due to differences in sample delivery, as other studies used the sip and expectorate technique (Lawless *et al,* 2004, Mojet et al, 2005, Simons et al., 2002, Moskowitz et al., 1976), whereas here samples were swallowed allowing a greater range of receptors to be coated. Additionally, assessors used in previous studies were

representative of the general population, whilst in the current study panellists were selected according to taste acuity and sensory performance. Stimuli are rarely unimodal and often stimulate multiple senses. NaCl (Green and Gelhard, 1989) and citric acid (Beidler, 1953, Gilmore and Green, 1993) elicit both gustatory and irritant responses, whilst sugars are thought to be non-irritant (Green and Gelhard, 1989). Sensitisation of NaCl increases the irritant effect over repeat exposure (Green and Gelhard, 1989), such as the delivery across ten replicates required during fMRI scanning. This study aimed to explore gustatory perception, making it important to minimise irritant properties where possible. Therefore it was important for the tastants to be strong, whilst minimising trigeminal responses. Tastant concentrations were adjusted and retested a number of times until they were rated at similar intensities between moderate and strong on the gLMS (**Fig 2.5**), and were not verbally reported to be irritant by the panellists.

The developed tastant stimuli (**Fig 2.5**) were then tested by 20 randomly selected semi-trained assessors used to identify intensity perception across individuals with a range of taste sensitivities. As with the sensory panel, mean intensity ratings were above moderate intensity for the prototypical taste stimuli. Metallic was just below moderate due to the high variation in sensitivity to $FeSO_{4,}$ where low intensity ratings reduced the mean value. Deionised water can exhibit bitter and/or a metallic sensations (Dalton et al., 2000), explaining why water was rated to have a taste between barely detectable and weak. Minor differences across the sensory panel and semi-trained assessor group may be due to general differences in taste sensitivity. Additionally, sensory assessors received minimal practice on use of the gLMS, which may have resulted in errors such a contrast effect where small differences between samples were exaggerated (Meilgaard et al., 2007). Importantly, tastants were detected above

the intensity of water for all assessors, and the mean ratings typically fell above moderate, whilst still being palatable for assessors with the highest taste sensitivity.

The gLMS identified overall differences in taste sensitivity across individuals, and in addition to this, the rank order of sample intensities (data not shown) also differed between each of the ten sensory panellists, and the 20 semi-trained assessors. This indicates variation across tastant qualities within and across assessors. Variation in sensitivity to bitterness is well evidenced, for example the range of sensitivities to perceive the bitterness of propylthiouracil (PROP) across PROP taster status groups (Bartoshuk et al., 1994), genotypes (Melis et al., 2013b), and fungiform papillae density phenotype (Miller and Reedy, 1990b). Evidence showing variation in sensitivity to other taste qualities is more limited. Sour and salt perception is affected by salivary composition (Matsuo, 2000), and dietary sodium intake influences NaCl perception (Piovesana et al., 2013). Lugaz et al (2002) identified significant differences in the ability to perceive MSG, and categorised those who were unable to detect it as 'non-tasters' (3.5%), whilst hypo-tasters detected it at a low sensitivity (10%), and tasters (81%) as more intense. The TAS1R3 gene mediates sweet taste in humans (Fushan et al., 2009), and the GNAT3 gene encodes for gustducin which is also involved in sweet taste perception (Fushan et al., 2010). Genetic variation of these genes influences sensitivity to sweet stimuli (Fushan et al., 2009, Fushan et al., 2010), another factor likely to be influencing the differences seen in response to the sweet tastant in the current study. Sensitivity to $FeSO_4$ also differs, and Epke et al (2009) categorised individuals as normosmic or anosmic to $FeSO_4$ depending on their ability to perceive this divalent salt. Although differences did not reach a significant level when later comparing intensity ratings across the groups in this instance, the observed trend for high

individual variation in response to metallic stimuli across studies indicates the need for further investigation. $FeSO_4$ causes lipid oxidation of tissue (Glinderman, 2006) in the oral cavity which releases volatiles detected as metallic via the retronasal pathway (Omur-Ozbek et al., 2012). Epke et al (2009) hypothesised that individual differences in lipid oxidation may explain the varied ability to perceive these metal salts, which was later supported when Omur-Ozbek et al (2012) identified individual variation in lipid oxidation after exposure to $FeSO_4$ in the oral cavity.

2.4.2 Measuring palate recovery time for taste stimuli

Figure 2.7 shows that the mean time taken for the palate to return to baseline ranged from 44-121 secs, with metallic, umami and bitter being the most persistent tastes. Interestingly, the highest variance in palate recovery time across assessors was also observed for these samples, indicating individual variation in the perceptual response. Metal salts are known to elicit a metallic aftertaste (Yang and Lawless, 2005) for more than two minutes after expectoration (Yang and Lawless, 2006), supporting the current finding. Umami perception is also reported to be persistent (Giovanni and Guinard, 2001) in excess of ten minutes in some instances (Lugaz et al., 2002). The persistence of umami after MSG exposure was only 105 secs in the current study. However, the taste was present >185 sec on 27% of responses. The bitterness of quinine persisted for 73 seconds, which is similar to previous studies reporting a lingering aftertaste (Naim et al., 2002) ranging from 60 (Leach and Noble, 1986) to 95 secs (Bajec et al., 2012). Palate recovery after glucose was 51 seconds, which is considerably less than the 90 secs previously reported at a similar sample concentration (Mahawanich and Schmidt, 2004), which may be due to sample delivery volumes of 5 or 15 ml administered across studies. NaCl persisted for 49 secs, which is slightly lower than the 61-77 seconds reported

when assessing table salt and a range of commercially available sea salts (Drake and Drake, 2011), where the low purity of table and sea salts may explain the variance. Citric acid had the shortest persistence of 44 secs, which is similar to the 54 secs previously identified (Bajec et al., 2012). These results provide an estimate for palate recovery times, although consideration should be given to method limitations. If the palate had not recovered after 185 seconds, panellists were instructed to record the results as >185 sec, and to move onto the next sample. Therefore the mean palate recovery time for some tastants is likely underestimated, and in these instances may also have caused carryover and interaction effects between tastants. Depending on the presentation order, this may include suppression, enhancement or synergistic effects (Kemp et al., 2009). Additionally, palate recovery time will vary across sample delivery techniques. Here, samples were sipped from cups when the participant was seated, whilst stimuli will be sprayed into the mouth of participants whilst they are supine in the MR scanner. These findings guided the order of sample delivery during the fMRI paradigm (Chapter 4) to minimise palate fatigue, allow the oral cavity to return to baseline between tastants, and minimise or eliminate sample interaction effects. To prevent sample carryover and interaction effects, MSG was positioned as the final sample delivered during fMRI scan sessions as it was one of the most persistent stimuli in the current study, and in other cases has been observed to linger in excess of ten minutes (Lugaz et al., 2002). Metallic and bitter were also persistent, and were therefore positioned before participants were given breaks during the fMRI scan to allow time for palate recovery. The compromise was that sample delivery could not be balanced and randomised across participants. Instead the samples were delivered in pairs (**Fig 2.3**); pair one (sweet and metallic), pair two (salt and bitter), and pair three (sour and umami) that were pseudo-randomised. The order of pair one and two was randomised across participants, whilst pair three was always delivered last

due to the excessive persistence of the MSG stimuli. Replicates of an individual taste stimuli were delivered every 12 secs during the fMRI delivery paradigm, and repeated over ten replicates before a water palate cleanser was delivered. Therefore, carryover between replicates of an individual tastant can be expected.

2.4.3 Comparison of sample delivery methods

The method of sample delivery can affect perceived intensity (Haase et al., 2009a, Meiselman, 1971). This study compared the influence of sample delivery on perceived taste intensity under two delivery conditions; sipping the sample when seated, compared to spraying the sample into the mouth when supine. Separating the influence of ingestion method (sip/spray) from body position (seated/supine) would require a more complex study design than was employed in this experiment, and was therefore beyond the scope of this study. As such, both of these factors may have contributed to tastant perception. Although not reaching a significant level, **Figure 2.8** shows a trend that perceived intensity was higher when samples are delivered by sipping from cups (when seated) compared to being sprayed into the mouth (when supine). This supports the findings from Haase et al (2009a) who reported tastants were perceived to be less intense when syringed onto the tongue compared to when sipped from a cup, or dorsal flow delivery. However, a limitation of comparisons made during the current study being that different sample volumes were delivered across the delivery methods. When sipping a tastant 5 ml was consumed, whereas only 3 ml was sprayed into the mouth as this mimics the volume delivered in an fMRI study. Additionally, when sipping from cups the tongue is lifted to the palate three times before swallowing, whereas the sample is swallowed soon after the spray is delivered, which will affect the number and range of oral receptors coated. Therefore, one possibility is that more replicates of spray are required

to coat the same number of oral receptors stimulated during fewer sipping replicates. Taste adaptation (reduction in sensitivity) can occur with repeated exposure (Halpern and Meiselman, 1980), which may have contributed to the trend for lower intensity ratings observed for the mean of ten spray replicates. Interestingly, when comparing the *Imax* little difference was observed across methods, supporting the hypothesis that more replicates of stimulus delivered by spray may be required to reach the same intensity that is perceived by fewer replicates when the stimuli is sipped.

Body position may also have influenced tastant perception. However, this is hypothesised to have had less of an impact in this study as no significant differences in taste thresholds (Vickers et al., 2001) or discrimination of retronasal flavours (Hort et al., 2008) have been observed when comparing sample delivery across seated and supine positions. Although the samples included in the current study are predominantly taste stimuli, volatiles released when ingesting $FeSO_4$ are thought to stimulate the retronasal aroma pathway (Omur-Ozbek et al., 2012). Interestingly, the ability to detect aromas at perithreshold concentrations when delivered orthonsally can be reduced when supine compared to sitting (Lundstrom et al., 2006; Lundstrom et al., 2008). It would therefore be interesting to further explore and understand if retronasal perception of $FeSO_4$ is influenced by body position at suprathreshold levels.

These results show a trend for the delivery method to influence intensity perception, but this result did not reach a significant level. Overall, a similar intensity is perceived across methods, and importantly, mean sample intensity ratings were typically between weak and strong.

2.5 CONCLUSION

A sensory panel was recruited and used to develop tastant stimuli which were rated at similar intensities between moderate and strong on the gLMS. Pilot testing of the perceived tastant intensity on a randomly selected group of sensory assessors who were representative of the general population identified that all stimuli were significantly more intense than the water control sample, and that prototypical tastes were typically rated between moderate and very strong in intensity. A series of further experiments were conducted to explore sample qualities which would guide the stimuli delivery paradigm used during the fMRI experiment detailed in Chapter 4. Palate recovery time differed across both taste qualities and panellists. Metallic, umami and bitter were the most persistent tastes, and had the highest variation of responses across panellists. These findings were used to inform an order of sample delivery during the fMRI paradigm in order to minimise sample carryover and interaction effects. Sample delivery methods have been reported to influence the perceived intensity of taste stimuli in the past. This study found a trend for tastants to be perceived more intensely when sipping from a cup when seated, compared to spraying into the mouth when supine. However, little difference was observed in the *Imax* across delivery methods, and mean intensity ratings during spray delivery were typically around moderate on the gLMS. These findings were used to guide the stimuli delivery paradigm in a way that would minimise sample carryover and interaction effects when measuring the cortical response to taste using fMRI in Chapter 4. Divalent salts, such as $FeSO_4$, elicit a range of taste, trigeminal and flavour qualities in addition to the metallic sensation reported. Due to the complex nature of metallic perception, further experiments detailed in Chapter 3 were conducted on a range of divalent salts previously reported to elicit a metallic quality.

3 INVESTIGATING THE ORONASAL CONTRIBUTIONS TO METALLIC PERCEPTION

The work detailed in this chapter was published in the International Journal of Food Science and Technology, April 2017, Issue 52, pages 1299-1306.

3.1 INTRODUCTION

Chapter 2 described the development of prototypical taste and metallic stimuli to be delivered to participants whilst being scanned using functional Magnetic Resonance Imaging (fMRI), as described in Chapter 4. The oronasal qualities involved in metallic perception are complex, yet not well understood. The debate as to whether it is a result of taste, aroma and/or trigeminal stimulation is ongoing, but so far inconclusive. A series of sensory experiments were therefore conducted on a range of divalent salts that are reported to elicit a metallic sensation, in order to better characterise the perceptual oronasal properties of the elicited metallic attribute. These results were also used to guide interpretation of the brain response to ferrous sulphate ($FeSO_4$) in Chapter 4.

3.1.1 Metallic perception

Metallic taints experienced when consuming food have negative implications for consumer acceptability, and therefore for food manufacturers. Such taints can arise from artificial sweeteners (Schiffman et al., 1979), when fortifying foods with compounds such as $FeSO_4$ (Hurrell, 2002), and when consuming food from metal serving utensils (Piqueras-Fiszman et al., 2012). This problematic sensation also extends beyond food, and is associated with some medications (Gould et al., 1988), can be reported as a phantom sensation by cancer patients

(Ravasco, 2005), those suffering from taste distortion (Nordin et al., 2004) and during burning mouth syndrome (Grushka, 1987). Developing strategies to mask this metallic sensation is therefore important, but to do this a better understanding of the mechanisms involved in its perception is needed. There are currently five widely recognised and accepted tastes (sweet, sour, salty, bitter and umami) with clearly identified receptor mechanisms (Chandrashekar et al., 2006). In the past metallic has been proposed as an additional taste quality (Bartoshuk, 1978), which is a controversial topic as evidence remains inconclusive.

Divalent salts (Lawless et al., 2004), electrical currents (Stevens et al., 2008, McClure and Lawless, 2007, Lawless et al., 2005) and solid metal (Lawless et al., 2005; Laughlin et al., 2011) have been found to stimulate a metallic sensation when placed on the tongue. Their presence in water supplies, and influence on nutritional status, have made divalent salts of interest, and their orosensory qualities are multisensory, with taste, astringent and metallic cues (Lim and Lawless, 2005a). Volatiles can stimulate the olfactory pathway via the orthonasal (nose) and retronasal (nasopharynx) routes (Visschers et al., 2006). Using a nose clip to occlude the nose is a well-recognised technique for blocking the retronasal pathway to isolate the taste and oral trigeminal components of a stimuli from the retronasal aspects (Murphy and Cain, 1980). Occluding the nose significantly reduces the frequency (Hettinger et al., 1990) and intensity (Lawless et al., 2004) at which metallic is reported after oral exposure to $FeSO_4$, indicating retronasal stimulation is involved. This retronasal metallic sensation is commonly perceived to originate in the mouth and can inaccurately be identified as a taste, a process termed gustatory referral (Lim and Johnson, 2012). Lipid oxidation in food and beverage releases metallic smelling compounds 1-octen-3-one and 4,5- epoxy-(E)-2-decenal (Hinterholzer et al.,

1998, Buettner and Schieberle, 2001). The predominant hypothesis relating to metallic perception states that lipid oxidation of the phospholipid bilayer in the oral cavity occurs after contact with divalent salts, releasing aldehydes and ketones which stimulate the retronasal pathway and elicits metallic perception (Omur-Ozbek et al., 2012). Phospholipids in cell membranes have been found to be oxidised by the iron redox cycle (Gorelik and Kanner, 2001). However, a reduction in metallic perception with nasal occlusion is not reported for $CuSO_4$, suggesting a taste or trigeminal mechanism is also involved (Epke et al., 2009). It is unknown whether volatiles released from the sample itself could also elicit lipid oxidation when coming into contact with the tissue in the nasal cavity via the orthonasal route, and to our knowledge sample headspace volatiles and orthonasal sensations related to divalent salts have rarely been investigated.

3.1.2 Headspace analysis

Gas chromatography coupled with mass spectroscopy (GC-MS) is the most common analytical technique used to separate, identify and quantify compounds in a sample. Solid-phase microextraction (SPME) can be used to extract a wide range of analytes from the air, water or solid matrices, and is often used in conjunction with GC-MS. During SPME an adsorbant-coated fibre with an extracting phase extracts analytes from the sample headspace, and the fibre is then dissolved in a solvent and injected into the inlet of the GC (Kataoka et al., 2000), which is used to separate the individual chemical components of the sample. The sample is vaporised and carried though a heated column by an unreactive carrier gas such as helium and partitioned between the stationary (column) and mobile (gas) phase. Sample substrates separate from one another and travel through the column at different speeds depending on their chemical properties such as volatility and mass, and their affinity for the

stationary phase. The most volatile components will be most partitioned to the mobile phase and so will elute first, and the least volatile components, or those with a higher affinity to the stationary phase, will elute last (Yolanda, 2012). A detector identifies the point at which each chemical component elutes from the column (retention time), and a chromatogram is produced. This provides information on how many components are in the sample (number of peaks), their abundance (height of peak), and retention times. The sample is next passed through a transfer line into the MS which is used to identify the sample components. Here the sample is ionised, fragmented, and components separated in order of their mass-to-charge ratio (M/z value). The mass of each fragment is displayed on the mass spectrum plot which is used for identification by comparing the mass with known compounds. Although GC accurately separates compounds from within a sample, it cannot reliably differentiate between molecules with the same retention time, whilst MS cannot accurately distinguish sample compounds from others with similar patterns of ionised fragments (Taylor and Linforth, 2009). Therefore, the GC retention times and MS mass spectra are often used in conjunction to reduce the risk of incorrect identification.

3.1.3 Aims

Metallic sensations are not well understood. Therefore, the overall aim of this study was to conduct sensory evaluation and headspace analysis on a range of divalent salts in order to better characterise the metallic quality, and add to the current understanding of how olfactory, trigeminal and gustatory aspects interact to elicit the overall metallic sensation perceived. This study therefore had several objectives:

- Identify if divalent salts can be detected orthonasally when smelling the sample headspace. Divalent salts were not expected to have a perceivable orthonasal quality, as previous literature indicates $FeSO_4$ solutions were not discriminated from water when sniffing the sample headspace (Lawless et al., 2004).
- Determine the sensory qualities perceived when assessing the divalent salts. A range of taste, trigeminal and metallic qualities were expected to be reported, as they typically exhibit complex sensory profiles (Yang and Lawless, 2005).
- Assess the impact of retronasal stimulation on sample perception by evaluating the samples under both the nose both open and occluded conditions. The influence of nasal occlusion on metallic perception is reported to differ across divalent salts (Epke et al., 2009), which was therefore expected in the current study.
- Establish whether perceptual differences were observed across the three anions of ferrous salts. Differences were expected, as previous researchers have reported that the anion influences the perceptual qualities of ferrous salts (Yang and Lawless, 2006).
- Use headspace analysis to determine if volatiles could be detected in the sample headspace. Only one other study reports measuring volatiles in the headspace of $FeSO_4$, suggesting low level volatiles may be present for this sample (Lubran et al., 2005).
- Better understand the modalities involved in the perception of $FeSO_4$ in order to inform interpretation of the brain response when it is delivered during the fMRI experiment detailed in Chapter 4. It was hypothesised that the metallic sensation elicited by this divalent salt would, at least in part, be attributed to retronasal flavour stimulation (Lawless et al., 2004).

3.2 MATERIALS AND METHODS

3.2.1 Sensory experimental

3.2.1.1 Participants

Participants included staff and students at the University of Nottingham (23 females and six males), 29 were recruited in line with the ISO standards for conducting a triangle test (ISO-4120, 2007). All were non-smokers, aged 18-45 years old, reported being healthy, and having no known taste or smell abnormalities when a health questionnaire was administered (Appendix 1). The study had ethical approval from the University of Nottingham Medical Ethics Committee (Q13112014 SoB Sensory Sci). Participants gave written informed consent and an inconvenience allowance was provided. Participants were instructed not to consume anything but water for at least one hour before testing.

3.2.1.2 Sensory stimuli

Divalent salts were dissolved in deionised water from a reverse osmosis unit at supra-threshold concentrations (**Table 3.1**). Pharmaceutical or food grade compounds were used where possible: $FeSO_4$, $CuSO_4$, and $CaCl_2$. Otherwise reagent grade was used: $FeCl_2$ and FeGlu.

Table 3.1: Sample, formula, source and concentration of the divalent salts that were sourced from Sigma Aldrich, USA or Spectrum Chemicals, UK.

Stimulus	Formula	Source	Concentration (M)
Calcium chloride dihydrate	$CaCl_2 \cdot 2H_2O$	Sigma Aldrich	0.015
Iron II chloride tetrahydrate	$FeCl_2 \cdot 4H_2O$	Sigma Aldrich	0.002
Iron II D gluconate dihydrate	$FeC_{12}H_{22}O_{14} \cdot 2H_2O$	Spectrum	0.001
Iron II sulphate heptahydrate	$FeSO_4 \cdot 7H_2O$	Sigma Aldrich	0.003
Copper II sulphate pentahydrate	$CuO_4S \cdot 5H_2O$	Sigma Aldrich	0.015

Pilot studies with researchers at the Sensory Science Centre, University of Nottingham showed the samples to be roughly equi-intense when assessed orally. Iron exists in more than one oxidation state, with ferrous (Fe^{2+}) and ferric (Fe^{3+}) being the most common forms. When $FeSO_4$ is exposed to air oxidisation occurs, resulting in the formation of ferric sulphate ($Fe(SO_4)_3$) (Glinderman et al., 2006). The latter does not impart the 'metallic' volatiles associated with retronasal metallic perception after exposure to the skin (Omur-Ozbek and Dietrich, 2011). Samples were made fresh every three hours to minimize such oxidation effects (Yang and Lawless, 2006). A deionised water control sample was evaluated so that any sensations elicited from the water itself could be decoupled from that of the divalent salts. Samples (5 ml) were presented according to a randomised balanced design in odourless plastic medicine cups at room temperature (22 ± 2°C), and were labelled with random three digit codes. Deionised water was provided for palate cleansing before and after all samples were consumed.

3.2.1.3 Sensory methods

All data were collected on FIZZ software (Biosystems, Cergy-Pontoise, France). Tests were conducted in an air conditioned room (20±1°C) in individual booths designed to ISO standards (ISO-8589, 2014). The experimental procedure was divided into two parts.

3.2.1.4 Experiment 1

In the first session five triangle tests (one for each divalent salt) were conducted to determine if the divalent salts could be differentiated from the water control. Order of presentation was randomised and balanced across participants following ISO standards protocols (ISO-6658, 2005). Red lighting was used in

the test area to disguise any potential visual cues. Samples were presented in lidded medicine cups, with the lid removed when assessing the sample. Participants were instructed to smell the headspace above the three samples and identify the odd one out.

3.2.1.5 Experiment 2

Participants attended two further sessions. Attribute qualities known to be associated with the divalent salts, as identified in previous literature or during benchtop testing, were selected for assessment when evaluating the samples. Before testing commenced, the attribute qualities were described to the assessors: sweet as the sweetness experienced from sugar; salty as the sensation from table salt; bitterness as found in coffee and tonic water; astringent as the 'drying or puckering' mouthfeel sensation experienced from red wine, green banana or strong tea; tingling as the mouthfeel sensation elicited by carbonated beverages; and metallic being like the taste of blood or metal. Reference samples to represent the attributes tested were not delivered, in order to avoid restricting the qualities reported to the constraints of that specific reference sample. This is particularly important when evaluating metallic, as the metallic quality is reported to differ across divalent salts (Schiffman, 2000). The option to report 'other' sensations was also given to reduce the occurrence of attribute dumping (Clark and Lawless, 1994). To ensure the full range of oral receptors were coated, participants were instructed to ingest the whole sample, hold it in the mouth, and lift the tongue to the palate three times before swallowing. They were asked to rate (on a 10-point continuous line scale) their perceived maximum intensity for sweet, salty, bitter, metallic, astringent, and tingling, as these attributes are commonly reported to be associated with divalent salts during preliminary testing or in previous

literature. A scale labelled from ‘none’ to ‘very intense’ was provided for each attribute. A 1 min inter-stimulus interval including palate cleansing with deionised water was compulsory. Samples were assessed under two conditions: (a) with the nose open, and (b) with the nose occluded using a swimming nose clip (Slazenger, Shirebrook, UK). Two repetitions were collected for each sample under each condition. During each session 50% of the participants tested samples with the nose open, and 50% with the nose occluded, with the condition being reversed during the second session. Data was collected under Northern Hemisphere daylight lighting.

3.2.1.6 Data analysis

To determine if the divalent salts could be detected orthonasally during the triangle test, the number of correct identifications was tested for significance using binomial statistics (α=0.05) (BS ISO 6658: 2005). A three factor (sample, nose condition, replicate) analysis of variance (ANOVA) with interaction (sample*nose condition) and Tukey’s Honestly Significant Difference (HSD) post-hoc test were undertaken to identify where any differences existed across sample intensity ratings, using SPSS, version 21 (SPSS IBM, USA) (α=0.05).

3.3 HEADSPACE ANALYSIS

Headspace SPME and GC–MS were used to explore whether any volatiles were present in the sample headspace.

3.3.1 Samples

Samples were prepared using the chemicals in **Table 3.1** (0.0, 0.003, 0.3M), and consisted of 8 ml of solution placed in 20 ml amber glass headspace vials

that were commercially clean, and capped with Teflon-lined silicone crimp caps. Stimuli were tested at room temperature (22 ± 2°C).

3.3.2 GC-MS analysis

Samples were tested in a Thermo Scientific Gas Chromatography Mass Spectrometer (Thermo Scientific, Hemel Hempstead, UK). A Supelco solid phase microextraction (SPME) sampling unit was used, with a 50/30 nanometer DVB/CAR/PDMS Stableflex fibre which was exposed to the headspace of the vial for 10 minutes to extract the volatiles using an out of tray method. Fibre was desorbed in the injection port at 230°C, for five minutes and in splitless mode. A Trace GC Ultra was used to run GC analysis using a ZB-wax GC column (Phenomenex), which was 30 metres in length, 0.25 ID mm, 1.00 film thickness and using a helium flow rate constant pressure at 18 PSI. The temperature programme was 40°C for one minute, then heated to 250°C at 8°C/min and held for one minute. Mass spectrometry Dual Stage Quadrupole (DSQ) was run with a full scan for mass range of *m/z* 15-200 and an ion source temperature of 200°C, and mass scan starting at 0.5 minutes. Each sample was run in triplicate, and sample presentation order was randomised to eliminate order effects.

3.3.3 Data analysis

The National Institute of Standards and Technology library was used to identify compounds that were likely present in the samples. Background subtraction was undertaken to identify compounds present in the sample headspace that were not in the water control. The same method was used to compare differences across divalent salts, as well as the low and high concentration samples. A specific search for the selected mass fragments of 1-octen-3-one (mwt 126g/mol) and 1-nonen-3-one (mwt 140g/mol) was undertaken, as they

have previously been reported in the headspace of divalent salts (Lubran *et al*., 2005). Differences across the three replicates for each sample were compared for consistency. GC-MS data was processed and analysed in conjunction with Sharon Lim, a specialist GC-MS technician in the department of Food Science at the University of Nottingham, and were interpreted by the author.

3.4 RESULTS

3.4.1 Sensory characterisation

3.4.1.1 Experiment 1

Table 3.2 lists the number of correct identifications and related probability values for the triangle tests investigating orthonasal stimulation. $FeCl_2$ and $FeSO_4$ were the only samples discriminated from the water control.

Table 3.2: Frequency of correct identifications and p values in triangle tests.

	$CaCl_2$	$FeCl_2$	FeGlu	$FeSO_4$	$CuSO_4$
Correct response	10	22	7	15	11
p value	0.55	<0.001	0.90	0.03	0.36

3.4.1.2 Experiment 2

ANOVA showed that global intensity ratings differed across replicates for metallic ($p<0.001$) and astringency ($p=0.019$) only, where replicate one was rated higher than two. However, Tukey results showed that this difference across replicates was not significant ($p>0.05$) when analysing intensity ratings for these attributes at the individual sample level. The only significant interaction between sample and nose condition occurred with the metallic attribute, which was due to a magnitude effect where the ferrous salts were rated significantly more intense ($p<0.001$) than all other samples under the nose open condition. ANOVA showed that nose condition had an effect on global attribute intensity

rating as all attributes except for tingling ($p=0.254$) were rated higher ($p<0.05$) with the nose open compared to occluded.

Figure 3.1 shows metallic intensity ratings for all ferrous salts were significantly higher than the water control sample with the nose open ($p<0.05$), but not with the nose occluded ($p>0.05$). $CuSO_4$ was perceived significantly more metallic than water with the nose open ($p<0.001$) and occluded ($p=0.038$). $CaCl_2$ was not rated more metallic ($p>0.05$) than water under either nose condition.

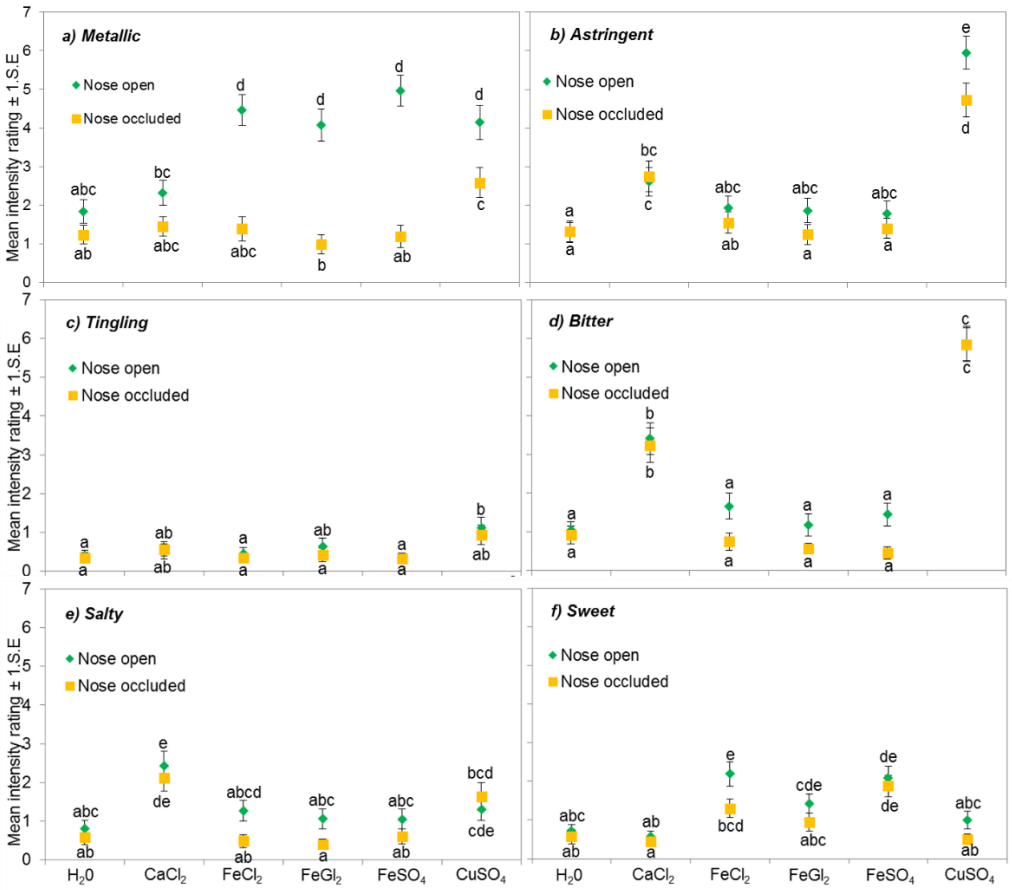

Figure 3.1: Attribute intensity ratings with the nose open and occluded. Mean intensity rating ± 1 standard error, for a) metallic, b) astringent, c) tingling, d) bitter, e) salty, and f) sweet. Bars with different letters ^{abcde} show significant differences ($p<0.05$) across samples and nose conditions according to the Tukey post-hoc test.

Tukey results for divalent salt attribute qualities significantly higher ($p<0.05$) than that of the water control are shown in **Table 3.3**. From these findings $CaCl_2$

was reported to be bitter, astringent and salty, whilst $CuSO_4$ was the most complex sample for which all attributes excluding sweet were reported. The only attributes rated significantly higher for ferrous salts than ratings for the water control were metallic and sweet. $FeSO_4$ was perceived to be sweet and metallic when the nose was open, and interestingly these attributes were rated no different to the water control when the nose was occluded. The option to report 'other' attributes was offered to avoid attribute dumping, and on occasion was used to report sourness for the FeGlu and $CuSO_4$ stimuli, although not frequently enough to report these ratings.

Table 3.3: Tukey post-hoc test results showing sample attribute intensity ratings compared to the water control under the nose open (NO) and nose closed conditions (NC) with significance level indicated; <0.05*, <0.01**, <0.001***.

Divalent salt	$CaCl_2$	$CaCl_2$	$FeCl_2$	$FeCl_2$	FeGlu	FeGlu	$FeSO_4$	$FeSO_4$	$CuSO_4$	$CuSO_4$
Nose condition	NO	NC	NO	NC	NO	NC	NO	NC	NO	NC
Metallic	2.32	1.46	4.47***	1.39	4.08***	0.99	4.97***	1.19	4.14***	2.58*
Astringent	2.61*	2.75**	1.94	1.55	1.87	1.24	1.79	1.41	5.95***	4.72***
Bitter	3.41***	3.24***	1.67	0.76	1.19	0.57	1.45	0.47	5.87***	5.84***
Tingling	0.52	0.57	0.46	0.33	0.64	0.42	0.32	0.33	1.12**	0.94
Sweet	0.57	0.45	2.19***	1.3	1.43	0.94	2.08***	1.88***	1	0.51
Salty	2.43***	2.11***	1.27	0.48	1.06	0.41	1.05	0.6	1.3	1.63**

3.4.2 Headspace analysis

Results across sample replicates were consistent, with the exception of $FeCl_2$ where ethyl ether, ethyl chloride, ethyl acetate, ethanol and ethyl chloroacetate were found in replicate one, but not replicate two or three. No compounds were identified in any other sample.

3.5 DISCUSSION

3.5.1 Orthonasal perception

Orthonasal stimulation by divalent salts has not been well researched, therefore its possible contribution to metallic perception is poorly understood. $FeSO_4$

solutions were not expected to be discriminated from the water control as they have previously been reported to produce little (Lubran et al., 2005) or no (Lawless et al., 2004) aroma, as they are not typically considered volatile. In contrast to Lawless et al. (2004) the current study found $FeSO_4$ was discriminated from water, indicating orthonasal stimulation is occurring (**Table 3.2**). This variance across studies could be due to the different sample concentrations of 1mM (Lawless et al., 2004) compared to 2.99 mM used in the current study, or the type of discrimination test used (Ennis et al., 2014).

Using SPME to collect volatiles in the sample headspace, and the human nose as a sensitive and selective detector of the odour active compounds using gas chromatography olfactometry (GCO), Lubran et al. (2005) identified the odorants 1-nonen-3-one and 1-octen-3-one, which were described by participants to be perceived as 'metallic' in the $FeSO_4$ sample headspace. Therefore, low level volatiles were expected to be identified during the headspace analysis, but were not detected in the current study. This may be due to differing sample temperature and purge times used across studies. Here GC-MS headspace analysis did not identify any volatiles present in the $FeSO_4$ or $FeCl_2$ sample that were not present in the water control, which could be because the GC-MS equipment is not sensitive enough to detect the compounds perceived by the human nose. Sample detection during the triangle test could arise from perception of low concentration volatiles released from the sample itself, or another hypothesis being that volatiles released from the sample cause lipid oxidation upon contact with tissue in the nasal cavity, and it is the by-products that are detected, as found in the oral cavity (Omur-Ozbek et al., 2012). When sniffed orthonasally $FeSO_4$ has been described as eliciting a 'tingling irritation' (Lubran et al., 2005), and so another question that arises is whether the reported sensation is due to an aroma and/or trigeminal response.

Volatiles activating nasal trigeminal receptors initiate sensations such as irritation, freshness, stinging, prickliness, burning and tingling, which collectively can be classified as 'pungent' (Cometto-Muniz and Hernandez, 1990). Decoupling olfactory and trigeminal responses is problematic as identical compounds commonly activate both sensory systems (Comettomuniz et al., 1989). Compounds which were not present in the water control were detected in the headspace of replicate one of the $FeCl_2$ sample. As they were not present in replicate two or three and are not typically associated with $FeCl_2$, this is likely due to some form of contamination.

Primary limitations relating to the headspace analysis conducted in this experiment were firstly that the sensitivity of the equipment may not detect volatiles that are perceived by the highly sensitive human nose, and secondly GC-MS only measured volatiles in the sample headspace, which does not take into account how they may interact with tissue in the nasal cavity. It would be of interest to explore theories relating to orthonasal perception of divalent salts in greater detail, which was unfortunately beyond the scope of this PhD project. One way to do this would be to use Atmospheric Pressure Chemical Ionisation-Mass Spectrometry (APC-MS), which allows volatiles in the nasal cavity to be sampled by inserting a tube into the nostril (Taylor et al., 2000). This instrument is typically used to detect volatiles that are exhaled via the retronasal pathway during ingestion of stimuli in the mouth, but could be inserted into the nostril immediately after orthonasal stimulation of the ferrous salt solution. In a similar way to GC, APCI separates the sample components, and MS identifies them. If volatiles which are not detected in the sample headspace by GC-MS are present in the nasal cavity this would indicate they are lipid oxidation by products. Further work should also be conducted to identify descriptive terms used to describe the sensation perceived when sniffing the sample headspace

of $FeSO_4$ and $FeCl_2$, in order to determine if the sensation is due to trigeminal and/or olfactory stimulation.

Results from the orthonasal sensory testing indicate that volatiles released from ferrous salts could impact metallic perception more than once thought, and thus highlights the need for more research investigating this quality. A description of the attribute quality detected when orthonasally sniffing the sample headspace was not collected, but further exploration is recommended.

3.5.2 Retronasal and oral metallic perception

The influence of nasal occlusion was hypothesised to differ across divalent salts. In line with previous research (Lim and Lawless, 2006), **Figure 3.1a** shows that occluding the nose significantly reduced ($p<0.05$) the intensity of the metallic sensation reported for ferrous salts, supporting the hypothesis that retronasal stimulation is the key driver of metallic for these salts. When $FeSO_4$ comes into contact with skin on the hand (Glinderman et al., 2006) and oral cavity (Omur-Ozbek et al., 2012) lipid oxidation occurs, causing the formation of C_6 to C_{10} *n*-alkanals, aldehydes (formaldehyde, acetaldehyde and proprionaldehyde) and ketones (with 1-octen-3-one accounting for about 1/3 of the total odour), which are thought to stimulate the retronasal pathway and elicit a metallic sensation. ANOVA showed the global intensity rating for metallic was higher ($p<0.001$) on replicate one than replicate two, although this was not seen with the Tukey post hoc analysis at the individual sample level. This global effect could be due to a reduced rate of lipid oxidation and subsequent metallic perception on replicate 2, therefore future testing could benefit from increased palate cleansing time between samples, or a reduction in the number of samples tested per session. Increased 'metallic' smelling volatiles were found

in the headspace of $FeSO_4$ samples at 37°C but not 22°C during GCO (Lubran et al., 2005), suggesting the temperature inside the mouth may stimulate the release of volatiles that are not associated with lipid oxidation, but may be detected retronasally and contribute to metallic perception. $FeSO_4$ (≥ 5mM) can be discriminated from water with the nose occluded (Lim and Lawless, 2005b), and when asked to describe the sensation assessors reported bitter, sour, sweet, astringent, metallic and electric. When applying the same sample to a non-gustatory part of the lip the solution could not be discriminated, suggesting there may be a gustatory component to metallic perception. The current study reports a different response for $CuSO_4$; whilst occluding the nose reduced the metallic rating ($p=0.006$), it remained higher ($p=0.038$) than that of the water control. $CuSO_4$ also induces lipid oxidation and the subsequent volatile release (Omur-Ozbek et al., 2012), which explains the difference observed across nose conditions. However, this does not explain the metallic quality reported under the nose occluded condition both here and in previous studies (Epke et al., 2009; Lawless et al., 2004). The same result is also seen for both solid metal stimuli and electrical stimulation of the tongue (Lawless et al., 2005), which has led to the hypothesis that different mechanisms may be involved in metallic perception reported across stimuli. Transient receptor potentials (TRP) are a family of cation channels involved in the transduction of chemical stimuli into taste, olfaction and trigeminal sensations, and have been associated with the perception of divalent salts (Riera et al., 2007). One possible mechanism is the involvement of TRPV1, TRPM5 and T1R3. When expressed in cultured cells in vitro, the TRPV1 was activated not only by artificial sweeteners which have been found to evoke a metallic quality, but also by solutions of $FeSO_4$, $CuSO_4$ and zinc salts, thus suggesting it may be involved in metallic perception (Riera et al., 2007). Comparing the behavioural response to divalent salts in wild type (WT), TRPV1 knockout (KO), TRPM5 KO and T1R3 KO mice, Riera et al (2009)

found these channels likely to influence perception as measured preference for divalent salts differed across the mice. However, divalent salts have multiple sensory attributes making it difficult to pinpoint which of these are affecting sample perception (Spence et al., 2015) and the hedonic differences observed.

No difference in metallic rating for $CaCl_2$ was reported when compared to water with the nose open or occluded ($p>0.005$). Although it has previously been reported as metallic, the rating has not always been compared to a water control (Lawless et al., 2003, Yang and Lawless, 2005) and the metallic perception could, at least in part, be attributed to the metallic quality reported for deionised water (Dalton et al., 2000). However, Lawless et al (2004) found that metallic intensity varied across different calcium anions, indicating a metallic component that was not observed in the current study may be present.

3.5.3 Oronasal qualities of divalent salts

An additional objective was to determine the non-metallic qualities reported for the samples, and differences across ferrous salts. A range of qualities were expected as divalent salts are complex in nature. Attributes discussed in this section were identified as those reported significantly more intense ($p<0.05$) than that in the water control sample, **Table 3.3**. Occluding the nose did not significantly affect bitter and astringency ratings, which is typically expected for gustatory and trigeminal stimuli (Lim and Lawless, 2006). A reduction in sweetness was reported with nasal occlusion for the $FeCl_2$ sample ($p=0.028$), but not for $FeSO_4$ ($p=1.00$). One hypothesis being that the perceived sweetness for $FeCl_2$ is a result of sweet 'smelling' volatiles that are perceived as taste due to gustatory referral (Lim and Johnson, 2012). Alternatively, volatiles detected from this sample enhanced sweet perception when the nose was open, as is

often seen with taste aroma interactions (Noble, 1996), including enhancement of sweetness (Pfeiffer et al., 2005). Saltiness was only reported for $CuSO_4$ under the nose occluded condition, perhaps because the intense metallic sensation dominates perception under the nose open condition. Tingling was reported for $CuSO_4$ with the nose open but not occluded, indicating the sensation originates from nasal stimulation as a result of pungency produced by volatiles that are detected retronasally (Cometto-Muniz and Hernandez, 1990). Volatiles could be released from lipid oxidation, or directly from the sample due to the temperature increase in the oral cavity, which would explain why they were not detected orthonasally.

Sweet was reported for the ferrous salts, whilst attributes reported in prior literature are bitter, astringent, sweet, sour, salty (Lim and Lawless, 2006), soapy and sulphurous (Hettinger et al., 1990). $CuSO_4$ was found to elicit bitter, astringent, salty and tingling. Prior research has found copper salts to be astringent, bitter (Lawless et al., 2004) and sour (Epke et al., 2009). $CaCl_2$ was found to be bitter, astringent and salty, whilst previously reported attributes are bitter, salty, sour, umami and astringent (Lawless et al., 2003; Yang and Lawless 2005). Potential reasons for the limited attribute qualities reported in this study compared to those evidenced elsewhere are multifactorial; here naïve participants were used, in comparison to a trained panel that has previously been used to provide detailed descriptive profiles (Yang and Lawless, 2005; Epke et al., 2009). The attributes which participants were asked to rate were limited to six to avoid them becoming overwhelmed. Although participants were given the option to report ‘other’ perceived attributes, this restricted list may have reduced the qualities reported as attributes are more likely to be rated when listed as opposed to free choice profiling (Lawless et al., 2005). Another consideration being that the sample concentration affected the qualities that

were perceived across studies (Murphy and Cain, 1980). Divalent salt attribute qualities change over time (Yang and Lawless, 2006), therefore the point at which the intensity rating is taken (immediate or aftertaste) may also have contributed to the variability.

The anion can affect the sensory qualities exhibited by divalent salts. Here differences across ferrous anions were observed; unlike FeGlu, $FeCl_2$ and $FeSO_4$ had a perceivable orthonasal aroma, and were sweet. Similar, but more detailed, anion effects for ferrous salts have been evidenced by a number of researchers (Lawless et al., 2003, Lim and Lawless, 2006, Yang and Lawless, 2005, Yang and Lawless, 2006). Greater differences were therefore expected across ferrous salts in the current study. One explanation being participants in the current study received only basic training on sensory attributes, compared to more detailed approaches adopted in other studies. It would be interesting to further explore, with attention focussed on differences in headspace volatiles.

The most prominent hypothesis relating to metallic perception associated with ferrous salts is that lipid oxidation of tissues in the oral cavity releases volatiles which are detected via the retronasal pathway as a flavour perception, a sensation which is eliminated by occluding the nose. However, the failure of nasal occlusion to eliminate the metallic sensation for $CuSO_4$ indicates a taste or trigeminal component may contribute to metallic perception. Utilising fMRI as a tool to measure brain activation associated with the $FeSO_4$ consumption is an innovative way to explore the origins of this sensation further, which could contribute to determining which sensory modalities are involved in the response.

3.6 CONCLUSIONS

These results show that both $FeCl_2$ and $FeSO_4$ were discriminated from water when orthonasally sniffing the sample headspace, indicating orthonasal stimulation may contribute to their perception more than previously recognised. This could be due to the detection of volatiles released from the sample itself. However, headspace analysis of the samples did not identify volatiles which could explain this. It is therefore possible that perception is due to the release of lipid oxidation by products that are produced when sample volatiles come into contact with tissue in the nasal cavity. Future work, which was beyond the scope of this PhD, should include using AP-CI-MS to explore whether volatiles that are not present in the divalent salt sample headspace are present in the nasal cavity after orthonasally sniffing the sample headspace, which would indicate if lipid oxidation is occurring in the nasal cavity. Additionally, descriptive terms should be acquired for the sensation/s perceived when orthonasally sniffing the sample headspace, to identify if this contributes to metallic perception. Occluding the nose when tasting ferrous salts reduces metallic perception, indicating retronasal stimulation is an important component of perception for these samples. However, metallic is still perceived for $CuSO_4$ when the nose is occluded, suggesting a second gustatory or trigeminal mechanism is involved. This work contributes to the ongoing debate over metallic being a taste, trigeminal or flavour response. Although metallic may have a gustatory component, particularly for $CuSO_4$, it is thought that defining it as a taste could be a misnomer, particularly when referring to the sensation which arises from $FeSO_4$, therefore metallic 'sensation' may be a more accurate description. These results guide the interpretation of the brain activation in response to $FeSO_4$ when sprayed into the mouths of participants being scanned using fMRI, as detailed in Chapter 4.

4 MEASURING VARIATION IN ORAL SENSITIVITY ACROSS TASTE PHENOTYPE AND GENOTYPE

4.1 INTRODUCTION

In Chapter 2, a set of prototypical (sweet, sour, salt, bitter, umami) and purported (metallic) taste stimuli were developed. Divalent salts elicit a metallic quality, and have complex sensory profiles that are thought to exhibit gustatory, trigeminal and flavour responses. The nature of these oronasal qualities was further explored in Chapter 3, where a range of divalent salts reported to elicit a metallic sensation were evaluated.

The aim of the current chapter was to use the tastant stimuli developed in Chapter 2 to measure the variation in taste perception across taste phenotypes and genotypes. Sensory evaluation and genotyping techniques were performed to explore the relationship between 6-n-propylthiouracil (PROP) taster status (PTS), fungiform papillae density (FPD), TAS2R38 and gustin rs2274333 genotypes and taste sensitivity. fMRI data was collected to explore variation in the cortical response to tastants across PTS groups.

4.1.1 PTS and oronasal sensitivity

Individual variation in the ability to perceive the bitterness of phenylthiocarbamide (PTC) (Blakeslee and Fox, 1932) and PROP is well evidenced. Individuals can be categorised as PROP non tasters (PNTs), PROP medium tasters (PMTs) or PROP supertasters (PSTs) according to the bitter intensity perceived (Bartoshuk, 1993, Bartoshuk et al., 1994). Increased

sensitivity to PTC/PROP is often considered a marker of oral sensitivity, and associated with increased sensitivity to gustatory stimuli including:

- Sweet (Gent and Bartoshuk, 1983, Lucchina et al., 1998, Clark, 2011, Drewnowski et al., 1997a, Bartoshuk, 1979, Yang et al., 2014, Bajec and Pickering, 2008, Yeomans et al., 2007, Melis and Barbarossa, 2017).
- Bitter (Yang et al., 2014, Ly and Drewnowski, 2001, Yackinous and Guinard, 2002, Bartoshuk et al., 1988; Hall et al., 1975; Masi et al, 2015, Melis and Barbarossa, 2017, Bajec and Pickering, 2008; Gent and Bartoshuk, 1983; Dinehart et al., 2006; Pickering et al., 2006).
- Salt (Miller and Reedy, 1990 b; Bartoshuk et al., 1998, Yeomans et al., 2007, Hayes et al., 2010, Yang et al., 2014, Bajec and Pickering, 2008).
- Sour (Bajec and Pickering, 2008, Yang et al., 2014, Webb et al., 2015, Melis and Barbarossa, 2017).
- Umami (Melis and Barbarossa, 2017) stimuli.

Conversely, in other studies no relationship has also been reported between the perceived intensity of PROP and the following stimuli:

- Sweet (Drewnowski et al., 1997b, Smagghe and Louis-sylvestre, 1998, Ly and Drewnowski, 2001)
- Bitter (Clark, 2011, Schifferstein and Frijters, 1991, Smagghe and Louis-sylvestre, 1998, Webb et al, 2015).
- Salt (Schifferstein and Frijters, 1991, Drewnowski et al., 1998, Clark, 2011, Melis and Barbarossa, 2017).
- Sour (Clark, 2011, Webb et al, 2015).
- Umami stimuli (Webb et al., 2015).

Increased sensitivity to PTC/PROP has been associated with increased sensitivity to trigeminal stimuli including:

- Capsaicin (Yang et al., 2014, Prescott and Swain-Campbell, 2000, Tepper and Nurse, 1997, Tepper and Nurse, 1998, Karrer and Bartoshuk, 1991).
- Temperature (Yang et al., 2014; Bajec and Pickering, 2008; Manrique and Zald, 2006; Clark, 2011).
- Aluminium sulphate (Bajec and Pickering, 2008; Pickering et al., 2006)
- Cinamaldehyde (Prescott and Swain-Campbell, 2000).
- Ethanol (Prescott and Swain-Campbell, 2000, Duffy et al., 2004a, Duffy et al., 2004b).
- Carbonation (Prescott et al., 2004).

Additionally, PSTs exhibit greater tactile acuity when the tongue is stimulated with Von Frey Filaments (Yackinous and Guinard, 2001), and are better at identifying letters placed on the tongue when measuring tactile acuity (Essick et al., 2003) compared to PNTs. Interestingly, this taster advantage is not always limited to the oral cavity, and in some cases has been observed for retronasal (Pickering et al., 2006, Yang et al., 2014) and orthonasal aroma (Yang et al., 2014) suggesting a central gain mechanism may be responsible.

PTS has also been associated with the perception of attributes in more complex products. For example PROP tasters rate the sourness of yoghurt (Prescott et al., 2004), saltiness of pretzels and cheese (Hayes et al., 2010), bitterness of grapefruit (Drewnowski et al., 1997c), sweetness of soft drinks (Zhao and Tepper, 2007), astringency of coffee (Masi et al., 2015), and the creaminess of dairy fats (Hayes and Duffy, 2007) as more intense than non-tasters, and are more able to discriminate between varying fat concentrations in salad dressings

(Tepper and Nurse, 1997, Tepper and Nurse, 1998). This has led to extensive exploration into the influence of PTS on food behaviour, weight status and health, as briefly discussed in Section 1.2.1.

Although a number of associations have been made between PTS, oronasal sensitivity, dietary behaviours and health, the lack of consistent evidence makes drawing definitive conclusions problematic. Taste sensitivity has been proposed as one of the key determinants for food preference (Tepper, 2008). In addition to PTS, other factors such as ethnicity, culture, taste genotype and phenotype, nutritional status and dietary intake also influence taste sensitivity. These factors are not always controlled for, and therefore contribute to the lack of consistent findings observed across studies. Another major source of variation is the diverse range of PTC/PROP taster status classification methods adopted.

Taster status has been assessed using both PTC and PROP, delivered in a range of mediums, including filter paper (Ly and Drewnowski, 2001), edible taste strips (Smutzer et al., 2013), solution applied to the tongue by cotton bud (Green and George, 2004) or as a whole mouth rinse (Prescott et al., 2004). Filter papers have been criticised due to the challenge of ensuring even distribution of solution across papers (Tepper, 2008). Identifying detection (Gent and Bartoshuk, 1983) or recognition (Hong et al., 2005) thresholds were traditionally considered the gold standard for separating non-tasters from tasters. However, the emergence of sub categorising tasters into PMTs and PSTs meant this was no longer the best way to divide groups as the distribution of detection thresholds for PMTs and PSTs overlapped, whilst the use of suprathreshold concentrations allows categorisation of PNTs, PMTs, and PSTs (Tepper et al., 2001). A single stimulus can be administered, or up to five different concentrations across multiple stimuli (Dinehart et al., 2006).

Alternatively, a combination of threshold and suprathreshold measurements can be used (Drewnowski et al., 1997a). Traditionally testing involved comparing PROP intensity ratings to that of a reference sodium chloride (NaCl) sample using magnitude estimation (Lucchina et al., 1998), as NaCl was believed to be independent of PROP sensitivity. However, this method has become controversial with the discovery that PROP tasters may have an overall heightened sensitivity to other oral stimuli, including NaCl. Audio sounds can be used as an alternative reference sample believed to be unrelated to PROP sensitivity (Tepper, 2008). Other researchers simply divided the population group into the percentage of each PTS groups expected in an average population, 25% PNTs, 50% PMTs, 25% PSTs (Prescott and Swain-Campbell, 2000). However, population groups can vary considerably (Reed et al., 1995), indicating this method may inaccurately categorise a group as it does not account for population variables (Tepper, 2008). More recently researchers have used numerical cut off points for intensity ratings of suprathreshold PROP stimuli. This has been adopted using the Labelled Magnitude Scale (LMS) where the upper limit is ‘strongest imaginable taste or oral stimulus’ (Tepper et al., 2001). However, this is again controversial when PTs are believed to have overall heightened oral sensitivity. Alternatively, the general labelled magnitude scale (gLMS) can be used as it has an upper anchor point of ‘strongest imaginable sensation of any kind’ which is thought to be independent of PTS (Lim et al., 2008, Clark, 2011, Yang et al., 2014). Ultimately, this lack of standardisation across studies contributes to the conflicting results reported across literature, and highlights the demand for more standardised approaches to be adopted across research groups.

A number of factors are associated with the variation in sensitivity to PROP, including TAS2R38 and gustin genotypes, and FPD, which are discussed below.

4.1.2 PTS and TAS2R38 genotype

The TAS2R38 gene expresses the TAS2R38 receptor responsible for detecting the N-C=S group of thiourea containing compounds such as PTC and PROP (Bufe et al., 2005). Genetic variation is associated with sensitivity to PROP, as receptor functioning is thought to be reduced when three single nucleotide polymorphisms (SNPs) on the TAS2R38 gene result in amino acid substitutions at positions 49 (alanine49proline), 262 (valine262alanine) and 296 (isoleucine296valine) (Kim et al., 2003). This results in two main haplotypes; the dominant allele PAV is associated with the taster variant, and PAV:PAV homozygotes with PSTs, whilst the recessive allele AVI is associated with non-tasting, and AVI:AVI homozygotes with PNTs (Kim et al., 2003, Prodi et al., 2004, Bufe et al., 2005, Duffy et al., 2010, Khataan et al., 2009, Calo et al., 2011, Melis et al., 2013b, Barbarossa et al., 2015, Yang, 2015, Shen et al., 2016, Barajas-Ramirez et al., 2016; Sollai et al., 2017). PAV/AVI heterozygotes are associated with a wider variance of sensitivity, and PMTs (Yang, 2015, Barajas-Ramirez et al., 2016, Bufe et al., 2005), and rare haplotypes AAV, AVV, PAI, PVI, AAI, and PVV also occur (Boxer and Garneau, 2015, Risso et al., 2016). Differences in the TAS2R38 genotype have been found to explain 50% (Melis et al., 2013b) - 85% (Kim et al., 2003) of the variance in response to PTC, and 43% (Bering et al., 2014) - 65% of the variance in PROP intensity ratings (Tepper et al., 2008). Further supporting these findings, cells that express the PAV receptor are activated by micromolar concentrations of PTC and PROP, whilst those expressing the AVI receptor do not respond to concentrations as

high as 1mM, and PVI, AAI and AAV halotypes were activated to a lesser degree indicating intermediate sensitivity (Bufe et al., 2005). Although an association between TAS2R38 genotype and PTS is frequently observed, the link to overall taste sensitivity is not clear as little or no difference is frequently reported across TAS2R38 genotypes (Yang, 2015, Barajas-Ramirez et al., 2016, Smutzer et al., 2013, Duffy et al., 2010, Melis and Barbarossa, 2017).

TAS2R38 genotype is consistently associated with PTS, but does not completely explain the differing sensitivity to PROP that is observed across groups. Therefore, other factors such as salivary protein composition and FPD may also be influential, and are discussed in the following sections.

4.1.3 PTS and Gustin genotype

Whilst salivary flow rate is not thought to be associated with PTS (Bajec and Pickering, 2008), associations have been made with PTS and the chemical composition and physical properties of salivary proteins. One that has received much attention is the zinc dependant metalloprotein gustin (carbonic anhydrase VI, or CA6) (Henkin et al., 1975), thought to be involved in CO_2 and ion transport in human tissue, and oral pH homeostasis (Kivela et al., 1999). Gustin acts on taste bud stem cells to promote growth and development (Henkin et al., 1999a), and is associated with gustatory functioning. Reduced gustin secretion in the saliva is associated with altered taste and smell functioning, and distorted taste bud anatomy (Henkin et al., 1999b). Interestingly, as zinc modulates the functioning of gustin at its active site, zinc treatment in individuals suffering from taste and/or smell disorders can be effective in increasing salivary gustin, and therefore improving gustatory functioning and taste bud morphology in deficient individuals (Shatzman and Henkin, 1981, Henkin et al., 1999a). The gustin gene

polymorphism rs2274333 (A/G) results in substitution of the amino acid at position Serine90Glycine, which is believed to result in structural changes and reduced functionality of the gustin protein, altering the active site and leading to both reduced zinc binding and gustatory functioning (Padiglia et al., 2010). This polymorphism has been associated with sensitivity to perceive PROP, with early studies showing that the AA genotype was more frequently carried by PSTs, the GG genotype by PNTs, and PMTs typically carry at least one A allele (Padiglia et al., 2010, Calo et al., 2011, Melis et al., 2013b). However, recent studies found no significant association between this polymorphism and PTS (Feeney and Hayes, 2014, Bering et al., 2014, Yang, 2015, Barbarossa et al., 2015, Shen et al., 2016, Shen et al., 2017). These contradictory findings may be caused by population based differences across studies (Barbarossa et al., 2015), and functional differences in genes between ethnic groups (Shen et al., 2017). Little or no difference in oral sensitivity is observed across gustin rs2274333 genotype (Yang, 2015, Feeney and Hayes, 2014, Shen et al., 2017).

4.1.4 PTS, FPD, and TAS2R38 and gustin genotypes

Variation in the perceived bitterness of PROP is not fully explained by the TAS2R38 and gustin genotypes, indicating other factors are involved. FPD is highly variable across individuals (Miller and Reedy, 1990a), and is thought to be associated with PTS. Taste buds, taste pores, and mechanoreceptors, are located in fungiform papillae, which are innervated by both gustatory and trigeminal nerve fibres (Whitehead et al., 1985). Therefore, FPD is thought to indicate innervation density, receptor number, and nerve activation, which has been associated with increased sensitivity to PROP (Bajec and Pickering, 2008, Tepper and Nurse, 1997, Yackinous and Guinard, 2001, Tepper and Nurse, 1998, Tepper, 1998, Yackinous and Guinard, 2002, Essick et al., 2003, Miller

and Reedy, 1990b,Yeomans et al, 2007, Hayes et al., 2008; Melis et al, 2013b, Sollai et al., 2017, Melis and Barbarossa, 2017, Nachtsheim and Schlich 2013; Hayes et al, 2007; Hayes et al., 2010, Bartoshuk et al., 1994, Duffy and Bartoshuk, 2000, Bakke and Vickers, 2008; Duffy et al., 2010; Duffy et al, 2004a; Duffy et al., 2004b; Essick et al, 2003). However, other studies have failed to identify a difference in FPD across PTS groups (Yang, 2015, Fischer et al., 2013, Delwiche et al., 2001, Bakke and Vickers, 2011, Masi et al 2015; O'Brien et al, 2010).

Higher FPD has also been associated with increased sensitivity to other oral stimuli (Delwiche et al., 2001, Masi et al., 2015, Zhang et al., 2009, Hayes et al., 2008, Hayes et al., 2010, Tepper and Nurse, 1997, Zuniga et al., 1993, Miller and Reedy, 1990b, Proserpio et al., 2016, Nachtsheim and Schlich, 2013, Prutkin et al., 2000, Duffy et al., 2004b, Essick et al., 2003), whilst others have not replicated these findings (Fischer et al., 2013, Feeney and Hayes, 2014, Webb et al, 2015).

The genetic basis for variation in FPD is unknown, but associations between TAS2R38 and gustin rs2274333 have been explored. Whilst no significant difference in FPD across TAS2R38 genotypes is frequently reported (Duffy et al., 2004b, Hayes et al., 2008, Duffy et al., 2010, Yang, 2015, Barbarossa et al., 2015), others have identified PAV homozygotes and/or heterozygotes to have a higher FPD (Melis et al., 2013b, Shen et al., 2016, Sollai et al., 2017).

Associations between FPD and the gustin rs2274333 polymorphism have also been made. The GG genotype has been associated with a lower FPD (Melis et al., 2013b, Barbarossa et al., 2015), and fungiform papillae that are larger in diameter and anatomically distorted in shape (Melis et al., 2013b). However,

Yang (2015) found the opposite, showing that GG had significantly higher FPD, and others identified no differences across groups (Feeney and Hayes, 2014, Yang, 2015, Shen et al., 2016, Shen et al., 2017). The gustin gene has been proposed to be more closely related to FPD and morphology than the TAS2R38 genotype, and in some cases has been found to predict 13-16% of the variance in FPD (Barbarossa et al., 2015, Melis et al., 2013b).

4.1.5 Central taste processing

Central processing of taste was detailed in Section 1.3.3. In summary, gustatory signals are transmitted via the cranial nerves and converge in the rostral nucleus of solitary tract (Verhagen and Engelen, 2006, Small, 2012, Rolls, 2016) before projecting to the ventral-posterior-medial (VPM) thalamus, and on to the anterior insula and/or adjoining frontal operculum (Small et al., 1999, Faurion et al., 2005, Verhagen and Engelen, 2006, Kurth et al., 2010, Veldhuizen et al., 2011, Small, 2012, Rolls, 2016, Yeung et al., 2017). Further projections transmit to the orbitofrontal cortex (OFC) (Small et al., 1999, Faurion et al., 2005, Verhagen and Engelen, 2006, Veldhuizen et al., 2011, Small, 2012, Rolls, 2016). Other areas associated with taste processing include the mid insula (Kurth et al., 2010, Veldhuizen et al., 2011, Small, 2012, Rolls, 2016, Yeung et al., 2017), thalamus, post central gyrus (Veldhuizen et al., 2011, Yeung et al., 2017), rolandic operculum, posterior insula, anterior cingulate cortex (ACC) (Veldhuizen et al., 2011, Rolls, 2016), amygdala (Faurion et al., 2005, Small, 2012, Rolls, 2016, Yeung et al., 2017), hippocampus and caudate (Yeung et al., 2017).

4.1.6 Variation in cortical processing across taste phenotypes

Despite the growing evidence exploring perceptual responses to oral stimuli across PTS, only two studies have compared the brain response across PTS phenotypes, both conducted using fMRI in a collaboration between the Sir Peter Mansfield Imaging Centre (SPMIC) and Sensory Science Centre, University of Nottingham (Clark, 2011, Eldeghaidy et al., 2012). A trend for PROP tasters to have higher brain activation than PNTs in response to gustatory-trigeminal (sweet-CO_2) stimuli has been observed, and reached a significant level of difference when comparing PSTs to PNTs in the SI, SII and ACC (Clark, 2011). PROP intensity ratings have also been positively correlated with cortical activation in areas associated with somatosensory (SI, SII, mid insula) and taste (anterior insula) processing in response to fat stimuli (Eldeghaidy et al., 2012). The observed differences across PTS groups in these studies supports the need to better understand the influence of PTS on the central processing in response to a wider range of oral stimuli.

4.1.7 Aims

The overall aim of this study was to combine sensory evaluation and genotyping techniques with fMRI, to explore variation in the perceptual and cortical response to taste across PTS groups, and to explore the relationship between PTS, FPD, TAS2R38 and gustin rs2274333 genotypes, and their influence on taste sensitivity. The specific objectives were to:

- Identify the perceived intensity of the tastant stimuli that were developed in Chapter 2, for each individual participant, and the average intensity across all participants. The average intensity (of the combined ratings from all participants) was expected to fall between moderate and strong

on the gLMS for each tastant, as this had been reported in the experiments detailed in Chapter 2.

- Determine the relationship between PTS and the TAS2R38 and gustin rs2274333 genotypes. It was hypothesised that the TAS2R38 genotype would be associated with PTS, as AVI homozygotes are typically associated with PNTs, PAV homozygotes with PSTs, and heterozygotes with PMTs. Whether a relationship would be identified between PTS and gustin rs2274333 was unknown, as previous findings associated with this genotype are inconsistent.
- Identify the relationship between FPD, PTS, and TAS2R38 and gustin rs2274333 genotypes. It was hypothesised that FPD would be higher in PSTs than PNTs, as this is frequently reported in previous literature. It was unclear whether a difference would occur between TAS2R38 or gustin rs2274333 genotypes as the evidence reporting these associations is more limited, and findings are conflicting.
- Compare perceived taste intensity across PTS groups, TAS2R38 and gustin rs2274333 genotypes, and FPD. PSTs were expected to rate stimuli more intensely than PNTs and/or PMTs, whilst it was unknown whether an association would be identified across genotypes, due to limited previous research exploring these associations. It was hypothesised that FPD and taste sensitivity would be positively correlated, as this is evidenced frequently in previous literature.
- Measure if there is an association between sensitivity to PROP, and cortical activation in the insula cortex. It was hypothesised that increased sensitivity to PROP would be associated with increased cortical activation in the anterior insula, which has been proposed as the primary gustatory cortex.

4.2 MATERIALS AND METHODS

This study was performed under the umbrella of the BBSRC and Unilever funded multidisciplinary project 'TASTEMAP' (BBSRC grant number BB/L000458/1), which is a collaboration between the Sensory Science Centre and Sir Peter Mansfield Imaging Centre (SPMIC) at the University of Nottingham. The experiments detailed in this chapter include a series of sensory evaluation tests to classify PTS, thermal taster status, FPD, identify TAS2R38 and gustin rs2274333 genotypes, and determine the perceived intensity of tastant stimuli. In addition, the brain response to tastants was measured using functional Magnetic Resonance Imaging (fMRI). The author's responsibilities within this study were recruitment and screening of participants, preparation of all samples, collection of sensory data and genotyping samples, analysis and interpretation of all sensory evaluation and genotyping data. Within the fMRI sessions, responsibilities also included assisting with setting up of the gustometer, preparation of all samples, providing an explanation of the fMRI scan session to participants, and setting participants up in the MR scanner. All fMRI data was acquired, processed and analysed by Dr Sally Eldeghaidy (SPMIC).

The study had ethical approval from the University of Nottingham Medical Ethics Committee (C13032014). A recruitment email requiring respondents to complete a basic health questionnaire (Appendix 1) and MR safety questionnaire (Appendix 2) was sent to staff and students at the University of Nottingham, and to participants who had participated in previous studies through the Sensory Science Centre. All participants were healthy non-smokers, age 19-41 years old, with no known taste or smell abnormalities or food allergies to the stimuli being tested. Written informed consent was given,

and an inconvenience allowance for participating was provided. Forty-one participants were recruited to complete the two sensory evaluation screening sessions which included phenotyping for PROP and thermal taster status. Of these, 30 participants (11 male) were recruited onto the study according to PTS and thermal taster status, and invited to attend the fMRI scan. Participants were instructed not to consume anything other than water for at least one hour prior to all test sessions.

4.2.1 Tastant stimuli

The tastant samples developed in Chapter 2 were used during the sensory evaluation and fMRI scan sessions. Samples were prepared on the day of testing using deionised water, and were delivered at room temperature (22 ± 2°C). The ferrous sulphate ($FeSO_4$) sample was made fresh every three hours to limit oxidation. Sample concentrations were: glucose (117.32g/L), citric acid (1.5 g/L), monosodium glutamate (MSG) (20 g/L), $FeSO_4$ (0.834 g/L), sodium chloride (NaCl) (10 g/L), and quinine sulphate (0.017 g/L). Deionised water was used for all palate cleansing.

4.2.2 Sensory Evaluation Session 1

4.2.2.1 gLMS training

Training on correct use of the gLMS was conducted following the methods described in Section 2.2.4.3, using the procedure detailed by Bartoshuk et al (2002). In summary, this involved participants rating the intensity of their ‘strongest imaginable sensation of any kind’, and 15 remembered or imagined sensations on a blank gLMS. This exercise improves understanding on scale

use (Hayes et al., 2013) and creates a reference gLMS which is presented during all subsequent testing, and used to guide sample intensity ratings.

4.2.2.2 Taste sensitivity

An exercise to measure taste sensitivity was conducted, where tastants were presented in plastic lidded medicine cups. Participants were first familiarised with known taste samples (sweet, sour, salt, bitter, umami, metallic) (15ml), which were labelled with the taste quality represented, for example glucose as 'sweet'. A one-minute inter-stimulus interval was given between tasting samples, to allow the palate to recover. During this time participants were instructed to cleanse the palate by rinsing with deionised water at least three times. Unknown tastant samples (5ml) were then presented with random three digit codes (two replicates), which were randomised and balanced across participants. To standardise testing participants were trained to consume the whole sample and lift the tongue to the palate three times before swallowing. They were instructed to rate the perceived intensity when it reached its maximum on a blank gLMS (Compusense Five 5.4, Canada). To allow the palate to recover, and prevent fatigue, participants were given a five minute break halfway through the test.

4.2.2.3 PTS classification

PTS was determined following methods described by Lim et al (2008). PROP solution (0.32mM) (Sigma Aldrich, UK) was presented (5ml) in a medicine cup labelled with a random three digit code. Participants were instructed to apply the solution by rolling a cotton bud (Boots pharmacy, UK) that had been saturated in the solution across the whole tongue for approximately three secs,

before retracting the tongue into the mouth and actively tasting the solution by moving the tongue across the hard palate using a 'smacking' motion. They were instructed to rate the perceived intensity when it reached its maximum on a blank gLMS (Compusense Five 5.4, Canada). A two minute break was given for palate recovery and palate cleansing with deionised water, before a second replicate was delivered. The mean of the two replicates of PROP intensity ratings was calculated for each participant, and used to identify PTS. Ratings below barely detectable (1.4% on gLMS) were classified as PNTs, ratings between barely detectable and moderate (1.4% -17% on gLMS) were classified as PMTs, and those rating anywhere above moderate (17% on gLMS) were classified as PSTs (Lim et al., 2008)

4.2.2.4 FPD measurement

A method to measure FPD from digital photographic images of the tongue was adapted from previous methods reported in the literature. Accurate results relied on consistent images being collected across both participants and replicates. Participants were seated, and rested their chin on a specially designed board which ensured images were collected from an equal distance and angle from the camera. Participants were trained to protrude their tongue in a standardised way by 'pursing' the tongue between the lips, and extending it to be flat against the chin. A ruler was placed vertically to the right hand side of the mouth, which was used to scale images against one another (**Fig 4.1**). Digital colour images of the tongue were collected using a Nikon 4DS camera (16.5 Mpixels, 5000 x 3300 pixels) fitted with a 100 mm macro lens. Multiple images were collected, until three images of adequate quality were obtained, and later downloaded onto a computer.

Figure 4.1: Standardised positioning of the tongue for image capture, with a ruler used to scale the images.

4.2.3 Sensory Evaluation Session 2

4.2.3.1 Thermal taste phenotyping

Participants were phenotyped for thermal taster status based on the methods described by Bajec and Pickering (2008). An intra-oral advanced thermal stimulator (ATS) peltier thermode (16 x 16 mm square surface) (Medoc Ltd., Israel) was used to deliver temperature stimulation to the anterior tip of the tongue. Before testing each participant, the thermode was cleaned with 99% ethanol (Fischer Scientific, UK) and covered with a fresh piece of tasteless plastic wrap (Tesco, UK). The researcher instructed participants to position the thermode firmly in contact with the tongue (Green and George, 2004) prior to the temperature trials being delivered. The 'warming trial' started at 35°C, reduced to 15°C, and was then re-warmed to 40°C where it was held for 1 sec (**Fig 4.2a**). A modified 'cooling trial' (discussed in Chapter 5, Section 5.2.1.2) delivered cooling stimulation. In this, the trial started at 35°C, was reduced to 5°C, and then held at this temperature for 1 s (**Fig 4.2b**) instead of the 10 s traditionally used (Bajec and Pickering, 2008).

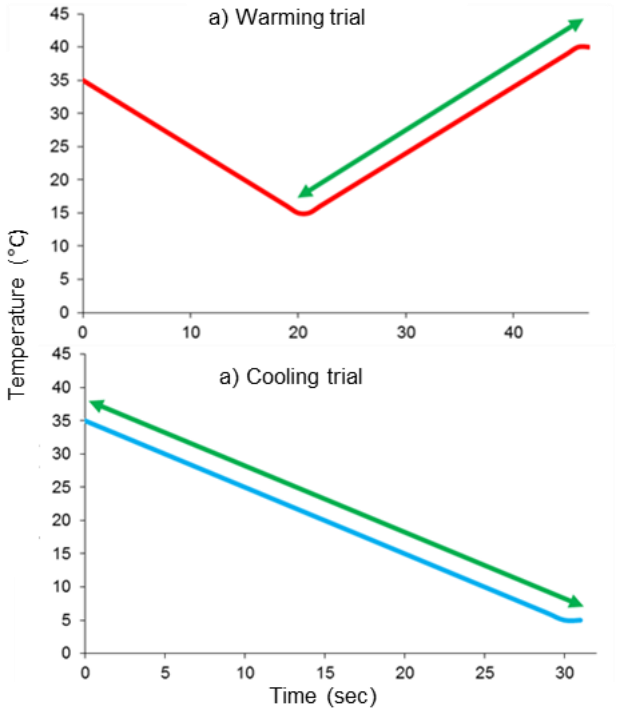

Figure 4.2: Thermode temperature across (a) 'warming', and (b) 'cooling' trials. Arrows (⟷) indicate when participants were instructed to 'attend' to the test; when the temperature increased from 15-40°C during the 'warming trial', and for the whole of the 'cooling trial'.

At the end of each trial participants rated the intensity of the temperature when it reached its maximum on a blank gLMS (Compusense Five 5.4, Canada). If a taste/s was perceived participants indicated the taste/s quality, and the taste intensity was rated on a second gLMS. Prototypical tastes (sweet, sour, salty, bitter, umami) and 'other' sensations (metallic, spicy and minty) were permitted to be associated with taste (Hort et al., 2016). Two replicates of each temperature trial were delivered, and a two minute palate recovery break was given between both replicates and temperature trials. Warming trials preceded cooling trials to prevent possible adaptation from the intense, sustained cold stimulation of the cooling trial (Green and George, 2004). Participants were not made aware of the purpose of the activity, and were informed that people do not always perceive taste, in order to reduce any false reporting of taste. Those who reported the same taste across both replicates of the warming and/or

cooling trial were classified as thermal tasters (TTs), whilst those who only perceived temperature were identified to be thermal non-tasters (TnTs). Those who did not meet the criteria for wither of these categories were assigned to an uncategorised group and were excluded from the study.

4.2.3.2 TAS2R38 and gustin genotyping

Genotyping buccal cell samples were collected using one buccal swab (Isohelix SK1 Buccal Swabs-1S) and silica gel capsule (IsohelixDri-Capsules) per person. Participants were instructed to rinse their mouth using deionised water prior to sample collection, and then to rub the buccal swab against the inside of the cheek with a firm pressure for one min (timed by the researcher). Upon completion, the participant placed the swab into the plastic tube, snapped the shaft above the swab head, added the dri-capsule, and sealed the tube with the cap provided. A sticker with the participant ID number was applied to each tube in order to retain anonymity when the samples were processed by an external company (LGC Genomics, Herts, UK) for genotyping. Participants were genotyped for three single nucleotide polymorphisms (SNPs) at base pairs 145 (C/G), 785 (C/T), and 886 (G/A) of the TAS2R38 locus, and for gustin (CA6) polymorphism rs2274333 (A/G), hereby referred to as ‘gustin’. The regions were amplified by PCR and sequenced, and the swabs destroyed after analysis.

4.2.4 Sensory evaluation data analysis

A total of 30 participants (18-41 years old) were recruited from the phenotyping sessions, and were selected to be balanced across PTS groups; 10 PNTs (6 male) 10 PMTs (2 male) 10 PSTs (3 male), and thermal taster status groups (7 TTs and 3 TnTs in each PTS group).

All gLMS intensity ratings of 0 were converted to 0.5, and the data was $\log_{10}$ transformed, as gLMS data is not normally distributed, before statistical analysis. All analyses were performed using SPSS, version 21 (SPSS IBM, USA) with an α-risk of 0.05 set.

4.2.4.1 Perceived intensity of tastants

Differences in the perceived intensity of tastants was explored using a two factor Analysis of Variance (ANOVA) (sample and replicate) with interaction, and Tukey's Honest Significant Differences (HSD) post-hoc testing were used to identify which samples were different for individual participants, and for the combined ratings of all participants (n=30).

4.2.4.2 Relationship between PTS, TAS2R38 and gustin genotypes

Analysis of the TAS2R38 genotype included AVI and PAV homozygotes, and PAV:AVI heterozygotes, whilst less common variants were excluded from the analysis. Cross tabulation and Chi-square tests were used to explore the relationship between both TAS2R38 and gustin genotypes with the distribution of PTS. Two factor ANOVA (replicate and genotype) with interaction, and where appropriate Tukey's HSD post-hoc tests, were used to identify if PROP intensity ratings differed across TAS2R38 and gustin genotypes.

4.2.4.3 Relationship between FPD, PTS, and TAS2R38 and gustin genotypes

From the multiple tongue images downloaded onto a computer, the highest quality image obtained for each participant was selected to measure FPD. To standardise measurement of FPD from the digital images across participants,

each image was cropped (Adobe Photoshop, version 10.1, USA) to include only the first 2 cm of the anterior tongue tip. Image J software (Image J, version 5.1, USA) was used to manually count the number of FP across the entire area of the tongue within the cropped image. Although FPD in a 6mm diameter circle at the anterior tongue tip has been reported to correlate with total FPD across the whole tongue (Shahbake et al., 2005), measuring across the anterior 2 cm portion of the tongue was hypothesised to be a more accurate representation of total FPD, and was adopted in the current study. Additionally, preliminary observations (data not shown) identified FP were not always located symmetrical across the left and right sides of the tongue, further supporting the decision to measure across a wider area. A separate one factor ANOVA was used to identify the individual impact of each PTS, TAS2R38 and gustin genotypes on FPD. The relationship between FPD and PROP intensity ratings was also measured using a Pearson's correlation coefficient.

4.2.4.4 Relationship between taste sensitivity and PTS, FPD, and TAS2R38 and gustin genotypes

A two factor ANOVA (replicate and group) with interaction, and Tukey HSD post-hoc testing where appropriate, was used to identify differences in the perceived intensity of each taste qualities, and global taste intensity ratings for combined stimuli (average of all tastes), across PTS, TAS2R38 and gustin genotype individually. The relationship between taste sensitivity and sensitivity to PROP was further explored using a Pearson's correlation coefficient to compare PROP and taste intensity ratings for individual taste qualities, and global taste intensity ratings. The relationship between FPD and taste sensitivity was also measured using a Pearson's correlation coefficient for intensity ratings of each tastant individually, and the global taste intensity rating.

4.2.5 Variation in taste related brain response across PTS

The 30 recruited participants were also invited to attend an fMRI scan session, which explored the association between taste related brain activation in the insula cortex and sensitivity to PROP.

4.2.5.1 fMRI scan session

As hunger/satiety can influence taste sensitivity (Haase et al., 2009b) participants were instructed to consume a light meal at least one hour before the scan commenced. To prevent lingering oral sensations influencing the results, participants were also asked not to consume anything but water for one hour prior to testing. The total scan session lasted for two hours, including an explanation of the session, with the duration of the fMRI scan lasting no more than one hour.

4.2.5.2 Tastant stimuli delivery

The tastant and metallic samples identified in Section 4.2.1 were delivered during the fMRI scan, and deionised water was used as both a control sample, and for palate cleansing. Stimuli were made on the day of scanning, and were delivered at (22 ± 2°C). Sample delivery was based on methods developed and described in Chapter 2, Section 2.2.7. The automated spray system, using a custom made gustometer, was used to deliver stimuli into the mouths of participants through nozzles positioned between the lips (Marciani et al., 2006). Due to the gustometer containing metal components, which must not enter the scan room, it was positioned in the MR control room and long plastic tubes were fed through to the participant who lay supine on the scan bed. For hygiene purposes, sterile nozzles were replaced between participants. The $FeSO_4$ sample was made fresh for each participant, and sample residue in the tube

was flushed away with deionised water before the fresh sample was added. Sample delivery was controlled by presentation software (Neurobehavioural System, San Francisco, US). Delivery flow rate was 1 ml/s, allowing precise and reproducible delivery of 3 ml stimuli over three seconds. As described in Chapter 2, Section 2.2.7 (**Fig 2.2**) the cycle length for stimulus delivery was 15 seconds, which included a 12 second break between replicates. Participants wore prism glasses which allowed them to view a screen at the end of the scan bed. A cross was presented on the screen, which changed colour to indicate when they should swallow after stimulus delivery. As described in Chapter 2, Section 2.2.7 (**Fig 2.3**), tastants were delivered across three tastant 'blocks', each containing 'pairs' of two tastants; pair one (sweet and metallic), pair two (salt and bitter), and pair three (sour and umami), to avoid sample carry over effects. Ten replicates of deionised water were delivered at the beginning of each tastant 'pair' block, as this was the control stimuli used when modelling the fMRI data. Ten replicates of each tastant were delivered, followed by five replicates of water to cleanse the palate. Delivery of each tastant 'pair', including the associated water control and palate cleanser, lasted for 14 minutes. A three minute break was given between tastant 'pair' blocks. As umami was the most persistent taste, pair three was always delivered last, whilst pair one and pair two were randomised across participants. **Figure 4.3** identifies a schematic representation of the sample delivery paradigm for one participant. To determine if tastants had been perceived, participants were asked to verbally identify which tastant qualities had been delivered after each tastant pair.

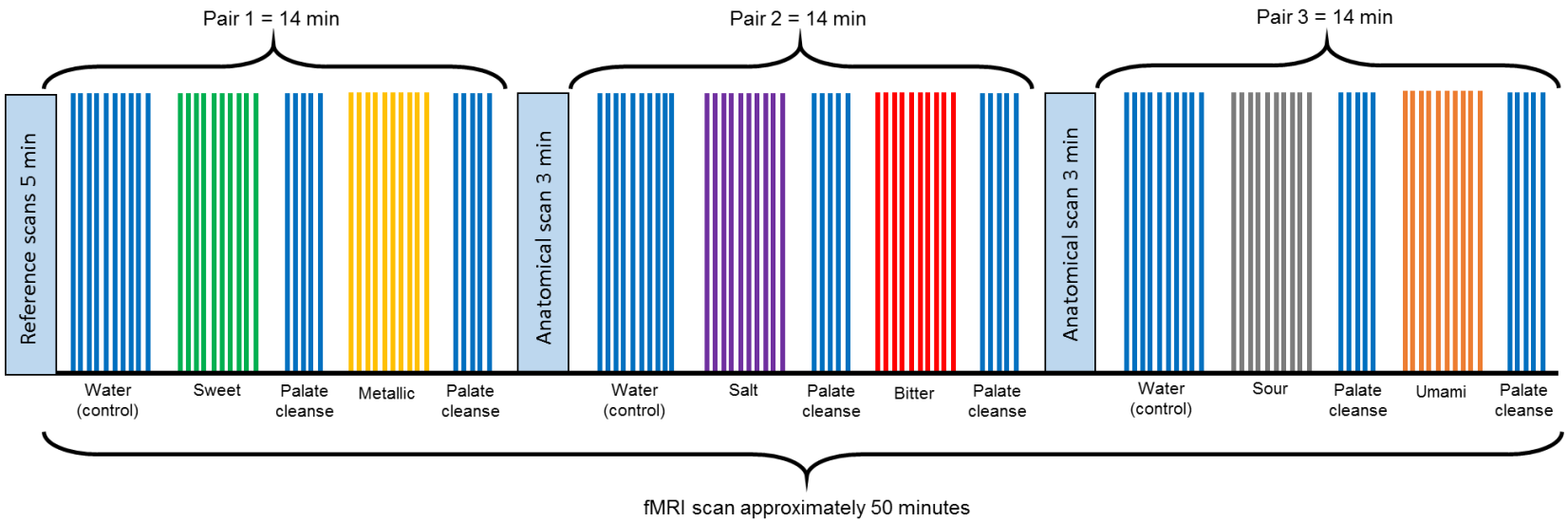

Figure 4.3: Schematic showing an example of the entire fMRI paradigm for one participant.

4.2.5.3 fMRI data acquisition and analysis

fMRI data were acquired on a 7T Philips Achieva scanner with head transmit and 32-channel receive coil using a Multiband (MB), also termed simultaneous multislice (SMS), gradient-echo, echo-planar-imaging (GE-EPI) acquisition: TE 30 ms, TR 2000 ms, flip angle (FA) 75°, 1.5 mm isotropic spatial resolution, 174 x 192 mm^2 field of view (FOV), SENSE factor 3 in the right-left (RL) direction, and 40 contiguous axial slices aligned parallel with the AC-PC plane with a MB factor of 2 (GyroTools LLC) to provide coverage of taste and oral somatosensory areas. Following the functional runs, a high-resolution T2*-weighted FLASH dataset was acquired with the same slice prescription and coverage as the functional data (0.5 × 0.5 × 1.5 mm3 resolution; TE/TR = 9.3/458 ms, FA = 32°, SENSE factor = 2), and a whole-head structural PSIR dataset (1 mm isotropic resolution, linear phase encoding order, TE/TR 3.7/15 ms, FA 8º, inversion time 1184 ms, (REF) was collected (four min).

fMRI data was analysed by Dr Sally Eldeghaidy. In summary, all data was pre-processed (realigned, normalised, and spatially smoothed) before statistical analysis. First level analysis was conducted to identify BOLD activation in response to each of the individual tastants for each individual participant. Second level analysis was conducted to identify group activation maps for the combined group of 30 participants for each individual tastant. Next, a spatial overlap of the group map responses to each of the six stimuli in the insula cortex was produced, to create a probabilistic map identifying overlapping areas activated across the tastant stimuli. Finally, a correlation analysis was conducted to identify the relationship between PROP intensity ratings and BOLD activation in the insula cortex.

4.3 RESULTS

4.3.1 Perceived intensity of tastants

The averaged tastant intensity ratings across all participants (n=30) were between moderate and strong on the gLMS, **Figure 4.4**. ANOVA identified a significant difference between sample intensity ratings ($p<0.001$), but no difference between replicates ($p=0.43$), and no interaction ($p=0.10$). Tukey HSD post-hoc testing identified a number of differences between sample intensities, including salty and sour being the most intense samples, and metallic being the least intense.

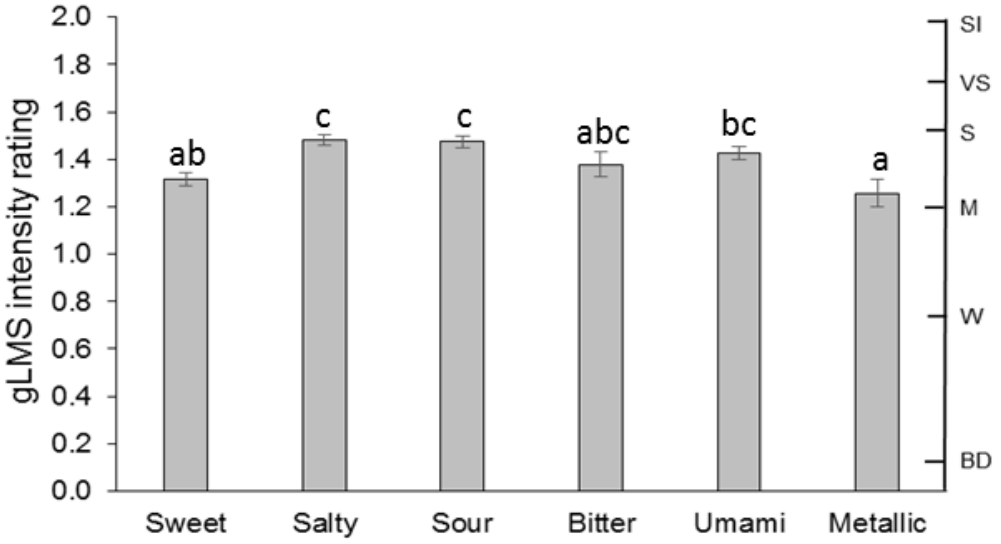

Figure 4.4: Mean taste intensity ratings across all participants (n=30). Bars represent mean ± S.E. Different letters above the bars ^{abc} indicate a significant difference between samples at $p<0.05$. BD - barely detectable, W - weak, M - moderate, S - strong, VS - very strong.

Examples of the variability in perceived taste intensity ratings by two participants are shown in **Figure 4.5**. Whilst no significant difference in intensity ratings were identified across taste qualities for some participants ($p>0.05$) (**Fig 4.5a**), for other participants the ratings across taste qualities were significantly different ($p<0.05$) (**Fig 4.5b**).

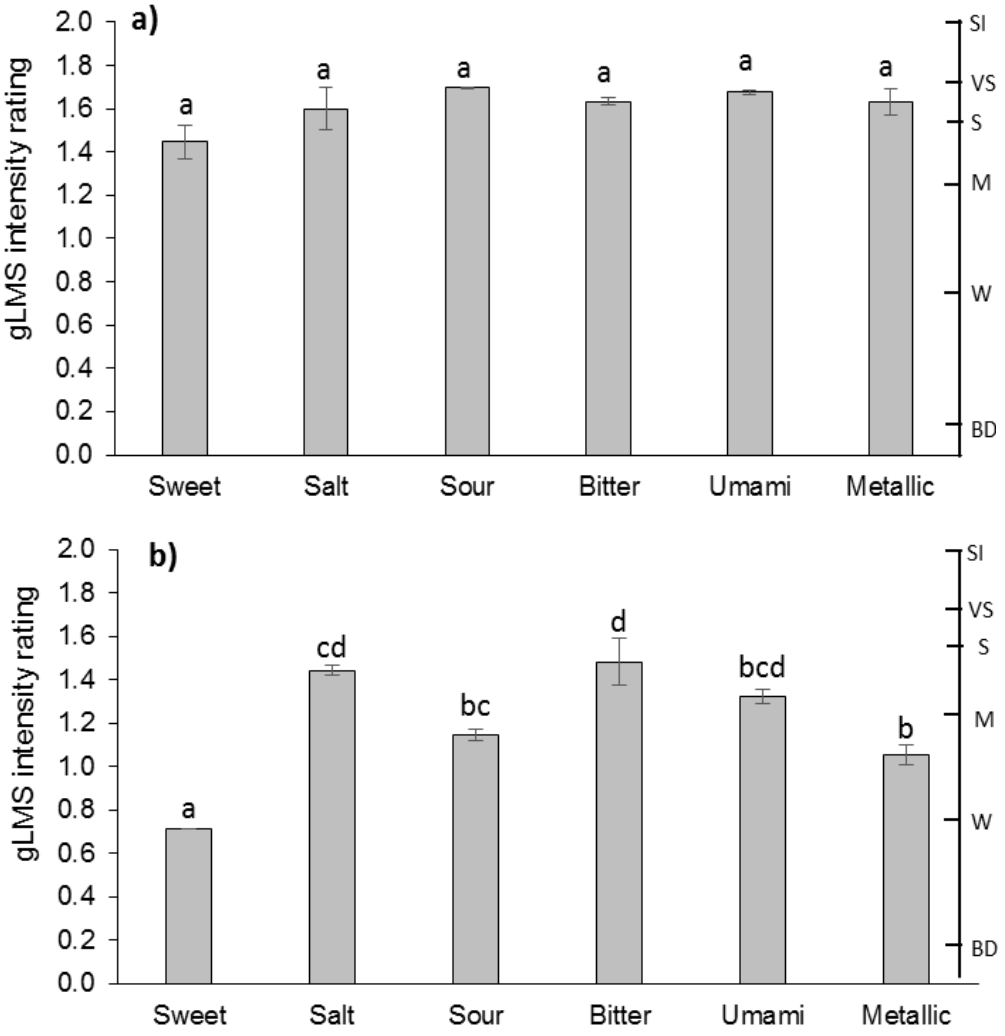

Figure 4.5: Examples of taste intensity ratings for two participants showing a) no significant difference in intensity ratings between taste qualities, and b) significant differences in intensity ratings between taste qualities. Bars represent mean ± S.E. Different letters above the bars [abcd] indicate a significant difference between samples at $p<0.05$ within a participant. BD - barely detectable, W - weak, M - moderate, S - Strong, SI – strongest imaginable.

4.3.2 Relationship between PTS, and TAS2R38 and gustin genotypes

TAS2R38 genotype could not be identified for two participants. Multiple factors can influence successful DNA extraction, such as bacteria in the mouth, use of an alcohol mouthwash prior to the swab being taken, and the preservation and transport of samples. Of the participants tested, 25% were AVI homozygotes, 50% AVI:PAV heterozygotes, and 21% PAV homozygotes. Interestingly, only one participant's genotype differed from these common variants, where a PNT was identified to be PAV:AAV. The cross tabulation of TAS2R38 genotype

against PTS phenotype (**Table 4.1)** identifies 71.4% of PNTs were AVI homozygotes, compared to only 20% of PMTs, and no PSTs. Conversely, 50% of PSTs were PAV homozygotes, compared to only 10% of PMTs and no PNTs, whilst 70% of PMTs were heterozygotes, compared to 50% of PSTs and only 28.6% of PNTs. Chi-square analysis showed PTS was significantly associated with TAS2R38 polymorphisms (p=0.004). ANOVA identified a significant difference (p<0.001) in PROP intensity ratings across TAS2R38 genotypes. Tukey HSD post-hoc test results identified a significant difference in ratings between all three PTS groups, where AVI homozygotes rated PROP as the least intense, and PAV homozygotes the most intense. No significant difference was identified between replicates (p=0.33), and no significant interaction occurred (p=0.40).

Table 4.1: Cross tabulation of TAS2R38 genotype and PTS phenotype.

TAS2R38	**PTS phenotype**						**[a]p value**
	PNT		**PMT**		**PST**		
genotype	n	%	n	%	n	%	
AVI:AVI	5	71.4	2	20	0	0	
PAV:AVI	2	28.6	7	70	5	50	0.004
PAV:PAV	0	0	1	10	5	50	

[a]p value associated with Chi-square analysis. n is number of participants. % is the percentage of TAS2R38 genotype in each column (PTS phenotype group).

Gustin genotype could not be identified for three participants, likely due to factors previously described. Of the participants tested, 48% were A:A, 30% A:G, and 22% G:G. Chi-square analysis did not find a significant relationship (p=0.60) between gustin genotype and PTS phenotype (**Table 4.2**). ANOVA identified no significant difference in PROP intensity ratings across gustin genotypes (p=0.40), replicates (p=0.88), and no interaction (p=0.92).

Table 4.2: Cross tabulation of gustin genotype and PTS phenotype.

Gustin genotype	PTS phenotype: PNT n	PNT %	PMT N	PMT %	PST n	PST %	[a]p value
A:A	5	62.5	4	44.4	4	40.0	
A:G	1	12.5	4	44.4	3	30.0	0.60
G:G	2	25.0	1	11.1	3	30.0	

[a]p value associated with Chi-square analysis. n is number of participants. % is the percentage of gustin genotype in each column (PTS phenotype group).

4.3.3 Relationship between FPD, PTS, and TAS2R38 and gustin genotypes

ANOVA identified no significant difference in FPD between PTS groups (p=0.41) (**Fig 4.6a**), and a Pearson's correlation identified no significant relationship between the number of FP and PROP intensity ratings (p=0.16). However, there was a trend for PSTs to have more FP than PNTs and PMTs (**Fig 4.6a**). ANOVA also identified no significant differences in FPD across TAS2R38 (p=0.11) (**Fig 4.6b**) or gustin (p=0.76) (**Fig 4.6c**) genotypes. There was also a trend for AVI:AVI genotypes to have lower FPD than AVI:PAV or PAV:PAV genotypes. However, because of the low standard error observed with the AVI:AVI results, the difference between groups is not as large as it appears.

4.3.4 Taste sensitivity

4.3.4.1 PTS phenotype

ANOVA identified no significant difference in intensity ratings for the individual taste qualities between PTS groups (p=0.09), replicates (p=0.93), and no interaction (p=0.97) occurred **Figure 4.7**. As the ANOVA result is p<0.1, the Tukey HSD post-hoc test results have been reported. Results indicated there may be an effect of PTS on taste sensitivity to some stimuli, as PSTs rated salt (p=0.006) and sour (p=0.005) stimuli significantly more intense than PMTs, but

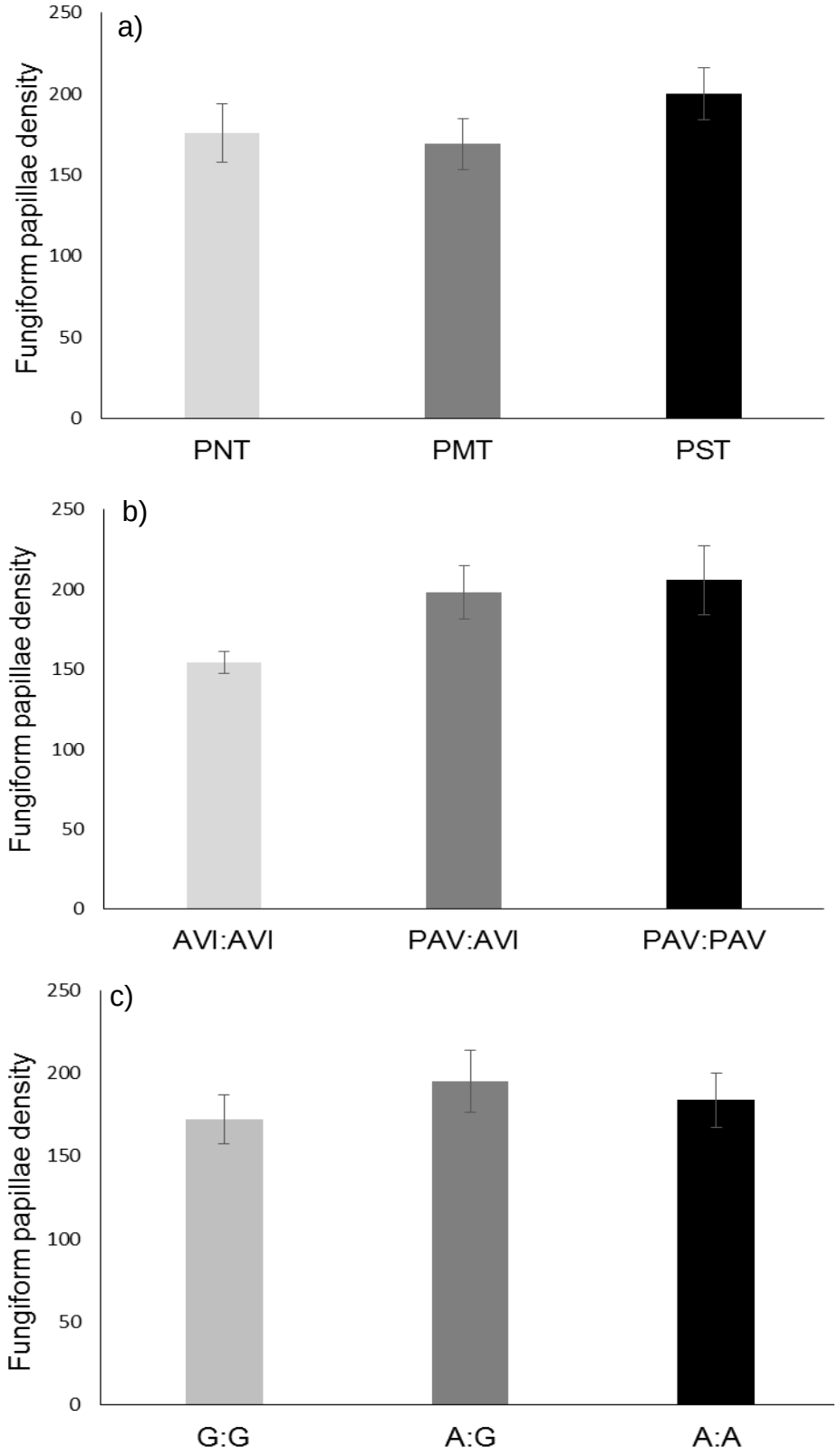

Figure 4.6: Comparison of FPD across a) PTS phenotype, b) TAS2R38 genotype, and c) gustin genotype. Data shown as mean FP count ± SE.

no different to PNTs. A separate ANOVA identified a significant difference (p=0.001) across PTS groups for the global taste intensity ratings (average of all tastants), for which no difference between replicates (p=0.43) or interaction (0.33) were identified (**Fig 4.7**). Tukey HSD post-hoc testing identified the rating by PMTs was significantly lower than that of PSTs, but not PNTs. Although not significantly different, there was a trend for PSTs to rate bitter and metallic stimuli more intensely than the other groups, and for PMTs to rate sweet and umami as less intense than both PNTs and PSTs.

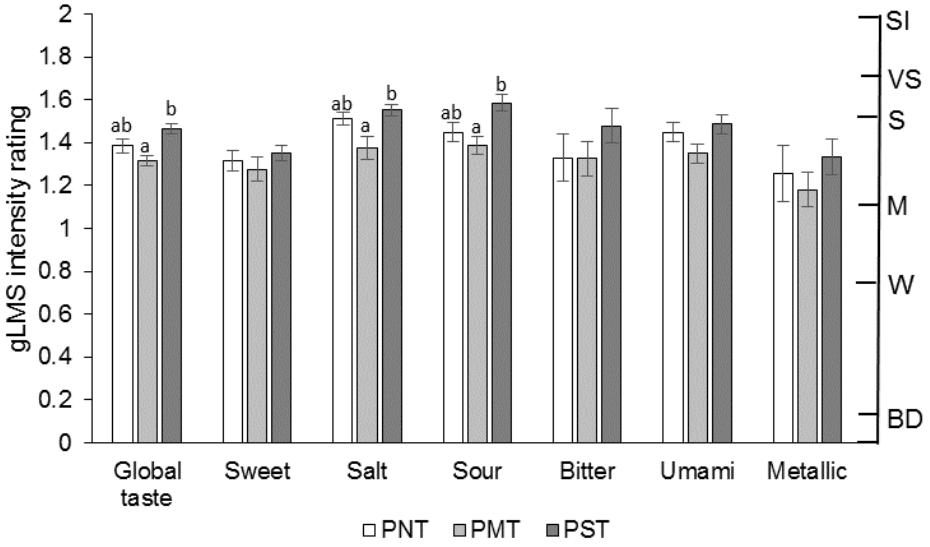

Figure 4.7: Mean taste intensity ratings across PTS groups. Bars represent mean ± S.E. Different letters above the bars [ab] indicates a significant difference between samples at $p<0.05$ (as identified by Tukey HSD). BD - barely detectable, W - weak, M - moderate, S - strong, VS - very strong, SI – strongest imaginable sensation.

PROP intensity ratings were not significantly correlated with taste intensity ratings for sweet (p=0.559), salt (p=0.920), sour (p=0.078), bitter (p=0.273), umami (p=0.859), metallic (p=0.478), or global taste intensity (average of all tastants) (p=0.175).

4.3.4.2 TAS2R38 genotype

ANOVA identified no significant difference in the taste intensity ratings between TAS2R38 genotypes (p=0.34), replicates (p=0.97), and no interaction (p=0.98)

(**Fig 4.8**). A separate ANOVA identified no significant difference across TAS2R38 genotypes for the global taste intensity ratings (average of all tastants) (p=0.88), replicates (p=0.47) or interaction (p=0.36).

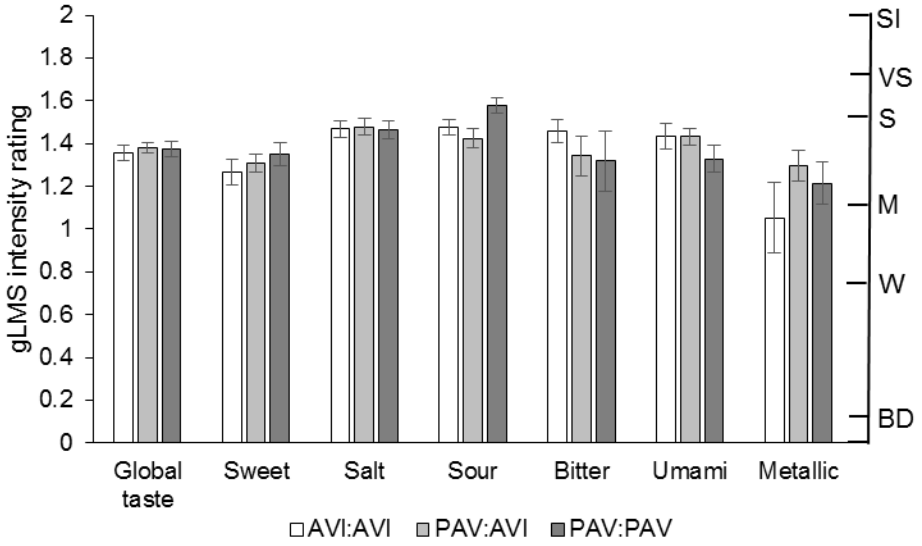

Figure 4.8: Mean taste intensity ratings across TAS2R38 genotypes. Bars represent mean ± S.E. BD - barely detectable, W - weak, M - moderate, S - strong, VS - very strong, SI - strongest imaginable sensation.

4.3.4.3 Gustin genotype

Although approaching significance, ANOVA identified no significant difference in the taste intensity ratings between gustin genotypes (p=0.058), replicates (p=0.86) or interaction (p=0.99), **Figure 4.9**. As the ANOVA result is approaching significance, the Tukey HSD post-hoc test results have been reported, and show those with GG genotype rated the umami stimuli to be significantly lower intensity (p=0.038) than those with AA or AG genotypes. A separate ANOVA identified no significant difference across gustin genotypes for the global taste intensity ratings for global taste intensity (average of all tastants) (p=0.61), replicates (p=0.76), and no interaction (p=0.54).

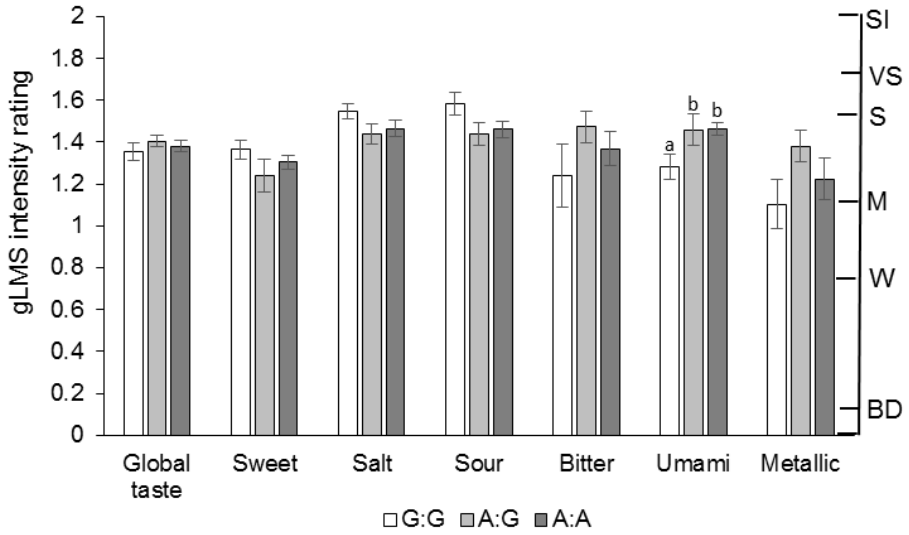

Figure 4.9: Mean taste intensity ratings across gustin genotypes. Bars represent mean ± S.E. Different letters above the bars [ab] indicates a significant difference between samples at $p<0.05$ (as identified by Tukey HSD). BD - barely detectable, W - weak, M - moderate, S - strong, VS - very strong, SI - strongest imaginable sensation.

4.3.4.4 FPD phenotype

Pearson's correlation coefficients identified no significant relationship between FPD and taste intensity ratings for sweet ($p=0.23$), salt ($p=0.10$), sour ($p=0.89$), bitter ($p=0.20$), umami ($p=0.89$) metallic ($p=0.49$) stimuli, or for the global taste intensity (average of all tastants) ($p=0.68$).

4.3.5 Cortical response to taste, and the relationship with PROP sensitivity

Cortical activation in response to each tastant was identified in the anterior insula (**Fig 4.10**). Greater extent of activation was observed in response to the sweet (**Fig 4.10b**) and metallic (**Fig 4.10f**) stimuli.

The spatial overlap of the group map responses to each of the six stimuli in the insula cortex is shown in **Figure 4.11**. This is represented as a probabilistic map of the number of tastants which activate the insula. It can be seen that a large area of the insula is activated by three or four tastants (see **Fig 4.10**). However, only a small region is active in response to five or six tastants.

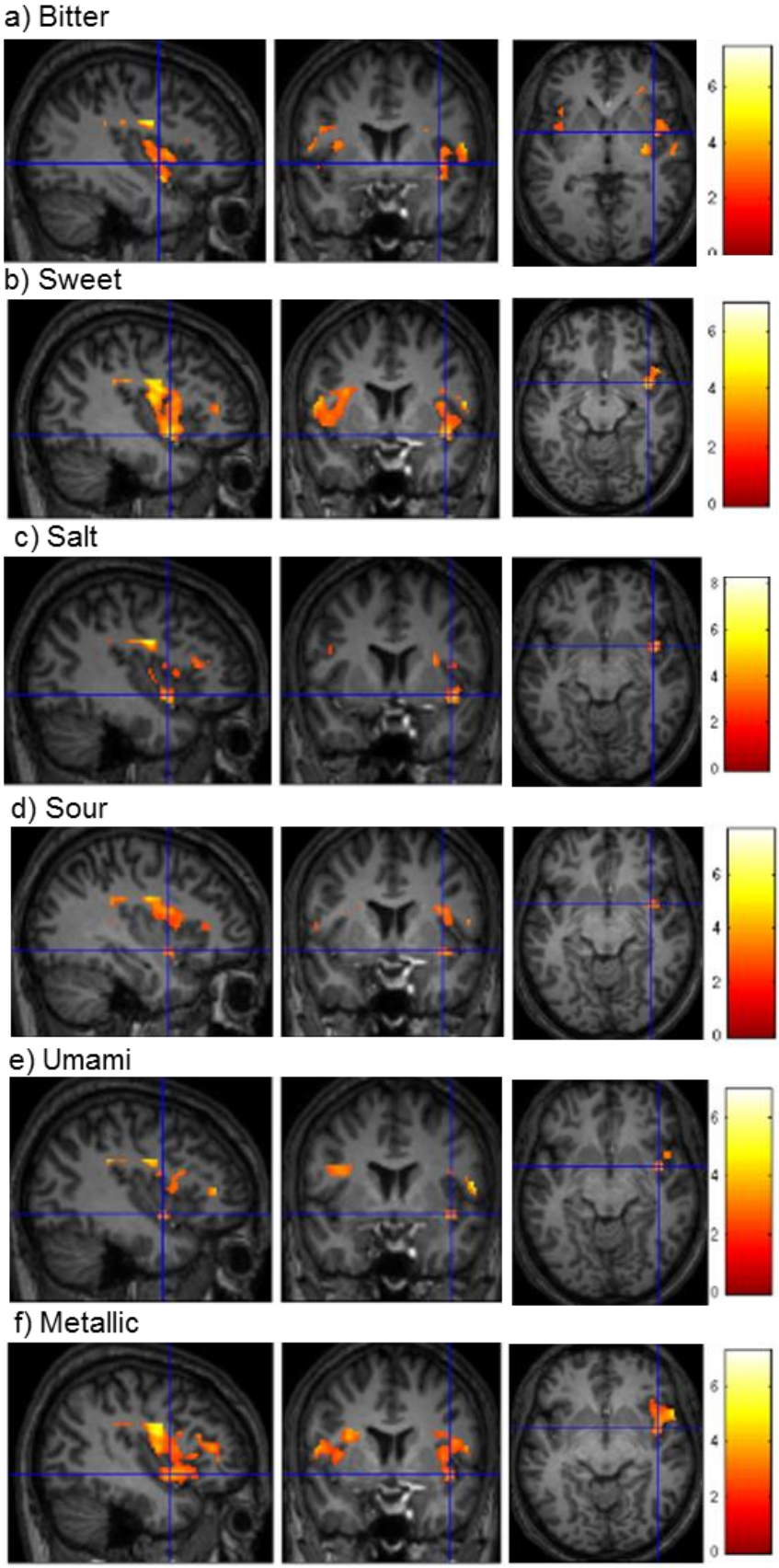

Figure 4.10: Group activation maps overlaid onto T_1-weighted images, showing activation in response to a) bitter, b) sweet, c) salt, d) sour, e) umami, and f) metallic stimuli. Cross-hairs indicate significant activation in the anterior insula.

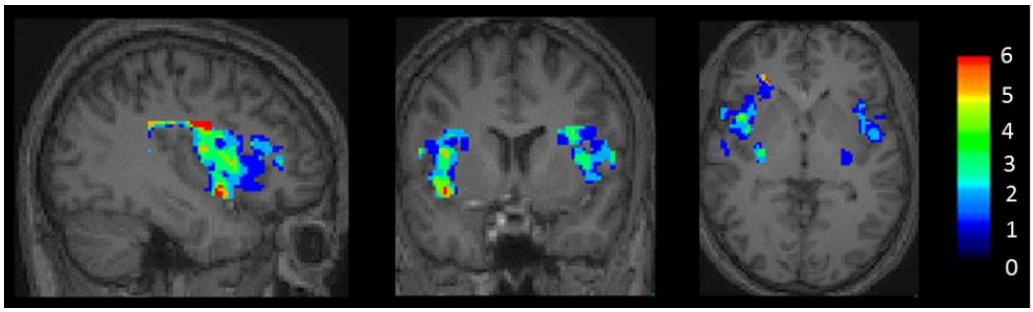

Figure 4.11: Probabilistic maps showing overlap in group maps to each of the six taste stimuli. Colours represent the number of tastants activating the anterior insula region, blue represents a single tastant and red all six tastants.

A greater extent of cortical activation was observed for the sweet and metallic stimuli (**Fig 4.10**), and a significant ($p<0.005$ uncorrected) linear correlation was observed between PROP intensity ratings and BOLD activation in the anterior insula in response to these stimuli (**Fig 4.12a & b**), as well the global response to combined stimuli (**Fig 4.12c**), which was not observed for the other tastants.

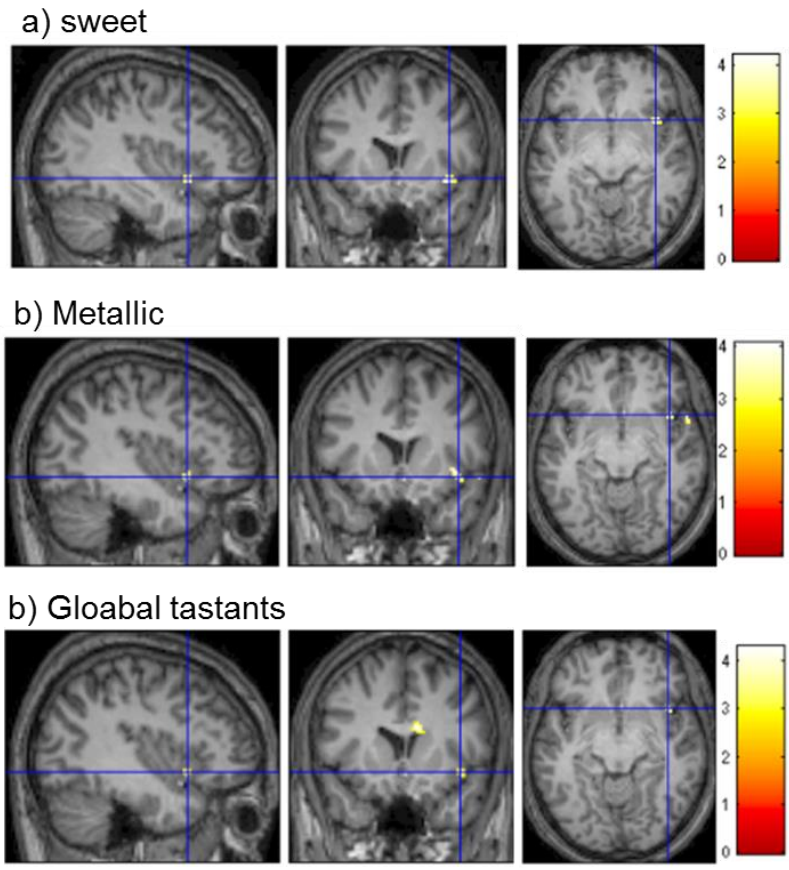

Figure 4.12: Group activation maps showing areas with a positive correlation of BOLD response with PROP intensity ratings, overlaid onto T1-weighted images ($p<0.005$ uncorrected). Cross-hairs indicate significant activation in the anterior insula in response to a) sweet, b) metallic, and c) global taste stimuli.

4.4 DISCUSSION

Compared to the large population groups typically used in sensory evaluation and genetics studies, fMRI experiments are restricted in the number of participants used, as data acquisition is time consuming and costly. The small population group used here (as required for the fMRI study) should be considered when interpreting the perceptual and genetics data presented, as associations that were not observed here may have been identified in a larger study population.

4.4.1 Perceived intensity of tastants

Averaged taste intensity ratings for the 30 participants (**Fig 4.4**) were similar to ratings made by the sensory panel (Section 2.3.2 **Fig 2.5**) and the 20 randomly selected assessors (Section 2.3.3, **Fig 2.6**) in Chapter 2. The metallic sample was rated at the lowest intensity of the six tastants, and sour and salt consistently high. Although averaged intensity ratings fell between moderate and strong on the gLMS, considerable variation in responses occurred within individual participant ratings. No significant difference in the perceived intensity across taste qualities was observed for some participants (**Fig 4.5a**), whilst ratings made by others were highly variable and reached a significant level of difference (**Fig 4.5b**). In some cases ratings ranged from around weak, to above strong intensity. Differences in scale use may be associated with some of these responses. For example, a contrast error occurs if the difference in rating across samples is exaggerated to highlight that a difference has been perceived (Kemp et al., 2009). Another explanation being variation in taste sensitivity across individual taste qualities. This variation highlights attempts to categorise overall taste sensitivity are too simplistic, and that responses to individual taste qualities should be considered.

4.4.2 Relationship between PTS and TAS2R38 and gustin genotypes

TAS2R38 genotype and PTS were significantly associated (**Table 4.1**), supporting the findings of many previous studies, and indicating PTS has a genetic component (Kim et al., 2003, Prodi et al., 2004, Bufe et al., 2005, Duffy et al., 2010, Khataan et al., 2009, Calo et al., 2011, Melis et al., 2013b, Garneau et al., 2014, Barbarossa et al., 2015, Yang, 2015, Shen et al., 2016, Barajas-Ramirez et al., 2016, Sollai et al., 2017). Interestingly, no PNTs were PAV homozygotes, and no PSTs were AVI homozygotes. However, there was an overlap of heterozygotes across all three PTS groups, which has previously been reported (Calo et al., 2011, Yang, 2015, Shen et al., 2016). Heterozygotes express different ratios of PAV and AVI alleles in taste receptor cells of FP, indicating gene expression introduces further variance which could explain the wider variety of sensitivity resulting in heterozygotes exhibiting 'AVI' like, or 'PAV' like sensitivity (Bufe et al., 2005). Additionally, issues when categorising PTS groups may result in some incorrect classifications, contributing to this overlap. Despite an association being observed between PTS and TAS2R38 genotype, these findings support the hypothesis that other factors, such as FPD, may also contribute to variation in PROP sensitivity and the crossover of TAS2R38 genotypes across PTS groups.

An association was not found between PTS and gustin genotype (**Table 4.2**), which supports the findings from a number of previous studies (Feeney and Hayes, 2014, Bering et al., 2014, Yang, 2015, Barbarossa et al., 2015, Shen et al., 2016, Shen et al., 2017). It is possible that in the few cases where a difference has been identified (Padiglia et al., 2010, Calo et al., 2011, Melis et al., 2013b) the difference may be attributed to the genetically similar cohort (Italian) used, and that these findings do not translate across genetically diverse population groups (Barbarossa et al., 2015) where functional differences in the

genes may exist (Shen et al., 2017). Other polymorphisms on the gustin gene (rs2274327, rs3737665, rs3765964) have been found to influence the perceived saltiness of sodium chloride and/or potassium chloride, which interestingly was not observed across variants of the rs2274333 genotype (Feeney and Hayes, 2014). This led to the hypothesis that the difference sometimes reported for the gustin rs2274333 genotype across PTS groups may be associated with one of the other polymorphisms, and the observed difference was a result of altered perception to the NaCl reference sample used when conducting magnitude estimation to categorise PTS groups. However, absolute PROP bitterness intensity ratings have also been reported to differ across gustin genotypes (Calo et al., 2011), therefore challenging this theory. This highlights the need for further research into gustin genotypes across diverse population groups.

4.4.3 Relationship between FPD, PTS, and TAS2R38 and gustin genotypes

FPD was not significantly different across PTS groups (**Fig 4.6a**), and was not significantly correlated with PROP intensity ratings in the current study. Whilst this has also been found by a number of other researchers (Yang, 2015, Fischer et al., 2013, Delwiche et al., 2001, Bakke and Vickers, 2011, Masi et al 2015; O'Brien et al, 2010, Garneau et al., 2014) the majority of literature reports PSTs and/or PMTs to have a higher FPD than PNTs (Bajec and Pickering, 2008, Tepper and Nurse, 1997, Yackinous and Guinard, 2001, Tepper and Nurse, 1998, Tepper, 1998, Yackinous and Guinard, 2002, Essick et al., 2003, Miller and Reedy, 1990b, Yeomans et al, 2007, Hayes et al., 2008, Melis et al, 2013b, Sollai et al., 2017, Melis and Barbarossa, 2017), or PROP ratings and FPD to be positively correlated (Nachtsheim and Schlich 2013; Hayes et al, 2007; Hayes et al., 2010, Bartoshuk et al., 1994, Duffy and Bartoshuk, 2000, Bakke

and Vickers, 2008; Duffy et al., 2010; Duffy et al, 2004a; Duffy et al., 2004b; Essick et al, 2003). The current study did identify a trend for PSTs to have a higher FPD compared to PNTs and PMTs. The fact that it did not reach a level of significance may be related to the small sample size. The trend for PSTs and PNTs to have higher FPD than PMTs supports the hypothesis that some PMTs may have been incorrectly classified as PNTs.

Although not reaching a significant level of difference between TAS2R38 genotypes, the current study found the average FPD was lowest in AVI homozygotes, compared to heterozygotes and PAV homozygotes (**Fig 4.6b**). Again, the fact this finding did not reach significance may be related to the small sample size used, as some researchers have reported differences across groups (Melis et al., 2017, Shen et al., 2016, Sollai et al., 2017). Alternatively, it may indicate that FPD is not associated with TAS2R38 genotype, which is also frequently reported (Duffy et al., 2004b, Hayes et al., 2008, Duffy et al., 2010, Garneau et al., 2014, Yang, 2015, Barbarossa et al., 2015). TAS2R38 genotype is associated with PTS, and it is possible that FPD further modulates taste sensitivity, although more research across larger population groups is required before conclusions can be drawn. Hayes et al (2008) found FPD did not affect PROP sensitivity in heterozygotes, but for both AVI and PAV homozygotes an increase in FPD was associated with increased bitter intensity ratings, suggesting FPD is associated with PROP perception. Yang (2015) found that AVI homozygotes with a high FPD were more sensitive to PROP and were therefore phenotyped as PMTs, whilst those of the same genotype but with a low FPD had lower sensitivity to PROP and were classified as PNTs, again suggesting FPD modulates sensitivity. Duffy et al (2004a) found that although TAS2R38 genotype was a better predictor of PROP sensitivity, FPD was an

independent contributor explaining an additional 5% of the variance in sensitivity.

The relationship between gustin genotype and FPD occasionally reported (Melis et al., 2013b, Barbarossa et al., 2015) was not identified in the current study where no difference was observed between gustin genotypes (**Fig 4.6c**). Other researchers have also frequently reported finding no differences across groups (Feeney and Hayes, 2014, Yang, 2015, Shen et al., 2016, Shen et al., 2017). Gustin genotype has also been associated with FP morphology. It has been suggested that those with GG genotype have FP that are larger in diameter, and anatomically distorted (Melis et al., 2013b). Exploring FP morphology was beyond the scope of this study, and should be explored in the future.

Although a large body of literature indicates an association between FPD and PTS, and some evidence suggests an association between FPD and TAS2R38 and gustin genotypes, others have found no relationship. Genetically diverse populations, the small population groups often used, and the widely variable methods used to determine FPD have likely contributed to discrepancies across studies. The latter is discussed in more detail next. Taste pores indicate the presence of taste buds within FP. The number of FP that contain taste pores, varies, where 0-35% do not contain taste pores (Miller and Reedy, 1990b). Additionally, those that do contain them may exhibit 0-22 (Miller and Reedy, 1990a) indicating high variation in gustatory functioning between FP. The gold standard measurement identifies the number of taste pores, taste buds and FP using videomicroscopy (Miller and Reedy, 1990a, Miller and Reedy, 1990b). However, this method is impractical for many researchers as it requires specialist equipment, and is time consuming (30 minutes per participant). FPD and taste pore density are correlated (Miller and Reedy, 1990a, Miller and

Reedy, 1990b; Bartoshuk et al, 1994), and current methods typically measure FPD as a proxy measure for taste pore and taste bud density. A number of approaches have been adopted, and include the following; FP can be counted visually directly from the tongue (Tepper and Nurse, 1997), a frequent approach is to manually count FP from an image of the tongue using software such as Image J (Nuessle et al., 2015), and in some cases specialised software has been designed to produce an automated count of FP (Sanyal et al., 2016). FPD is associated with the number of taste buds, but without taking into account variation in taste pore/bud density across FP, it is possible that these methods are not making accurate assessments of gustatory functioning. FPD within a circular area of the anterior tongue tip (to the left and/or right side of the midline) is often assessed as a representative region of the tongue (Yackinous and Guinard, 2001) as it has been shown to correlate with FPD across the whole tongue (Shahbake et al., 2005). The size of the area counted from is variable, including 6 (Melis et al., 2013b) to 10 mm diameter circles (Nuessle et al., 2015), a 5x5 mm^2 area (Bakke and Vickers, 2008), or the whole tongue can be measured (Miller and Reedy, 1990a, Miller and Reedy, 1990b, Sanyal et al., 2016). How these differences influence the conclusions drawn regarding gustatory functioning is unknown, but likely explain some of the discrepancies observed across studies, and highlight the need for more standardised approaches to be adopted.

4.4.4 Taste sensitivity

4.4.4.1 PTS

Limited differences in the perceived taste intensity was observed across PTS groups (**Fig 4.7**). Although PSTs rated salt and sour stimuli significantly more intensely than PMTs, no difference was observed when comparing responses

between PSTs and PNTs. This is surprising as PNTs are typically expected to have the lowest sensitivity. The same response was also observed for the global taste intensity ratings for the six taste qualities combined. General considerations when interpreting this data will be discussed, before focusing attention on the individual taste qualities.

As discussed in Section 4.1.1, PTS classification methods are particularly problematic. The unexpected high intensity ratings observed for PNTs may have been influenced by the classification method used, as three of the PNTs rated PROP intensity close to the cut-off point between PNTs and PMTs. It is therefore possible that they were PMTs incorrectly classified as PNTs. However, PROP and tastant intensity ratings were not significantly correlated, indicating PTS classification did not explain the lack of differences observed across PTS groups for most stimuli, and showed PNTs were generally rating tastant stimuli at a high intensity.

Although some minor differences in taste sensitivity were observed across PTS groups, a strong relationship does not appear to be present in this population group. One interpretation being that PTS is not a good marker of overall taste sensitivity, especially at stronger intensities that may be found in everyday food and beverage products. The variation in perceived intensity across taste qualities within individual participants, as discussed in Section 4.4.1, indicates using PTS as a marker for overall taste sensitivity is too simplistic. Multiple factors, independent of PTS, are likely influencing sensitivity. Examples of this include genetic variation in sweet perception (Fushan et al., 2009, Fushan et al., 2010), varied ability to perceive umami stimuli (Lugaz et al., 2002), saliva composition influencing sour perception (Matsuo, 2000), dietary intake of NaCl impacting NaCl sensitivity (Piovesana et al., 2013), and variation in the ability

to perceive a metallic sensation from ferrous salts, thought to be associated with individual variation in tissue lipid oxidation capacities (Epke et al., 2009, Omur-Ozbek et al., 2012).

Another possibility is that a concentration effect occurred. It is already documented that the taster advantage observed for suprathreshold stimuli is not always identified at detection threshold, as increased sensitivity to PROP has not been associated with a lower detection threshold for sucrose, caffeine, (Yang et al., 2014, Melis and Barbarossa, 2017), quinine (Pasquet et al., 2002), NaCl (Yang et al., 2014, Melis and Barbarossa, 2017, Pasquet et al., 2002), citric or tanic acid (Pasquet et al., 2002) or trigeminal stimuli including capsaicin and N-ethyl-2-isopropyl-5-methylcyclohexane carboxamide (Yang et al., 2014). In contrast, increased sensitivity to PROP has in some cases been associated with lower detection thresholds for sucrose (Chang et al., 2006, Hong et al., 2005, Pasquet et al, 2002), fructose (Pasquet et al., 2002), quinine (Chang et al., 2006, Hong et al., 2005), and citric acid (Melis and Barbarossa, 2017). These variances suggest that stimuli intensity likely influences the perceptual differences observed across PTS groups (Yang et al., 2015). It is therefore possible that the PTS advantage observed by researchers when using stimuli at weaker concentrations is lost when delivering the strong intensity samples used in the current study. Tastants were developed to be strong in order to elicit good cortical activation during the fMRI scan, and were at a higher concentration than used in many sensory studies. Some researchers have reported stimuli at similar, or even higher concentrations. However, sample delivery methods could have caused lower overall perceived intensity. For example stimuli applied to the tongue using a saturated cotton swab (Clark, 2011, Yang, 2015) delivers a lower volume of stimulus, and coats fewer oral receptors when compared to the whole mouth rinse used in the current study.

Observed differences in taste sensitivity across PTS vary dependent on the concentration of suprathreshold tastants delivered (Yang, 2015, Melis and Barbarossa, 2017), and detection and suprathreshold perception are not correlated (Webb et al., 2015), supporting the theory that a concentration effects may have occurred. Results for each taste quality will be discussed individually.

Bitter, sweet, and salt stimuli are the most widely researched taste qualities when exploring the relationship between PTS and taste perception. It is interesting that the perceived bitterness of quinine did not differ across PTS groups, as the intensity of caffeine (Yang et al., 2014, Ly and Drewnowski, 2001, Yackinous and Guinard, 2002, Bartoshuk et al., 1988; Hall et al., 1975; Masi et al, 2015, Melis and Barbarossa, 2017), quinine (Bajec and Pickering, 2008; Gent and Bartoshuk, 1983; Dinehart et al., 2006; Masi et al, 2015), epicatechin (Pickering et al., 2006), and the bitterness of saccharin (Bartoshuk, 1979) potassium chloride, caffeine (Bartoshuk, 1993) and benzoate (Bartoshuk et al., 1988) are frequently perceived more intensely by PSTs and/or PMTs than PNTs. However, no difference has also been reported across PTS groups for quinine (Clark, 2011, Schifferstein and Frijters, 1991) caffeine (Smagghe and Louis-Sylvestre, 1998, Webb et al., 2015) or potassium chloride (Schifferstein and Frijters, 1991), and in one case the perceived bitterness of naringin was more intense in non-tasters than tasters (Smagghe and Louis-Sylvestre, 1998).

The current study found no significant differences in perceived intensity of glucose across PTS groups. PSTs and/or PMTs frequently perceive sucrose (Gent and Bartoshuk, 1983, Lucchina et al., 1998, Clark, 2011, Drewnowski et al., 1997a, Bartoshuk, 1979, Yang et al., 2014, Bajec and Pickering, 2008, Yeomans et al., 2007, Melis and Barbarossa, 2017) and artificial sweeteners (Gent and Bartoshuk, 1983, Bartoshuk, 1979) more intensely than PNTs, whilst

other studies have reported no association for sucrose (Drewnowski et al., 1997, Smagghe and Louis-Sylvestre, 1998, Ly and Drewnowski, 2001, Webb et al., 2015) or saccharine (Smagghe and Louis-Sylvestre, 1998).

PSTs rated the intensity of NaCl more intensely than PMTs, but no different to PNTs. Other researchers frequently report increased sensitivity to PROP is associated with increased sensitivity to NaCl (Miller and Reedy, 1990b, Bartoshuk et al., 1998, Yeomans et al., 2007, Hayes et al., 2010, Yang et al., 2014, Bajec and Pickering, 2008, Webb et al., 2015). However, a difference across groups is not always identified (Schifferstein and Frijters, 1991, Drewnowski et al., 1998, Clark, 2011, Melis and Barbarossa, 2017). This association is controversial due to the popular method of categorising PTS using NaCl as a reference standard, as it was believed to be unrelated to PROP taste sensitivity, an assumption now under scrutiny. These findings support the recommendation to avoid the use of NaCl reference samples when classifying PTS, as it may be associated with PROP sensitivity.

PSTs rated the 0.0078M sour sample more intensely than PMTs, which has also been reported with lower concentration citric acid samples (0.0013 and 0.0052M) when delivered as a whole mouth rinse (Melis and Barbarossa, 2017). However, a stronger concentration (0.056M) solution applied to the tongue using a saturated cotton swab was not perceived differently across PTS groups (Clark, 2011, Yang, 2015), and is not correlated with PROP intensity ratings (Lim et al., 2008), indicating concentration influences the observed differences. It would be interesting to further explore the relationship between sensitivity to PROP and sourness over a range of sour stimuli at increasing concentrations, and using a larger population group.

The impact of PTS on umami perception is not well evidenced, and, as with Webb et al (2015), no association was observed in the current study. A concentration effect has previously been evidenced, as no difference in perceived intensity was observed across PTS groups in response to a 0.01M sample, whilst PSTs rated a 0.08M sample more intensely (Melis and Barbarossa, 2017). The sample delivered in the current study was at a higher concentration (0.12M) which could indicate a concentration effect and explain the lack of difference observed.

Intensity perception of $FeSO_4$ and PTS were not associated in this study. To the authors knowledge, only one other study has reported the association between PTS and metallic perception, where PSTs rated $FeSO_4$ more intensely than PNTs or PMTs (Bajec and Pickering, 2008). The current study used 5.46mM, compared to 0.3mM and 3mM used by Bajec and Pickering (2008), which again may explain the differences across studies.

Overall, these results indicate the PROP taster advantage frequently observed in previous studies, may not be evident here due to the strong concentration of the tastants used. In support of this hypothesis, the current study found perceived temperature intensity of thermal stimuli delivered during thermal taster status phenotyping to be associated with PTS (data not shown). Both warm and cool intensity ratings were significantly correlated with PROP intensity ratings ($p<0.05$). Therefore, the intensity of tastant stimuli used in the current study may more closely mimic attributes perceived in food and beverage, compared to weaker stimuli used in some studies, which could explain why the difference in perceived intensity of tastant solutions across PTS groups does not always translate into differences with more complex products. PTS has been proposed as a marker for overall taste sensitivity, but the variance observed

across taste qualities, as discussed in section 4.4.1, shows sensitivity is more complex than this; an individual may be highly sensitive to one taste quality, whilst exhibiting low sensitivity to another, thus indicating taste sensitivity is influenced by multiple factors. As indicated by many previous studies, it seems in some cases a relationship between PTS and oral sensitivity can be observed. However, the association is complex and differs across population groups and stimuli, suggesting it may not be a good marker of overall sensitivity. It is likely that the multiple factors determining oral sensitivity can often over-ride associations arising as a result of PTS.

4.4.4.2 TAS2R38 genotype

No significant differences in taste intensity ratings were observed across TAS2R38 genotypes (**Fig 4.8**). Despite a link between TAS2R38 and PTS frequently being identified, and PTS often associated with taste sensitivity, literature reporting the influence of TAS2R38 genotype on taste sensitivity is not clear. Yang (2015) found PSTs rated a range of taste, trigeminal and aroma stimuli significantly more intense than PNTs and/or PMTs, but found few significant differences across TAS2R38 genotypes, despite showing PTS and TAS2R38 genotype were significantly associated. Where differences were observed, the direction of the difference was the opposite of that expected, as PAV homozygotes typically rated stimuli less intensely than the other haplotypes. Similarly, no significant differences have been reported for detection threshold or supra-threshold concentrations of sucrose, capsaicin, or NaCl stimuli (Barajas-Ramirez et al., 2016), supra-threshold NaCl (Smutzer et al., 2013) or the bitterness of quinine (Duffy et al., 2010). Interestingly, one study did report MSG was rated significantly more intense by PAV homozygotes, and caffeine by heterozygotes when compared to the other genotypes (Melis and

Barbarossa, 2017). It is evident that further research is required to better understand the relationship between taste sensitivity and TAS2R38 genotype.

4.4.4.3 Gustin genotype

Those with GG genotype rated umami significantly less intense than those with AG or AA genotype (**Fig 4.9**). This is interesting as, to the author's knowledge, umami perception has not been reported across gustin genotypes before. Although a number of researchers have explored the association between gustin and the perceived bitterness of PROP, few report perception for other oral stimuli. Yang (2015) explored the effect of gustin genotype on sensitivity to low and high concentration taste (sucrose, NaCl, caffeine) trigeminal (capsaicin) and orthonasal and retronasal aroma (ethyl butyrate), and the only differences showed those with GG genotype rated the high concentration sucrose, and low concentration ethyl butyrate significantly more intense than those with AG genotype. No significant difference across gustin genotype has also been reported for bitter intensity of potassium chloride (Feeney and Hayes, 2014), intensity of NaCl (Shen et al., 2017), and little or no association with brassica vegetable (Shen et al., 2016) or ice cream intake (Shen et al., 2017). However, in some cases the conclusions drawn should be treated with caution as the percentage of GG genotype is typically small (Yang, 2015, Shen et al., 2016, Shen et al., 2017). Other factors may also be influential in these findings, for example zinc is involved in gustin functioning, and zinc deficiency has been estimated to effect 17.3% of the population, and prevalence is variable across countries ranging from <15%->25% of a population thought to be deficient in zinc (Wessells and Brown, 2012). Together, these findings indicate this genotype may be less influential on oral sensitivity in a genetically diverse

population than was initially reported in genetically similar Italian cohorts (Padiglia et al., 2010, Calo et al., 2011, Melis et al., 2013b).

4.4.4.4 FPD

The current study did not find FPD and taste sensitivity to be significantly correlated for individual taste qualities, or for global taste intensity ratings for combined stimuli. Previous literature frequently reports FPD is associated with increased sensitivity to gustatory (Masi et al., 2015, Zhang et al., 2009, Hayes et al., 2008, Hayes et al., 2010, Tepper and Nurse, 1997, Zuniga et al., 1993, Miller and Reedy, 1990b, Proserpio et al., 2016) and trigeminal stimuli (Nachtsheim and Schlich, 2013, Prutkin et al., 2000, Duffy et al., 2004b, Essick et al., 2003), whilst others have not replicated these findings at detection, recognition (Webb et al, 2015) or suprathreshold taste stimuli (Delwiche et al., 2001, Webb et al, 2015) including suprathreshold concentrations in large scale studies (Fischer et al., 2013, Feeney and Hayes, 2014). This, combined with the lack of association between the perceived bitterness of PROP and FPD frequently reported (Yang, 2015, Fischer et al., 2013, Delwiche et al., 2001, Bakke and Vickers, 2011, Masi et al 2015; O'Brien et al, 2010, Garneau et al., 2014) questions how robust the relationship is (Garneau et al., 2014). As previously discussed, the variance across studies may be attributed to multiple factors including study population size and demographics, method of determining FPD, type of stimuli and the concentration used.

4.4.5 Cortical response to taste, and the relationship with PROP sensitivity

As expected, cortical activation in response to each of the stimuli was observed in the anterior insula (**Fig 4.10**), which has been proposed as the primary gustatory cortex, involved in the identification and perceived intensity of taste

(Small et al., 1999, Faurion et al., 2005, Veldhuizen et al., 2011, Rolls, 2016a). In some cases, activation was also observed in the mid insula and posterior insula, both of which have also been associated with taste processing (Small et al., 2003, Small, 2010, Faurion et al., 2005, Veldhuizen et al., 2011, Rolls, 2016a).

Interestingly, some areas of the insula were activated by all tastants, whilst other areas only showed activation in response to certain stimuli (**Fig 4.11**). Possible explanations for these differences include variation in the perceived intensity of the stimulus, as the mid insula has also been associated with taste intensity perception (Small et al., 2003, Small, 2010, Spetter et al., 2010). The experiments detailed in Chapter 1 aimed to develop a set of stimuli that were roughly equi-intense, but individual variation in taste perception across taste qualities (**Fig 4.5**) prevented stimuli being equi-intense within and across participants, and taste qualities, which could support this theory. However, in comparison to the other tastants, greater overall activation was observed in response to the sweet and metallic stimuli, whilst perceived intensity ratings for these stimuli were, on average, at a lower intensity than the other tastants (**Fig 4.4**), indicating this may not be driving the difference.

Another consideration is that taste adaptation in the perceived intensity of the tastants may have occurred with repeat exposure during the fMRI scan, as is frequently reported in perceptual literature (McBurney and Pfaffmann, 1963, Bornstein et al., 1993, Schiffman et al., 1994, Theunissen et al., 2000). The degree of adaptation varies across taste qualities (Bornstein et al., 1993, Theunissen et al., 2000). Stimuli eliciting less adaptation would translate to a higher mean BOLD response occurring across the 10 replicates, which could also contribute to the increased activation observed for the sweet and metallic

samples. Future analysis should include using a parametric modulation across replicates to explore the rate of adaptation across the different taste qualities to better understand the impact of adaptation on the cortical responses observed.

Another theory is that the increased cortical activation is associated with the hedonic response to taste. Activation associated with the pleasant and aversive qualities of gustatory stimuli are typically associated with the OFC, amygdala (O'Doherty et al., 2001, Zald et al., 2002), and ACC (de Araujo and Rolls, 2004, Grabenhorst and Rolls, 2008). However, the insula has been proposed as an integration area (Kurth et al., 2010) which may also be involved in this response. The experiments in Chapter 3 characterised the perceptual attributes associated with ferrous salts, and identified $FeSO_4$ to have a sweet quality. It is therefore possible that the increased activation to the sweet and metallic stimuli identified here is associated with the perceived sweetness. The metallic quality elicited by $FeSO_4$ is thought to arise due to volatiles released by lipid oxidation in the oral cavity, which stimulate the olfactory system via the retronasal pathway (Omur-Ozbek et al., 2012). It is possible that sample delivery when supine in the scanner reduces retronasal aroma delivery, compared to when in a seated position, causing a reduction of metallic perception and increased sweet taste. Evidence for the effect of body position on aroma perception is sparse; whilst orthonasal aroma perception is reduced when supine (Lundstrom et al., 2006, Lundstrom et al., 2008), body position has no significant effect on the ability to discriminate between flavours involving retronasal aroma delivery (Hort et al., 2008). To the author’s knowledge, the influence of body position on retronasal aroma intensity perception, as opposed to discrimination, has not been explored, but would be interesting to understand when interpreting these results.

A further possibility is that the increased cortical activation observed for these samples is associated with other non-gustatory qualities. For example, in some cases $FeSO_4$ is reported to exhibit astringency (Lim and Lawless, 2005b, Lim and Lawless, 2006), and it is possible that the strong sweet sample elicited some viscosity. The posterior insula has been associated with oral somatosensory processing (Rolls, 2016b) which supports this possibility.

The prototypical taste qualities (sweet, sour, sat and bitter) have been shown to have different spatial representations in the gustatory cortex of rodents, where a gustoptopic map has been outlined (Chen et al., 2011). Although cortical activation across taste qualities have frequently been compared in the human brain (e.g. O'Doherty et al., 2001, Iannilli et al., 2012, Schoenfeld et al., 2004) whether taste qualities have topographical representation remains a controversial topic and, so far, a gustotopic map in the human brain has not been identified. Where differences in activation across tastants have been identified, it is unclear whether differences are associated with taste intensity, hedonic response, or variation in anatomy of the brain (Small and Faurion, 2015, Schoenfeld et al., 2004). The differing activation observed between taste qualities in the current study may indicate towards a chemotopographic map of chemical taste in the human brain. However, this theory requires much more extensive exploration, and should be the focus of future analysis in this area.

Of the samples delivered, the sweet and metallic stimuli elicited the strongest BOLD response. The PROP intensity ratings and BOLD activation in response to these stimuli were significantly correlated in the anterior insula (**Fig 4.12**), which was driving the significant correlation also observed for the global taste response of combined stimuli. Interestingly, this response was not observed with the perceptual data, where PROP and tastant intensity ratings were not

significantly correlated. Possible explanations include the gLMS not being sensitive enough to detect perceptual differences (Yang, 2015) that have been identified cortically. Additionally, other factors including variation in scale use, attention and focus during the test, and emotional state may have influenced the perceptual ratings (Kemp et al., 2009). These findings therefore highlight the power and importance of utilising multidisciplinary approaches when exploring perceptual and sensory responses.

Another possibility being that the differing sample delivery method used inside the scanner compared to outside the scanner influenced the perceived intensity of the stimuli. Although not reaching a significant level of difference, the experiments in Chapter 2 (Section 2.4.3) identified a trend for stimuli to be rated as less intense when delivered by spraying into the mouth when supine, compared to sipping from a cup when seated, and (Haase et al., 2009a) also found tastants were perceived to be less intense when syringing stimuli onto the tongue compared to when sipping from a cup. It is therefore possible that the stimuli were perceived at a lower intensity during the fMRI scan than when delivered during the sensory evaluation session. In Section 4.4.4.1 it was proposed that the PTS perceptual advantage to taste may be lost at the intensity of stimuli delivered in the current study. If the stimuli were therefore perceived at a lower intensity inside the scanner, this could explain why a significant correlation between PTS and cortical activation was observed, whilst no such association was identified in the perceptual testing.

Only two other studies have compared cortical activation to oral stimuli across PTS groups, and it is worth noting that these both used multisensory gustatory-trigeminal stimuli, as opposed to the prototypical gustatory stimuli used in the current study (with the exception of the $FeSO_4$ used here). In response to oral

fat stimuli, the first study also showed PROP intensity ratings were positively correlated with activation in the anterior insula, and saw additional activation in areas associated with somatosensory (SI, SII, mid insula) and taste (ACC) (Eldeghaidy et al., 2011). The second study (Clark, 2011) delivered sweet, cold stimuli at varying levels of carbonation (no CO_2, low CO_2, high CO_2), and did not observe a difference in activation across PTS groups in the anterior insula. Interestingly, as with Eldeghaidy et al (2011), PSTs again had higher activation in the SI, SII and ACC compared to PNTs. The data acquisition method used in the current study was designed to provide high spatial resolution mapping of the insula, and as such did not provide whole brain coverage. In future, an interesting extension to this work would be to collect data at coarser spatial resolution to image the whole brain, particularly given the hypothesis that activation may be associated with a hedonic response to the stimuli, which would be evident in areas such as the amygdala and OFC.

Overall, these findings support previous literature indicating an association between cortical activation and sensitivity to PROP. However, the current study found fewer associations with cortical activity and PROP sensitivity than previously reported. This is hypothesised to be due to the strong intensity tastants used in the current study, resulting in a reduction in the PTS sensitivity advantage to oral stimuli. This is of interest as it could indicate that the effect of PTS on oral sensitivity is less important in relation to consuming everyday food and beverage items.

4.5 CONCLUSION

The experiments detailed in this chapter included a diverse range of measures used to explore the perceptual and cortical response to taste. Perceptually, a

high level of variation in the perceived taste intensity was observed within individual participants, as well as across participants and taste qualities. This indicates the need to consider responses not only at the study population level, but also at the individual subject level.

In agreement with previous literature, PTS was associated with TAS2R38 genotype, supporting the likelihood that this phenotype has a genetic component. In contrast, PTS was not associated with gustin genotype, and requires exploration over a larger population group. FPD has frequently been associated with PTS in the past. Interestingly, the current study found no difference in FPD across PTS, TAS2R38 or gustin genotype groups. This could be due to the small sample size, as a trend for PSTs to have a higher FPD was observed.

Few or no differences were identified when exploring the relationship between perceived taste intensity and PTS, FPD, and TAS2R38 and gustin genotypes. A positive correlation was expected between taste sensitivity, and both sensitivity to PROP and FPD as this is frequently reported. A concentration effect was hypothesised as the main factor explaining the lack of difference observed. The heightened oral sensitivity associated with PTS may be lost when administering the strong intensity samples used in the current study. This indicates PTS may have less importance when consuming everyday food and beverages, in comparison to the lower intensity gustatory stimuli often used to explore this phenotype in the laboratory setting.

Activation was observed in the anterior insula in response to all stimuli. Interestingly, greater activation was observed for the sweet and metallic stimuli compared to the other tastants. Similarly, when exploring the effect of PTS on the cortical response to taste, a significant correlation was identified between PROP intensity ratings and activation in the anterior insula in response to the sweet and metallic stimuli, but not for the remaining tastants. This supports the findings of previous studies indicating a difference in brain responses occurs across PTS groups. Possible explanations for the difference observed for the sweet and metallic stimuli only include differing degrees of spatial representation within the insula across taste qualities, activation being associated with the hedonic response, variation in the degree of adaptation across taste qualities, and other sensory qualities such as astringency or viscosity that were only present in these samples. It is interesting that PROP and gLMS intensity ratings were not correlated, but a correlation was identified with cortical activation. This highlights the value of using a multidisciplinary approach when exploring sensory responses.

5 MEASURING VARIATION IN TEMPERATURE RESPONSES ACROSS THERMAL TASTE PHENOTYPES

5.1 THERMAL TASTER STATUS

The thermal taste phenotype identifies thermal tasters (TTs) who perceive phantom taste sensations, in the absence of gustatory stimuli, when the tongue is thermally stimulated using a temperature thermode. Those individuals who only perceive temperature are termed thermal non-tasters (TnTs) (Cruz and Green, 2000). The prevalence of TTs has been reported to be from 20% (Bajec and Pickering, 2008) to 50% (Cruz and Green, 2000) of participants. In some cases, TTs are also observed to report heightened sensitivity to chemical taste stimuli delivered at suprathreshold concentrations (Green and George, 2004, Green et al., 2005, Bajec and Pickering, 2008, Yang et al., 2014), as well as sucrose (Yang et al., 2014) at detection threshold, and tartaric acid (Pickering and Kvas, 2016) at difference threshold, when compared to TnTs. Observed intensity ratings for astringency, metallic (Bajec and Pickering, 2008) and temperature (Green and George, 2004, Bajec and Pickering, 2008; Yang et al., 2014) are more intense for TTs than TnTs, whilst an advantage is not reported for capsaicin and menthol (Green et al., 2005, Yang et al., 2014). Evidence for altered sensitivity to olfactory stimulation is contradictory (Green and George, 2004, Yang et al., 2014).

TTs perceptual advantage has been supported in a recent study showing increased cortical activation in multiple brain regions in response to gustatory-trigeminal (sweet-carbonated) stimuli in TTs compared to TnTs (Hort et al.,

2016). Some evidence suggests that thermal taster status may also influence food preference (Bajec and Pickering, 2010, Pickering et al., 2016). However, the heightened oral sensitivity that TTs exhibit to attributes in alcohol and some food products does not always translate to a difference in overall preference (Pickering et al., 2010a, Pickering et al., 2010b, Pickering et al., 2016, Pickering and Klodnicki, 2016).

5.1.1 Proposed mechanisms for phantom taste in TTs

Little is understood about the mechanism responsible for thermal taste phenotype. One hypothesis is that TTs have temperature sensitive neurons in the chorda tympani and glossopharyngeal nerves which encode taste when the tongue is thermally stimulated, thus resulting in a phantom taste response (Cruz and Green, 2000). An alternative theory is that TTs have a central nervous system gain mechanism, which results in increased excitability in sensory integration areas where trigeminal, gustatory and olfactory inputs merge to produce a flavour perception (Green and George, 2004, Bajec and Pickering, 2008). A genetic mechanism is possible, and transient receptor potential (TRP) cation channels involved in the transduction of chemical stimuli into taste, temperature, irritant and pungent sensations may be involved. The TRPM5 cation channel is a potential candidate for thermal taste as it is involved in the taste transduction of sweet, umami and bitter chemical tastes, and has been found to be temperature sensitive and activated between 15-35°C in the absence of gustatory stimuli (Talavera et al., 2005). Other cation channels associated with taste transduction are possibly involved in the perception of other phantom tastes (sour, salt, bitter) (Talavera et al., 2007) and oral sensations (metallic, spicy, mint). Synaesthetes perceive stimuli from one sensory modality, such as sound, as a different modality, such as vision

(Bargary and Mitchell, 2008). As this 'joining of the senses' can be experienced across different modalities, it is possible that TTs ability to experience taste from thermal stimulation is a new type of temperature-gustatory synaesthesia that, to date, has not been characterised (Yang, 2015). Another theory is that there is co-innervation of the gustatory and trigeminal nerve fibres that innervate the fungiform papillae, and that cross wiring allows them to activate one another (Clark, 2011). This would explain the lack of difference in the perceived intensity (Yang et al., 2014) and cortical response to aroma in TTs compared to TnTs (Eldeghaidy et al., 2015, Yang, 2015) reported in some studies.

5.1.2 Phenotyping to identify thermal taster status

Traditional classification methods to identify thermal taster status are restrictive, and stipulate that TTs must rate phantom tastes above weak in intensity on the general labelled magnitude scale (gLMS). It is probable that TTs perceive taste at a greater range of intensities than is currently represented. Due to this restriction, and inconsistent reporting across the standard two replicates of a temperature trial, current thermal taste phenotyping protocols result in as many as 42% of tested individuals being assigned to an uncategorised (*uncat)* group, thus excluding a large percentage of the study population (Yang et al., 2014). Together, these factors highlight the need to review traditional phenotyping to characterise thermal taster status, and it is hypothesised that an improved methodology may reduce the number of individuals assigned as *uncat*.

5.1.3 Variation in the phantom taste response across TTs

Research to date has focussed on the differences in orosensory perception between TTs and TnTs, whilst little attention has been given to exploring individual differences between TTs, which may help elucidate the mechanism/s

involved. The quality of the perceived phantom taste differs across TTs, with sweet, sour, salty, bitter (Cruz and Green, 2000), metallic, mint (Hort et al., 2016) and spicy (Yang et al., 2014) all reported. The number of tastes, and the temperature at which they are perceived in response to warming/cooling also appears to vary, but evidence is limited. For example, sweet taste is more frequently reported when warming the tongue from 20 to 40°C, whilst cooling the tongue from 35 to 10°C evokes sourness, and saltiness has been reported as the temperature decreases from 10 to 5°C (Cruz and Green, 2000). However, the specific temperature range within which tastes are perceived, and how this varies across TTs, has not yet been quantified. The tongue area which is thermally stimulated has also been shown to influence taste perception, with sweet more frequently reported when stimulating the anterior tip, bitter when stimulating the posterior, and sour by stimulating the lateral edges of the tongue (Cruz and Green, 2000). Phantom taste intensities for individual TTs are not reported in current literature, and group means have ranged between weak (Hort et al., 2016) to above strong (Pickering et al., 2016).

A decrease in perceived taste intensity over time on prolonged exposure to a stimulus, termed adaptation, is often reported for simple tastant solutions (McBurney and Pfaffmann, 1963, Bornstein et al., 1993, Schiffman et al., 1994, Theunissen et al., 2000). The method of stimulus delivery greatly influences the degree to which adaptation occurs, with no change, an increase, or a decrease in perceived intensity being reported for the same stimuli (Halpern and Meiselman, 1980, Halpern et al., 1986). Changes in perceived phantom taste intensity with repeated exposure to thermal stimulation in TTs has not yet been characterised.

In summary, it is important to gain insights into the variation in the characteristics of phantom tastes perceived by TTs as this may influence their wider oral sensitivity and perception, and may help to develop understanding of the mechanisms involved in phantom taste responses.

5.1.4 Variation in temperature perception across TTs and TnTs

As already discussed, orosensory differences have been reported between TTs and TnTs, including the response to temperature. TTs are often observed to rate thermal stimulation applied to the tongue as more intense than TnTs (Green and George, 2004, Bajec and Pickering, 2008; Yang et al, 2014). However, this does not always reach significance (Hort et al., 2016). As no difference in perceived intensity is identified when temperature is delivered to non-gustatory regions of the lip and hand, this indicates the advantage is limited to the oral cavity (Green and George, 2004). It is possible that the tongue location tested influences perceived temperature intensity, as TTs rate cool stimuli delivered to the lateral edges of the tongue as more intense than TnTs, whilst a significant difference was not seen when the same stimuli were delivered to the anterior tip (Bajec and Pickering, 2008). Interestingly, the same effect was not seen when warming the tongue as TTs rated the intensity of this stimuli as more intense when it was applied to both tongue locations. Surprisingly the responses to multimodal temperature/taste stimuli typically yields less consistent results. Bajec and Pickering (2012) identified no significant differences between groups when rating the perceived taste intensity of gustatory stimuli delivered at warm (35°C) and cool (5°C) temperatures. Interestingly, Yang (2015) identified the temperature of gustatory stimuli to have a different effect on perceived taste intensity across thermal taster groups. No difference was observed between TTs and TnTs to ambient gustatory stimuli, whilst TTs reported the same stimuli

delivered cold (5°C) as more intense than TnTs, indicating that temperature modulates taste perception more in TTs than TnTs.

5.1.5 Peripheral temperature processing

Whilst the ability to detect temperature in the oral cavity works as a safety mechanism to avoid burning, the temperature of both the oral cavity and stimuli also influences flavour perception (Engelen et al., 2003). In a review, Gardener and Johnson (2013) identify that temperatures deviating below 31°C are perceived as cool or cold, until the temperature drops below 10-15°C when this is experienced as pain. Temperatures above 36°C are perceived as warm or hot, until they reach above 45°C when pain is perceived. Thermoreceptors, responding to innocuous (non-painful) temperature, and nociceptors, responding to noxious (painful) temperature, are located on free nerve endings of different types of afferent fibres. Warming receptors located in terminals of C-fibres in the dermis are activated by innocuous temperature (36-45°C), and conduct slowly because the fibres are unmyelinated. Temperatures above 45°C activate nociceptors, which are innervated by myelinated Aδ fibres that have faster conductivity and therefore a short latency to signal burning pain. Low and high threshold cold receptors are predominantly located in terminals of Aδ fibres located in the epidermis, and are activated by innocuous temperature 10/15-31°C. Temperatures below 10/15°C activate cold nociceptors which are innervated by C-fibres and elicit a dull pain response. Interestingly some cold receptors respond to high temperatures above 45°C to give rise to a paradoxical response to heat, the mechanism or function of this response is unknown (Long, 1977). Depending on the nature of thermal stimulation, a range of these fibres will be activated and involved in the perceptual response.

TRP channels are a large group of ion channels with involvement in a range of physiological functions such as gustatory, chemesthesis and temperature perception. This group includes 28 TRP members that are divided across seven sub-categories: TRPC (classical or canonical), TRPPP (Polycysteine), TRPML (mucolipin), TRPN (no mechanoreceptor), TRPV (vanilloid), TRPM (melastatin), TRPA (Ankyrin-like), the latter three of which are involved in thermoreception (Ferrandiz-Huertas et al., 2014). **Figure 5.1** outlines the two TRPs which are known to respond to cooling; TRPM8 and TRPA1, and the six responding to warm or hot temperatures; TRPM3, TRPV4, TRPV3, TRPV1, TRPV2, TRPM5, (Ferrandiz-Huertas et al., 2014). Temperature activation thresholds differ across these, and they may also bind to chemical ligands such as toxins or venoms, as reviewed by Ferrandiz-Huertas et al (2014) and Gardener and Johnson (2013), and summarised next.

Both the TRPM8 and the TRPA1 are expressed in high threshold cold receptor terminals, while only TRPM8 is expressed in low threshold cold receptor terminals. The TRPM8 is activated at temperatures <25°C (perceived as cool or cold), and by menthol which gives rise to the familiar perception of a cooling sensation associated with mint/menthol. The TRPA1 is activated at <17°C, is perceived as cold or freezing, and is activated by allium compounds associated with garlic and radish.

Of the warming channels, the least is known about the TRPM3 channel which is expressed in nociceptors and detects noxious heat. TRPV4 is activated in temperatures >27°C. TRPV3 responds to temperatures >33°C to give rise to sensations ranging from warmth to heat, and also responds to camphor. TRPV1 and TRPV2 are expressed in nociceptors and involved in the perception of pain. TRPV1 is activated at temperatures >42°C and is also activated by capsaicin

which is the compound responsible for the burning sensation experienced when the skin comes into contact with chilli. TRPV2 responds to noxious heat >52°C, and derivatives of cannabis sativa like cannabidiol. TRPM5 is activated downstream of the taste receptor, is involved in the transduction of sweet, bitter and umami tastes, and is temperature sensitive and activated in the absence of gustatory stimuli at temperatures ranging from 15-35°C.

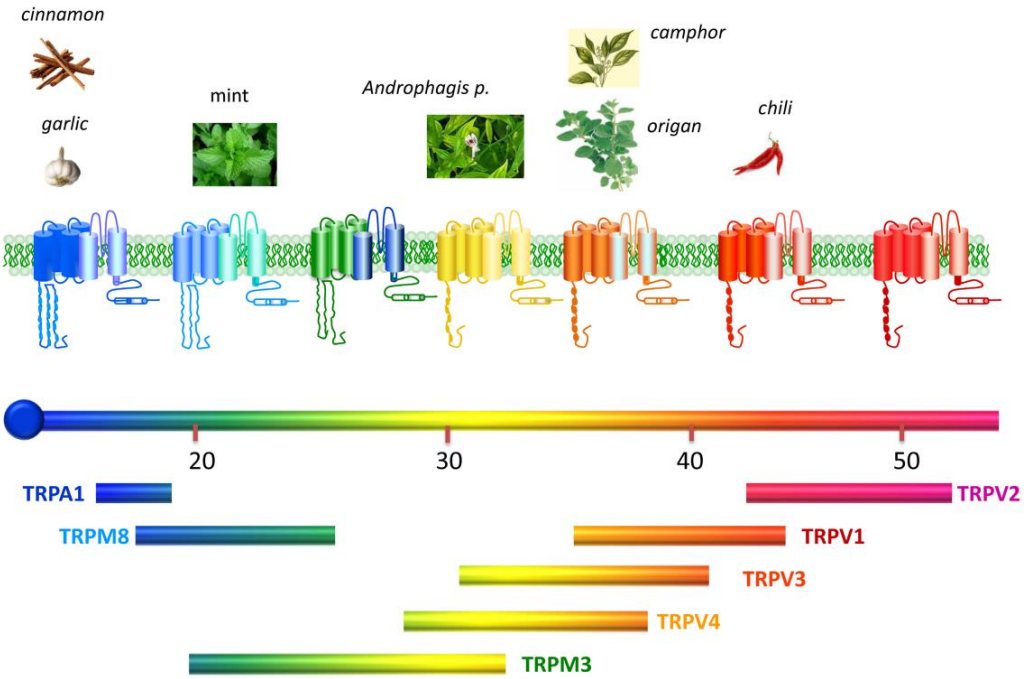

Figure 5.1: TRP channels have distinct temperature thresholds from noxious cold to noxious hot temperatures, and are also activated by chemical compounds (Source: Ferrandiz-Huertas et al., 2014).

5.1.6 Central processing of temperature

Innocuous thermal stimuli presented to the hand or arm are represented in the brain within the thalamus (Davis et al., 1998), somatosensory cortex and posterior insula (Rolls et al., 2008), whilst the subjective pleasantness of the stimuli is correlated with activation of the mid orbitofrontal cortex (OFC), pregenual cortex and ventral stratium, and the lateral/anterior OFC is correlated to subjective unpleasant perception (Rolls et al., 2008). As is seen with the other sensory modalities, this indicates that the hedonic response to temperature is

represented in different areas to its intensity and identification. Nociceptors are typically activated and elicit a pain response at temperatures above 45°C and below 10-15°C (Gardener and Johnson, 2013). Noxious thermal stimuli to the hand or arm show activation in the cerebral vermis, ipsilateral thalamus, premotor cortex, anterior cingulate cortex (ACC) and anterior insula (Casey et al., 1996), posterior insula and putamen (Brooks et al., 2005). Intensity of stimulation has been correlated with activation in the anterior insula and OFC (Craig et al., 2000) SII, lateral medial thalamus (Davis et al., 1998), and cingulate cortex (Maihofner et al., 2002). The oral response to temperature was first evidenced in the brain of the macaque monkey with areas of the anterior insula/frontal operculum (Verhagen et al., 2004, Kadohisa et al., 2005b), OFC (Kadohisa et al., 2004) and amygdala (Kadohisa et al., 2005a, Kadohisa et al., 2005b) responding to warm and cool temperatures. In the human brain, thermal stimuli delivered orally activate the anterior insula (primary gustatory cortex), somatosensory cortex, OFC, ACC, and the ventral stratium, while the OFC and pegenual cingulate cortex areas have been shown to correlate with the pleasantness of stimuli (Guest et al., 2007).

5.1.7 Variation in brain response to oral stimuli across thermal taste phenotypes

Despite growing evidence reporting differing perceptual responses to oral stimuli across taste phenotypes, only two studies (both conducted using functional Magnetic Resonance Imaging (fMRI) at the Sir Peter Mansfield Imaging Centre (SPMIC), University of Nottingham) have compared brain response across this taste phenotype. Hort et al (2016) reported increased brain activation in somatosensory (SII and rolandic operculum) and reward (ACC and DLPFC) areas in response to gustatory-trigeminal (sweet-cold) stimuli in TTs

compared to TnTs. The response between groups differed when delivering sweet/cold stimuli of increasing CO_2 (no, low and high CO_2); a positive correlation between activation in somatosensory areas (SI and SII) and increasing CO_2 levels was observed across both groups. However, increased activation was also identified in primary taste (anterior insula) and reward areas (ACC) in TnTs, whilst decreased activation in these areas was reported for TTs. The authors hypothesised that gustatory and trigeminal nerves are able to activate one another in TTs, and so are both highly stimulated already, whereas the increased cortical activation in TnTs is likely to be a result of stimulation of the trigeminal nerve associated with increasing CO_2. Increased cortical activation has also been observed in somatosensory areas (SI, SII and mid insula) in response to gustatory-trigeminal (sweet-cold) stimuli in TTs when compared to TnTs, whilst no differences were observed for olfactory (ethyl burate) or flavour (sweet/ethyl burate) stimuli, indicating that the TT advantage may be limited to oral responses (Yang, 2015, Eldeghaidy et al., 2015). These findings highlight differences in brain processing between taste phenotypes, therefore indicating a demand for extended research to further explore such effects.

The mechanisms behind thermal taste are not yet understood, with theories suggesting that both peripheral and central mechanisms may be involved (Cruz and Green, 2000). To date there is limited evidence detailing differences in the phantom taste response across TTs, or differences in brain activation across the phenotype. The overall aim of this study was to utilise sensory evaluation and fMRI techniques to explore the phantom taste response across TTs, as well as the temperature related responses across TTs and TnTs. The specific objectives were to:

- Investigate variability in taste qualities reported whilst warming/cooling the anterior tongue in TTs, as performed in traditional thermal taste phenotyping protocols. TTs have been found to perceive a range of different phantom sensations, which are expected to be reported during the phenotyping sessions.
- Characterise the phantom temporal taste response to temperature trials in TTs, identifying the taste quality, intensity and temperature at which tastes are perceived. It is hypothesised that TTs will perceive a range of taste qualities, at variable intensities, and that certain tastes will be perceived at specific temperatures, for example sweet as the tongue is warmed and bitter and sour when it is cooled.
- Determine if adaptation in reported taste intensity occurs with repeated temperature stimulation in TTs. Repeated exposure to chemical stimuli can result in a reduction in perceived intensity. Therefore, it was hypothesised that a reduction in the perceived phantom taste intensity would occur with repeat temperature stimulation.
- Identify if the primary gustatory cortex, and other brain regions associated with taste processing, are activated at the time when phantom taste is perceived in TTs. It is hypothesised that perceived phantom tastes will activate the gustatory cortex, an area which is active in response to 'true' chemical tastants.
- Determine if the perceptual and brain response to temperature differs between TTs and TnTs when thermally stimulating the anterior tongue tip. TTs have been observed to report temperature more intensely than TnTs in a number of previous studies (Green and George, 2004, Bajec and Pickering, 2008, Hort et al., 2016). Therefore, the perceptual, and cortical response was predicted to be greater in TTs than TnTs.

5.2 MATERIALS AND METHODS

The experiments presented in this chapter comprise two parts; Part I used sensory evaluation techniques to explore variation in the perceptual phantom taste response across TTs. Part II measured the effect of thermal taster status on the perceptual and brain responses to temperature through a combination of sensory evaluation and fMRI techniques. The study had ethical approval from the University of Nottingham Medical Ethics Committee (C13032014). All participants gave written informed consent, and an inconvenience allowance for participating was provided. All participants completed a basic health questionnaire (Appendix 1) and MR safety questionnaire (Appendix 2). In Part I, 85 individuals who reported being healthy, non-smokers, aged 19-41 years, with no known taste or smell abnormalities or tongue piercings were phenotyped to identify 37 TTs who then participated in experiments to explore variation in the perceptual phantom taste response. In Part II, 12 TTs and 12 TnTs were scanned using fMRI to measure their brain response to temperature stimulation. Participants were instructed not to consume anything other than water for at least one hour prior to all test sessions, which were individually conducted with each participant.

5.2.1 Part I: Variation in perceptual phantom taste response in TTs

An initial phenotyping session was conducted to identify TTs. These individuals were then invited to attend two further study sessions. During Session 1 (90 min), TTs were trained to use the gLMS, rate their temporal response to the intensity of taste/s perceived in response to thermal stimulation, and identify the associated taste qualities. During Session 2 (60 min), reproducibility of the temporal taste response to thermal stimulation was measured using 10 replicates of each temperature trial.

5.2.1.1 Phenotyping thermal tasters

In total, 85 individuals volunteered to be phenotyped for thermal taster status, based on the methods described by Hort et al (2016). An intra-oral Advanced Thermal Stimulator (ATS) peltier thermode (16 x 16 mm square surface) (Medoc Ltd., Israel) was used to deliver temperature stimulation to the anterior tip of the tongue, as it has the highest fungiform papillae density (Shahbake et al., 2005), and has been shown to be most responsive to thermal taste (Cruz and Green, 2000). Before testing each participant, the thermode was cleaned with 99% ethanol (Fischer Scientific, UK) and covered with a fresh piece of tasteless plastic wrap (Tesco, UK). Prior to the temperature trials being delivered, participants were instructed to position the thermode firmly in contact with the tongue (Green and George, 2004). The traditional warming trial started at 35°C, reduced to 15°C, and then re-warmed to 40°C at which it was held for 1 s (**Fig 5.2a**). The traditional cooling trial started at 35°C, was reduced to 5°C and then held at this temperature for 10 s (**Fig 5.2b**). All temperature changes occurred at a rate of 1°C/s. Participants were instructed to 'attend' to the temperature increasing from 15 to 40°C during the warming trial, and to the whole of the cooling trial. At the end of each trial, the participant was instructed to rate the intensity of the temperature when it reached its maximum on a general labelled magnitude scale (gLMS). If a taste(s) was perceived, a second gLMS was presented so that each of the perceived taste qualities could be rated. Prototypical tastes (sweet, sour, salty, bitter, umami) and 'other' sensations such as metallic, spicy and minty were permitted to be associated with taste perception (Hort et al., 2016). The gLMS consisted of a vertical line, which was 230 mm high. Considering the line to be 100 units, unequal quasi-logarithmic spacing between word descriptors; 'no sensation,' 'barely detectable,' 'weak,' 'moderate,' 'strong,' 'very strong' and 'strongest imaginable sensation of any kind', were placed at 0, 1.4, 6, 17, 35, 53 and 100% of the scale respectively

(Green et al., 1996). Two replicates of each temperature trial were delivered, and where the taste quality or presence of phantom taste was inconsistent across replicates, a third trial was conducted to confirm classification. A two minute palate recovery break was given between replicates and warming/cooling trials. Warming trials preceded cooling trials to prevent possible adaptation from the intense, sustained cold stimulation of the cooling trial (Green and George, 2004). Participants were not made aware of the purpose of the activity, and were informed that people do not always perceive taste in order to reduce any bias of falsely reporting a taste.

Traditional thermal taste phenotyping classifies TTs as those individuals who report taste above weak in intensity, while those who report below weak are classified as *uncat*. To explore the range of sensitivities reported across TTs, this study defined TTs as those individuals who reported taste at any intensity, but that the same taste/s was reported across two replicates of the warming and/or cooling trials. This approach enabled the inclusion of those perceiving low intensity tastes who would traditionally be classified as *uncat*. Participants who only perceived temperature were classified as TnTs, and those who reported taste inconsistently (taste quality or presence of taste) across ≥ 2 replicates were assigned to the *uncat* group. This resulted in 24 participants being classified as TTs. These participants, together with 13 TTs identified from the University of Nottingham Sensory Science Centre database or recruited in Chapter 4, then took part in two additional sessions to further investigate the thermal taste phenomenon.

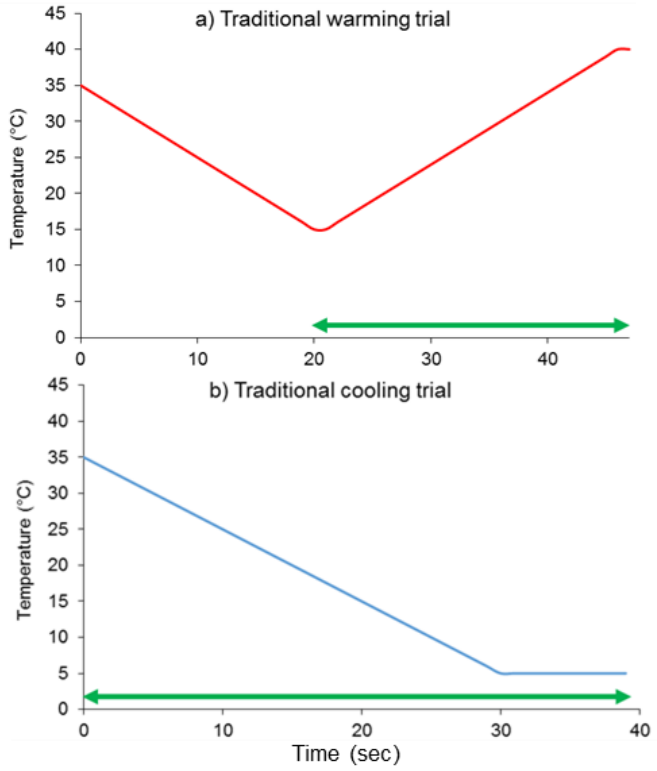

Figure 5.2: Thermode temperature across traditional (a) warming, and (b) cooling temperature trials. Arrows (⟷) indicate when participants were instructed to 'attend' to the test.

5.2.1.2 Modification of temperature trials

During preliminary testing, some individuals reported numbing of the tongue, and occasional pain when the traditional cooling trial was held at 5°C for 10 s, which is expected during this temperature range (Gardener and Johnson, 2013). The cooling trial was therefore modified to hold at 5°C for a shortened period of 1 s instead of 10 s. To compare responses across the traditional cooling trial with that of the modified trial, TTs were phenotyped again using the traditional warming trial, and a modified cooling trial in which the temperature was now held at 5°C for only 1 s. This testing was completed after participants had been trained on correct use of the gLMS during the Sensory Evaluation Session 1 detailed in Section 5.2.1.3, using the testing protocol described in Section 5.2.1.1. The thermal taster status of all participants remained the same

when re-phenotyping, and so the modified cooling temperature trial was used for all further testing.

For subsequent testing, both temperature trials were also extended to return to 35°C after reaching their destination temperature of 40°C (warming) or 5°C (cooling) to aid palate recovery between replicates. This was felt important when delivering a large number of replicates to measure reproducibility and adaptation, and when imaging the brain response to the temperature trials. As the modified temperature trials thus contained both warming and cooling components they are subsequently termed according to the temperature extremes that were reached during each trial; the '40°C trial' (modified warming trial) lasting for 52 s (**Fig 5.3a**), and the '5°C trial' (modified cooling trial) lasting for 61 s (**Fig 5.3b**).

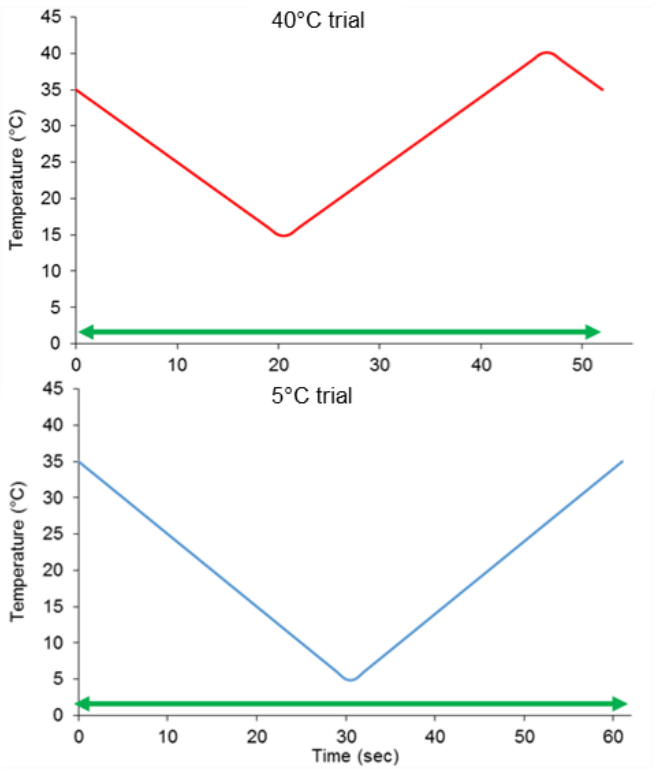

Figure 5.3: Thermode temperature across modified (a) 40°C and (b) 5°C temperature trials. Arrows (⟷) indicate when participants were instructed to 'attend' to the test.

Traditional thermal taste phenotyping requires a perceptual response to be taken only during the 'warming' (15-40°C of the warming trial) or 'cooling' (35-5°C of the cooling trial) component of the temperature trial. Here, this was expanded such that all taste responses were collected across the entirety of each modified temperature trial (35-35°C) to capture the complete temporal taste response to temperature stimulation. A specialised thermode holder/mouthpiece was engineered and used to standardise the positioning of the thermode on the tongue across both replicates and assessors (**Fig 5.4**).

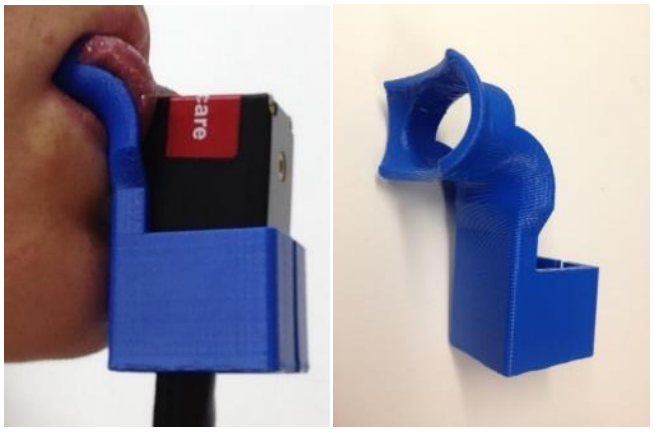

Figure 5.4: Mouthpiece used to guide the positioning of thermode on the tongue.

5.2.1.3 Session 1: Identifying taste qualities perceived during modified temperature trials

The aim of Session 1 (90 minutes) was to familiarise participants with the use of the gLMS, study protocols, and record the nature of the taste(s) they perceived using the modified temperature trials.

<u>gLMS familiarisation:</u>

Participants were trained on the correct use of the gLMS (Bartoshuk et al., 2002) as described in Section 2.2.4.3. In summary, a blank gLMS was provided, and participants instructed to add their strongest imaginable sensation at the top of

the scale, before rating the perceived intensity of 15 remembered or imagined sensations on their scale. This created each participants' individualised reference gLMS which was presented during all subsequent testing to guide the level of intensity of the sensations perceived.

Temporal taste protocol familiarisation:

To collect taste and temperature evaluations in response to the thermode temperature in real time, participants used an on-screen gLMS (Presentation Software, Neurobehavioral System, San Francisco, US) to which they responded by rating using a rollerball. Participants were initially familiarised with the procedure by running a small number of practice trials rating the perceived temperature, and then any perceived tastes separately.

Identifying taste qualities associated with the temporal response:

Temperature trials were delivered again to identify which taste(s) were associated with which elements of the temporal taste response, as more than one taste can be reported per trial (Yang, 2015). A list of tastes (sweet, sour, salty, bitter, umami), metallic, and the option to report 'other' were presented to participants on a sheet prior to the test commencing. Two replicates of each temperature trial were conducted, during which the participant was instructed to point to the relevant word descriptors on the sheet in real time to indicate whether 'no taste,' 'taste,' or 'other' sensations were perceived across the trial. If the 'other' option was selected, the participant was asked which sensation they had perceived once the trial had finished. More than one sensation could be reported at any one time. Both the taste quality and the temperature range at which it was perceived were recorded.

5.2.1.4 Session 2: Measuring the temporal taste response over repeat replicates

The aim of Session 2 was to explore the variability in taste response across TTs, its reproducibility within a TT across a large number of replicates, and to identify whether any adaptation in perceived intensity occurred.

A block of 10 repetitions of the 40°C trial was followed by a block of 10 repetitions of the 5°C trial. The inter-stimulus-interval between replicates was reduced to 10 s as preliminary testing (data not shown) found this duration to be long enough for the tongue to recover. The 40°C trial block preceded the 5°C trial block to prevent adaptation from the intense cold stimulation delivered during the 5°C trial. A five minute palate recovery break was given between blocks. Participants were instructed to use the rollerball to rate the intensity of any perceived taste/s on the gLMS for each replicate of all trials. At the end of each block of temperature trials participants verbally reported if any taste/s were perceived and these were recorded by the researcher.

5.2.1.5 Data analysis

The percentage of individuals phenotyped as TT/TnT/*uncat* was determined, and the frequency of taste sensations reported during the traditional warming and cooling trials used during the initial phenotyping session were computed. The frequency of taste sensations reported when re-phenotyping TTs using the cooling trial that held at 5°C for 1 second instead of 10 seconds, was also identified.

The taste qualities perceived by TTs were recorded from the ‘taste identification temperature trials’ performed at the end of Session 1, and the block of 10

replicate trials during Session 2. The 10 temporal taste response replicates were visually checked for consistency. If the rating of one individual replicate was not within one standard deviation of the remaining 9, that replicate was defined as an outlier and removed. Temporal response curves were analysed using GraphPad Prism version 7.02 (GraphPad software, USA). The mean maximum intensity (*Imax*) for each taste reported across the 10 replicates for each participant was calculated. TTs who reported inconsistently across the 10 replicates were not included in this analysis. Here, and for all subsequent gLMS intensity ratings, a value of 0 was converted to 0.5, and the data was $\log_{10}$ transformed before statistical analysis, as the data is not normally distributed.

Variation in the temporal taste responses was explored. Consistency of the temporal taste response across the 10 replicates was visually assessed, and where the ratings were consistent a mean intensity rating was calculated for each TTs taste response for each temperature trial. The shape/duration of these response curves was compared across TTs to explore variability in ratings. The temporal curves for TTs were then visually grouped by shape and duration to categorise the different types of responses that were observed for each temperature trial (40°C and 5°C).

Graphpad Prism software was used to identify the onset and offset temperature at which taste/s were reported by each TT during each replicate of the temporal response from Session 2, and the means (+/-stdev) calculated. In cases where two temporal taste peaks were reported during a single temperature trial, but for which the taste intensity rating did not return to zero between peaks, the second taste was identified from the onset of the second taste peak.

To determine if adaptation in perceived taste intensity occurred across replicates, a one-way (replicate) repeated measures Analysis of Variance (ANOVA) was conducted on the log *Imax* (log value) across each temperature trial. Analyses were performed using SPSS, version 21 (SPSS IBM, USA) with an α-risk of 0.05. Participants inconsistently reporting across replicates, or perceiving taste on <10 replicates were excluded from this analysis (40°C (n = 8/34), 5°C (n = 7/34)).

5.2.2 Part II: Measuring variation in temperature and phantom taste related brain response in TTs and TnTs

In Part II a second set of experiments were conducted to measure phantom taste-related brain activity in TTs, and to compare differences in the perceptual and brain response to temperature across thermal taste phenotypes. In order to compare the perceptual and brain response, these experiments combined both sensory evaluation and fMRI imaging techniques. It was necessary to collect these combined measures as the perceptual responses were used in the fMRI data analysis to model the fMRI response, and interpret the fMRI results. This study formed part of the multidisciplinary 'TASTEMAP' BBSRC funded project, a collaboration between the Sensory Science Centre and SPMIC, University of Nottingham. The authors responsibilities within this project included participant recruitment and screening, collection of all sensory data, including providing an explanation of the fMRI scanning sessions to participants, setting participants up in the MRI scanner, and analysis and interpretation of all sensory data. All fMRI data was processed and analysed by Dr Sally Eldeghaidy (SPMIC), with the sensory data then used to together with the fMRI analysis to interpret findings.

5.2.2.1 Participants

12 TTs and 12 TnTs were recruited from those who had already been phenotyped for thermal taster status, and trained on correct use of the gLMS, during experiments detailed in Part I of this chapter, (n=9) or Chapter 4 (n=15). All were non-smokers aged between 19-41 years (13 female/8 male), who reported as being healthy, having no known taste or smell abnormalities or tongue piercings, and had passed an MR safety questionnaire. PROP taster status had been determined during phenotyping sessions in Section 4.2.2.3, or during previous studies conducted through the Sensory Science Centre, using the same method. Oral sensitivity is proposed to vary across PROP taster status groups. To control for this variance, when making comparisons across the thermal taster status phenotypes, all participants were PROP tasters, except for one PROP non-taster in each of the TT and TnT groups.

5.2.2.2 Temperature stimulation

The peltier thermode and mouthpiece (**Fig 5.4**) were used to deliver temperature stimulation to the anterior tip of the tongue during both the sensory and fMRI sessions. The modified 40°C and 5°C temperature trials described in Section 5.2.1.2 were used for all testing. During fMRI scan sessions the thermode system was situated in the MR control room, and a long MR compatible thermode attachment was attached to the penetration panel with an RF filter and then to the participant in the MR room. Trials were controlled by a PC and triggered by the MR scanner during each fMRI block.

5.2.2.3 Sensory evaluation of temperature stimulation

Participants recruited from Part I of this chapter were not required to attend any further sensory evaluation sessions, as their temporal taste responses had

already been collected. TTs recruited from Chapter 4 were required to attend an additional 60 minute Sensory Evaluation Session where their temporal phantom taste response was measured.

5.2.2.4 Sensory evaluation session: Protocol familiarisation, and measuring reproducibility of the temporal taste response

Participants were familiarised with using the rollerball to rate the temporal intensity of temperature and phantom taste using the method described in Section 5.2.1.3. The taste qualities associated with each temporal taste response were identified using the method described in Section 5.2.1.3 'Identifying taste qualities associated with the temporal response'. The temporal phantom taste response was also collected across 10 replicates of each temperature trial, using the method described in Section 5.2.1.4. This provided a measure of consistency across replicates, and allowed the comparison of the responses collected outside the scanner with measures collected inside the scanner (detailed in Section 5.2.3.1).

5.2.2.5 Sensory evaluation data analysis

The frequency of different phantom taste sensations reported by TTs during their phenotyping were ascertained. All phantom taste and temperature intensity ratings were $\log_{10}$ transformed. The mean taste intensity perceived by TTs was calculated for each taste quality reported across the warming and cooling trial separately. Perceived temperature intensity ratings for TTs and TnTs were averaged. To determine if TT status had a significant effect, a two-way ANOVA (TT status and replicate) was applied for gLMS temperature intensity ratings collected for each temperature trial. All analyses were performed using SPSS, version 21 (SPSS IBM, USA) ($\alpha = 0.05$). The 10 replicates of the temporal

phantom taste rollerball responses made by TTs were visually checked for consistency. If the rating of an individual replicate was an outlier and did not match that of the remaining nine replicates made by the participant, then the anomalous replicate was removed.

5.2.3 fMRI method: Phantom taste and temperature related brain activation

5.2.3.1 fMRI scan session

All TTs and TnTs attended a fMRI scan session (60 min). As hunger/satiety can influence taste sensitivity (Haase et al., 2009b) participants were instructed to consume a light meal at least one hour before the scan commenced. All participants were scanned during thermal stimulation of the tongue. Blocks of 10 repetitions of 40°C trials followed by 10 repetitions of 5°C trials were delivered, as described in Section 5.2.1.4. A control task followed, during which participants simply moved the rollerball up to and down the gLMS. This was employed to model any confounding effects (e.g., due to motor activation) of the rollerball rating on the cortical response. The fMRI scan session is represented schematically in **Figure 5.5**. To allow the tongue to recover between the 10 replicates of the 40°C and 5°C trials, an anatomical T_1-weighted image was acquired.

At the beginning of each block of temperature trials participants (TTs and TnTs) were instructed to use the rollerball to rate the intensity of perceived phantom taste(s) on the gLMS, and to leave the rollerball rating at ‘no sensation’ if they only perceived temperature during all replicates of the temperature trials. At the beginning/end of each temperature trial block, participants were verbally instructed to insert or remove the mouthpiece holding the thermode. Visual cues were delivered (Presentation Software, Neurobehavioral System, San

Francisco, US) on a screen at the end of the scan bed, which the participants could see using a mirror attached to the head coil. The word ‘rest’ directed participants to remove the tongue from being in contact with the thermode, the word ‘ready’ directed them to place the tongue in contact with the thermode as the temperature trial was about to begin. When the gLMS was displayed on the screen the rollerball could be used to rate the intensity of any perceived phantom taste throughout the trial. At the end of each temperature trial block participants who had rated taste intensity were asked which taste(s) had been perceived, and this was recorded by the researcher. All participants were asked to rate the maximum intensity of any temperature-related pain elicited from thermal stimulation on a blank gLMS. During the control task participants were instructed to insert the mouthpiece whilst making sure the tongue was not in contact with the thermode. Visual cues were delivered that were identical to those delivered during temperature trials, however, participants were instructed to use the rollerball to rate up to ‘strong’ and back down again each time the gLMS was presented, over 10 replicates.

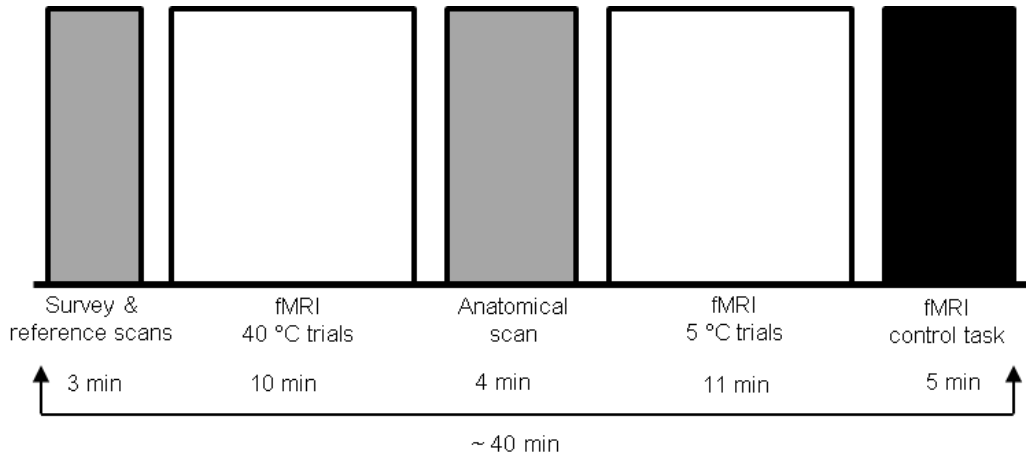

Figure 5.5: Schematic representation of the fMRI scan session. In each temperature block, 10 replicates of either the 40°C or 5°C trials were delivered.

5.2.3.2 fMRI data acquisition

Data were acquired on a 3T Philips Achieva scanner with a 32-channel receive head coil. fMRI data was collected using a double-echo gradient-echo, echo-planar-imaging (GE-EPI) acquisition: TE = 20/45 ms, TR = 2500 ms, flip angle (FA) 85°, 3 mm isotropic spatial resolution, 240 x 240 mm^2 field of view (FOV), SENSE factor 2 in the right-left (RL) direction, and 36 contiguous axial slices aligned parallel with AC-PC plane. A total of 250 volumes (10 min) were acquired for the 40°C trials, 284 volumes (11 min) for the 5°C trials, and 100 volumes for the control task (4 min). A T_1-weighted MPRAGE image (1 mm isotropic resolution; TE/TR = 8.3/3.8 ms, FA = 8°, SENSE factor = 2, 160 slices, 256 x 256 matrix) was collected (4 min) to aid registration of fMRI data to MNI space.

5.2.3.3 fMRI data analysis

The double echo fMRI images were combined using a weighted summation. These weighted fMRI images were then processed using SPM12 (www.fil.ion.ucl.ac.uk/spm). Images were realigned, slice-timing corrected, normalized, and spatially smoothed using a 6-mm full-width half-maximum Gaussian kernel. A first level GLM analysis was formed to model both the 40°C trials and 5°C trials to identity brain areas responding to 1) phantom taste "phantom taste mapping" in TTs. 2) thermal stimulation of the tongue in TTs and TnTs "temperature response mapping".

<u>Phantom taste mapping in TTs</u>

For phantom taste mapping in TTs, the onset and duration of the perceived phantom taste was identified from the rollerball rating continually collected during the fMRI acquisition of the 40°C and 5°C trials for each participant. This

rollerball time series was convolved with a canonical haemodynamic response function (HRF), and motion parameters included as covariates of no interest. In this model the temperature change (identified from thermal stimulation waveform during the 40°C and 5°C trials) was also included as covariate of no interest. A high-pass frequency filter (cut-off 128 s) and autocorrelation correction were applied to the time series. To identify brain activation in response to phantom taste, a contrast vector of (phantom taste > control taste) was formed. Thermal tasters who perceived a phantom taste during anytime point of the 40°C or 5°C trials were combined at the second level using a one-sample t-test random effects group (RFX) group analysis to form combined group maps to phantom taste.

TTs rated the temporal intensity of the perceived phantom taste across 10 replicates of each temperature trial during the Sensory Evaluation Session (outside the scanner, Section 5.2.2.4), and across the 10 replicates of each temperature trial collected during the fMRI session inside the scanner (Section 5.2.3.1). To examine the relationship between responses across sessions for each TT, interclass correlation coefficients (ICC) were calculated on the mean temporal taste ratings from the ten replicates of each temperature trial outside of the scanner, and compared to the mean ratings collected inside the scanner.

Temperature response mapping across TTs and TnTs

A second model was generated to assess the brain's temperature response to thermal stimulation of the tongue in TTs and TnTs. In this model the time series of the temperature change during the 40°C and 5°C trials was convolved with canonical HRF. Motion parameters were included as covariates of no interest. A high-pass frequency filter (cut-off 140 s for the 40°C trial, and 160 s for the 5°C trial) and autocorrelation correction were applied to the time series. To

identify the brain activation to temperature change during the 40°C and 5°C trials, a contrast vector of (temperature response > control) was formed. A one sample t-test RFX analysis was first performed to determine the brain's response to thermal stimulation of the tongue in each of the TT and TnT group. Second, the difference in the brain's response between TTs and TnTs to temperature change during the 40°C and 5°C trials was assessed, using a two-sample *t*-test. A binary mask of temperature response in TTs and TnTs *(*p<*0.05, uncorrected*) was included in the model. For all RFX analysis, statistical parametric maps (SPM) were threshold at a false discovery rate (FDR) corrected probability of p<0.05, k> 10 voxels. Additional activations at lower thresholds are reported when relevant.

5.3 RESULTS

5.3.1 Part I: Variation in perceptual phantom taste response in TTs

5.3.1.1 TTS and taste qualities perceived by TTs during phenotyping

Of the 85 participants attending the initial phenotyping session, 28% were TTs, 51% TnTs, and 21% *uncat*. Notably seven participants classified as TTs would have been classified as *uncat* if using the traditional phenotyping method delivering only two, rather than three, replicates of each temperature trial. The current protocol permitted TTs to report the same taste on only two of the three replicates administered. Of the 37 TTs recruited, data from one participant was removed due to contradictions between temporal taste ratings and what was reported verbally, leaving 36 (13 male) participants for analysis. During the initial phenotyping, the tastes most frequently reported during the traditional warming trial were sweet (42%), metallic (13%) and spicy (13%) (**Fig 5.6a**). During the traditional cooling trial they were sour (25%), bitter (25%) and metallic (17%) (**Fig 5.6b**).

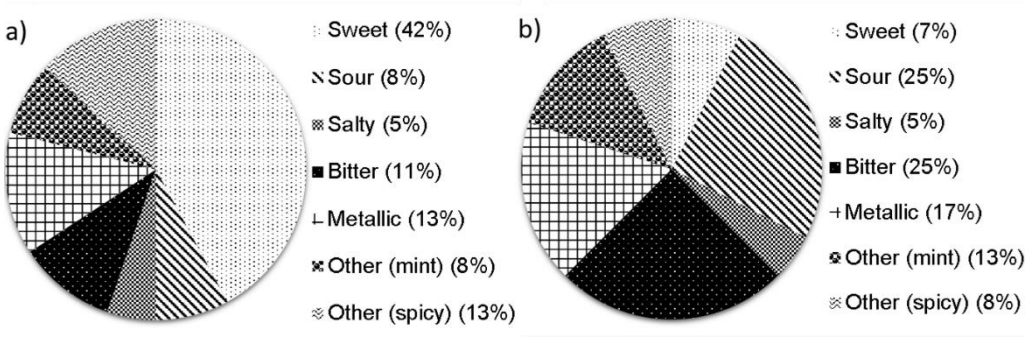

Figure 5.6: Taste qualities (%) reported by 36 TTs when screening to classify TT status during the traditional warming (a) and cooling trial which holds at 5°C for 10 seconds (b).

Figure 5.7 shows the frequency of taste qualities reported when TTs were re-phenotyped using the cooling trial held at 5°C for 1 s instead of the traditional 10 s. Minor variations in the frequency of taste qualities reported were observed. However, no TTs were re-classified during the second phenotyping session. As before, sweet was most frequently (45%) reported when warming the tongue (**Fig 5.7a**), and bitter (28%) and sour (25%) when cooling it (**Fig 5.7b**).

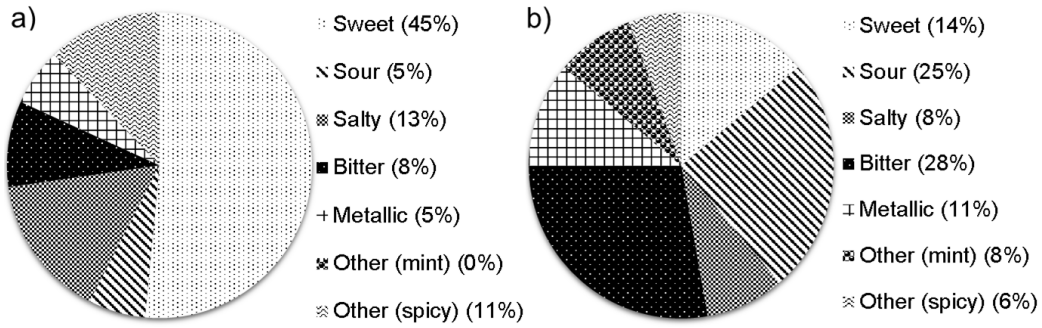

Figure 5.7: Taste qualities (%) reported by 36 TTs when re-phenotyping TT status during the traditional warming trial (a) and the modified cooling trial which holds at 5°C for 1 second (b).

5.3.1.2 Variation in temporal taste response

Variation was observed across TTs in terms of the taste quality, intensity, number of tastes perceived, the reproducibility and category of temporal taste response, and temperature range of perception, detailed next.

5.3.1.3 Taste qualities and intensities perceived during modified temperature trials

A number of different taste qualities were reported by TTs during the temporal taste testing performed using the modified temperature trials (**Table 5.1**). Tastes also varied in the level of intensity at which they were perceived, with *Imax* ranging from 0.19 (below barely detectable) to 1.94 (above very strong) on the gLMS. Two TTs (participant 5 and 17) reported taste intensity below weak on the gLMS, and ordinarily would have been classified as *uncat* if using the traditional phenotyping protocols. Only four TTs (participant numbers 3, 5, 35 and 36) reported 'no taste' across one of the two temperature trials, and the number of perceived tastes perceived by an individual ranged from 0-4 during one trial. In the majority of cases (69%) one individual taste was reported alongside one temporal response. However, in 31% of responses, multiple tastes (two to four) were associated with a single temporal peak, or were reported inconsistently across replicates. This had not been anticipated, and meant that in these latter cases it was not possible to assign a particular temperature range to a given taste perceived.

5.3.1.1 Categories and reproducibility of temporal taste response

Examples of two TTs reporting reproducible temporal taste responses across 10 replicates of temperature trials during the sensory evaluation Session 2 are shown in **Figure 5.8**.

Table 5.1: Taste/s and mean intensity (stdev) reported during temperature trials. *Indicates inconsistent temporal taste reporting across replicates and mean intensity not calculated.

	Taste/s reported during the 40 °C trial				*Taste/s reported during the 5 °C trial*			
Participant	First Taste/s	Mean intensity	Second Taste/s	Mean intensity	First Taste/s	Mean intensity	Second taste/s	Mean intensity
1	Spicy	0.92 (0.41)			Spicy	0.91 (0.98)		
2	Bitter	1.35 (0.68)			Bitter	1.35 (0.62)		
3	Sweet	1.08 (0.02)			No taste			
4	Sweet	0.82 (0.39)			Sweet	0.86 (0.36)		
5	Sweet	0.77 (0.36)			No taste			
6	Sweet	1.41 (0.25)			Bitter, sweet	1.06 (0.86)		
7	Salty, sweet	1.20 (0.62)			Salty	1.62 (0.65)	Sweet	1.08 (0.49)
8	Salty, sweet	1.64 (0.93)			Bitter, salt, sweet, umami	*		
9	Bitter, salty, umami	0.84 (0.55)			Bitter	0.59 (0.12)		
10	Bitter	1.60 (0.50)	Bitter	1.54 (0.72)	Bitter	1.56 (1.89)	Bitter	1.48 (0.50)
11	Bitter	1.29 (0.50)	Sweet	1.26 (0.39)	Bitter	1.16 (0.49)	Sweet	1.15 (0.68)
12	Mint	1.56 (1.07)	Sweet	1.18 (0.73)	Mint, salt	1.20 (0.88)	Sweet	0.98 (0.47)
13	Sour	1.65 (0.76)	Sweet	1.49 (0.60)	Sour	1.67 (0.56)	Sweet	1.41 (0.80)
14	Mint	1.33 (0.64)	Sweet	1.50 (0.83)	Minty	1.14 (0.67)	Sweet	0.69 (0.30)
15	Bitter	1.53 (0.62)	Salty	1.19 (0.81)	Bitter	1.66 (0.50)		
16	Sour	1.22 (0.64)	Sweet	1.11 (0.56)	Sour	1.42 (0.82)	Sweet	1.13 (0.57)
17	Mint	0.23 (0.27)	Spicy	0.36 (0.03)	Metallic, mint	0.19 (0.03)		
18	Minty	1.31 (0.78)	Sweet	1.12 (0.76)	Minty	1.21 (0.65)	Sweet	1.05 (0.52)
19	Metallic	0.94 (0.44)	Spicy	0.66 (0.04)	Metallic, sour, bitter	1.18 (0.84)		
20	Bitter	1.09 (0.30)	Spicy	1.00 (0.36)	Bitter	1.12 (0.50)		
21	Mint, sweet	1.37 (0.53)	Spicy, sweet	1.42 (0.64)	Sweet, mint, salt	1.48 (0.36)	Sweet	1.24 (0.67)
22	Minty	1.13 (0.72)	Bitter, spicy	0.96 (0.61)	Minty	1.24 (0.73)		
23	Metallic, bitter	0.83 (0.42)	Metallic, bitter	0.77 (0.54)	Metallic	1.14 (0.81)		
24	Bitter, sour	1.77 (0.98)	Sweet	1.72 (0.42)	Sour, bitter	1.87 (0.88)	Sweet	1.43 (0.61)
25	Bitter	1.43 (0.78)	Sweet	1.44 (0.68)	Bitter	1.59 (0.52)	Sweet	1.35 (0.85)
26	Mint, bitter	1.76 (1.10)	Sweet	1.72 (0.94)	Minty, bitter, sour	1.78 (0.89)	Sweet	1.76 (0.86)
27	Salty, sweet	1.31 (0.86)			Salty, sweet	1.32 (0.68)		
28	Bitter, sweet	1.50 (0.73)			Bitter	1.51 (0.54)	Sweet	1.13 (0.55)
29	Metallic, sweet	1.37 (0.78)			Metallic	1.46 (0.62)		
30	Sour, bitter, sweet	1.43 (0.73)			Sour	1.49 (0.41)	Bitter, sweet	1.24 (0.74)
31	Mint, sweet	*			Minty	1.52 (0.89)	Sweet	1.15 (0.65)
32	Sour, salt, sweet, spicy	*			Sour	1.65 (0.60)	Sweet	1.11 (0.45)
33	Bitter, sour, sweet	*			Bitter, sour, sweet	1.28 (0.95)		
34	Bitter	*	Sweet	*	Bitter, sweet	*		
35	No taste				Sour	1.94 (0.27)		
36	No taste				Sour, spicy	1.17 (0.88)		

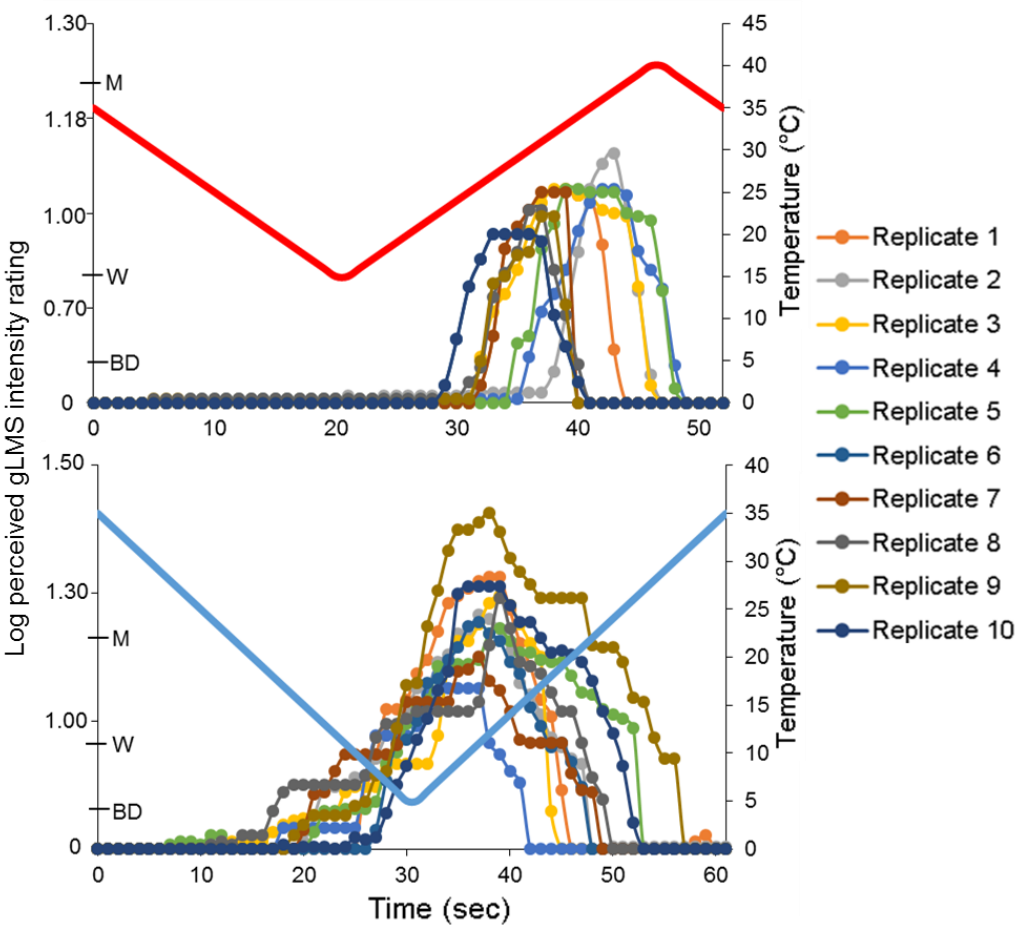

Figure 5.8: Examples of two TTs temporal taste rollerball rating for a block of 10 repetitions of a) 40°C and b) 5°C temperature trials during the sensory evaluation Session 2.

A number of different trends in temporal taste response were evident, indicating the variability of responses across TTs. For demonstration purposes, **Figure 5.9** shows variation in the types of temporal taste responses for the 40°C trial only. No taste was reported on 5% of responses (5% of 5°C trials). One temporal peak, occurring over a short temperature range (**Fig 5.9a**), was reported during 25% of trials (33% of 5°C trials). For 42% of trials, two temporal peaks were reported, again across short temperature ranges (**Fig 5.9b**) (33% of 5°C trials). Of these two types of responses (a & b) there were a small number of occasions (four of the 40°C trials and two of the 5°C trials) where a taste was reported on ≤7 of the 10 replicates (**Fig 5.9c**). These were usually reported at weak intensities, indicating that some TTs experience a very subtle taste in response

to temperature stimulation but that it is generally repeatable. For a further 6% of trials, two peaks were reported, but the intensity rating did not return to zero between (**Fig 5.9d**), indicating one taste may merge into another as the temperature changes (11% of 5°C trials). A taste was reported throughout most of the temperature stimulation for 11% of trials (**Fig 5.9e**) (11% of 5°C trials). An inconsistent temporal response across replicates, (an example shown in **Fig 5.9f**), was reported for 11% of trials (6% of 5°C trials).

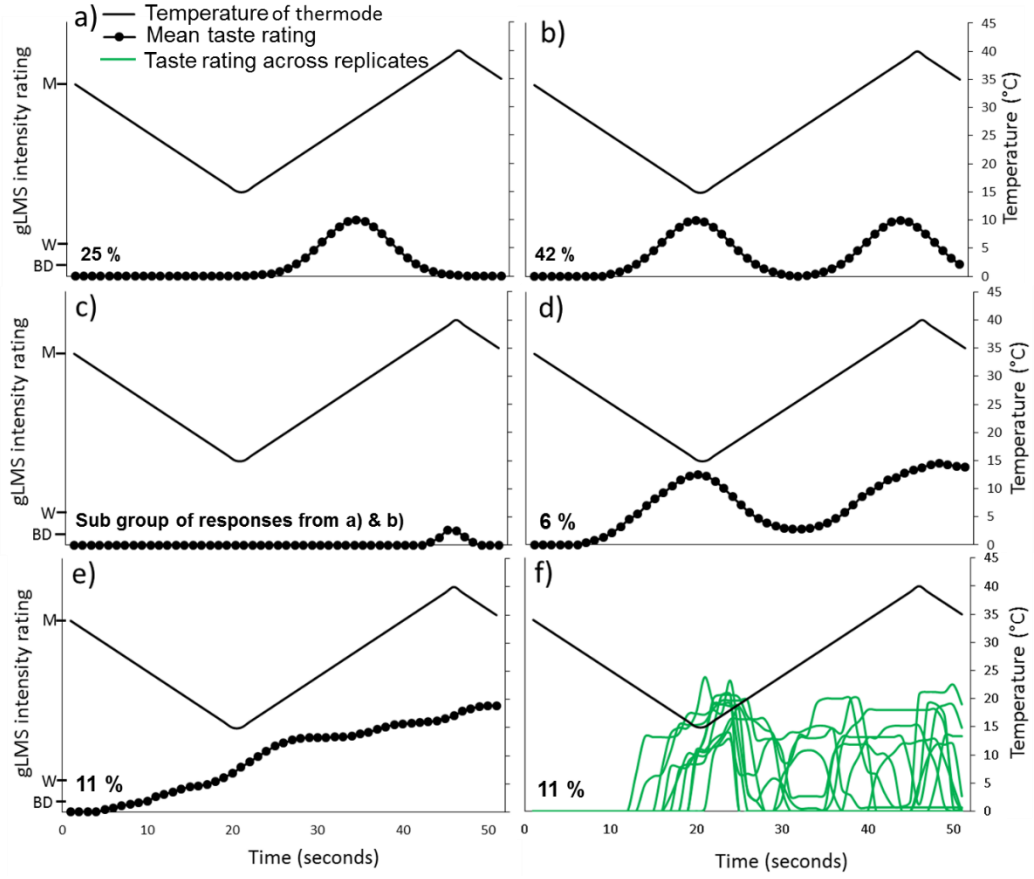

Figure 5.9: Schematic diagram showing categories of taste responses identified by the author, including frequency of response type (%). Responses are displayed for the 40°C trial only, but apply equally to the 5°C trial. BD-barely detectable, W-weak, M-moderate. a) A single temporal taste peak. b) Two temporal taste peaks. c) Minimal temporal taste peak (a small subset of TTs categorised in a) and b) perceived taste on ≤7 of the 10 replicates, with intensity often barely detectable or weak). d) Two temporal taste peaks but no return to zero between the peaks. e) Prolonged temporal taste response across most of trial. f) Example of taste inconsistently reported across 10 replicates by one TT.

5.3.1.2 Temperature range of the taste responses

During both the 40°C (**Fig 5.10**) and 5°C (**Fig 5.11**) trials, tastes (**Table 5.1**) were reported at variable temperature ranges across TTs. Some individuals reported taste associated with only warming (n=5), or cooling (n=2) temperatures, whilst most TTs perceived taste during both warming and cooling (n=29). In line with the phenotyping results, sweet was most frequently reported when warming the tongue, and bitter when cooling. Interestingly, sweet was reported alone during 28% of total responses, and always during warming when the temperature was increasing. Onset of the sweet taste ranged between 22-38°C. Bitter was reported alone during 17% of total responses, and the temperature range of its perception was more variable. Although the onset of bitterness predominantly occurred when the tongue was being cooled (between 32 and 18°C), onset did occur as temperature increased on three trials (between 19 and 25°C). Two of these responses were made by the same TT (participant 10) who reported two temporal taste peaks on each temperature trial, and the third response was made by a TT (participant 9) who perceived bitterness only when the tongue was being warmed and not cooled.

Sourness alone was reported during 7% of total responses, and onset was always during cooling of the tongue (between 27-17°C). During 9% of the total responses, mintiness was reported, and again the onset was always when the temperature was decreasing (between 27-13°C). The temperature range of individual taste qualities could not be determined when multiple tastes were associated with one temporal response. Other phantom sensations (salt, umami, metallic and spicy) were not generally reported alone, and thus the temperature range of perception was not isolated or discussed in detail.

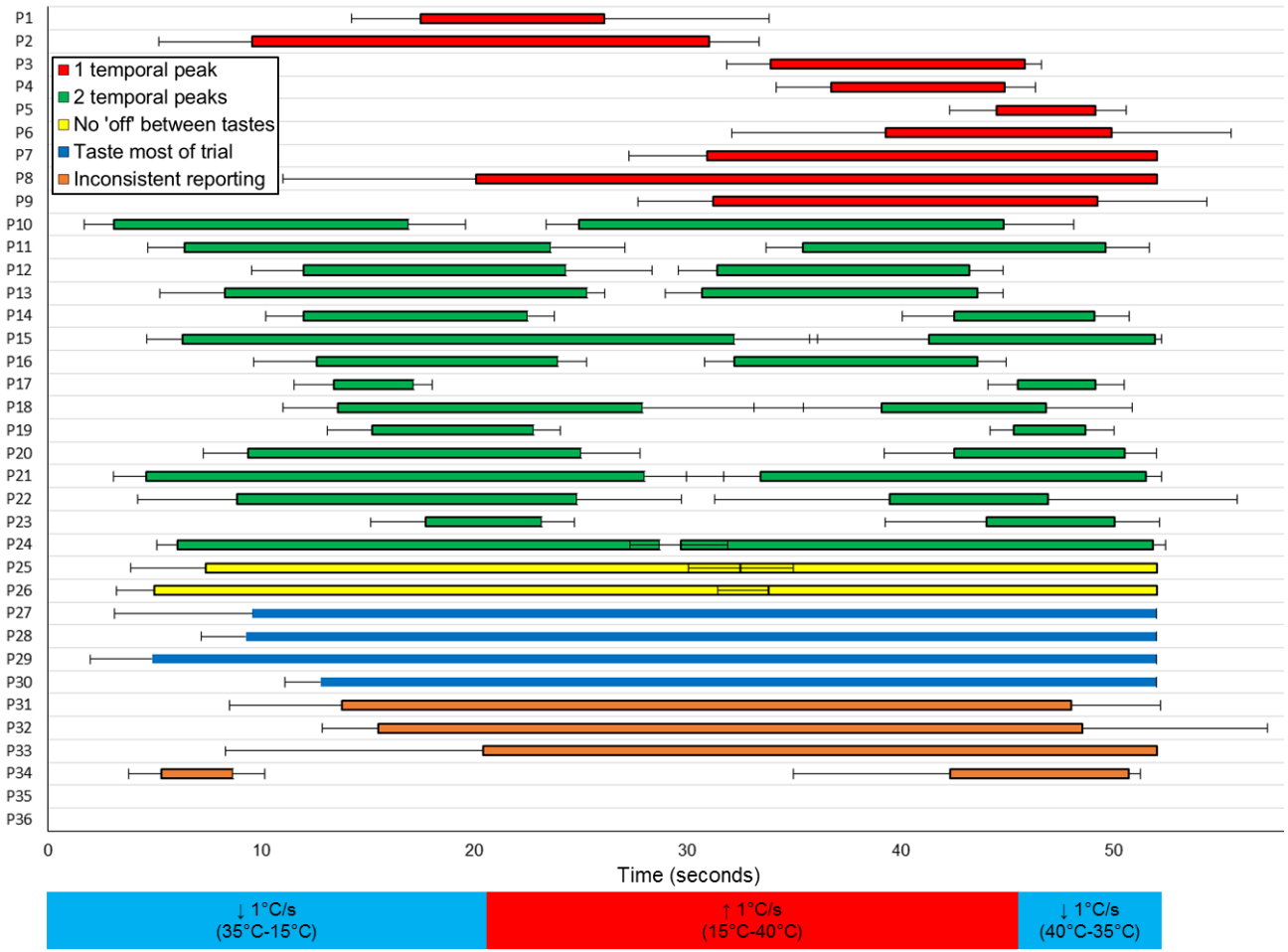

Figure 5.10: Mean temperature range of temporal taste responses reported by each participant (P) during the 40°C trial. Error bars show ± 1 S.D of mean onset and off of taste. Coloured boxes indicate when the temperature of the thermode was warming (↑) or cooling (↓) (± 1°C/s).

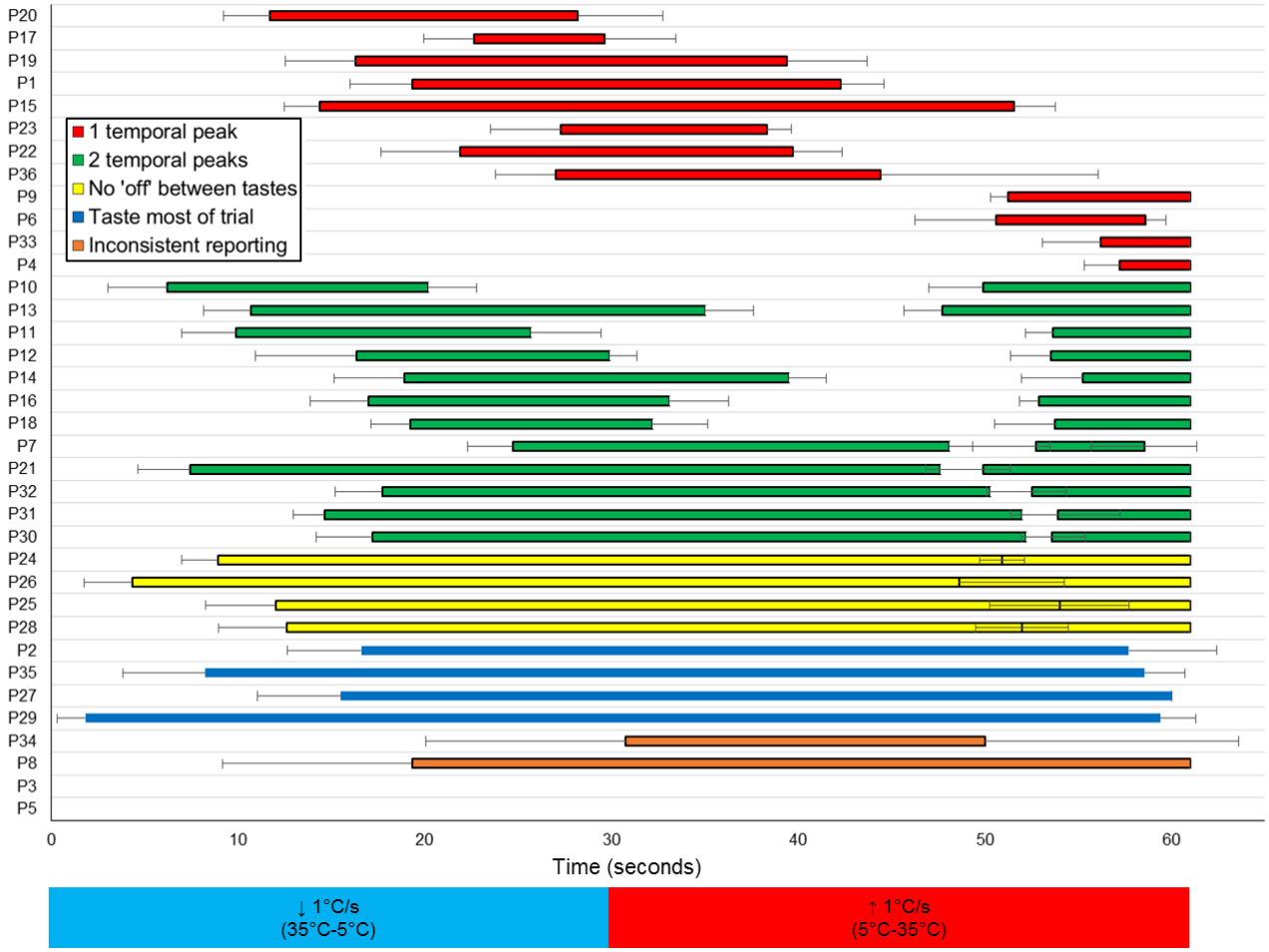

Figure 5.11: Mean temperature range of temporal taste response reported by each participant (P) during the 5°C trial. Error bars show ± 1 S.D of the mean onset and off of taste. Coloured boxes indicate when the temperature of the thermode was warming (↑) or cooling (↓) (± 1°C/s).

Tastes were associated with a brief temperature range (as little as 3.3 sec, equivalent to 3.3°C of the trial) for some TTs, whilst others perceived taste(s) for a longer duration spanning most of the trial (as long as 58 sec, equivalent to 58°C of the trial), which includes both a warming and cooling spell. This highlights the different taste/temperature specificities across TTs. When taste was reported over a short duration (and therefore over a small temperature range) the response was frequently associated with individual taste qualities (e.g. participants 16-20 on the 40°C trial, **Table 5.1** and **Fig 5.10**), whilst responses that lasted for longer durations or most of the trial (and therefore over a long temperature range), were frequently associated with more than one taste quality (e.g. participants 27-30 on the 40°C trial, **Table 5.1** and **Fig 5.10**). Those reporting individual tastes associated with individual temporal peaks predominantly showed responses as illustrated in **Figure 5.9 a & b**, whilst those reporting multiple taste qualities were frequently associated with rating across most of the trial (**Fig 5.9e**), or inconsistent reporting across replicates (**Fig 5.9f**).

It is also important to note that some tastes elicited during cooling persisted as the temperature increased during the subsequent warming component of the trial, which has not previously been observed due to the nature of when participants are traditionally asked to attend to the temperature stimulus.

5.3.1.1 Adaptation

The mean log transformed taste *Imax* was 1.39 during the 40°C trial, and 1.44 during the 5°C (both between moderate and strong on the gLMS). Repeated measures ANOVA found no significant differences in the *Imax* of perceived taste by a participant across their 10 replicates during either the 40°C ($p=0.166$) or the 5°C ($p=0.503$) trial, showing consistency in the taste *Imax* reported.

5.3.2 Part II: Measuring variation in temperature and phantom taste related brain response in TTs compared to TnTs

5.3.2.1 Perceptual phantom taste and temperature results

During phenotyping sweet and salty were most frequently reported during the 40°C trial, and bitter and sour during the 5 °C trial, **Fig 5.12**. Taste intensity was rated as being between weak and very strong, and the mean intensity was 1.17.

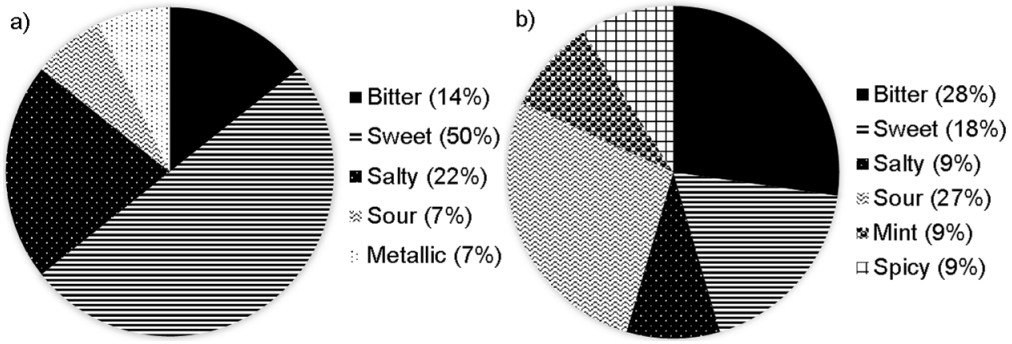

Figure 5.12: Percentage of taste qualities reported by the 12 TTs during the a) 40°C and b) 5°C temperature trials when phenotyping to classify TT status.

Perceived intensity varied across taste qualities, where metallic was rated the least intense, and spicy and mint the most intense, **Figure 5.13.**

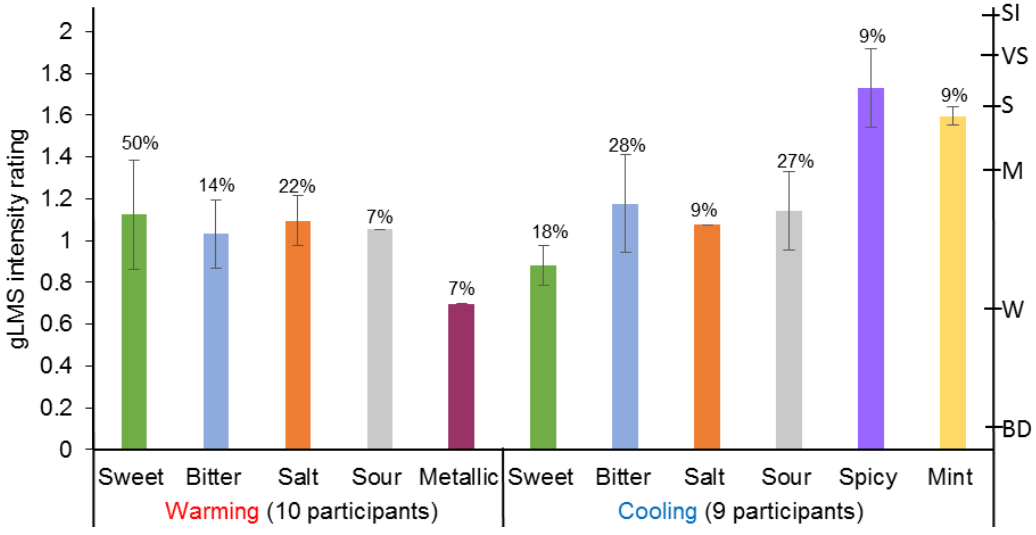

Figure 5.13: Taste quality and intensity reported by 12 TTs during phenotyping to classify thermal taster status. Note: some participants perceived taste on both temperature trials. Bars indicate mean ± S.E. BD – barely detectable, W – weak, M – moderate, S – strong, VS – very strong, SI-strongest imaginable sensation. The percentage of times each taste quality was reported is indicated above bars.

Participants rated the perceived temperature intensity during the phenotyping session. On average TTs reported both warming and cooling stimuli more intensely than TnTs. However, ANOVA showed it did not reach a significant level of difference for either the 40°C (p=0.544) or 5°C (p=0.406) trial (**Fig 5.14**), and no difference was observed between replicates (p=0.615).

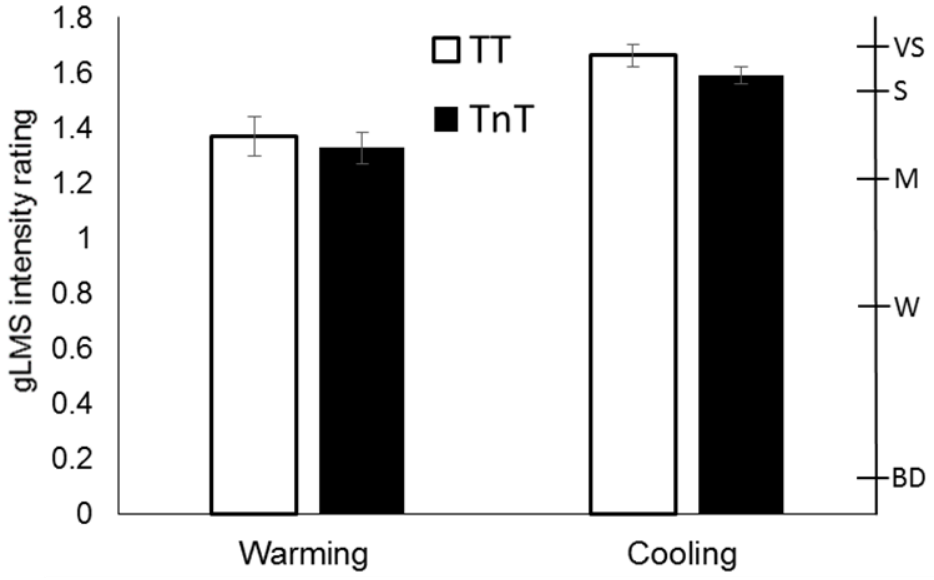

Figure 5.14: Mean temperature intensity ratings made across TT and TnT groups during the phenotyping session. Bars represent mean ± S.E. BD - barely detectable, W - weak, M - moderate, S - strong, VS - very strong.

Figure 5.15 identifies the mean temporal taste ratings from 10 replicates for each TT during the fMRI scan session. These findings indicate taste intensity, number of temporal peaks, shape of the curve, and the temperature range at which a taste was perceived differed greatly across TTs. Some TTs perceived taste during both temperature trials, whilst others only perceived taste during the 40°C (participant 01 and 03) or 5°C (participant 11 and 12) temperature trials.

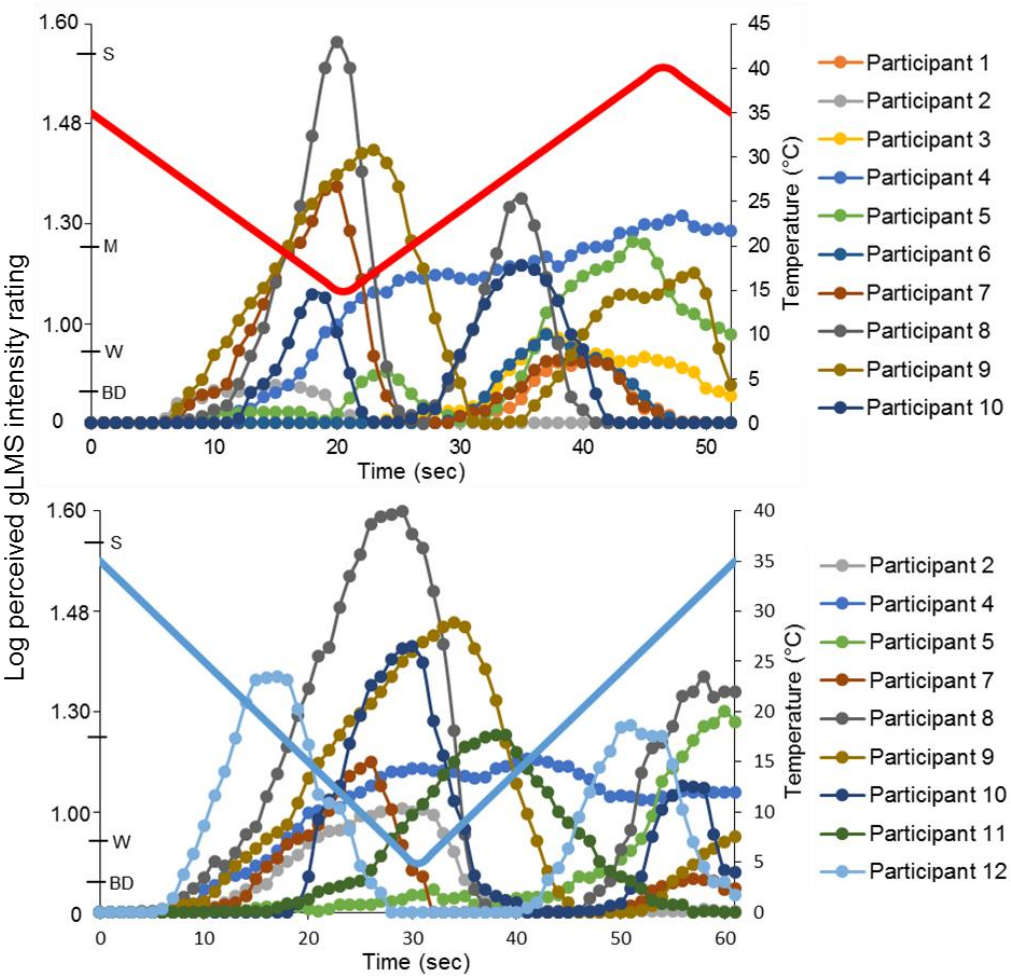

Figure 5.15: Mean temporal taste intensity responses reported by TTs during the fMRI scan during a) 40°C trial and b) 5°C trial. BD - barely detectable, W - weak, M - moderate, S - strong, VS - very strong.

The mean temporal taste response (of 10 replicates) reported using the rollerball during the Sensory Evaluation Session 2 outside the scanner was correlated with the mean temporal taste response (of 10 replicates) collected inside the scanner during the fMRI session for each TT separately, and across both of the temperature trials. Responses across the two sessions were significantly correlated (ICC's ranged from 0.562-0.975) (**Table 5.2**) for all participants except for participant 02 during the 40°C trial, therefore indicating the mean temporal phantom taste curves were similar inside and outside the scanner for most participants.

Table 5.2: ICC for each individual TTs mean temporal phantom taste response derived from the 10 replicates of template trial (40°C and 5°C separately) delivered inside the scanner, compared to the mean of 10 replicates delivered outside the scanner. *** indicates a significant correlation at p<0.001.

Participant	40°C trial	5°C trial
01	0.576***	no taste
02	0.151	0.568***
03	0.928***	no taste
04	0.928***	0.965***
05	0.711***	0.975***
06	0.83***	no taste
07	0.765***	0.797***
08	0.814***	0.878***
09	0.868***	0.813***
10	0.562***	0.917***
11	no taste	0.713***
12	no taste	0.658***

5.3.2.2 fMRI: Measuring phantom taste related brain activation in TTs

fMRI activation maps to phantom taste (**Fig 5.16a**) and **Table 5.3** show significant brain responses in areas associated with taste processing (including anterior insula, ACC, inferior and mid frontal gyrus and superior parietal gyrus) and somatosensory areas (mid insula, supramarginal gyrus, precentral gyrus) to phantom taste during temperature trials (40°C and 5°C trials combined). Oral somatosensory areas (including pre and post central gyrus, rolandic operculum mid and posterior insula) (**Table 5.4**) are activated in response to thermal stimulation (**Fig 5.16b**). The differential spatial representations of phantom taste and temperature related activation are demonstrated in **Figure 5.16c**. Responses associated with the control task (movement of the rollerball but no thermal stimulation) were limited to somatosensory, not taste, areas.

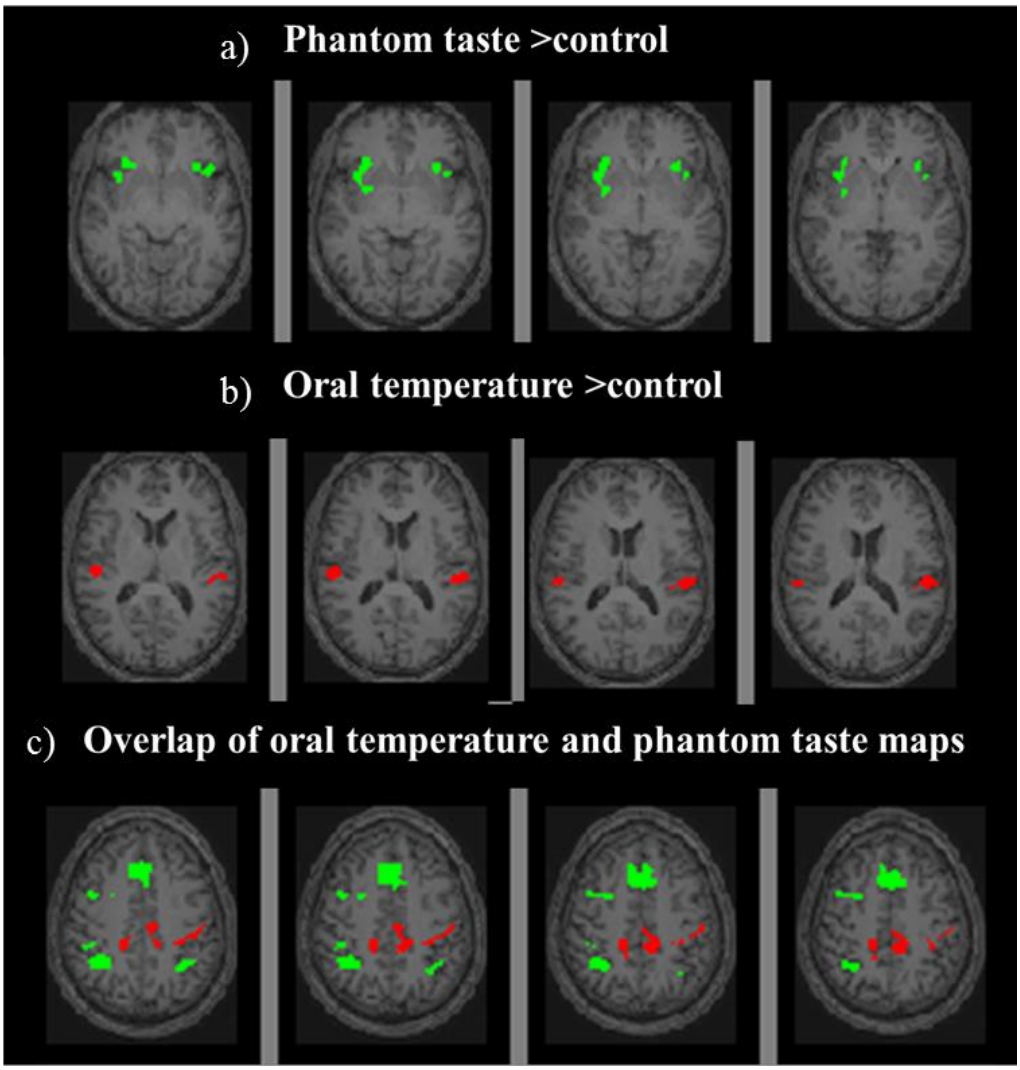

Figure 5.16: Group brain activations maps to phantom taste perceived in TTs (threshold at $p<0.005$ for illustration). a) Phantom taste versus control task showing activation in bilateral anterior insula, ACC, mid frontal gyrus and superior parietal gyrus b) temperature response versus control showing activation in oral somatosensory areas (including post central gyrus), C) overlap of group activation maps for phantom taste (green) and temperature response (red) in TTs.

Table 5.3: Areas of the brain showing a significant response to phantom taste versus control in TTs.

Area	**Side**	**MNI[1]**	**Z-score**	**P-value**	**Cluster size[2], k**
Anterior insula	R	30, 26, -6 36, 14, -8	5.87 3.82	<0.001 0.009	267 sub
	L	-30, 20, -6 -38, 28, 0 -40, 18, -8 -30, 24, 10	4.54 3.31 3.31 3.59	0.010 0.012 0.015 0.012	235 sub sub sub
Mid insula	R	36, -2, 8 32, 2, -6	3.73 3.00	0.011 0.019	25 35
ACC	R	4, 16, 46 2, 32, 40	3.65 3.80	0.010 0.010	874 sub
	L	-6, 16, 54	3.82	0.010	sub
Inferior Frontal gyrus	R	32, 56, 10	4.34	0.006	115
	L	-36, 34, 24	3.78	0.01	111
Mid Frontal /precentral gyrus	R	42, 8, 46 28, 4, 46	4.05 4.07	0.012 0.015	276 sub
Supramarginal gyrus	R	46, -40, 40	3.43	0.013	42
Inferior parietal gyrus	R	34, -50, 42	5.08	0.003	269
	L	-30, -54, 40	4.34	0.007	105

Table 5.4: Brain response to oral temperature versus control task in TTs.

Area	Side	MNI[1]	Z-score	P-value	Cluster size[2], k
Postcentral gyrus	R	48, -16, 34 48, -10, 42 20, -34, 66	3.97 3.89 3.32	<0.001 <0.001 0.001	122 Sub 97
	L	-36, -26, 38 -22, -38, 58 -44, -22, 35	5.20 4.52 4.56	<0.001 <0.001 <0.001	692
Precentral gyrus	L	-38, -12, 38 -44, -14, 54 -48, -10, 44	4.66 3.79 3.76	0.001 0.001 0.001	157 sub sub
Rolandic operculum	L	-40, -8, 28	4.35	<0.001	65
Posterior insula	L	-48, -30, -14	3.98	<0.001	238
Posterior cingulate cortex	R	16, -34, 42 12, -44, 48	4.32 3.72	0.001 0.001	183 sub
	L	-4, -22, 42 -4, -34, 45	4.32 4.14	<0.001 <0.001	408 sub
Superior temporal gyrus	R	56, -22, 10 58, -25, 20	4.02 3.33	0.001 0.003	124 sub
	L	-54, 4, -18	3.78	0.001	79

5.3.2.3 fMRI: Comparing temperature related brain activation across TTs and TnTs

Statistical parametric maps (SPMs) for temperature related brain activation are shown for TTs **(Fig 5.17a)** and TnTs (**Fig 5.17b**). Interestingly, activation was observed in the anterior insula in response to temperature stimulation in TnTs (**Fig 5.17b**) [(-34, 24, -2), Z=3.60, p=0.02, k=17]. TTs shows higher cortical activation in oral somatosensory areas (including pre and post central gyrus, posterior insula) in TTs than TnTs (**Figure 5.17c and Table 5.5**).

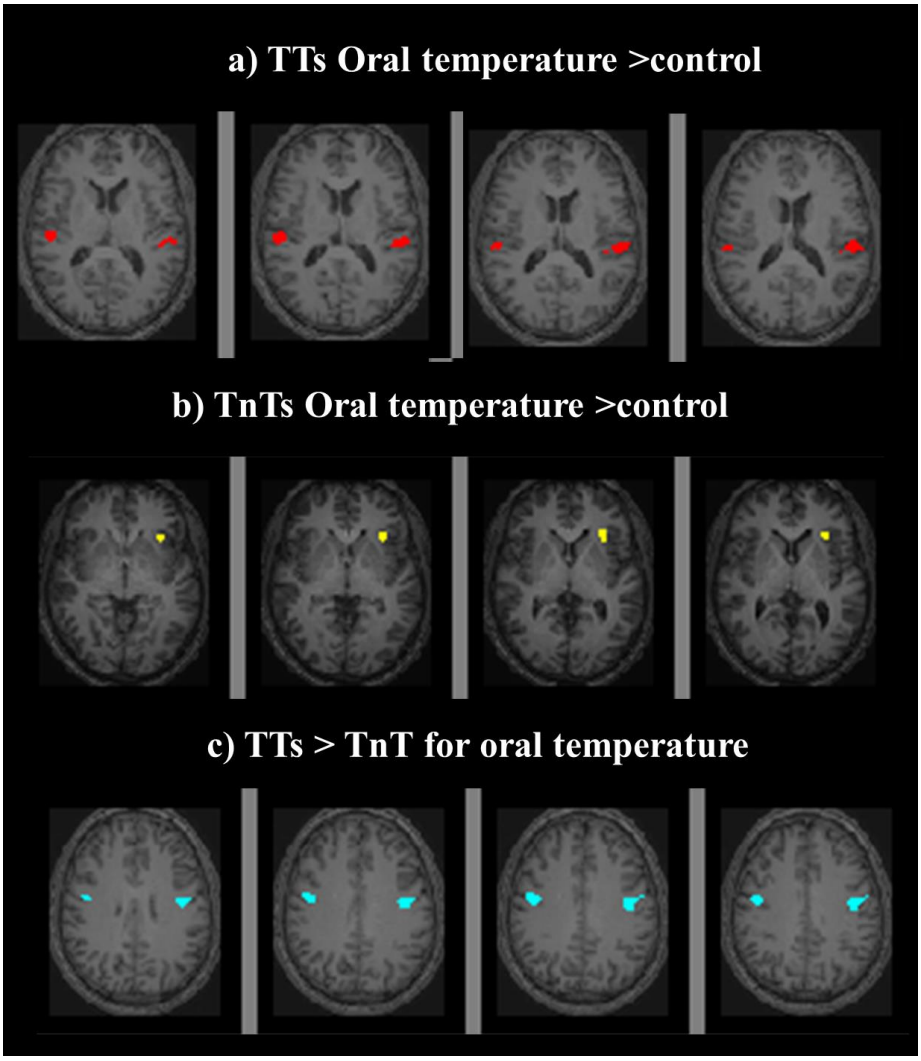

Figure 5.17: Group brain activations maps for oral temperature response versus control for combined (40°C and 5°C) temperature trials (threshold at p<0.005 for illustration). a) TTs, b) TnTs, and c) Differential group activation maps for TTs versus TnTs.

Table 5.5: Brain areas showing greater BOLD response to oral temperature simulation in TTs compared to TnTs.

Area	Side	MNI[1]	Z-score	P-value	Cluster size[2], k
Post/pre-central gyrus	R	46, -6, 32	3.35	0.025	112
	L	-40, -10, 30	4.45	0.017	232
		-55, -5, 22	3.30	0.026	sub
		-44, -12, 52	3.11	0.032	sub
		-38, -26, 38	3.51	0.021	36
Posterior insula	L	-46, -30, 14	3.30	0.04	40

5.4 DISCUSSION

5.4.1 TTS and taste qualities perceived during phenotyping

Of the participants initially phenotyped for this study (in Part 1), 28% were TTs, which is within the 20% (Bajec and Pickering, 2008) to 50% (Cruz and Green, 2000) range previously reported. Of the participants, 51% were classified as TnTs, again within the range previously reported 29% (Yang et al., 2014) to 77% (Hort et al., 2016), but higher than the typical 35-40% identified in most studies (Bajec and Pickering, 2008, Bajec and Pickering, 2010, Pickering et al., 2010a, Pickering et al., 2010b, Pickering et al., 2016). In total, 21% of participants were *uncat*, which is lower than previous findings which range from 23% (Pickering et al., 2016) to 42% (Yang et al., 2014), and considerably lower than the 33-42% typically reported (Bajec and Pickering, 2008, Bajec and Pickering, 2010, Bajec et al., 2012, Yang et al., 2014). This variation across studies is likely due to differences in the classification methods used, indicating the need for a more standardised approach (Yang et al., 2014). Considerations include whether an individual should be classified as a TT if they perceive only prototypical tastes

or ‘other’ sensations, the intensity at which taste is perceived, how many replicates of the temperature trials are delivered, and the number of tongue locations tested (see Section 5.4.7 for recommendations).

Phenotyping using the traditional temperature trials resulted in sweet, metallic and spicy being most frequently reported during the warming trial (**Fig 5.6a**), and sour and bitter during the cooling trial (**Fig 5.6b**). The classification of thermal taster status did not change when re-phenotyping the recruited TTs using the cooling trial which only held at 5°C for 1 second instead of the traditional 10 seconds. Minor changes in the taste qualities reported across phenotyping sessions occurred (**Fig 5.7**), which were due to some TTs perceiving taste qualities inconsistently across trials (discussed in section 5.4.4). Additionally, it is hypothesised that a learned response occurs over time, where TTs become better able to identify and articulate what they are perceiving with repeated exposure. Early literature on TTs failed to report which taste qualities were perceived, and more recently researchers have grouped tastes perceived across both trials together (Pickering et al., 2016, Pickering and Klodnicki, 2016). When tastes have been identified across separate trials, sweet, metallic and bitter are most frequently perceived when warming the tongue, and sour, bitter, metallic and salt when cooling (Cruz and Green, 2000, Yang et al., 2014, Hort et al., 2016, Pickering and Kvas, 2016), as found in the current study. Cruz and Green (2000) identified sweet as most frequently reported when warming the anterior tongue tip, sourness when cooling the lateral edges and bitterness when cooling the posterior of the tongue. Only the anterior tongue tip was stimulated in the current study. This may explain the high prevalence of sweet (42%) compared to bitter (11%) tastes during the warming trial, in comparison to 27% of tastes being sweet, and 33% bitter when testing all three tongue locations (Pickering and Kvas, 2016). However, Yang et

al (2014) also only tested the anterior tip of the tongue and found sweet reported less frequently (22%), suggesting perceived sensations are variable across sample population groups. Some studies have classified TTs as those reporting only prototypical taste qualities (sweet, salty, sour and bitter) (Cruz and Green, 2000, Green and George, 2004, Bajec et al., 2012), whilst others including the current study, have permitted 'other' attributes thought to be associated with taste (minty, metallic, spicy) (Yang et al., 2014, Hort et al., 2016, Pickering and Klodnicki, 2016, Pickering and Kvas, 2016, Pickering et al., 2016). In the past, metallic has been proposed as an additional taste (Bartoshuk, 1978), and although evidence remains inconclusive, some studies indicate it may have a taste component (Epke et al., 2009, Lawless et al., 2004, Lawless et al., 2005), and the data presented in Chapter 3 of this thesis. Sweetness is an important aspect of mintiness, which may be reported due to the combined trigeminal cooling and sweet taste perceived when cooling the tongue (Hort et al., 2016). Spicy and sweet were frequently reported in conjunction as the tongue was warmed, but exactly how these attributes are associated is currently unknown.

5.4.2 Variation in the perceptual taste response across TTs

The predominant focus of previous research on thermal taster status has been comparing orosensory perception between TTs and TnTs. This is the first study to evidence detailed differences in the taste response across TTs. It has been demonstrated that TTs not only perceive different taste qualities, but that the number of tastes perceived, their intensity, the temperature range at which they are detected, and the reproducibility of the temporal taste profile also varies. Little is known about the mechanisms which could be responsible for phantom tastes experienced by TTs, and these novel findings provide a valuable contribution to this ongoing debate, as discussed in Section 5.4.5. They also

provide an excellent foundation to guide future experiments, which should be conducted in order to improve the current understanding of this phenotype.

5.4.3 Taste qualities and intensities perceived during modified temperature trials

A range of different taste qualities were perceived during the modified temperature trials (**Table 5.1**). The number of taste qualities detected by each TT during each temperature trial has rarely been detailed (Yang, 2015). Participants perceived between zero and four tastes across a trial. These findings demonstrate that different mechanisms may be involved in the phantom taste responses, and that the mechanism responsible may vary across TTs and taste qualities. Sweet was the taste most frequently reported alone with an associated temporal rating, followed by bitter. However, as many as three tastes were reported within one temporal peak by some TTs, indicating for the first time that tastes may arise together, or merge from one to another. Perceived taste intensity varied considerably from 0.19 (< barely detectable) to 1.94 (> very strong) on the gLMS, showing a diverse spectrum of sensitivity to temperature induced taste perception, as seen with chemical tastants (Garcia-Bailo et al., 2009). Current phenotyping practices are not inclusive of this range of sensitivities, highlighting the need to revise current phenotyping classification. **Figures 5.10**, **5.11**, and **Table 5.1** illustrate the variability of the taste response across TTs.

5.4.4 Categories of temporal taste response and reproducibility

Analysis of the temporal taste data indicated variation in responses across TTs, and on occasions a different category of response was observed during the 40°C trial compared to the 5°C trial within an individual TT. Sometimes a single

taste peak was perceived on either cooling (e.g. participants 1 and 2 during the 40°C trial), or warming (e.g. participants 3-9 during the 40°C trial) components of the trial, whereas in other cases a taste was detected on each of the warming and cooling elements leading to two peaks (e.g. participants 10-24 during the 40°C trial). These responses occurred over a short temperature range within the trials, showing temperature specificity in the mechanism driving these perceptions. On a subset of these responses (four of the 40°C trials and two of the 5°C trials) taste was reported on ≤7 replicates, and usually at low intensities. This highlights the perceived intensity of taste is lower in some TTs than others, supporting the need for more liberal TT classification criteria as well as indicating considerable variation in the intensity of response across TTs. For others, taste was more persistent and apparent between the two temporal taste peaks (**Fig 5.9d**). Most of these TTs verbally identified the first taste being merged into the second with no 'off' period in between. In all cases a different taste was reported for each temporal peak, and the first taste (associated with cooling) was always more intense than the second taste (associated with warming). For the first time this study has evidenced that tastes can, for some TTs, arise in parallel and/or interchangeably with other tastes. It was hypothesised that certain taste qualities would be associated with specific temperature ranges, such as sweet when the tongue was warmed and bitter and sour when it was cooled. However, this interesting observation that multiple taste qualities may arise in parallel/interchangeably had not been anticipated. Furthermore, in contrast to taste being reported across short temperature ranges by many TTs, taste was perceived across most of the temperature trial for a small number (12%) of all trials (**Fig 5.9e**), showing temperature specificity of the taste response differs across TTs**.** Temporal taste curves were reported consistently across replicates by most TTs, with inconsistent reporting across the 10 replicates of the temperature trial only observed during 11% of 40°C

trials, and 6% of 5°C trials (**Fig 5.9f**). These inconsistent responses were often associated with multiple (two to four) tastes (**Table 5.1**). Interestingly, participants verbally reported the tastes arising interchangeably across the trial, and stated that more than one taste may be perceived at one time, however, the way in which they interact remains unknown. These novel findings indicate phantom taste perceptions, and the mechanisms responsible, are more complex than perhaps previously thought. This complex category of response explains why the temporal profile is less consistent for these TTs, and multiple tastes perceived in this manner indicates more than one mechanism is likely to be involved in eliciting the different taste qualities, which occur in parallel for some TTs. The interaction between these mechanisms may explain why the response is varied across replicates.

5.4.5 Temperature range of the taste responses

Sweet taste was frequently reported alone, which allowed an associated temperature range to be identified. TRPM5 is a likely mechanism for phantom sweet taste as it is temperature sensitive and activated by temperatures between 15-35°C in the absence of gustatory stimuli, and also modulates sensitivity to sweet taste (Talavera et al., 2005). Warming the tongue has been hypothesised to activate the TRPM5, causing phantom sweet taste perception. However, this does not explain the selectivity for sweet when the TRPM5 is also involved in the transduction of bitter and umami tastes. Here, the onset of sweet taste ranged between 22-38°C as the temperature increased, thus supporting the hypothesis of the TRPM5 being involved, as it is temperature activated between 15-35°C. The sweet onset only occurred at a temperature > 35°C on one occasion, which may be due to a latency effect in responding to the stimulus when using the rollerball. Bitterness was also frequently reported alone, with the

taste onset occurring predominantly when the tongue was cooled, (ranging between 31.9-18.4°C), which is in agreement with bitter being frequently reported during the traditional cooling trial when phenotyping (Cruz and Green, 2000, Yang et al., 2014, Pickering and Kvas, 2016). However, during three temperature trials the onset of bitterness occurred when warming the tongue (between 19-25.3°C). Two of these responses were made by the same TT who reported two temporal taste peaks on each temperature trial. The onset of a first bitter taste arose at around 30°C as the temperature decreased (persisting for approximately 15 seconds), then a distinct off period occurred before the onset of a second bitter taste arising at around 23°C as the temperature increased (and persisted for approximately 20 seconds during the 40°C trial, and until the end of the 5°C trial). Interestingly, this suggests that for this TT the mechanism responsible for eliciting taste is activated at a similar temperature during both warming and cooling of the tongue. The third response was made by a TT who perceived bitterness only when the tongue was being warmed and not cooled, again highlighting that one response does not fit all, and that a different mechanism may be involved. One possible mechanism being a TRP channel that has not yet been associated with bitter taste transduction (Talavera et al., 2007). Interestingly, bitter has frequently been reported during the traditional warming trial (Pickering and Kvas, 2016, Hort et al., 2016). It is worth noting that traditional phenotyping specifies that participants 'attend' to the whole of the cooling trial (35-5°C), but only part of the warming trial as the temperature increases (15-40°C). Here, temporal taste responses were collected across the entirety of both the modified temperature trials (35-35°C) which each contained both a 'warming' and 'cooling' component. **Figure 5.10** and **Figure 5.11** showed that tastes elicited during 'cooling' components of the trials often persisted as the temperature increased during the 'warming' part of the trials. Some of the tastes reported during the warming component of the traditional warming trial

when phenotyping may therefore be associated with the pre-cooling temperatures. This could explain, at least in part, why some tastes typically associated with cooling of the tongue are reported during the traditional warming trial when phenotyping (such as bitter, sour and salty). This study demonstrates that sweet is most frequently associated with true warming of the tongue, after the pre-cool taste has diminished. Bitter was occasionally reported during warming of the tongue, but this response was infrequent. Mintiness was reported alone on only nine occasions, and the onsets always occurred when the temperature decreased between 26 and 13°C. TRPM8 responds to cool temperatures <25°C, and is activated by menthol which gives the perception of 'mintiness' and cooling (Ferrandiz-Huertas et al., 2014). Its involvement in taste processing is unknown, however, it is expressed in taste buds in the soft palate and pharynx of rats (Sato et al., 2013). These findings support the hypothesis that the TRPM8 may be involved in mint perception when cooling the tongue, and as mint is usually sweet this may then it be associated with sweet perception (Hort et al., 2016). The temperature range during which metallic and spicy were perceived could not be isolated as they were only reported alone on only three or five responses respectively. The TRPV1 cation channel is activated by divalent salt solutions which are also reported to elicit a perceivable metallic sensation, and so the possible involvement in metallic perception has been proposed (Riera et al., 2007). Spicy sensations are typically associated with the burning of capsaicin, a response known to be mediated by the TRPV1. However, this cation channel is only temperature sensitive and activated at temperatures >43°C (Caterina et al., 2000), thus making it an unlikely candidate for the mechanism behind phantom metallic or spicy sensations during these temperature trials. TRPV3 is activated at >33°C (Latorre et al., 2009) and has been implicated in the perception of carvacrol, eugenol and thymol, compounds derived from plants including oregano, savoury, thyme and clove, all of which

elicit multisensory flavour perception combining aromatic odour with bitterness, pungency, sharpness, and warming sensations (Xu et al., 2006). TRPV3 could be a candidate for the mechanism behind spicy perception when warming the tongue. It is not currently believed to be involved in taste transduction, however it remains one of the most poorly characterised cation channels. It is of interest that a phantom cinnamon sensation has been reported by TTs (Pickering and Kvas, 2016) as it is a relative of camphor which also activates TRPV3. Whether the spicy sensation has a taste component remains to be determined and would be of interest to further explore in future studies. These findings provide the first insight into the temperatures associated with the range of phantom sensations perceived by TTs, and the similarities and variability across both taste qualities and individuals.

An original objective of this study was to isolate the temperature range associated with each taste quality. However, this was not possible with the more complex responses; interestingly, multiple tastes were sometimes reported with one temporal response (**Table 5.1**), indicating that they arose together and/or interchangeably. In other instances, up to four tastes were perceived during a temperature trial, and were associated with multiple temporal peaks reported inconsistently across replicates (**Fig 5.9f**). Better characterisation of these complex responses would aid in elucidating the mechanism (s) driving the taste response. In particular, the possible involvement of the TRP cation channels. Taste was perceived during a narrow temperature range by some TTs, and for most of the trial by others (**Fig 5.10** and **5.11**), thus showing great variability in the specificity of the response across TTs. This variance may not only be influenced by different latency in responding to the stimuli; another theory is that individual variation in temperature sensitivity of the mechanisms driving taste perception is occurring.

Characterisation of TT subtypes, for example those reporting sweet compared to those reporting bitter, has been proposed as a way to explore differences across TTs (Bajec and Pickering, 2010). However, only one paper reports such sub-categorisation (Bajec et al., 2012). Although this provided an interesting start, further research and sub-categorisation of TTs would be beneficial in understanding the mechanisms behind each taste quality. A temporal check all that apply (TCATA) approach could be used to capture the temperature range at which each taste is perceived during temperature trials, which may further elucidate the potential TRP cation channels involved in taste responses. Additionally, this methodology may enable further understanding and categorisation of the more complex responses exhibited by some TTs.

The variability observed across TTs in this study indicates that a number of mechanisms in addition to the TRPs may also be involved in eliciting different taste qualities. One possible explanation is individual variation in the physiology of fungiform papillae. The degree by which gustatory and trigeminal nerve fibres innervate these papillae, and the occurrence of cross wiring between them may influence phantom taste perception (Clark, 2011). An alternative theory is that the temperature response mechanisms differ, as gustatory nerve fibres are often bimodal and also activated by temperature stimulation (Kadohisa et al., 2005a). Those responsive to cold temperatures are typically sensitive to sour and salt, while those responsive to warm stimulation are sensitive to sweet and bitter (Ogawa et al., 1968). The transduction of bitter, sweet and umami tastes are mediated by G-protein coupled receptors (GCPRs), whilst Na^+ and H^+ ion channels are involved in salt and sour taste transduction respectively (Chandrashekar et al., 2006). Cruz and Green (2000) hypothesised that GCPRs are temperature sensitive and activated by warming, whilst Na^+ and H^+ ion

channels are activated by cooling, which would explain the occurrence of sweet or occasional bitterness when warming the tongue, and sour and salt with cool temperatures. They also suggest that variability in both the incidence and distribution of the temperature sensitive gustatory neurons across TTs may explain the variety of taste responses reported. Although taste qualities are more frequently associated with a certain temperature, for example sweet with warming and bitter with cooling, this is not exclusively the case. It is therefore likely that a combination of different mechanisms interplay in the variety of responses reported by TTs.

5.4.6 Adaptation

Adaptation in the perceived intensity of gustatory stimuli can occur with repeated exposure. It was therefore of interest to see if such a response was observed to phantom taste in this study, when the stimulation is not chemical in nature. Neither a significant difference nor a trend in the taste *Imax* was reported across replicates of the 40°C ($p=0.36$) or 5°C ($p=0.73$) temperature trials, indicating no adaptation. The degree of adaptation to gustatory stimuli ranges from none (Halpern and Meiselman, 1980) to 84% (Schiffman et al., 1994), with differences observed across tastant qualities and method of stimulus delivery (Theunissen et al., 2000). Responses have traditionally been measured by delivering a continuous flow of tastant solution using a gravity flow system, with intensity ratings frequently made (e.g. every 15 sec) (Dubose and Meiselman, 1979), or repeated stimuli delivered in quick succession (e.g. every 30 sec) which can allow a degree of recovery between replicates (Schiffman et al., 1994). Similarly, adaptation may not have occurred in the current study as the duration between taste(s) allows the tongue to recover between replicates. Although the inter-stimulus-interval between temperature trials is only 10 s, the

duration between taste(s) perceived was often considerably longer as taste was reported for as little as 3.3 s within a trial. Although these findings give an insight into the intensity of the taste over time, adaptation could be further and better investigated by delivering continuous temperature stimulation at the temperature at which a taste is elicited for each individual TT, with an intensity measure taken at regular intervals to mimic the continuous flow delivery system.

In this study, the first 20 seconds of each of the temperature trial (as the temperature decreased from 35-15°C) was the same. Tastes perceived during this temperature range were therefore expected to have the same mean onset within an assessor across temperature trials. Instead, a trend for the onset to be reported earlier during the 40°C trial than the 5°C trial was observed. One explanation for this is that the tongue becomes less sensitive to temperature stimulation after exposure to the 10 replicates of the 40°C trial. It is interesting that this delayed onset occurred, whilst no difference was observed in the *Imax* across replicates. Traditionally the warming trial always precedes the cooling trial to avoid possible adaptation from the intense, sustained cold stimulation (Green and George, 2004). More recently, trials have been conducted in a randomised manner (Pickering and Klodnicki, 2016, Pickering and Kvas, 2016, Pickering et al., 2016) which is recommended in response to these findings.

5.4.7 Recommendations for TT phenotyping

The findings of the current study raise questions over the phenotyping classification currently used, and highlight the need to review these protocols. A standardised approach across research groups is required in order to accurately make across study comparisons. Agreement on the number of tongue locations tested is advisable. The possible advantage of identifying more

phantom sensations when stimulating three tongue locations may not be necessary when delivering temperature stimuli using the large thermode in the current study, as it likely covers (and therefore stimulates) the three tongue areas captured by the smaller thermode probe used in other studies reporting testing across the three locations. It would be interesting to explore this hypothesis by making direct comparisons of responses elicited by each thermode. Agreement on the phantom sensations permitted when phenotyping for thermal taster status is required. The findings of the current study indicate practices should be inclusive of no-prototypical phantom sensations; metallic, spicy and minty, and future research should investigate the origin of these sensations. Some researchers specify that the same taste must be perceived across replicates of a temperature trial (Pickering and George research groups and the current study), whereas others have permitted different taste qualities across replicates (Yang et al., 2014, Hort et al., 2016). This study evidences that a variety of different responses are reported by TTs, suggesting that classifying only those who report the same taste over replicates may be too restrictive. Traditional methods require taste intensity to be reported above weak on the gLMS. This is the first study classifying individuals (n=2) who report taste as below weak in intensity as TTs. These individuals continued to perceive reproducible taste sensations across 10 replicates of the temperature trials, which would not be experienced by TnTs. Classifying them as *Uncat*, as traditional methods stipulate, results in the TT group containing only those with high phantom taste acuity. This likely creates a bias which works favourably towards a TT advantage when making comparisons between phenotypes. Prevalence estimates are likely skewed to show a lower percentage of TTs than is representative of those perceiving tastes. Using this gLMS cut-off point is subjective, and as many as 42% of a study population have been *Uncat* when using this criteria (Yang et al., 2014). It is not unreasonable to assume that some

of these individuals are TTs who are at the lower end of the temperature-induced taste sensitivity spectrum, and should be included in the TT group. Additionally, further distinction between TTs and *Uncat* can be made by administering a third replicate of a temperature trial when taste is reported inconsistently across the first two replicates. Using this method in the current study resulted in seven participants who traditionally would have been classed as *Uncat* to be assigned to the TT group. In this study the traditional temperature trials were modified so each returned to 35°C after reaching its destination of 40 or 5°C, and therefore each contained a warming and cooling component. Of the 36 TTs, only four perceived no taste on one of the temperature trials. It appears unnecessary to use two separate trials, and that one single trial reaching both extremes of temperature could be used to effectively capture the range of tastes elicited by both warming and cooling. However, more research to validate an alternative trial and to ensure all tastes are detected with a single trial is required. It would also be of interest to explore a greater range of temperature stimuli on the tongue (< 5°C and > 40°C). Little has been reported on the rationale behind the use of the temperature trials initially deployed by Cruz and Green (2000), which have continued to be implemented with only minor modifications since (Green and George, 2004). Re-evaluation of these trials is required in order to utilise the most effective stimuli for eliciting the range of tastes perceived by TTs. Overall phenotyping protocols need to be adapted to best capture the wide range of phantom taste responses observed and characterised across TTs in this study.

5.4.8 Perceived phantom tastes, and associated brain activation in TTs

The 12 TTs scanned using fMRI reported a range of phantom taste sensations which included prototypical (sweet, sour, salty, bitter) and ‘other’ sensations

(spicy, minty, metallic), (**Fig 5.12**). As in the experiments detailed in Part 1, the perceived intensity varied, with ratings ranging between weak and very strong, and the mean intensity rating being 1.17 on the gLMS. Metallic was rated the least intense during the warming trial, and spicy and mint the most intense during the cooling trial, **Figure 5.13**. It is interesting that the spicy and mint sensations were perceived more intensely than the prototypical tastes, possibly indicating greater interaction between the perceived 'taste' sensation and temperature of the stimulus. The temperature range at which tastes were perceived were variable across TTs (**Fig 5.15**). The mean temporal taste response for each TT from 10 replicates of the temperature trial collected outside the scanner (sensory evaluation session) was significantly correlated to the mean rating from the 10 replicates rated by the same TT inside the scanner (fMRI session) (**Table 5.2**). This suggests that the temporal phantom taste response was consistent with repeat exposure across test sessions, and that the scanning environment did not alter the responses.

This is the first ever study to thermally stimulated the tongue of TTs to elicit phantom taste sensations whilst imaging the brain. Activation maps identified areas associated with taste processing, including the anterior and mid insula, mid frontal gyrus, and parietal lobule were activated at the time point when TTs reported perceiving a phantom taste using the rollerball to rate intensity during temperature trials (**Fig 5.16** and **Table 5.3**). A control task was used to account for activation related to the movement of the rollerball, where activation was not observed in taste areas, but limited to somatosensory areas. Although mechanisms behind this phenotypic trait are not yet understood, phantom taste has been hypothesised to arise at either the central or peripheral level (Cruz and Green, 2000). These findings support the theory that phantom taste may occur due to thermal stimulation activating temperature sensitive gustatory

nerve fibres at the peripheral level in TTs, which encodes taste instead of temperature. This would explain the observed activation in the anterior insula, which has been proposed as the primary gustatory cortex, and is thought to be involved in the identification and intensity perception of taste (Small et al., 1999, Verhagen and Engelen, 2006, Veldhuizen et al., 2011), with further projections to the ACC which is thought to be associated with the hedonic response to taste (Veldhuizen et al., 2011). Although most literature suggests the PGC is located in the anterior insula, the mid insula has also been proposed as a possible candidate (Small et al., 2003, Small, 2010). It is therefore interesting that activation was seen here in response to phantom taste in TTs. It would be interesting to compare the cortical response to pleasant and unpleasant phantom taste perceptions to identify if they are represented differently, as with chemical taste (Small et al., 2003). As previously discussed, TRP channels are a feasible mechanism that may be involved in thermal stimuli activating gustatory nerve stimulation at the peripheral level (Talavera et al., 2005, Talavera et al., 2007). A central gain mechanism where TTs have increased integration between sensory modalities (such as gustation and temperature) has been proposed as another possible mechanism involved in both the phantom taste response, and increased sensitivity to oral stimuli (Green and George, 2004, Bajec and Pickering, 2008). The findings of the current study suggest this is less likely to be responsible for phantom taste perceptions as increased activation in areas such as the OFC, typically associated with sensory integration, were not observed either here, or in previous fMRI studies comparing the cortical response across thermal taste phenotypes (Yang, 2015, Hort et al., 2016). Some synasthetes experience taste and flavour sensations in response to written and verbal words (Jones et al., 2011). A wider range of neural networks are activated in these individuals when stimulated with words that elicit gustatory sensations, compared to non-synasthetes presented with

the same stimuli (Jones et al., 2011). Interestingly, the anterior insula was activated at the time point when gustatory sensations were perceived. Therefore it cannot be ruled out that activation of the anterior insula observed in the current study occurs due to a temperature-gustatory form of synaesthesia (Yang, 2015). Another possibility being that activation is associated with attending to the stimulus, as increased activation is observed in this area when trying to detect taste in a 'tasteless' solution (Veldhuizen et al., 2007). These findings add to the growing body of evidence characterising the phantom taste response in thermal tasters, and contributes to better understanding the possible mechanisms which may be involved in these responses.

5.4.9 Temperature response in TTs compared to TnTs

On average TTs were observed to report perceiving both warming and cooling stimuli applied to the tongue more intensely than TnTs (**Fig 5.14**). However, this did not reach the level of significance which is often reported (Green and George, 2004, Bajec and Pickering, 2008, Yang et al., 2014). This may be due to the small sample size used in the current study. Interestingly, TTs have reported cooling stimuli delivered to the lateral edges of the tongue as more intense than TnTs, but this did not reach significance when testing the anterior tip (Bajec et al., 2012), the area tested in the current study. TTs perceive taste stimuli delivered at warm (35°C) and cool (5°C) temperatures more intensely than TnTs, but the variance in responses is higher in TTs than TnTs, and the authors indicated that the overall group difference was driven by higher intensity ratings reported by a subset of TTs as opposed to it being representative of the whole group (Bajec et al., 2012). A higher variance in intensity ratings were also seen for TTs compared to TnTs in the current study, indicating a demand to further investigate this on a larger population group. Interestingly, this

perceptual advantage is thought to be limited to the oral cavity as temperature stimulation on non-gustatory regions of the lip and hand have yielded no significant differences in intensity ratings between phenotypes (Green and George, 2004). Some studies have reported that an increased perceptual response (Yang et al., 2014) and brain activation (Yang, 2015) are not observed in TTs in response to aroma, further supporting the likelihood of the TT advantage being limited to the oral cavity. However, Green and George (2004) did report TTs to have a heightened sensitivity to aroma delivered via both the orthonasal and retronasal routes, highlighting further investigation into this is required.

Activation maps (**Fig 5.17**) show greater activation in oral somatosensory areas (post and pre central gyrus, and posterior insula) (**Table 5.5**) in TTs compared to TnTs when thermally stimulating the tongue. Although a number of studies have compared the perceptual response to trigeminal stimuli across TT phenotypes, only two other studies have investigated differences in the brain response. Higher cortical activation in somatosensory (SI, SII and mid insula) and taste (anterior insula) areas were identified in TTs than TnTs when comparing the brain response to gustatory-trigeminal (sweet/cold) stimuli with ambient sweet stimuli (Yang, 2015, Eldeghaidy et al., 2015). Hort et al (2016) also showed TTs to have greater brain activation in oral somatosensory (SII, rolandic operculum) and reward (ACC) areas in response to gustatory-trigeminal (sweet, cold) samples when compared to TnTs. Combined results from these studies indicate that TTs and TnTs exhibit differing cortical activation in response to oral stimuli, and that TTs have increased cortical activation to thermal stimuli. Interestingly, activation was observed in the anterior insula in response to temperature stimulation in TnTs, but not TTs. In addition to being the purported PGC, the anterior insula is also thought to be an integration area

that has been implicated in response to thermal stimulation (Guest et al., 2007), highlighting the need to explore this further. These findings also support perceptual results showing that observed ratings by TTs are more intense than TnTs to not only temperature, but also to other oral trigeminal stimuli including astringency (Pickering et al., 2010b, Bajec and Pickering, 2008), carbonation (Pickering et al., 2010a, Hort et al., 2016) and metallic (Bajec and Pickering, 2008). Interestingly, the same result is not observed for the trigeminal response elicited by capsacin or menthol (Green et al., 2005, Yang et al., 2014), indicating that the TT advantage is not consistent across all trigeminal stimuli, thus warranting the need for further investigation, especially at the central level. Temperature and gustatory nerve fibres innervating the fungiform papillae may be cross wired and able to activate one another to elicit both phantom taste sensations in response to thermal stimulation, and increased perceptual and cortical activation to orall stimuli in TTs (Clark, 2011).

5.5 CONCLUSION

This is the first study to explore and evidence how highly variable the phantom taste response within TTs is, and to measure the brain response in TTs compared to TnTs whilst thermally stimulating the tongue. TTs not only perceived different taste qualities, but the number of tastes perceived and their intensity also varied. A number of different categories of temporal taste responses were identified, and the reproducibility of the temporal taste profile across the 10 replicates differed across TTs. Interestingly, the temperature range at which tastes were elicited was variable across TTs and taste qualities. Whilst some TTs perceived taste for a short temperature range within a trial, others perceived taste across most of the trial. The onset of sweet taste was frequently reported as the temperature increased between 22-35°C, supporting

the hypothesis that the TRPM5 may be involved in phantom sweet taste perception. Adaptation in the perceived intensity of taste was not seen during repeated exposure to the temperature trials across 10 replicates, which may be due to the duration of time between perceived tastes. In light of these new findings, this raises questions over the phenotyping classification currently used, and highlights the need to review protocols. This includes the use of one temperature trial to capture extremes of both warm and cool temperatures, and implementing methods to reduce the number of individuals uncategorised due to inconsistent reporting across replicates of temperature trials, or for reporting taste at a low intensity. These findings highlight the vast perceptual differences in taste perception across TTs in response to thermal stimulation of the tongue, indicating different mechanisms including the involvement of TRPs, variation in fungiform papillae density and temperature sensitive gustatory neurons.

Activation maps identified that the anterior insula (thought to be the PGC) and other areas associated with taste processing (ACC, anterior and mid frontal gyrus, and inferior parietal lobule) were activated at the time point when TTs reported perceiving phantom taste. This supports the theory that phantom taste may occur due to thermal stimulation activating temperature sensitive gustatory nerve fibres at the peripheral level in TTs, thus causing activation in the primary gustatory cortex. Activation maps identified greater activation in oral somatosensory areas (post and pre-central gyrus and rolandic operculum) in TTs compared to TnTs when thermally stimulating the tongue. This study reinforces evidence that central processing of oral stimuli differs between TTs and TnTs, and aids in elucidating the mechanisms which may be responsible for the unusual phenomenon of phantom taste experienced by TTs. It also supports the growing body of evidence which indicates a heightened perceptual and brain response to oral stimuli in TTs compared to TnTs. It is likely that the

differences observed between this phenotype will influence food and beverage preference and consumption habits which may in turn impact on nutrition and health outcomes. Up to 50% of individuals have been reported to perceive phantom taste sensations (Cruz and Green, 2000), therefore, better understanding of the thermal taste phenotype may contribute to the current understanding over food preference.

6 CONCLUSIONS, RESEARCH IMPLICATIONS, AND RECOMMENDATIONS FOR FUTURE WORK

The overall aim of this thesis was to explore the effect of taste phenotype and genotype on oral sensitivity, focusing primarily on gustatory and thermal stimuli. Although evidence for variation in perceptual responses is growing, only three studies had previously reported measuring differences in brain activation across taste phenotypes (Clark, 2011, Eldeghaidy et al., 2011, Yang, 2015). Here, in this novel experimental approach, detailed sensory evaluation was combined with functional MRI (fMRI) techniques to explore both the perceptual and brain response to stimuli, to bridge the gap between stimulation at the peripheral level and the perceived sensation. Responses were explored across PROP taster status (PTS), thermal taster status (TTS), and fungiform papillae density (FPD) phenotypes, and TAS2R38 and gustin genotype, and the variation in the cortical response was reported across PTS and TTS groups.

6.1 CONCLUSIONS

6.1.1 Considerations for stimuli development for fMRI experimental design

Initial experiments were conducted to develop stimuli that would be used to explore individual variation in oral perception, and inform the experimental design of the fMRI experiment. The work in Chapter 2 highlighted a number of considerations that should be made when designing and delivering oral stimuli in fMRI experiments, including adopting an experimental protocol that minimises interaction effects between stimuli, and the effect that delivery method and body position may have on stimulus perception. Limited evidence is available to determine the extent that stimulus delivery in the MR scan environment has on

perceived stimulus attributes, highlighting the need for further research to explore this in greater depth.

6.1.2 Understanding metallic perception

The origin of the metallic sensation associated with divalent salts is not well understood, and was studied in Chapter 3 of this thesis and published in Skinner et al. (2017). This work showed that ferrous salts are detected orthonasally when sniffing the sample, but GC-MS analysis did not detect any volatiles in the sample headspace. One theory is that low level volatiles released from the sample interact with tissue in the nasal cavity to produce the perceived sensation, or alternatively that they cause lipid oxidation with tissues in the nasal cavity, and it is the lipid oxidation by products that are perceived. These findings show orthonasal stimulation may play a part in the metallic sensation perceived during sample consumption. When ingesting ferrous and copper salts, a metallic sensation was elicited. For ferrous salts, occluding the nose to block retronasal stimulation reduced this sensation such that it was no different to the water control, indicating retronasal flavour is driving the response. Interestingly, the same was not observed for copper sulphate, where it remained significantly more metallic than water when the nose was occluded, suggesting a taste or trigeminal component is involved, and should be further explored in order to understand the underlying mechanisms involved with each response.

6.1.3 The relationship between PTS, TAS2R38 and gustin genotype, and FPD

In Chapter 4, PTS, FPD, and TAS2R38 and gustin genotype of the 30 participants who were scanned using fMRI was determined. A relationship between PTS and TAS2R38 genotype was identified, with AVI homozygotes

being associated with PNTs, PAV homozygotes with PSTs, and heterozygotes with PMTs. This supports previous literature indicating PTS has a genetic component. An association between PTS and gustin genotype was not observed. This may be due to the genetically diverse population included here, compared to the Italian cohorts which have previously shown an association (Calo et al., 2011; Melis et al., 2013; Padiglia et al., 2010). Interestingly, no difference in FPD was identified between PTS groups, or TAS2R38 and gustin genotypes, which may be influenced by to the small sample size used, as an association between FPD and PTS has predominantly been identified in previous studies.

6.1.4 The relationship between taste phenotype and genotype and perceived taste intensity

Few differences in perceived taste intensity were observed between PTS groups. The primary hypothesis for this finding being that a concentration effect occurred, where the PTS advantage frequently reported for PSTs when administering lower concentration stimuli (Melis et al., 2017) that was lost in the work shown in this thesis when delivering the strong intensity tastants. Interestingly, few or no differences were also observed for taste sensitivity across TAS2R38 and gustin genotypes, or FPD.

6.1.5 The relationship between sensitivity to PROP and the cortical response to taste

A correlation between PROP intensity ratings and brain activation in the anterior insula cortex was observed in response to metallic and sweet stimuli (but not for bitter, salt, sour and umami). This may have been associated with the hedonic qualities of these samples. This supports findings from the only

previous studies exploring variation in cortical brain processing across taste phenotype (Clark, 2011, Eldeghaidy et al., 2011), which indicate that cortical processing differs across taste phenotype. Interestingly, for the perceptual data collected, PROP and gLMS taste intensity ratings were not correlated, which is likely due to variation in scale use across participants, or the gLMS not being sensitive enough to detect subtle individual variation. This highlights the importance and value of using multidisciplinary approaches to measure sensory perception.

6.1.6 Evaluating thermal taste phenotyping

Chapter 5 of this thesis adopted a modified thermal taste phenotyping approach. 28% of participants were classified to be TTs, which is within the 20% (Bajec & Pickering, 2008) to 50% (Cruz & Green, 2000) range previously reported. Interestingly, permitting phantom taste ratings below weak intensity, and administering three replicates of the temperature trials, instead of the traditional two, resulted in nine individuals who would traditionally have been assigned to the uncategorised (*uncat*) group, to be assigned as TTs. These findings support the growing evidence indicating that a large percentage of the population are TTs. However, it is likely that the strict classification criteria used in traditional phenotyping does not take into consideration the range of thermal taste responses. In light of these findings, TT phenotyping methods should be revised and modified to include the diverse range of responses reported.

6.1.7 Variation in perceived phantom taste responses across TTs

Previously, limited attention has been devoted to exploring the diversity of phantom taste responses across TTs. In line with other researchers, the results shown in Chapter 5 showed TTs perceive different taste qualities, at different

intensities, and the number of tastes reported varies across TTs. Work presented in this thesis has also been able to identify different categories of temporal taste responses, and shown the reproducibility of the temporal taste differed across TTs. Most interestingly, phantom tastes were perceived at variable temperature ranges across both TTs and taste qualities. Taste was perceived across a short temperature range by some TTs, whilst others report a taste across most of the temperature trial. Importantly, the onset of sweet taste occurred as the temperature increased between 22-35°C. This supports the hypothesis that the transient receptor potential cation channel, TRPM5, may be involved in sweet phantom taste responses (Talavera et al., 2005).

6.1.8 Measuring temperature related responses across thermal taste phenotypes

Despite thermal taster status being identified nearly 20 years ago, the novel experiment detailed in Chapter 5 is the first to report the brain activation associated with thermally stimulating the tongue of TTs to elicit phantom taste. Interestingly, at the time when TTs reported perceiving phantom taste, brain activation was identified in the anterior insula, the area thought to be the primary gustatory cortex and shown to elicit chemical taste in the same individuals. Other brain regions typically associated with taste processing were also activated, including the anterior cingulate cortex (ACC), inferior frontal gyrus, and inferior parietal lobule. This indicates that thermal stimulation may activate temperature sensitive gustatory nerve fibres in TTs, resulting in cortical activation in the primary gustatory cortex and areas associated with taste processing. It supports the hypothesis of cross wiring between gustatory and trigeminal nerves in TTs (Clark, 2011).

Interestingly, the trend shown in previous studies for TTs to rate temperature more intensely than TnTs when thermally stimulating the tongue, did not reach significance in the current study. However, greater cortical activation was observed in oral somatosensory areas (post and pre-central gyrus and posterior insula) of the brain in TTs compared to TnTs, again highlighting the value of adopting a multidisciplinary approach to measure responses. These findings support the evidence suggesting that TTs have a heightened sensitivity to oral stimuli, and that cortical processing differs across this phenotype (Hort et al., 2016).

6.2 IMPLICATIONS

6.2.1 Understanding the basis of metallic perception

Understanding metallic perception is of interest to food manufacturers and those working in health and nutrition fields, as metallic taints are frequently perceived when consuming food and beverage, and are problematic for food fortification. In order to develop strategies to eliminate or mask this sensation, understanding of the mechanisms involved in its perception is required. This research provides a valuable contribution to forward current knowledge, as it highlights divalent salts elicit orthonasal stimulation, which may contribute to metallic perception, and the findings also support the hypothesis that metallic perception may have a gustatory component.

6.2.2 Challenging the use of PTS as a marker of oral sensitivity

Although it is becoming more common to include multiple measures when exploring individual variation, attempts to use PTS as a single marker of sensitivity are still made. The limited effect that PTS had on both the perceived and cortical response to gustatory stimuli challenges this approach for being too

simplistic, and indicates the demand for research that considers the multiple variables inflicting oral sensitivity. It also highlights the need for the individual factors influencing oral perception to be understood, before determining how they interact, and the overall impact on sensitivity.

6.2.3 Challenging the importance of PTS on product perception

The few associations identified between PTS and the perceptual and cortical response to the strong tastants used here, poses questions over the true association relationship between PTS gustatory sensitivity. These findings would need to be replicated on a larger population group to draw robust conclusions, but may indicate PTS is not as influential on the perception of more complex products as frequently proposed. This would raise questions over the demand for individualised food and beverage products to cater for differences in oral sensitivity across PTS phenotype, which has previously been proposed.

6.2.4 The impact of thermal taste phenotyping methods on the reported incidence of TTs

Improving thermal taste phenotyping protocols is beneficial for those working in research in this field, as it could result in an increase in the number of individuals assigned as TTs, and therefore reduce the number of subjects excluded from a study population. Current methods are likely underestimating the number of TTs identified within a given population. As current testing criteria only defines those subjects with the highest phantom taste sensitivity (above weak intensity) and reproducibility as a thermal taster, it is possible that this has biased the impact of thermal taste on oral sensitivity, and the differences between TTs and TnTs. Resolving these questions is of interest to food manufacturer's when determining the true impact of thermal taster status on product perception.

6.2.5 Elucidating mechanisms associated with thermal taste phenotype

Isolating the temperature range at which phantom tastes are perceived by thermal tasters is important for two reasons. Firstly, to aid in elucidating the mechanism/s which may be responsible for the phantom tastes reported, such as temperature sensitive TRP channels. Secondly, little is known about whether TTs perceive these tastes when consuming food and beverage ingested at differing temperatures. This phenomena could explain why some individuals report off taints in products, where others do not. Better understanding of the temperature range over which tastes are perceived could aid in developing appropriate testing methodologies to explore if phantom tastes are arising during food consumption. For example, it will be of interest to understand whether those TTs who perceive a phantom mint sensation with an onset of 5°C of thermal stimulation applied to the tongue using a Medoc ATS peltier thermode also perceive this same sensation when water is delivered to the tongue at 5°C.

6.2.6 Identifying the demand for individualised food and beverage

Differences in oral sensitivity across thermal taste phenotypes may alter food and beverage preference, and consumption habits. Therefore, understanding of these differences could provide valuable insights into consumer demand based on thermal taste phenotype, leading to more individualised healthier products that are better accepted by thermal taste phenotypes to improve nutritional status and health outcomes.

6.3 FUTURE WORK

6.3.1 Utilising methods to better explore taste phenotype and genotype

To better understand the impact of taste phenotypes and genotypes on oral sensitivity, and the associated influence on dietary behaviours and nutrition and health outcomes, large scale population based studies such as the Italian Taste Project (Monteleone et al., 2017) should be conducted. Additionally, multidisciplinary studies should also be utilised. The work outlined in Chapters 4 and 5 of this thesis highlights the sensitivity of techniques such as fMRI in identifying differences in study populations which may not detected during perceptual testing. This is particularly important when exploring fundamental sensory processing, and understanding the mechanisms involved in responses.

6.3.2 Understanding the relationship between taste sensitivity and PTS

Chapter 4 of this thesis showed that differences in taste sensitivity across PTS may be reduced or eliminated when delivering strong intensity tastants. Therefore, future work should explore the effect of taste phenotype and genotype on both the perceptual and cortical response to tastants at a range of concentrations. In the fMRI study performed, high spatial resolution fMRI data primarily covering the insula cortex was acquired, since one primary aim of the study was to assess whether a gustotopic map exists within the insula (work currently being explored by Dr Sally Eldeghaidy). Chen et al (2011) identified a gustotopic map in the rodent brain, but it is still under debate whether this also occurs in humans. In future studies, coarser spatial resolution fMRI data providing coverage of the whole brain should be collected to allow activation associated with the hedonic qualities of the samples to be identified, since this was proposed as a possible factor influencing the differences observed in relation to sensitivity to PROP in the current study.

6.3.3 Improving thermal taste phenotyping methods

An improved and standardised method of thermal taste phenotyping should be developed, which can be adopted across research groups to enable large group comparisons, and reduce the number of individuals assigned to the *uncat* group. This should explore the use of one temperature trial reaching the extremes of both warm and cool temperatures, as well as the delivery of three replicates of the temperature trials, and inclusion of phantom taste intensity ratings below weak intensity. The Medoc peltier thermode currently used for thermal taste phenotyping is expensive, limiting those able to perform such thermal taste phenotype sessions. Alternative cheaper and more accessible methods of eliciting phantom taste in TTs should be explored, such as applying liquids at variable temperatures to the tongue. In addition, to aid in defining an optimised approach, it would be beneficial to understand if the different thermodes used across research groups (and the number of tongue locations tested) influences the taste qualities reported, or classification of a participant when phenotyping for thermal taster status.

6.3.4 Understanding the true incidence of thermal taste

Updated phenotyping protocols should be used to explore the true incidence of thermal taste when the number of individuals assigned to the *uncat* group has been reduced. As with the other taste phenotypes, the incidence of TTs over different ethnicities should be explored, including large scale across culture studies. Incidence of thermal taste should be explored within families to explore if this phenotype has a genetic component.

6.3.5 Variation in phantom taste perceptions across TTs

This study isolated the temperature range over which phantom tastes were perceived, but a method limitation meant the temperature range could not be determined in the cases when multiple tastes were associated with one temporal rating. Future work should therefore utilise Temporal Check All That Apply (TCATA) methods to isolate the temperature range over which phantom tastes are perceived within temperature trials. Comparing responses across taste qualities may aid in elucidating TRP channels, or other temperature sensitive mechanisms, involved in phantom taste responses. Comparisons should also be made across TTs to improve the current understanding of those who exhibit more unusual phantom taste responses, such as inconsistent reporting across replicates

6.3.6 Identifying how phantom taste is represented in the brain

The fMRI data collected to measure the brain response to phantom taste in TTs was performed at 3 Tesla rather than 7 Tesla, due to the limitation of functioning of the Medoc thermode within the head coil at 7 T. To ensure sufficient SNR for the fMRI data collected at 3 T, this resulted in the 3 mm isotropic spatial resolution being used in the current phantom taste study. Thus the spatial mapping of each individual phantom taste within the anterior insula could not be compared. Future work could utilise high resolution 7 Tesla fMRI to measure the cortical response to both chemical and phantom taste activation in TTs, if an alternative method to elicit phantom taste is found, such as applying liquids at variable temperatures to the tongue. This would allow direct comparisons of cortical activation across chemical and phantom tastes, and identification of the similarities and differences could provide valuable insight into the possible mechanism/s involved in phantom taste.

Non-prototypical sensations (metallic, spicy and minty) have not always been permitted when classifying TTs during phenotyping. Better understanding of the nature of these sensations would aid in defining appropriate criteria to adopt during future phenotyping. The cortical representation of prototypical phantom tastes (sweet, salt, sour, and bitter) could be compared to that of metallic, spicy and minty sensations using high resolution 7T fMRI to determine if the latter are represented in areas of the brain responding to the prototypical phantom tastes. Cortical activation of phantom sensations could also be compared to that of the equivalent chemical stimuli (iron sulphate, capsacin and menthol) to again determine similarities/differences in processing.

6.3.7 Beyond gustatory responses

Evidence detailing the effect of taste phenotype and genotype on cortical processing is limited, and future work should explore this across a wider range of oral stimuli. This should include both stimuli delivering an individual sensory response (such as gustatory), as well as those that elicit a multimodal response (such as gustatory-olfactory flavour). This work indicates the effect of taste phenotype on taste sensitivity may be reduced for stronger tastants, and hypothesises this may translate into reduced differences observed across food and beverage products. It would therefore be of interest to explore the effect of taste phenotype and genotype on the perceptual and cortical response to more complex stimuli that can be delivered in an MR scanner.

Despite PTS being identified nearly a century ago, and being the topic of hundreds of research papers, understanding of both its effect on oral sensitivity, and the mechanisms responsible, remain poorly understood. This research offers new insights into associations between PTS and the perceptual and

cortical response to gustatory stimuli. Interestingly it suggests that PTS may not be a good marker for oral sensitivity, and confirms cortical processing does differ with PTS. Although a number of mechanisms are hypothesised to be involved in the thermal taste response, few studies provide any evidence to support these theories. This novel research has successfully revealed how variable phantom taste responses are across TTs, and has provided valuable contributions towards elucidating the mechanisms which may be involved. This innovative research should be used to guide future studies which continue to explore the mechanisms involved across these fascinating taste phenotypes, as well as the true impact on product perception and dietary choices.

APPENDIX

Appendix 1: Participant health questionnaire

TASTEMAP HEALTH QUESTIONNAIRE

1. Please indicate (with 'X's) if you have any of the following:

☐ Dentures

☐ Oral/gum disease

☐ Diabetes

☐ None of the above

2a. Are you presently, or have you ever been, a smoker? Delete as appropriate:

Yes/No

2b. If yes please provide details of when and how often below.

3a. Do you have any medical condition that may impair your sensory ability? Delete as appropriate.

Yes/No

3b. If yes please provide details below

4a. Do you have any food allergies? Delete as appropriate.

Yes/No

4b. If yes, please provide details below.

5a. Are you currently on any long-term medication? Delete as appropriate.

Yes/No

5b. If yes, please provide details below.

6. Are you, or do you intend to become, pregnant in the next year? Delete as appropriate.

Yes/No

7. Do you have any metal amalgam (not white/porcelain) tooth fillings?

Yes/No

8. **Do you have the rare inherited condition haemochromatosis which causes your body to absorb too much iron from the diet?**

Yes/No

9. **Are you short tongued?** *(The medical name for being short tongued is frenulum of tongue, which means the thin strip of tissue that connects the underside of the tongue to the floor of the mouth causes a more restricted movement of the tongue than in those without the condition).*

Yes/No

10. Please give details if you have any other health related issues that you think may affect your eligibility to participate in the study:

NAME _____

Appendix 2: MR safety questionnaire

The University of Nottingham **Sir Peter Mansfield Magnetic Resonance Centre**

MR Volunteer Safety Screening Questionnaire:

NAME	Date of Scan	Date of Birth
ADDRESS	Volunteer Number	
	Ethics Code: C13032014 SoBS TASTEMAP fMRI	
Phone number	Weight	Height

MR scanning uses strong magnetic fields. For your own safety and the safety of others it is **very important** that you do not go into the magnet halls with any metal in or on your body or clothing. Please answer the following questions carefully and ask if anything is not clear. All information is held in the strictest confidence.

1. Do you have any implants in your body? e.g. replacement joints, drug pumps Y/N
2. Do you have aneurysm clips (clips put around blood vessels during surgery)? Y/N
3. Do you have a pacemaker or artificial heart valve? Y/N
4. Have you ever had any surgery? Please give brief details over. Y/N

   (We do not need to know about uncomplicated caesarean delivery, vasectomy or termination of pregnancy)
5. Do you have any foreign bodies in your body (e.g. shrapnel)? Y/N
6. Have you ever worked in a machine tool shop without eye protection? Y/N
7. Do you wear a hearing aid or cochlear implant? Y/N
8. Could you be pregnant? (Pregnancy tests are available in the female toilets) Y/N
9. Have you ever suffered from tinnitus? Y/N
10. Do you wear dentures, a dental plate or a brace? Y/N
11. Are you susceptible to claustrophobia? Y/N
12. Do you suffer from blackouts, epilepsy or fits? Y/N
13. Do you have any tattoos? (If yes, you may be asked to read and sign another form) Y/N
14. Do you have any body piercing jewellery that cannot be removed? Y/N
15. Do you have any skin patches (trans-dermal patches)? Y/N
16. Do you have a coil in place (IUD) for contraception? Do you know what type? Y/N
17. Do you have any condition that may affect your ability to control your temperature? Y/N

    (e.g. Do you have a fever, cardiovascular disease, hypertension, diabetes or cerebrovascular disease?)
18. Will you remove all metal including coins, body-piercing jewellery, false-teeth, hearing Aids etc. before entering the magnet hall? *lockers available by the changing rooms* Y/N
19. Is there anything else you think we should know? Y/N

I have read and understood all the questions **Signature:**	**Date:**
Verified by: **SPMMRC Staff Signature:**	**Date:**

Appendix 3: Achievements

Refereed publications

Published: Skinner, M., Lim, M., Tarrega, A., Ford, R., Linforth, R., Thomas, A. & Hort, J. 2017. Investigating the oronasal contributions to metallic perception. International Journal of Food Science and Technology, doi:10.1111/ijfs.13417, 1-8.

Under review: Skinner, M., Eldeghaidy, S., Ford, R., Giesbrecht, T., Thomas, A., Francis, S., Hort, J. Variation in illusionary taste response across thermal tasters. *Journal of Physiology and Behaviour*.

Symposia presentations and awards

Award: Giract Young scientist awarded for innovative flavour research (2013)

Poster: Unilever and BBSRC PhD symposium, Colworth, UK (2014)

Flash poster: Nursten Flavour Symposium, University of Nottingham (2014)

Award: IFST SSG award for best flash poster presentation

Poster: IFST Sensory Science Group conference, Southampton, UK (2014)

Poster: Neuroscience at Nottingham, Nottingham, UK (2015)

Flash poster: Trends in Food Flavour, Nottingham, UK (2015)

Flash poster: IFST SSG conference, Nottingham, UK (2015)

Oral: Nursten Flavour Symposium, Northumbria, UK (2015)

Oral: Link 15 Postgraduate Research Symposium, Nottingham, UK (2015)

Poster: Pangborn Sensory Science Symposium, Gothenburg, Sweden (2015)

Award: Pangborn Young Scientist Travel Award

Award: WVS Kesteven Travel Award, University of Nottingham

Poster: Neuroscience at Nottingham, Nottingham, UK (2016)

Award: Best poster presentation in the human research category

Oral: Nursten Flavour Symposium, Reading, UK (2016)

Oral: Eurosense symposium, Dijon, France (2016)

Award: SCI A J Banks student travel award

Award: Lamming travel award, University of Nottingham

Poster: Neuroscience at Nottingham, Nottingham, UK (2017)

Guest lecture: Bath Spa University, UK (2017)

Oral: Nursten Flavour Symposium, Belfast, Ireland (2017)

Award: SCI award for best overall presentation & contribution to flavour science

Oral: Pangborn Sensory Science Symposium, Rhode Island, USA (2017)

Award: Pangborn Young Scientist travel award

Award: IFST SSG travel award

Award: University of Nottingham graduate travel award

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