INDIVIDUAL VARIATION ACROSS PROP AND THERMAL TASTE PHENOTYPES

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Doctor of Philosophy

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ABSTRACT

PROP taster status (PTS) has long been investigated, but thermal taster status (TTS), where thermal tasters (TTs) perceive a ‘phantom taste’ from temperature stimulation is less well understood.

This research aimed to understand the impact of both taste phenotypes (TTS and PTS), together with other potential markers, on oronasal sensitivity, whilst also considering temperature effects. Over 200 volunteers were screened for these taste phenotypes, and a subset assessed for TAS2R38 and gustin genotypes and fungiform papillae count. Sensitivity to a range of oronasal stimuli was measured and compared within and across taste phenotypes. In addition, fMRI was applied to investigate cortical activations to sensory stimuli (including temperature effect) among TTS.

Both PTS and TTS impacted on oronasal sensitivity, however, TTS had a greater impact when testing the anterior tip of the tongue. TTs demonstrated a greater sensitivity to temperature compared to TnTs. For PTS, neither TAS2R38 nor gustin rs2274333 genotype could explain the heightened sensitivity in PROP tasters.

Although PTS and TTS were shown to be independent phenotypes, the intensity advantage gained by TTs was more apparent in pMTs than already highly sensitive pSTs.

The mechanism driving TTS is unknown, the fMRI study showed that TTs had an increased cortical activation in the somatosensory cortex with cold sucrose stimulation compared to TnTs. This finding together with the sensory data

added weight to a proposed hypothesis of cross-wiring between taste and trigeminal nerves in TTs.

This research also looked at the relationship between taste phenotypes and personal traits. Interestingly, TTS was associated with food behaviour, whereas PTS was not. In addition, both TTS and PTS were shown to be associated with personality features, in particular ‘openness’ and ‘Conscientiousness’ dimensions respectively.

This original research advances current understanding concerning how combinations of different taste phenotypes affect oral sensitivity and presents novel findings concerning a link between some personal traits and taste phenotypes.

PREFACE

6-n-propylthiouracil (PROP) is widely used for sensitivity tests. Based on individual variation in PROP perception, individuals can be further grouped as PROP supertasters (pSTs), medium tasters (pMTs) and non-tasters (pNTs). The perceptual difference to PROP was found to extend to other taste and trigeminal compounds, which was speculated to be related to fungiform papillae density. So far, much is known about PROP taster status (PTS), but limited research has been done regarding the newly discovered taste phenotype 'thermal taster status' (TTS) and the relationship between these two taste phenotypes. In addition, the mechanism behind TTS is unknown.

This fundamental research investigates the impact of both PTS and TTS on taste, trigeminal and olfactory stimuli, in order to gain some understanding of the mechanisms behind them. This research also investigate the relationship between these two taste phenotypes (PTS and TTS) and personal traits, which is a novel investigation to test if taste phenotypes can be used as a predictor of personal traits.

To do this, Chapter 1 gives a general introduction to sensory perception and how sensory perception can be measured. It also introduces individual variation in perception, specifically taste phenotypes (TTS and PTS) and genotypes (TAS2R38 and Gustin 2274333), as well as food choice behaviour. Chapter 2 to 6 present the empirical work in this PhD, and each chapter contains a detailed introduction that relevant to the specific investigation. In chapter 2, TTS and PTS screening and classification over 200 subjects is described. These subjects are used in subsequent experimental chapters.

Chapter 2 also investigate the links between taste phenotypes and personal traits. Chapter 3, 4 and 5 describe a series of studies investigating the impact of taste phenotypes and genotypes on oronasal sensitivity. The studies described in Chapter 3 and 4 measure the oral sensitivity at the anterior tip of the tongue, and the study presented in Chapter 5 use the whole mouth consumption protocol. Chapter 3 reports the impact of both TTS and PTS respectively, as well as the relative effect of TTS and PTS across a range of gustatory, trigeminal and olfactory stimuli. Chapter 4 details the investigation into the reason behind PROP tasters' heightened sensitivities by examining the relationship between PTS phenotype and TAS2R38 genotype, gustin rs2274333 genotype and fungiform papillae counts. In chapter 5, the study focuses on investigating the impact of TTS and PTS on perceived intensity with varying serving temperatures of two beverages, as well as the impact of taste phenotypes on overall liking. Chapter 6 describes a collaborative fMRI study with the Sir Peter Mansfield Magnetic Resonance Centre (SPMMRC) at the University of Nottingham to investigate the cortical responses to sensory stimuli across TTS groups, in order to gain some evidence concerning the mechanism involved in TTS. Finally, the main findings of this thesis, general conclusions, implications and further work are summarised in Chapter 7.

1. GENERAL INTRODUCTION

1.1. SENSORY PERCEPTION

Sensory properties are perceived when our sensory receptors interact with stimuli around us, and are understood through the five senses: vision, audition, gustation, olfaction and somatosensation (Kemp *et al.*, 2009). Sensory receptors then convert this energy into neural impulses that are sent to the brain. Perception itself occurs when the brain organises the information and translates/interprets it into something meaningful (Goldstein, 2009).

Flavour perception is the sensation realised when a food/beverage is placed in the mouth, and the overall sensation of flavour results from responses form receptors present throughout the oral and olfactory cavity. These sensory receptors produce signals in the nervous system and enable us to differentiate between products and environments in sensory terms (Woods, 1998). **Figure 1-1** shows a diagram of various stages of flavour perception.

During the eating process, a variety of chemical stimuli (volatiles and non-volatiles) are released. Saliva facilitates the movement of non-volatile components to reach the taste and trigeminal receptors in the oral cavity (Laing & Jinks, 1996), while the volatile components are transported retronasally from the mouth to the roof of the nasal cavity, where odour receptors are located (Kemp *et al.*, 2009). All five senses are associated with different types of receptors and play a role in the sensory evaluation of food and beverage products. Combinations of some or all the senses and other accompanying sensations are associated with flavour perception, further influencing product palatability and acceptability (Woods, 1998). In order to

better understand the eating experience, it is essential to understand the basic mechanism behind each modality. Thus, the gustatory, olfactory and trigeminal perceptions are discussed below.

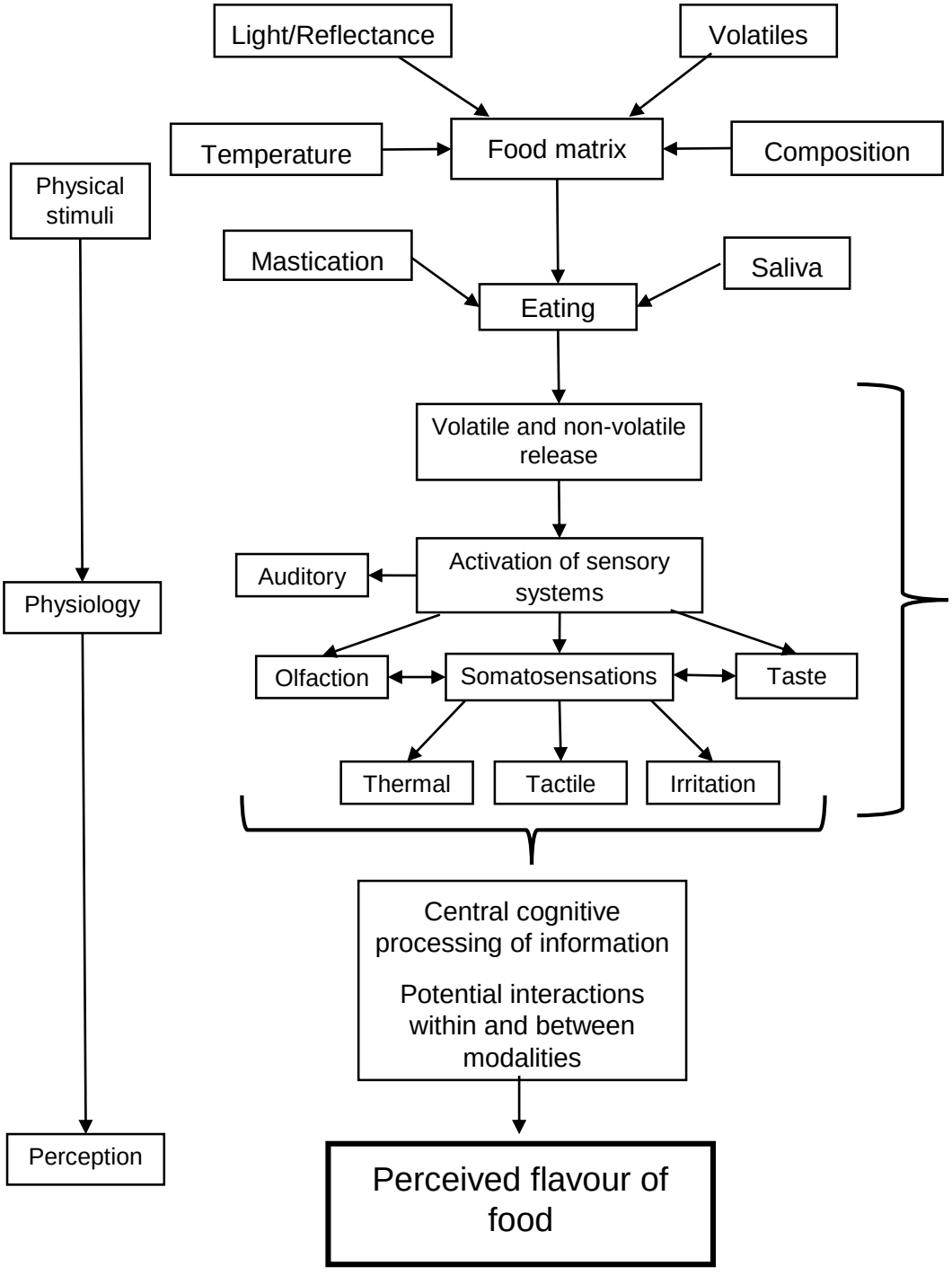

Figure 1-1: Flow diagram outlying various stages and factors that influence flavour perception (Source: Keast *et al.* (2004)).

1.1.1. Gustatory Perception

Most researchers agree that there are five types of taste qualities with specific receptor types: sweet, salty, sour, bitter and umami (Iwata *et al.*, 2014; Kemp *et al.*, 2009). Taste stimuli are detected by taste receptors, which are located in taste buds throughout the oral cavity, including the tongue, palate and throat. Taste buds are onion-like structures (**Figure 1-2 (a)**) and each taste bud contains 50-100 taste cells (Chandrashekar *et al.*, 2006). When a food or drink is consumed, some substances dissolve in saliva, and enter the taste pore to contact the taste cells. They interact either with taste receptors on the surface of the cells or through ion channels. These interactions cause electrical changes in the taste cells, triggering them transmit signals that ultimately result in impulses to the brain (Smith & Margolskee, 2006).

The majority of taste buds are located within taste papillae on the tongue. There are four types of papillae: filliform papillae, fungiform papillae, foliate papillae and circumvallate papillae. Filliform papillae are normally found across the surface of the tongue and do not contain any taste buds, and however are involved in tactile sensation (Smith & Margolskee, 2006). Fungiform papillae are ‘mushroom-like’ papillae that appear as pinkish spots, located on the front part of the tongue, containing one or more taste buds at the surface of the papillae. Foliate papillae are ‘leaf-like’ papillae, folded on the sides at the rear of the tongue and the taste buds are located deeply in the folds of these papillae (Chandrashekar *et al.*, 2006). Circumvallate papilla are ‘wall-like’ papillae, seated at the very back of the tongue, and taste buds are located deeply in the trenches of these papillae (Hoon *et al.*, 1999). **Figure 1-2 (b&c)** show the shape and location of the three papillae that contain taste buds.

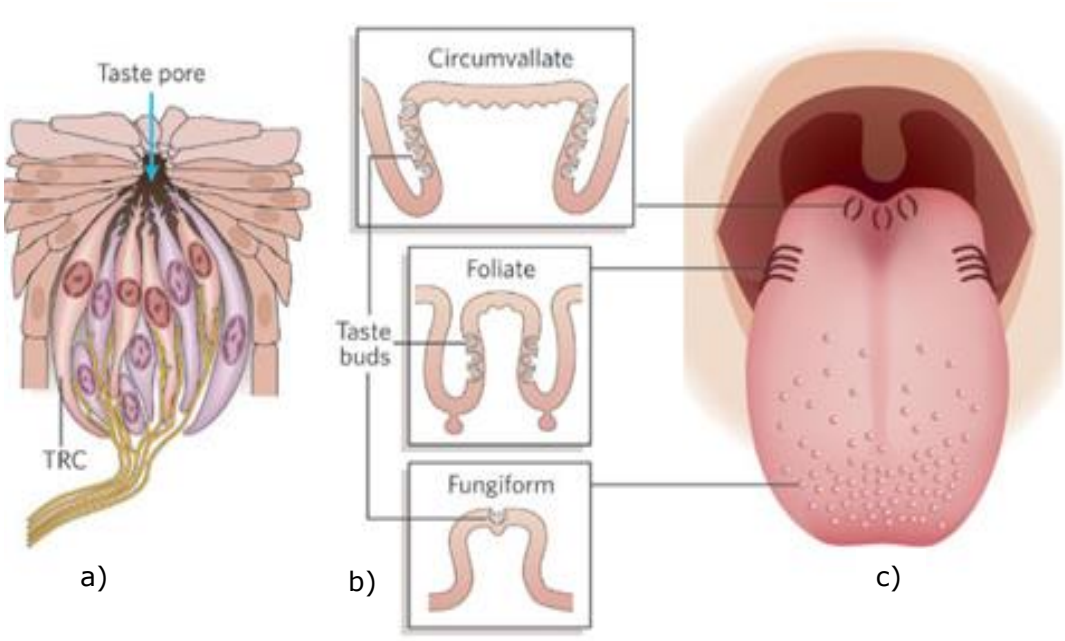

Figure 1-2: Functional anatomy of the human tongue: a) Taste bud anatomy, b) Shape of different papillae, c) Papillae location on the tongue (Source: Chandrashekar *et al.* (2006)).

In general, there are three theories concerning how different tastes are detected in the taste bud. The first model, the labelled-line model, involves receptor cells (TRCs) responding to a single taste modality, such as sweet, bitter, sour, salty or umami – and are innervated by individually tuned nerve fibres. In this case, each taste is specified by the activity of non-overlapping cells and fibres (**Figure 1-3 (a)**). The prevailing models for the past two decades suggest an across-fibre model. It is proposed that individual TRCs express different families of taste receptors, and consequently the same afferent fibre carries information for more than one taste modality (**Figure 1-3 (b)**), or that TRCs are still tuned to single taste qualities but the same afferent fibre carries information for more than one taste modality (**Figure 1-3 (c)**) (Chandrashekar *et al.*, 2006).

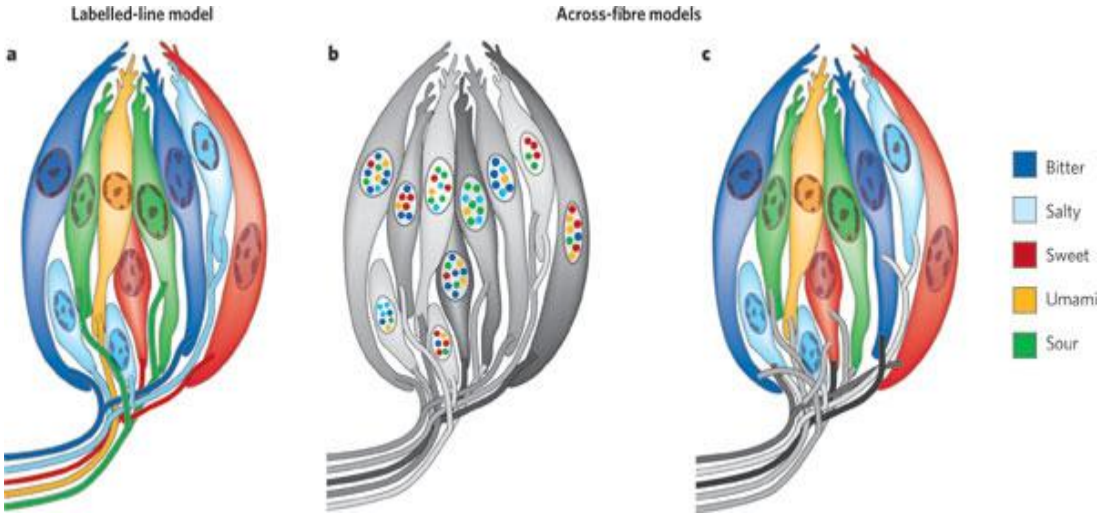

Figure 1-3: Encoding of taste qualities at the periphery. a) Labelled-line model, b & c) Across-fibre models (Source: Chandrashekar *et al.* (2006)).

Taste buds in the fungiform papillae located on the anterior two-thirds of the tongue are innervated by sensory neurons of the chorda tympani, a branch of the facial nerve (cranial nerve VII) (Walker, 1990b). Taste buds located on the posterior third of the tongue, where foliate and circumvallate papillae are seated, are innervated by the glossopharyngeal nerve (cranial nerve IX). Taste buds on the palate are innervated by a branch of cranial nerve VII. And finally, taste buds on the epiglottis and esophagus are innervated by cranial nerve X (Walker, 1990c). The sensory fibres that receive sensory input from the taste cells run in cranial nerves VII, IX and X, which then enter into the nucleus of solitary tract (NST). The neurons in NST further project to the thalamus and gustatory cortex (anterior insula and frontal operculum) (Buck & Bargmann, 2013), as illustrated in **Figure 1-4.**

Different tastes involve different transduction mechanisms, sour and salty permeate the taste cell wall through ion-gated channels, but sweet, bitter and umami tastes are mediated by G protein-coupled receptors (GPCRs) (Rawson

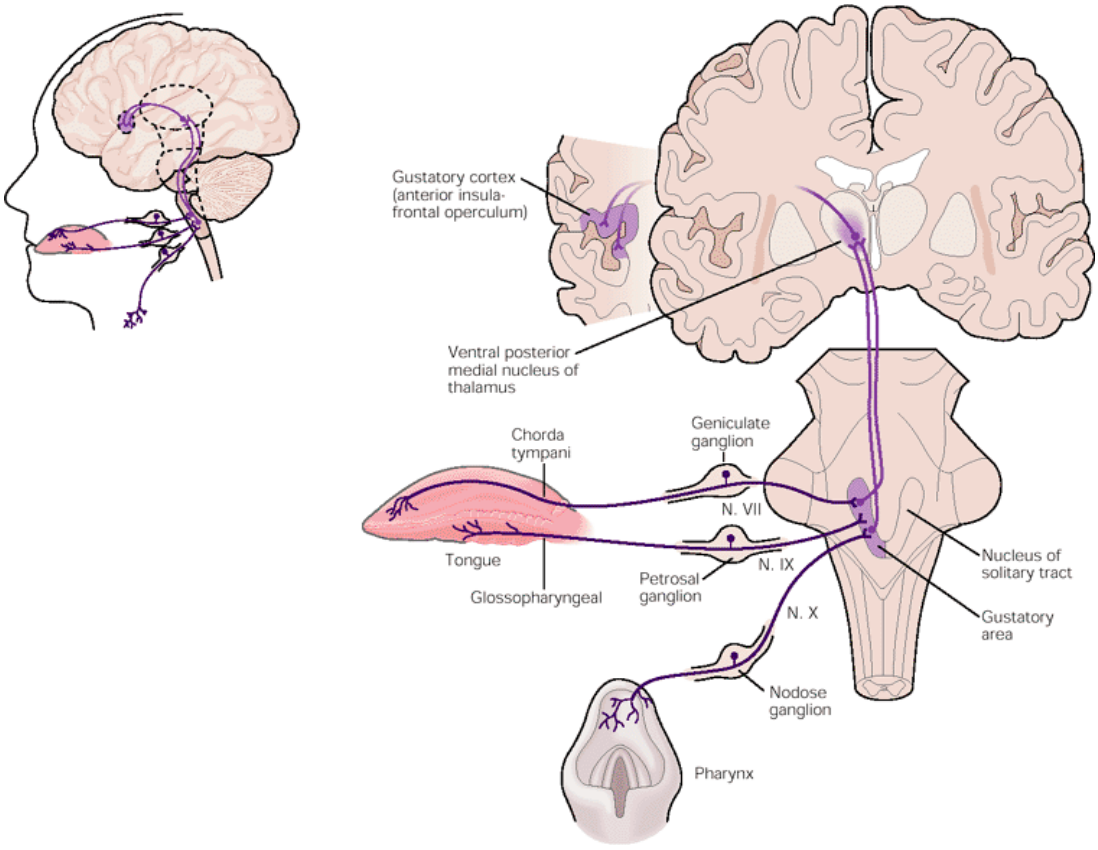

Figure 1-4: Taste information is transmitted from the taste buds to the cerebral cortex via synapses in the brain stem and thalamus (Source: Buck and Bargmann (2013).

& Li, 2004). The detailed pathways of the five basic tastes are discussed below.

For salty taste, the positive ions (e.g. Na^+) of salt (sodium chloride and other salts) enter into the cell through ion channels on microvilli (**Figure 1-5 (a)**). In addition, sodium ions can also enter via channels on the basolateral cell. The accumulation of sodium ions causes an electrochemical change called depolarisation that results in calcium ions (Ca^{2+}) entering the cell. The calcium triggers transmitter release, which is received by neuron, and further signalling the brain. Taste cells repolarise, or 'reset' themselves by opening potassium ion channels so that potassium ions can exit (Halpern, 1997; Rawson & Li, 2004; Smith & Margolskee, 2006).

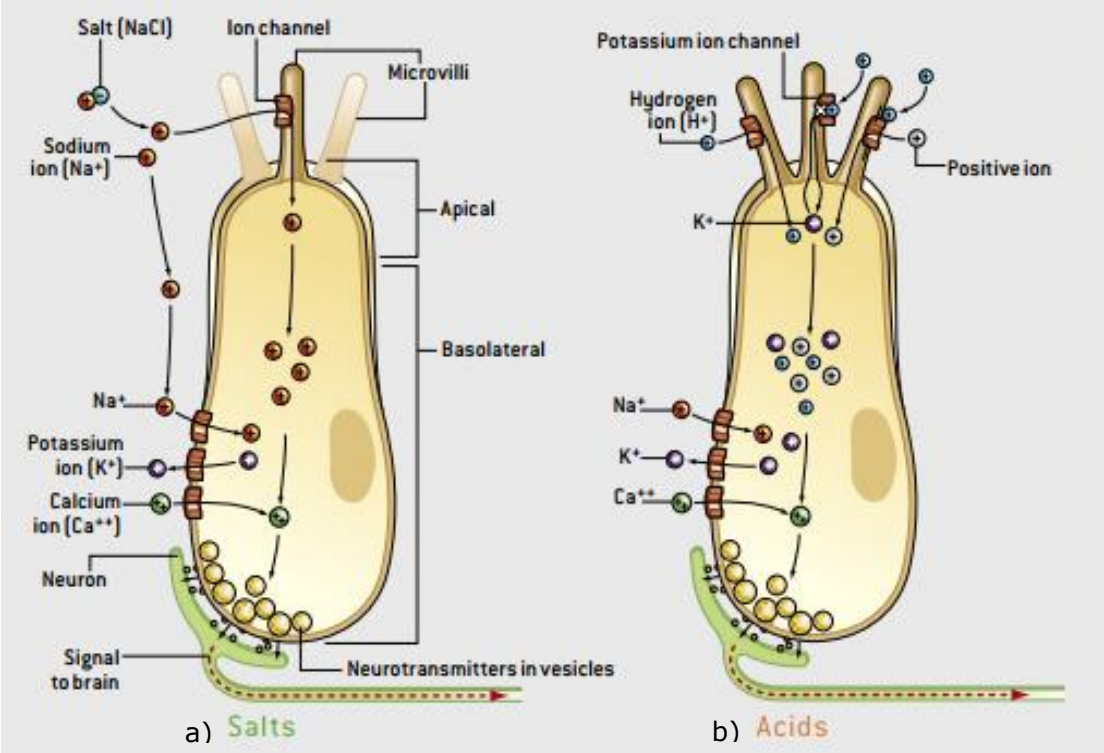

Figure 1-5: Mechanism of a) Salt and b) Acids (sour) transduction pathways (Source: Smith and Margolskee (2006)).

Sour tastes are perceived due to the accumulation of hydrogen ions (H^+). It has been suggested that these ions act on taste cells in different ways: 1) by directly entering the cell; 2) by blocking K^+ channels on the microvilli; 3) by binding to and opening channels on the microvilli that allow other positive ions to enter the cells (Smith & Margolskee, 2006), as illustrated in **Figure 1-5 (b)**. The accumulation of positive charges depolarises the cell and leads to neurotransmitter release. It has been suggested that a broad range of receptors and mechanisms might be responsible for sour taste (Chandrashekar *et al.*, 2006). Recent evidence has identified at least one specific sour taste receptor protein, PKD2L1, it was found that genetically modified mice, lacking PKD2LI, showed a complete loss of response to acids (Huang *et al.*, 2006). Sour taste perception is only beginning to be explored and a lot more research is needed to understand its complexity.

Sweet stimuli, such as natural or artificial sweeteners do not enter into taste cells, instead, they bind to the GPCRs on the taste cell surface. This prompts the subunits, which activate other cellular processes, including the enzymes that generate second messengers (Smith & Margolskee, 2006). It has been suggested that sweet tastants activate taste cells through at least two transduction pathways. For natural sweeteners – sugars appear to activate adenyl cyclase, elevating intercellular levels of cAMP or cGMPs, whereas for artificial sweeteners, a different secondary messenger is produced (IP_3). Both pathways may block potassium channels indirectly, resulting in cell depolarisation, Ca^{2+} enter the cells through activated Ca^{2+} channels, and an electric current is produced (Rawson & Li, 2004; Smith & Margolskee, 2006), as shown in **Figure 1-6 (a)**. T1R2 and T1R3 are taste-specific GPCRs that functions in combination as a heterodimeric sweet taste receptor (Hoon *et al.*, 1999). The T1R2+3 combination has been found to respond to all classes of sweet tastants, including natural sugars, artificial sweeteners and intensely sweet proteins (Jiang *et al.*, 2005; Li *et al.*, 2002; Nelson *et al.*, 2001).

For umami taste, the transduction pathway is similar to sweet taste. Amino acids such as glutamate binds GPCRs and activate secondary messengers (Smith & Margolskee, 2006). Currently, the intermediate steps between secondary messengers to transmitter release are still unknown (**Figure 1-6 (c)**). However, evidence suggests that the combination of T1R1 and T1R3 are responsible for umami taste (glutamate) (Li, 2009; Li *et al.*, 2002).

Bitter taste transduction pathways are similar to sweet and umami tastes, acting through GPCRs and secondary messengers (**Figure 1-6 (b)**). In this case, the secondary messengers cause the release of calcium ions from the

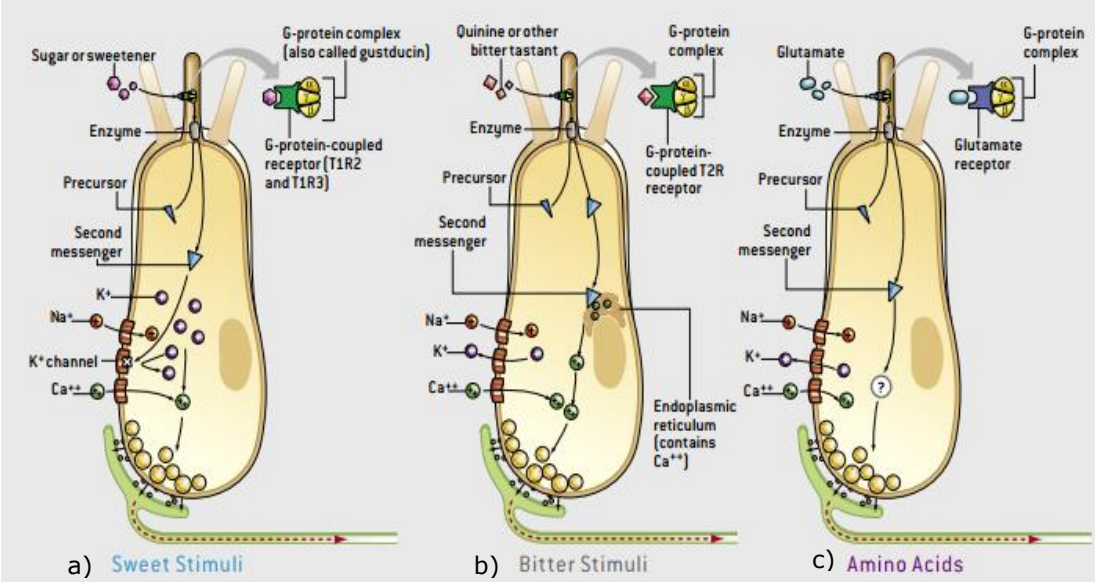

Figure 1-6: Mechanism of a) Sweet, b) Bitter and c) Umami transduction pathways (Source: Smith and Margolskee (2006)).

endoplasmic reticulum, resulting in depolarization and neurotransmitter release (Smith & Margolskee, 2006). A family of approximately 30 GPCRs called T2Rs detect bitter tastants. These receptors vary in protein sequence and are consistent with their ability to recognise bitter compounds with diverse chemical structures. Indeed, different T2R receptors recognise different bitter compounds (Chandrashekar *et al.*, 2000).

Additional qualities such as fat, calcium, kokumi and metallic have also been considered as basic tastes by some researchers (DiPatrizio, 2014; Ohsu *et al.*, 2010; Tordoff, 2010). Metallic sensation was considered as a primary taste by Bartoshuk (1978). It was later work that showed metallic could be evoked both by metal salts and electrical tongue stimulation. Two mechanisms behind metallic sensation have been proposed: i) it is a multimodal perception including gustatory olfactory and possibly trigeminal pathways (Epke *et al.*, 2009), ii) it is a true gustatory mechanism and were not affected by nasal

occlusion (Lawless *et al.*, 2005). Metallic sensation is complex and more studies are certainly required to determine the mechanism behind metallic sensation.

1.1.2. Olfactory Perception

Odorants (volatile chemicals) are detected by olfactory sensory neurons in the nasal cavity. Humans and other mammals are capable of discriminating a great variety of odours. In the olfactory system, the olfactory sensory neurons are embedded in a specialised olfactory epithelium distributed with gila-like supporting cells. They are located in the back of the nasal cavity over approximately $5cm^2$ in humans (Goldstein, 2009).

Volatile compounds can arrive at the olfactory epithelium through two distinct pathways: orthonasal or retronasal pathways. Orthonasal stimulation can be achieved by sniffing a flavour solution or a food product. During the inhalation process, odorants travel inwards from the anterior nares (nostrils) towards the olfactory mucosa, and if an adequate number of molecules reach the receptors, an orthonasal stimulus delivery is provoked. In contrast, retronasal stimulation is caused by the ascent of odorants through the posterior nares of the nasopharynx, which occurs during respiratory exhalation or after swallowing (Diaz, 2004; Pierce & Halpern, 1996).

Odorant receptors are proteins encoded by a multigene family that belong to the GPCR superfamily. Odorant receptors have seven hydrophobic regions that are likely to serve as transmembrane domains (Buck & Axel, 1991). The amino acid sequences of odorant receptors are especially variable in several transmembrane domains. This provides a possible basis for variability in the

odorant binding pocket that could account for the ability of different receptors to recognise structurally diverse ligands (Buck & Bargmann, 2013). Humans have approximately 350 different odorant receptors, and are capable of detecting more than 10,000 different volatile chemicals. Every olfactory receptor cell has only one type of receptor. Each receptor type can detect a small number of related molecules and responds to some with greater intensity than others (Buck & Bargmann, 2013). During the consumption of foods, odorant molecules arrive at the olfactory receptors either orthonasally or retronasally and bind to olfactory receptor, as indicated in **Figure 1-7**.

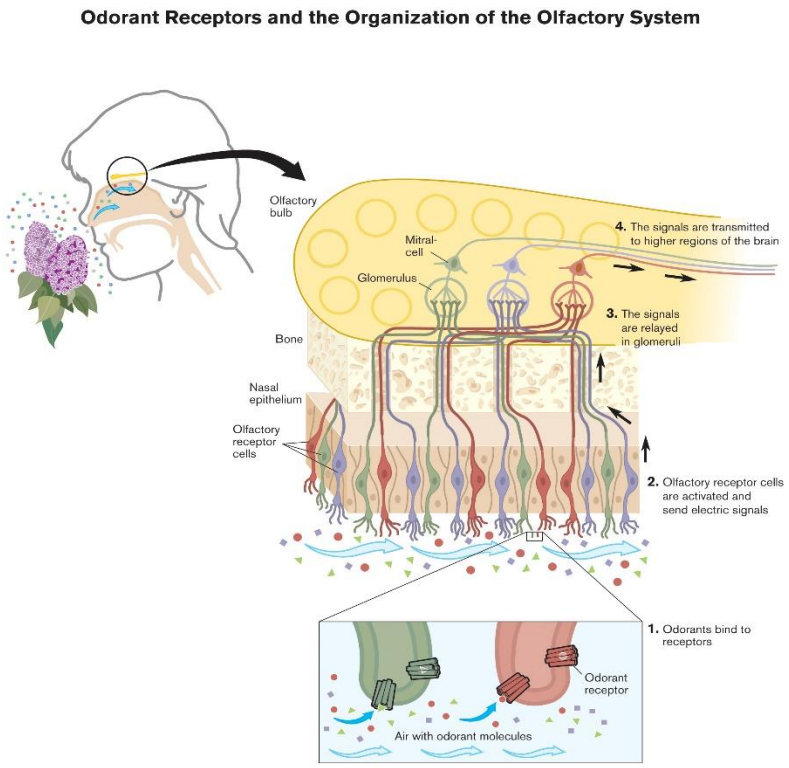

Figure 1-7: Human odour receptors ad the organisation of the olfactory system (Source: Buck and Axel (2004)).

Olfactory receptor cells are then activated and send electric signals, which are relayed in glomeruli. Receptor cells of the same type are randomly distributed

in the nasal mucosa but converge on the same glomerulus. In the glomerulus, the receptor nerve endings excite mitral cells that forward the signal to higher regions of the brain – olfactory cortex (Buck & Axel, 1991; Rinaldi, 2007).

The olfactory cortex, defined roughly as the portion of the cortex that receives a direct projection from the olfactory bulb, is comprised of five main areas: the anterior olfactory nucleus; the anterior and posterior cortical nuclei of the amygdala; the olfactory tubercle; part of the entorhinal cortex and the piriform cortex which is the largest and is considered as the major olfactory cortical area (Buck & Bargmann, 2013). From these areas, olfactory information is transmitted directly to other areas of the brain as well as indirectly via the thalamus, as illustrated in **Figure 1-8**. Beyond the primary olfactory cortex, the neocortex including frontal and orbitofrontal areas are also involved (Wilson & Rennaker, 2009). These pathways to higher cortical areas are thought to be important in odour discrimination (Gottfried, 2010).

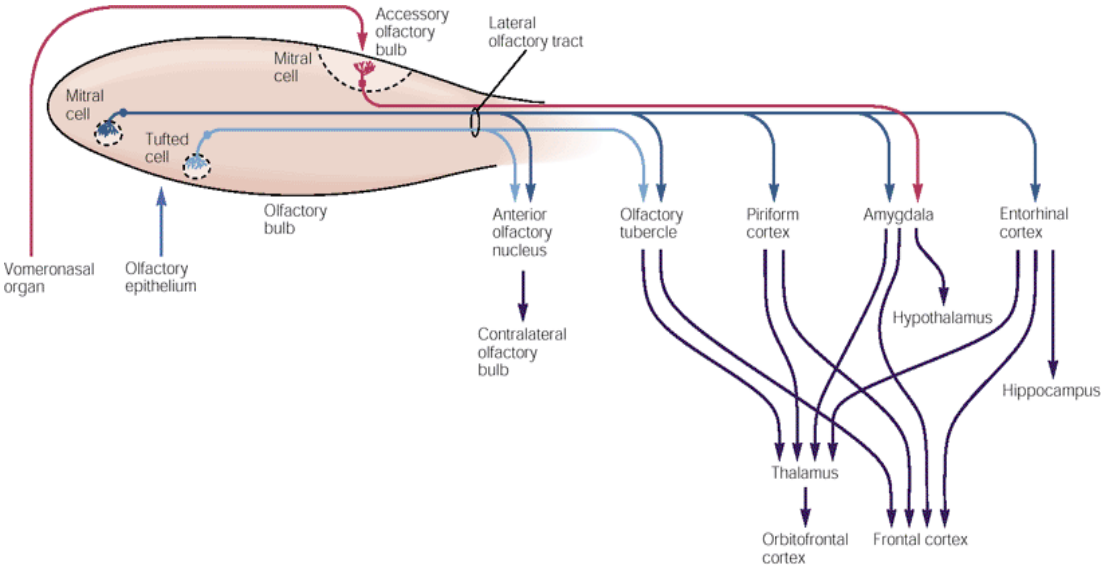

Figure 1-8: The olfactory cortex (Source: Buck and Bargmann (2013)).

1.1.3. Trigeminal Perception

The three major branches of trigeminal nerves are: ophthalmic nerve (V1), maxillary nerve (V2) and mandibular nerve (V3). The three branches leave the skull through three separate foramina: the superior orbital fissure (forehead and eye), the foramen rotundum (cheek) and the foramen ovale (lower face and jaw) (Walker, 1990a) (**Figure 1-9**).

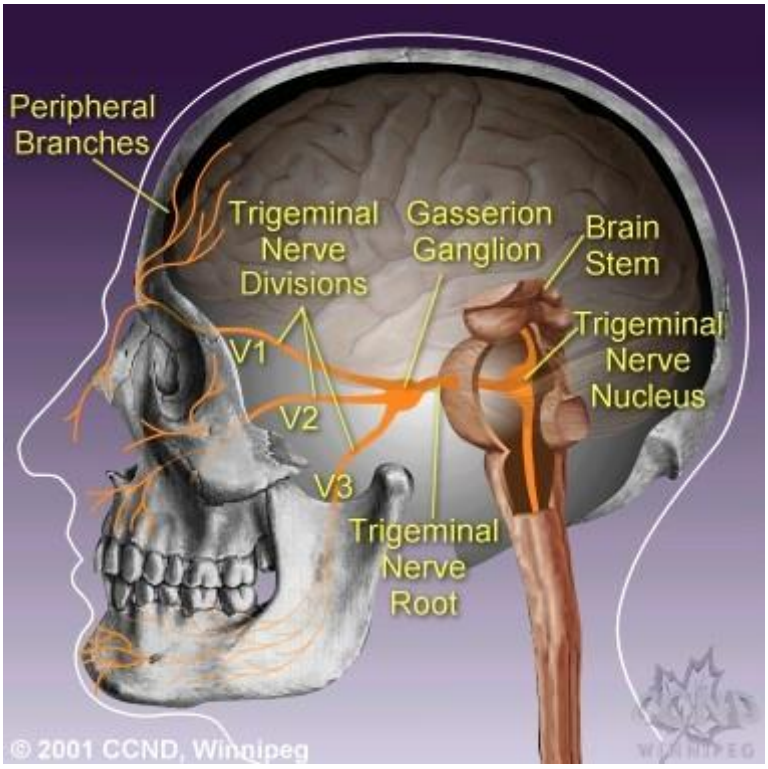

Figure 1-9: The three branches of the trigeminal nerve (Source:. Kaufmann and Patel (2001).

Trigeminal nerves are responsible for the sensation of tactile, proprioceptive, temperature and pain stimuli, providing information on the texture, consistency and chemical irritation of foods and beverages (Gardner & Johnson, 2013). The mandibular nerve (V3) provides the main source of nerve innervation to the mouth. The lingual nerve is a branch of the mandibular division, which

supplies general somatic afferent innervation of the anterior two thirds of the tongue. It also carries fibres from the facial nerve (chorda tympani nerve), which returns taste information from the anterior two thirds of the tongue (Cichero, 2006), as shown in **Figure 1-10**.

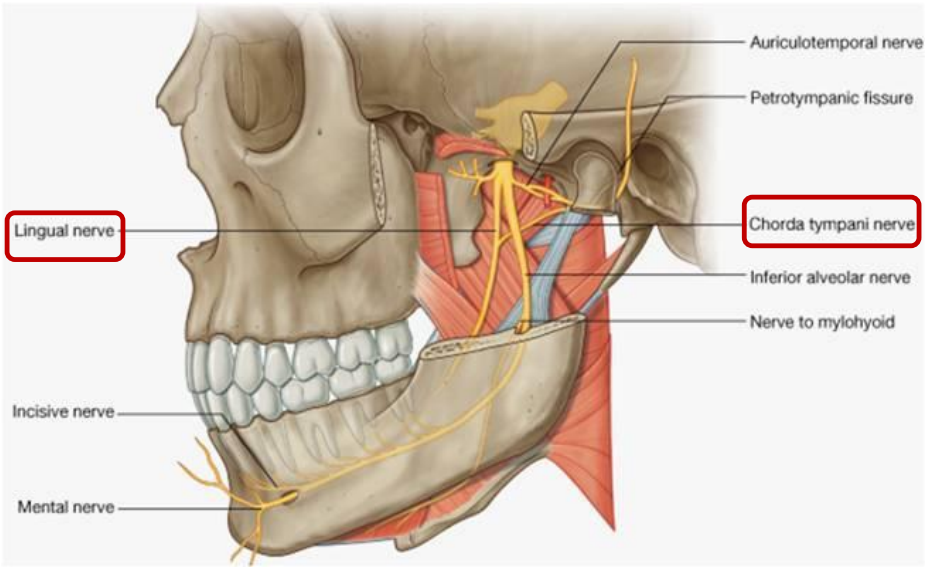

Figure 1-10: Example of lingual nerve carries fibres from chorda tympani nerve (Source: https://www.studyblue.com/notes/note/n/1101200).

Trigeminal information from the face or mouth is carried in the first order neurons of the trigeminal nerve in the trigeminal ganglion. The trigeminal nerve enters the brainstem at the level of the pons to terminate on second order neurons in the trigeminal brainstem complex. This complex has two major components: the principal nucleus, which is responsible for processing mechanosensory stimuli, and the spinal nucleus, which is responsible for processing thermal and painful stimuli (Purves *et al.*, 2001). The secondary fibres cross the midline and ascend to the third order neurons in the ventral posterior medial (VPM) nucleus of the thalamus by way of the trygeminothalamic tract (Engelen, 2012; Walker, 1990a). The axons arising

from neurons in the VPM project mainly to the primary somatosensory cortex (SI) and is then distributed from the SI to 'higher-order' cortical fields, such as the adjacent secondary somatosensory cortex (SII) which sends projections to limbic structures, such as the amygdala and hippocampus (Dougherty, 1997; Engelen, 2012).

1.1.3.1. Temperature Sensation

The temperature of foods and drinks can be very important for its acceptance (Cardello, 1996). Humans have strong preferences for the temperature of some products. The experience of eating and drinking is made up of a variety of sensations, such as taste, smell, texture and temperature. The temperature of products can also affect the way that food is perceived. For example, the temperature can affect flavour release, as well as changes in the physical characteristics of foods, such as melting jelly at high temperatures and the separation of fat-containing sauces upon cooling. Oral temperatures have also shown a significant effect on oral perception (Engelen, 2012), where cooling the tongue temperature to 20ºC reduced the perceived sweetness (sucrose) and bitterness (caffeine) intensity (Green & Frankmann, 1987).

Most of the current knowledge concerning temperature perception is limited to cutaneous thermoreceptors, but little is known about oral temperature receptors. Current evidence found that thermal sensations result from the combined activity of six types of afferent fibres: low-threshold and high-threshold cold receptors, warm receptors, and two classes of heat nociceptors (Gardner & Johnson, 2013). If skin temperature changes slowly, a person is unaware of the changes in the range between 31 to 36ºC. Below 31ºC, the sensation progressively changes from cool to cold and finally, to pain at 10ºC

to 15ºC. Above 36ºC, the sensation progressively change from warm to hot and then, to pain at 45ºC (Gardner & Johnson, 2013).

Evidence was found that transient receptor potential (TRP) channels (contribute to peripheral temperature sensation. Several TRP channels from the TRP ion channel superfamily (the TRP vanilloid channels (TRPV), the melastatin TRP channels (TRPM), and the ankyrin (TRPA)) are equipped to detect thermal changes (Ferrandiz-Huertas *et al.*, 2014). All TRP channels are gated by temperature and various chemical ligands, but different types respond to different temperature ranges and have different activation thresholds. At least six types of TRP receptors have been identified in sensory neurons (Gardner & Johnson, 2013).

As illustrated in **Figure 1-11**, TRPM8 receptors respond to cold stimuli below 25ºC. TRPM8 receptors can also be activated by menthol and other 'minty' chemicals. TRPA1 receptors respond to temperature below 17ºC and also can be activated by 'alliums' compounds such as garlic and radishes. Both TRPM8 and TRPA1 receptors are expressed in high-threshold cold receptor terminals, but only TRPM8 is expressed in low-threshold terminals. TRPV4 receptors are activated by temperatures above 27ºC and respond to normal skin temperatures, which may also play a role in touch sensation. TRPV3 receptors are expressed in warm fibres, which respond to warming above 35ºC and also bind camphor. TRPV1 and TRPV2 receptors respond to heat (exceeding 45ºC), and mediate sensations of burning pain; they are expressed in heat nociceptors. TRPV1 but not TRPV2 receptors bind capsaicin, which mediates the burning sensations evoked by chili peppers (Engelen, 2012; Gardner & Johnson, 2013; Green, 2004).

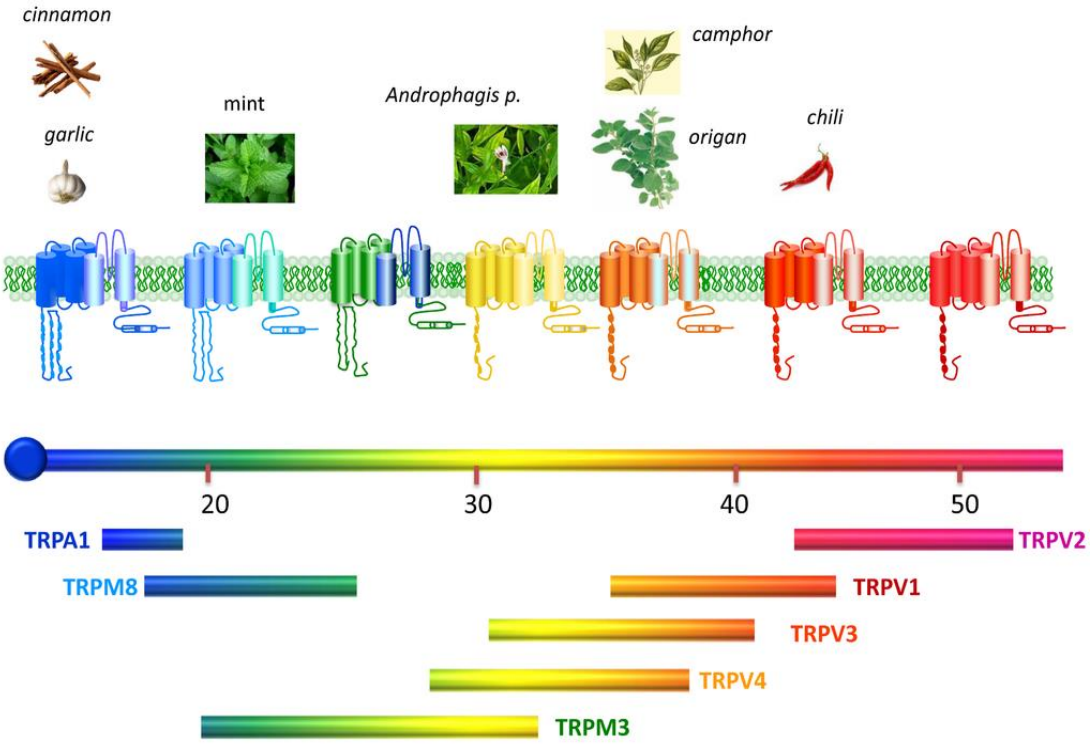

Figure 1-11: Temperature response profiles of different TRP channels (Source: Ferrandiz-Huertas *et al.* (2014)).

1.2. MEASURING PERCEPTION

1.2.1. Sensory Measurement

Perception is a conscious sensory experience, and a perceptual process allowing us to experience the world around us. The perceptual process, shown in **Figure 1-12**, is a sequence of processes that work together to determine our experiences and reactions to stimuli in the environment. The process can be divided into four categories: stimulus, electricity, experience and action, and knowledge (Goldstein, 2009). Stimulus refers to what is out there in the environment, what we actually pay attention to, and what stimulates our receptors. Electricity refers to the electrical signals that are created by the

receptors and transmitted to the brain. Experience and action refers to the perception, recognition and reaction to the stimuli. Knowledge refers to the knowledge we bring to the perceptual situation. The perceptual process is continual, and no time is spent thinking about the actual process that occurs when you perceive multiple stimuli that surrounds you at any given moment (Goldstein, 2009). There are a number of perceptual responses linked to a stimulus, such as detection, recognition, discrimination and perceiving magnitude (intensity). In this research project, detection threshold and perceived intensity were measured among individuals.

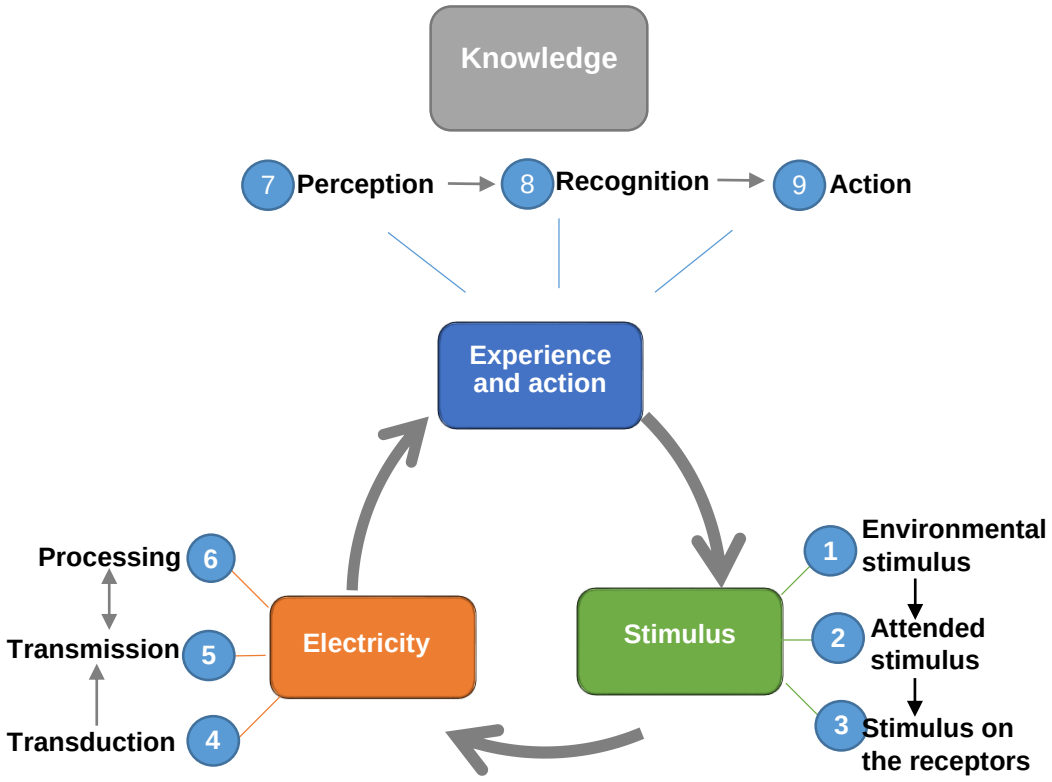

Figure 1-12: The four categories of perceptual process (Source: Goldstein (2009)).

1.2.1.1. Detection

Detection threshold or absolute threshold is defined as the smallest amount of stimulus energy necessary to detect a stimulus (Lawless & Heymann, 2010b). For example, the smallest amount of light energy that enables a person to just barely detect a flash of light would be the absolute threshold for seeing that light. A variety of different techniques have been used to measure absolute thresholds so far. The three main categories of threshold methods are: method of constant stimuli; adaptive testing and method of limits (Lawless & Heymann, 2010b).

In method of constant stimuli, the stimuli are randomly presented by researchers, minimally the effect of adaptation or expectation (Gescheider, 1997). The staircase method, sits under the adaptive testing category and begins with high intensity stimuli, as it is the easiest to detect. The stimuli intensity is decreased after the subject reports a detection ('yes'), and is increased after reporting no detection ('no') (Lawless & Heymann, 2010b). There are different types of staircase procedures, using different decisions and termination rules. The threshold value is calculated by the average of peaks and valleys obtained. This threshold technique is time consuming and fatiguing for subjects (Lawless & Heymann, 2010b; Wales & Blake, 1970).

The method of limits is one of the most common approaches to measure thresholds. Stimuli of different magnitudes are presented in either ascending or descending order by the experimenter (Lawless & Heymann, 2010b). One of the method of limits is described in British Standards (BS-5929-7, 1992), which requires a series of solutions to be presented at the same time in ascending order, starting with water, and assessors indicate when they can

detect a stimulus. Other methods of limits include two of the ASTM International methods, which employ a three-alternative forced choice (3-AFC) technique to identify the odd sample in a set of three samples, where two of the samples are the controls (ASTM-E679, 2004; ASTM-E1432, 2011).

ASTM-E679 calculates each individual's best estimate threshold (BET) by taking the geometric mean of the concentrations at which the assessor's response changes from incorrect to consistently correct. The group BET is calculated by the mean of each individual's BET (ASTM-E679, 2004). ASTM-E1432 determines each individual's sensory threshold from the correct percentage above chance response of 50% using linear regression, the data set is typically for more than 20 to 40 3-AFC presentations per individual (ASTM-E1432, 2011). However, in practice it is common for panellists to 'get the signal' on one trial but temporarily lose it on the next. This could be due to excessive exposure to the testing stimulus, introducing fatigue, adaptation, reduced motivation or other types of interference, all of which could be sources of bias in threshold estimation (Peng *et al.*, 2012; Stevens *et al.*, 1988). Therefore, one of the challenges for using ASTM-E679 is to choose an appropriate stopping rule.

1.2.1.1.1. Stopping rule in ASTM E679

So far, different stopping rules have been used in different studies, such as stopping rule '2', '3', as well as last reversal rule. Stopping rule '2' calculates individual's BET immediately after two consecutive correct responses occur; and stopping rule '3' calculates the threshold after three consecutive correct responses. The last reversal rule normally examines the whole set of samples,

and calculate individual's BET from the last incorrect to correct response (Peng *et al.*, 2012). The threshold theory that ASTM-E679 is based on is the all-or-non assumption that characterises the threshold as a sharp transitional point that separates no detection to detection. However, the modern threshold has demonstrated that detection performance increases monotonically as a function of stimulus concentration (Peng *et al.*, 2012), which measures detection threshold by proportion correct (p(c)). The correct proportion gradually increases as the concentration increases, known as the psychometric function.

Lawless (2010) proposed an alternative analysis of the data generated by ASTM-E679, which involves a simple interpolation of chance-corrected 50% detection for group threshold based on the theory of psychometric function. This analysis takes into account the possibility of guessing correctly, which is not considered in the ASTM calculations. Additionally, it does not discount the correct response early in the series, and no stopping rule is needed. This method is typically useful for group threshold measurement, but it does not give the results of each individual's threshold level (Lawless, 2010). Peng *et al.* (2012) compared threshold results following different stopping rules for ASTM-E679 with group psychometric functions, and suggested that stopping rule '3' provides threshold estimates consistent with those estimated by the method-independent approach, and was therefore recommended for future research.

1.2.1.2. Discrimination

The discrimination threshold or just noticeable difference (JND) is the smallest

difference between two stimuli that a person can detect (Martin *et al.*, 2007). According to Weber's law: ΔI/I = K (ΔI is the JND; I is the reference stimulus; K is a constant, the Weber fraction). The smallest detectable increment in the stimulus parameter of interest is always a constant percentage of the reference value. If the reference value (I) is small, a small increase (ΔI) can be detected. However, if the reference value is large, then only large increase will be noticed (Goldstein, 2009).

In sensory studies, discrimination tests are commonly used to determine if two products or more are perceptibly different such as triangle test, duo-trio test, paired comparison, 3-AFC, Two out of five, A or Not A, Same/different and tetrad (Lawless & Heymann, 2010a).

1.2.1.3. Scaling

Scaling techniques require assessors to assign a word or number to express the intensity of a sensation (Meilgaard *et al.*, 2007). There are three main types of response scale: category scaling, magnitude estimation scaling and line scaling.

Category scales are normally presented as horizontal or vertical scales, offering choices of integer numbers, simple check boxes, or word phrases. Magnitude estimation requires assessors to assign numbers to sensations in proportion to how strong the sensation seems. With a line scale, assessors rate the intensity of a given stimulus by making a mark on a line. Line scales are usually labelled with words 'very poor or weak' on the left end and 'excellent or strong' on the right end, if presented horizontally. (Meilgaard *et al.*, 2007).

Humans cannot share each other's perceptual experience. In order to directly study the perceptual differences between subjects, the labelled magnitude scale (LMS) was developed by Green *et al.* (1993), which is another format of a line scale. The LMS scale consists of a vertical line with quasi-logarithmic spaced verbally descriptors (barely detectable, weak, moderate, strong, very strong and strongest imaginable of oral sensations). It assumes that the descriptors have the same meaning across individuals within a specific sensory modality. For example, two subjects rate a given amount of sucrose as 'very sweet' on the LMS, the scale assumes the 'very sweet' are equal intense between these two individuals. In addition, LMS scale also assumes the top of the scale (oral pain) would be equivalent across individuals. However, studies have found that PROP tasters have an overall enhanced sensitivity to oral sensations (Bartoshuk *et al.*, 1998; Tepper & Nurse, 1997), and was associated with fungiform papillae density (Bartoshuk *et al.*, 1994). Consequently, the top of the LMS was speculated to vary among individuals (e.g. PROP taster status) (Bartoshuk *et al.*, 2002). Bartoshuk*, et al.* (2004) have addressed that the heart of the problem with the LMS is whether or not individuals are experiencing the same intensity is unknown. Hence, Bartoshuk*, et al.* (2004) suggested a solution to this problem, which was having a standard that is unrelated to the stimuli of interest.

The general labelled magnitude scale (gLMS) was created by stretching the LAM scale to its maximum, by labelling the top scale as 'strongest imaginable sensation of any kind'. People rarely choose taste sensations as the most intense sensations they have ever experienced, so the 'strongest imaginable sensation of any kind' is unlikely to be related to taste (Bartoshuk *et al.*, 2005).

Most of the cases, the top of the gLMS is associated with strongest pain. Thus, gLMS is thought to provide a valid group comparison.

Hayes *et al.* (2013) suggested that it is essential to provide training on how to use the scale properly before collecting intensity ratings. In order to do that, the experimenter needs to clearly explain the nature of the gLMS, and direct participants to think about their own individual's strongest imaginable sensation of any kind. In addition, a warm up procedure to practice using the scale can help subjects to understand how to proper use the scale. A range of sensations across different modalities (e.g. the strongest oral pain, the loudness of a whisper, the brightness of a well lit room) are provided for participants practicing rating intensity using the gLMS, in comparison to their own strongest imaginable sensation of any kind (Bajec & Pickering, 2008; Hayes *et al.*, 2013). The gLMS scale was used throughout this research for collecting intensity results.

1.2.1.4. Description

Recognition threshold is when a person categorises a stimulus by naming it, it is to determine a person's ability to recognise objects and provide information about what a person is perceiving (Goldstein, 2009). BS-5929-7 (1992) has provided a standard for measuring recognition threshold, where a series of samples in ascending concentration order are presented, the point at which the stimulus is correctly identified is treated as this individual's recognition threshold.

In sensory studies, sometimes it is not enough to only find out if the two products are the same or different, it may also be important to determine what

the differences are. Hence descriptive analyses are needed, such as Quantitative Descriptive Analysis (QDA) using trained sensory panel to help sensory scientist obtain detailed sensory descriptions of the products.

1.2.2. Measuring Cortical response

Brain imaging techniques are popular and are used by medical d\octors to check problems in the human brain. Over the last decade, researchers have started to use brain imaging techniques to review brain function from the level of individual molecules to the whole brain, and to identify the neural networks involved in performing a specific task (Rolls, 2005; Rolls, 2012; Small & Prescott, 2005). In a typical neuroscience experiment, participants perform a specific tasks (e.g. tasting a stimulus) whilst the researcher examines brain activation using appropriate brain imaging techniques. Areas of the brain associated with these specific tasks can be highlighted (Grabenhorst *et al.*, 2010). A number of techniques are available to investigate how and where in the brain particular perceptual and cognitive processes occur. Various common methods of functional neuroimaging include: Computerised Tomography (CT), Multichannel Electroencephalography (EEG), Positron Emission Tomography (PET), Functional Magnetic Resonance Imaging (fMRI) (Bandettini, 2009). CT scan technique use X-rays to reflect the relative density of the tissue, it is useful for identifying problematic brain tissue, but gives little insight into how the brain functions. EEG records electrical activity along the scalp, which measures voltage fluctuations resulting from ionic current flows within the neurons of the brain, however, the spatial resolution is poor in comparison to other imaging techniques (Asbury, 2011). PET involves a low activity, short lasting radioactive label to compounds (glucose or oxygen) in

the brain. (Demitri, 2007). fMRI measures brain activity by detecting associated changes in blood flow. This technique uses the fact that the cerebral blood flow and neuronal activation are coupled. When participants are performing a task, the associated brain areas are more active, consuming more oxygen to meet this increased demand, and blood flow increase to the active area. Thus, fMRI uses the blood-oxygen-level dependent (BOLD) contrast (active condition ─ control condition) to monitor brain functions (Astolfi *et al.*, 2004). In addition, fMRI provides valuable information on activation maps showing parts of the brain involved in a particular mental process and the spatial patterns and intensities of activation associated (Demitri, 2007). Although the neuroimaging techniques do not indicate what the subject perceives, it is a useful tool to understand the mechanisms behind perception.

1.3. INDIVIDUAL VARIATION IN SENSORY PERCEPTION

Individual sensitivity to taste and other oral sensations vary greatly between individuals, and may be one of the most important determinants of food preference and consumption, affecting the nutritional and health status (Stewart *et al.*, 2010; Tepper *et al.*, 2014; Ullrich *et al.*, 2004; Villarino *et al.*, 2009). Many factors can affect oral sensitivity such as health (Sasano *et al.*, 2015), age (Mojet *et al.*, 2001), gender (Hirokawa *et al.*, 2006), genetic (Prodi *et al.*, 2004) and phenotypic (Gent & Bartoshuk, 1983) differences. Smell and taste disorders, such as anosmia, hyposmia, dysosmia and ageusia, are common in the general population, with loss of smell occurring more frequently, affecting about 5% of the population (Bromley, 2000). The most common causes of these diseases are nasal and sinus disease, upper respiratory

infection and head trauma, and oral infections. In addition, aging has been associated with the ability to smell and taste, usually described as decreased sensitivity to smell and taste (Doty *et al.*, 1984; Heft & Robinson, 2014; Mojet *et al.*, 2001). Research on gender effects for taste and smell sensitivity have shown conflicting results, some studies have found that women tend to be more sensitive to taste compared to men (Curtis & Contreras, 2006; Hirokawa *et al.*, 2006; Mojet *et al.*, 2003), whilst some studies suggest no difference (Chang, *et al.*, 2006; Mojet *et al.*, 2001). Interestingly, it has also been shown that women have more fungiform papillae and are more likely to be supertasters (Bartoshuk *et al.*, 1994). Evidence has also found that the olfactory perception can be modulated by hormonal changes, for example, women at luteal phase have higher odour thresholds (Derntl *et al.*, 2013). Taste phenotypes (thermal taster status and PROP taster status (Bajec & Pickering, 2008) and taste genotypes (TAS2R38, gustin) (Calo *et al.*, 2011) were also shown to influence oral sensation perception, which are described below.

1.3.1. Thermal Taster Status

Thermal taster status is a newly discovered taste phenotype. Cruz and Green (2000) reported that part of the population could perceive 'phantom taste' sensations when their tongue was either warmed or cooled. Interestingly, thermal sweetness was the most common tastes that was perceived when the tongue was re-warmed from 15 to 40ºC, whereas bitter and sour were more frequently reported when the tongue was cooled to 5ºC (Cruz & Green, 2000). However, not everyone had the ability to perceive 'phantom taste' from thermal stimulation: those who had the ability to perceive 'phantom taste' were named

thermal tasters (TTs), those who could not perceive any tastes from temperature stimulation were named thermal non-tasters (TnTs). Green and George (2004) revealed that approximately 50% of the US population were TTs, who can perceive 'phantom taste' from either a warming or a cooling trial. Whereas Bajec and Pickering (2008) have used an amended classification criteria, revealing that around 20% of the population could perceive the same 'phantom taste' within replicates. Subsequent studies revealed that in comparison to TnTs, TTs not only perceived 'phantom taste' from temperature, but also had heightened responsiveness to some basic tastes (sweet, bitter, sour and salty) and some trigeminal stimuli (temperature), other oral sensations (metallic and astringent), and an aroma stimuli (sensed both retronasally and orthonasally) (Bajec & Pickering, 2008; Cruz & Green, 2000; Green & George, 2004). However, the ratings of burning, stinging and prickling evoked by capsaicin and menthol did not differ between TTs and TnTs (Green *et al.*, 2005). Bajec *et al.* (2012) also examined the influence of stimulus temperature across TTS groups using time intensity measurements, but no significant differences in any TI parameters were observed for any stimuli examined across TTS groups. Two additional studies examined difference between TTs and TnTs on overall liking of alcoholic beverages (beer and wine). Although TTs were shown to have an overall increased intensity perception to oral sensations elicited by beer and wine, no differences on overall likings was found across TTS groups *(Pickering, Bartolini et al. 2010, Pickering, Moyes et al*. 2010)

So far, only nine papers from three labs have published looking at thermal taster status. The mechanism behind thermal taster status is unknown,

apparently more research is now needed to investigate the impact of TTS on oronasal perception, in order to understand the mechanism behind TTS.

1.3.2. PROP Taster Status

PROP taster status (PTS) is a well-known taste phenotype in the field of sensory science, and has been studied extensively. The discovery of this taste phenotype dates back to the 1930s when a chemist in the United States was trying to synthesise a very bitter compounds called phenylthiocarbamide (PTC), accidently flew some of it into the air. Surprisingly, he found that some of his colleagues could barely tolerate the bitterness, whilst himself and others could not sense anything (Welland, 2008). Researchers have since discovered that the degree of sensitivity to PTS varies greatly among individuals, for some, PTC tastes shockingly bitter, but for some minority, PTC had no taste at all (Blakeslee & Fox, 1932). Due to concerns over PTC's safety, scientists began to use 6-n-propylthiouracil (PROP), a synthetic compound used in thyroid medicine instead of PTC. Scientists then classified individuals as nontasters if they found PROP had no taste; medium tasters if they found PROP is unpleasant and moderately intense; and supertasters if they perceived PROP as extremely bitter (Bartoshuk, *et al.*, 2004). The distribution of non-tasters varied greatly among ethnic origin. The percentage of non-tasters is 25 – 40% in European (Clark, 2011; Macht & Mueller, 2007; Nachtsheim & Schlich, 2013; Padiglia *et al.*, 2010) and American Caucasians (Ullrich *et al.*, 2004; Yackinous & Guinard, 2002), 5-15% in Asian population (Chang, *et al.*, 2006; Sato *et al.*, 1997; Villarino *et al.*, 2009) and 6% in African population (Barnicot, 1950).

Much researches have been carried out to examine the link between PTC/PROP sensitivity and taste sensitivity, trigeminal irritation, food preference, as well as dietary intake (Bajec & Pickering, 2008; Keller *et al.*, 2002; Tepper & Nurse, 1998). Conflicting results on the link between PROP sensitivity and oral sensitivity (Bartoshuk, 1979; Bartoshuk *et al.*, 1998; Schifferstein & Frijters, 1991), and food preference (Baranowski *et al.*, 2011; Keller *et al.*, 2002) have been observed so far and there are many factors that may have contributed to the inconsistencies in these results. The lack of a standardised method for measuring PTC/PROP sensitivity and the approach to PTS classification are important examples. Researchers have used a variety of classification methods, ranging from PTC/PROP threshold measurement (Gent & Bartoshuk, 1983; Kranzler *et al.*, 1996) to suprathreshold measurement with different concentrations. The latter approach has also varied in the number of PROP concentrations used, ranged from five (Bartoshuk *et al.*, 1994; Tepper & Nurse, 1997), to one (Prescott & Swain-Campbell, 2000; Tepper *et al.*, 2001). Some researchers have used a reference standard, such as NaCl (Tepper *et al.*, 2001; Tepper & Nurse, 1997), with assumption that salt perception should be unrelated to PROP status. Some researchers have used the ratio of PROP to NaCl ratings to differentiate PROP tasters (Drewnowski *et al.*, 1997; Horne *et al.*, 2002), while some segment their subjects by visually comparing their PROP and NaCl psychophysical functions (Yackinous & Guinard, 2001). Yet others have simply classified their subjects on the basis of the PROP distribution they obtained (25% / 50% / 25%) (Prescott & Swain-Campbell, 2000). Recent studies have simply classified subjects into different groups by setting the cut-

off point on the gLMS scale, which has shown to be a reliable method and was chosen to be used in this study (Lim *et al.*, 2008).

Despite some contradictory and negative findings, most researches have shown variations in sensitivity to PROP bitterness to be associated with variations in sensitivity to other compounds. For example, PROP tasters (pTs) rated the intensity of quinine (Derntl *et al.*, 2013), caffeine (Yackinous & Guinard, 2002), sucrose (Gent & Bartoshuk, 1983), saccharin (Bartoshuk, 1979), astringent, metallic (Bajec & Pickering, 2008), a range of capsaicin concentrations (Karrer & Bartoshuk, 1991; Tepper & Nurse, 1997) as more intense than PROP nontasters (pNTs). Additionally, the saltiness of NaCl (Bartoshuk *et al.*, 1998), sourness of citric acid (Bajec & Pickering, 2008), irritation of ethanol (Prescott & Swain-Campbell, 2000), creaminess intensity (Tepper & Nurse, 1997; Yackinous & Guinard, 2001) and discrimination ability (Tepper & Nurse, 1998) of fat have also been reported to be significantly associated with PROP bitterness ratings. Interestingly, several studies have found that PROP sensitivity is associated with fungiform papillae density, which provides a possible explanation to the association between PTS phenotype and the sensitivity to other oral sensations (Bartoshuk *et al.*, 1994; Tepper & Nurse, 1997).

1.3.3. TAS2R38 genotype

As mentioned previously, considerable individual variation occurs in sensitivity to PTC/PROP bitterness. This has been found to have a genetic component. Research found that PROP/PTC bitterness was mainly determined by alleles at a putative bitter receptor gene on chromosome 7q (TAS2R38), a member

of the bitter taste receptor family (Kim *et al.*, 2003). The alleles in TAS2R38 gene differ at three nucleotide potions resulting in amino acid changes in the protein (Proline48Alanine, Alanine262Valine, and Valine296Isoleucine), with amino acid combination proline-alanine-valine (PAV) identifying taster variant, and alanine-valine-isoleucine (AVI) identifying the non-taster variant (Duffy *et al.*, 2004). The TAS2R38 gene codes for a receptor that responds to compounds containing N—C═S. However, TAS2R38 could not fully explain the PROP bitterness, it was shown to account for 55 to 85% of the variations in PROP taste sensitivity (Kim *et al.*, 2003; Prodi *et al.*, 2004; Sandell & Breslin, 2006) indicating other factors may also contribute to PROP perception.

1.3.4. Gustin rs2274333 genotype

Gustin, also referred to as carbonic anhydrase VI (CA6), is a zinc dependant metallo-proteinase salivary protein that catalyses reversible hydration of carbon hydroxide in saliva, and makes up 3% of human parotid saliva protein (Henkin *et al.*, 1975). Gustin has been identified in taste bud and olfactory mucus (Henkin *et al.*, 1999a; Okamura *et al.*, 1996). Additionally, gustin has been suggested to not only have a mucosa-protective role in the gastrointestinal tract, but also in the respiratory tract (Leinonen *et al.*, 2004), suggesting that gustin may contribute to both taste and smell functions.

Recently, studies on Italian cohorts have found that the rs2274333 polymorphism in the gene encoding gustin was strongly associated with PROP taste sensitivity and papillae density (Calo *et al.*, 2011; Melis *et al.*, 2013). The alleles in rs2274333 polymorphism (A/G) differ at position 90, resulting in amino changes in the protein (Serine90Glycine). Consequently, A allele

indicates serine and G allele indicates glycine. According to Calo *et al.* (2011), gustin AA homozygotes have a lower PROP detection threshold and higher intensity PROP bitterness perception than AG heterozygotes and GG homozygotes. Data from systemic studies have observed pSTs were more frequently to be AA homozygotes, while pNTs were more frequently to be GG homozygotes (Calo *et al.*, 2011; Padiglia *et al.*, 2010). Interestingly, the same research group also found that subjects with GG homozygotes had a lower fungiform papillae density and showed more distorted shape, than subjects with A allele (Melis *et al.*, 2013).

1.4. FOOD CHOICE BEHAVIOUR

Food choice is a complex human behaviour, and can be affected by many interrelating factors. The decisions that people make everyday concerning what they choose to eat are not only based on physiological (satiety, hunger, etc.) and nutritional needs, but also depend on sensory appeal (taste appearance, etc.), psychological factors (personality, experience etc.), economic and social factors (price, brand, culture etc.) (Shepherd, 1999). A numbers of studies have been carried out to understand how the above factors contribute to food choice behaviour. In generally, low-income groups have been shown to have a greater unbalanced diet and less consumption of fruit and vegetables (Irala-Estevez *et al.*, 2000). In addition, family element is widely recognised as an important factor in food decisions. Research showed that parents' diet habits have a strong impact on children's food intake (Roos *et al.*, 2012), and an individual's diet plans can have an effect on the eating habits of others at home (Anderson *et al.*, 1998). Although the influence of stress in food choice is complex, in general, scientists found that some people

eat more and some eat less than normal when experiencing stress (Oliver & Wardle, 1999).

Interestingly, personality has also found to play a role in food choice behaviour. Examining the association between personality (Neuroticism-Extroversion-Openness Personality Inventory (McCrae & Costa, 1997)) and food choices (Food Frequency Questionnaire (Hartmann *et al.*, 2013; Paalanen *et al.*, 2006)) found that high openness to experience were associated with lower meat and soft drink consumption but higher fruit and vegetable consumption (Keller & Siegrist, 2015; Tiainen *et al.*, 2013) Conscientiousness mainly encouraged fruit consumption and prevented meat consumption by restrained eating, and neuroticism and extraversion promoted consumption of sweet and savoury foods influenced by emotional and external eating (Keller & Siegrist, 2015).

The impression people get from the sensory properties perceived from foods play a significant role in the way people select food and how much they eat (Sorensen *et al.*, 2003). However, taste and aroma perception varies greatly among individuals. Many factors affect taste and smell sensitivity such as age (Heft & Robinson, 2014), gender (Donkin *et al.*, 1998), experience (Ludy & Mattes, 2012), taste genotype (Prodi *et al.*, 2004) and phenotype (Bajec & Pickering, 2008). A Pan-European survey has reported that female, older and more educated people considered health aspects to be particularly more important (Lappalainen *et al.*, 1998). However, how taste genotype and phenotype are driving food choice behaviour is less researched, and only a few studies have looked at this area (Duffy *et al.*, 2004; Forrai & Bankovi, 1984; Ullrich *et al.*, 2004). Taste genotype (TAS2R38), the dominant gene that

contributes to PROP perception, was found to have an impact on alcohol intake, with AVI/AVI homozygotes consuming more alcohol (Duffy *et al.*, 2004). There are also a few papers looking at taste phenotype (PROP taster status) and food choice behaviour. PROP tasters perceive tastants (Bajec & Pickering, 2008; Bartoshuk *et al.*, 1998; Gent & Bartoshuk, 1983) and trigeminal sensations (Pickering *et al.*, 2004; Tepper & Nurse, 1997) as more intense than PROP non-tasters. Some studies have found that the pSTs dislike cruciferous and green vegetables (Forrai & Bankovi, 1984), whereas pNTs more like high fat content food (Tepper & Nurse, 1997). It has been hypothesised that the increased bitter perception in pSTs leads to a reduced vegetable intake, which could be associated with higher risk of colon cancer (Basson *et al.*, 2005; Dinehart *et al.*, 2006). pNTs' lower discrimination ability among fat content (Tepper & Nurse, 1998) may lead to higher fat consumption, hence inducing a higher cardiovascular disease risk (Duffy, 2004). However, the data was not always consistent, and other studies failed to find the link between PROP sensitivity with a robust pattern of food likings (Baranowski *et al.*, 2011).

Apart from PROP taster status, the newly discovered taste phenotype 'thermal taster status' has also been reported to play a role in taste, trigeminal and olfactory perception (Bajec & Pickering, 2008; Green & George, 2004). However, how TTS links to food choice behaviour and attitude to food has not been researched.

One of the interests of this PhD was to investigate if taste phenotypes (PTS and PTS) can be used as a predictor of attitude to food, eating behaviour and

food preference. Understanding how taste phenotypes link to food choice behaviour would contribute to the food choice behaviour model (Köster, 2009). A number of well-established questionnaires can be used to investigate the attitude to food and food choices, including Food Neophobia Scale (Pliner & Hobden, 1992), Food Involvement Scale (Bell & Marshall, 2003), Food Choice Questionnaire (Steptoe *et al.*, 1995), and Health and Taste attitudes (Roininen *et al.*, 1999).

1.5. MAIN OBJECTIVES OF THE THESIS

It is important to understand individual variation in sensory perception, as it may shape food behaviour. Both PROP and thermal taster status have shown to impact on oral perception, which in turn, may affect food behaviour. However, the reason behind why PROP tasters and thermal tasters have heightened responses to oral sensations are not well understood. Until now, little research has been conducted on thermal taster status, especially on the UK population.

The overall aim of this research was to investigate taste phenotypes, thermal taster status, PROP taster status, the taste genotypes, TAS2R38 and gustin rs2274333, and their impact on oronasal sensitivity across a range of gustatory, trigeminal and olfactory modalities, whilst also attempt to understand their relationship to personal traits. In addition, working with colleagues in the SPMMRC provided an opportunity to investigate cortical responses.

- The first objective was to examine the incidence of TTS and PTS in the UK population and to investigate the relationship of both taste

phenotypes to a certain personal traits (e.g. food neophobia, food involvement and personality). This is presented in Chapter 2.

- The second objective was to investigate the effect of TTS and PTS on oronasal sensitivity, including temperature sensitivity, and to determine the relative impact of these two taste phenotypes. This is presented in Chapter 3.

- The third objective was to understand why PROP tasters have heightened responses to oral sensations by examining the relationship between PTS phenotype and TAS2R38, gustin (rs2274333) genotype and fungiform papillae count. This work is described in Chapter 4.

- The fourth objective was to investigate the impact of TTS and PTS on perceived intensity of sensory attributes at varying serving temperatures of a strawberry flavoured drink and water. The effect of taste phenotype on overall liking was also examined. This is presented in Chapter 5.

- As a partner in a multi-school project on TTS, in collaboration with colleagues at the SPMMRC, the final objective was to examine the relationship between sensory and cortical response to a selected range of stimuli across TTS phenotype. This is the topic of Chapter 6.

2. CHARACTERISING INDIVIDUAL VARIATION ACROSS THERMAL AND PROP TASTER STATUS, AND INVESTIGATE PHENOTYPIC RELATIONSHIPS WITH PERSONAL TRAITS

2.1. INTRODUCTION

2.1.1. Effect of taste phenotypes in sensory perception

Taste perception plays a key role in determining individual food preference and food choice behaviour and consequently affects food consumption and thus a range of health and disease outcomes (Stewart *et al.*, 2010; Ullrich *et al.*, 2004; Villarino *et al.*, 2009). Recently, Cruz and Green (2000) discovered a new marker of phenotypic variation - thermal taster status (TTS). Green and co-workers published evidence that sweet taste could be induced by warming the tip of the tongue from 20 to 35 ℃, and that cooling to ≤ 20 ℃ could elicit a sour taste in some individuals, with one of the participants even experiencing saltiness at temperatures below 10 ℃ (Cruz & Green, 2000). They also tested the 'phantom taste' response at different locations on the tongue, finding that thermal sweetness was perceived most and strongest near the tongue tip when re-warmed from an initial cooling period, and that thermal sourness was perceived more laterally during cooling. Between 20 to 50% of the population have been shown to perceive a 'phantom taste' and were named thermal tasters (TTs) (Bajec & Pickering, 2008; Green & George, 2004). Cruz and Green (2000) also suggested the anterior tip of the tongue is the most sensitive area for 'phantom taste'. So far, only three research groups (including the sensory lab in UoN) have been working on thermal taster status, and the mechanism behind thermal taster status has not been well understood.

These systematic studies of TTS revealed that TTs did not only have the ability to perceive 'phantom taste' from temperature stimulation, but also perceive oral sensations including temperature more intensely than thermal non-tasters (TnTs) (Bajec & Pickering, 2008; Green & George, 2004). An additional study on self-reported preferences for foods on a food preference checklist revealed TTs demonstrated greater disliking of cooked fruits and vegetables over TnTs, suggesting that differences among TTS groups might be texturally driven (Bajec & Pickering, 2010). To this author's knowledge, no research has examined the association between TTS taste phenotype and food behaviours.

The taste phenotype discussed above is a new discovery, but PROP taster status is a well-known taste phenotype that has been widely studied over the last eight decades. Individuals can be grouped as PROP supertasters (pSTs), PROP medium-tasters (pMTs) and PROP non-tasters (pNTs) in order of descending responsiveness to PROP bitterness. The distribution of non-tasters varied greatly among ethnic origin. The percentage of non-tasters is around 25 – 40% in European (Clark, 2011; Macht & Mueller, 2007; Nachtsheim & Schlich, 2013; Padiglia *et al.*, 2010), but the PROP taster status proportion varies among ethnic group, with lower prevalence of pNTs in Asian and African populations (Barnicot, 1950; Chang, *et al.*, 2006). Bartoshuk *et al.* (1994) published evidence that there were gender differences among PROP taster status, where females are more likely to be supertasters. However, further research failed to find a clear association between PROP taster status and gender (Chang, *et al.*, 2006; Von Atzingen & Pinto e Silva, 2012)

A number of studies have reported that PROP tasters (pSTs and/or pMTs) perceived increased intensity to other tastants (e.g. sucrose, salt and other bitterants such quinine) (Bajec & Pickering, 2008), and trigeminal stimuli such as capsaicin, as well as greater discrimination ability with regard to fat content (Tepper & Nurse, 1998). It has been hypothesised that bitter taste evolved as a defence mechanism to detect harm in the environment; hence individuals with different sensitivities to bitter compounds might react differently by protecting themselves from ingestion of potentially poisonous bitter compounds, and consequently influence vegetables likings. Sensitivity to PROP bitterness had been associated with likings of bitter vegetables, with higher responsiveness to PROP being associated with lower liking of bitter vegetables such as Brussel sprouts, cabbage and spinach and high fat content food (Bajec & Pickering, 2010; Chang, *et al.*, 2006; Tepper & Nurse, 1998; Ullrich *et al.*, 2004).

2.1.2. Personal traits

Food neophobia is defined as reluctance to eat unfamiliar foods and is often measured by the Food Neophobia Scale (FNS) (Knaapila *et al.*, 2007). High FNS scores indicate a low anticipated liking of unfamiliar foods and foreign cuisines. Evidence has shown food neophobia could affect diet variety, notably by reducing fruit and vegetable consumption in children – food neophobic children have been shown to have lower vegetable intake and higher intake of saturated fat than children without food neophobia (Falciglia *et al.*, 2000; Galloway *et al.*, 2003). It has been suggested that neophobia is largely determined by genetic factors and part of this complex phenomenon could also be explained by external (environmental) factors (Cooke *et al.*, 2007;

Knaapila *et al.*, 2007; Wardle & Cooke, 2008). As discussed above, both food neophobia and individuals with different taste sensitivity (e.g. PTS) appear to affect vegetable liking and intake, therefore, examining the relationship between individual variation (e.g. PTS) and food neophobia is one of the interests in this study.

In the consumer behaviour literature, food involvement is another behavioural factor that may be an important mediator for food choice behaviour (Bell & Marshall, 2003). It is described as an indicator of 'the level of importance of food in a person's life' and level of food involvement was shown to vary across individuals. Levels of food involvement could be assigned either as personal or social characteristics. These often relate to the time invested in making decisions, the perceived social risk of using or not using a product, and the financial risk relative to one's ability to pay for the product; food motivation such as health and pleasure, enjoyment of food (Somers *et al.*, 2014). Bell and Marshall (2003) suggested that highly involved individuals might pay more attention to foods themselves during procurement, preparation, cooking and eating. Thus, the increased attention might lead to a greater ability to differentiate between products from a purely sensory perspective. As taste phenotypes (both PTS and TTS) were demonstrated to have an impact on oral sensitivity (Bajec & Pickering, 2008), hence may result in differences in food enjoyment, as well as food pleasantness, which might further influence their food involvement behaviour. However, little research has been done to investigate the link between taste phenotypes and food involvement so far.

Studies have also started to investigate the relationship between personalities and food preference in recent years. Interestingly, published research has provided evidence of the link between personalities and taste preference, with participants high in novelty seeking showing a strong preference for salty tastes, whereas participants high in reward dependence having a strong preference for sweet tastes (Day *et al.*, 2008). Another study has found that sweet taste preference was associated with a higher level of impulsiveness but lower openness in a white wine study (Saliba *et al.*, 2009). Besides taste perception, studies have also shown that sensation seeking and rewarding traits were positively related to the enjoyment and frequency of eating spicy food (Byrnes & Hayes, 2013). In addition, food neophobia has demonstrated to be associated with spicy food, with adventures more enjoyed and tolerance for spiciness (Törnwall *et al.*, 2014). Chatterjee *et al.* (2004) has reported that emotion and personality could affect olfactory perception in a complex way, with personality being found to modulate reaction time and olfactory intensity. Although the reason behind the l between personality traits and food choice and sensory perception is unknown, these studies provide a new insight into how personality variables may play a role in food preference and behaviour.

For this PhD research project the relationship between personality traits and taste phenotypes was of interest. Therefore, this study has set up a preliminary experiment to test the relationship between taste phenotypes (PTS and TTS) and the Big Five Personality Test (John & Srivastava, 1999), as well as the Toronto Alexithymia Scale (Bagby *et al.*, 1994). The Big Five Personality Test is widely used in the field of psychology, and it has been described as ‘Currently the most popular approach among psychologists for studying

personality traits is the Five-Factor Model or Big Five dimensions of personality' (Scott Acton). The Toronto Alexithymia Scale is designed to examine the ability to identify and describe emotions, as well as minimise emotional experience and external attention focus, which have previously been shown to relate to food consumption and distress (van Strien & Ouwens, 2007).

Only a few studies have been conducted on thermal taster status, and there is no published work on the UK population. In addition, to the author's knowledge, no research has been carried out investigating the association between PTS and TTS and personal traits. Consequently, whether or not these two taste phenotypes are good indicators of personal traits is currently unknown. The objectives of the study reported in this chapter were to:

- Investigate the incidence of TTS and any gender effect.
- Examine the quality and intensity of 'phantom taste' perceived by TTs (sub -objectives are also to look at whether the tongue tip is more sensitive to 'phantom taste' than the lateral edge of the tongue).
- Verify the previously reported heightened response to temperature (both warm and cold) in TTs.
- Examine the incidence of PTS and gender effect, as well as the influence of PTS on PROP bitterness and temperature perception.
- Investigate the relationship between TTS and PTS classification.
- Explore the relationship between taste phenotypes (PTS and TTS) with personal traits measured using the Food Neophobia Scale, Food Involvement Scale, Toronto Alexithymia Scale and Big Five Inventory Personality Test.

2.2. MATERIALS AND METHODS

2.2.1. 'Phantom taste' responsiveness

2.2.1.1. Subjects

204 volunteers (132 females, 72 males with a mean age 42 yrs, range 16-75 yrs) were recruited from Sensory Dimensions Ltd (Nottingham, UK) consumer database, and students and staff from the University of Nottingham. All subjects signed to say they had given informed consent and were given an incentive for participating.

2.2.1.2. gLMS scale used and training

The gLMS scale was developed by (Bartoshuk, *et al.*, 2004) and was popularly used to collect perceived intensity data in individual variation studies. The gLMS scale used in this study consisted of a veritical line 13.7cm long when printed as FIZZ form (A4 sized paper). The extreme bottom was labeled 'no sensation' (0%) and the extreme top was labeled' strongest imaginable sensation of any kind' (100%). Inbetween the scale was labeled with quasi-logarithmic spacing between descriptors, including 'barely detectable' (1.4%), 'weak' (6%), 'moderate' (17%), 'strong (35%), and 'very strong' (53%).

To familiarise subjects with the gLMS scale and facilitate its correct use, a gLMS scale reference sheet was given to each participant. Prior to data collection, all participants were trained in the use of the gLMS scale to measure the intensity of perceived sensations. To emphasise the general nature of the top scale, participants were asked to think of the strongest sensation of any kind they had experienced previously or the strongest sensation they could imagine happen to them, and then write them down on

the top of the gLMS scale. They were then asked to rate the intensities of 15 remembered sensations, as shown in **Table 2-1** (Bartoshuk *et al.*, 2002) relative to their own strongest sensation. Their reference sheet was always provided in subsequent experiments, and subjects were encouraged to refer back to their reference sheet.

Table 2-1: 15 remembered or imagined sensations for rating practice

	15 remembered or imagined sensations
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	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 candy floss
12	The bitterness of grapefruit
13	The strongest taste ever experienced
14	The strongest oral burn experienced
15	The strongest oral pain ever experienced

2.2.1.3. Evaluation of 'phantom taste' responses from thermal stimulation

A circular intra-oral ATS (advanced thermal stimulator) thermode (Medoc, Israel) was used to heat and cool a small area of the tongue (**Figure 2-1 (a)**), this was controlled by a PATHWAY pain and sensory evaluation system (**Figure 2-1 (b)**). For hygiene purposes, the thermode was wiped with 99%

ethanol (VWR International, UK) between subjects and covered with a fresh piece of tasteless and odour free plastic wrap (Tesco, UK) for each subject.

Subjects were asked to extend their tongue, and then gently place the thermode on the tongue themselves with the guidance of the researcher (**Figure 2-1 (c)**). Subjects were instructed to hold the thermode firmly in place during the temperature trials. Three locations were examined: the anterior tip of the tongue and the left and right lateral edges of the tongue. Duplicate data were obtained on the anterior tip of the tongue, as it had been reported to be the most sensitive area for 'phantom taste (Cruz & Green, 2000), and the data on the anterior tip of tongue was further used for thermal taster status classification. One measurement was conducted on the left and right lateral edges of the tongue to test which area of the tongue was most sensitive to 'phantom taste'. Data on left and right edges of the tongue were combined for further data analysis.

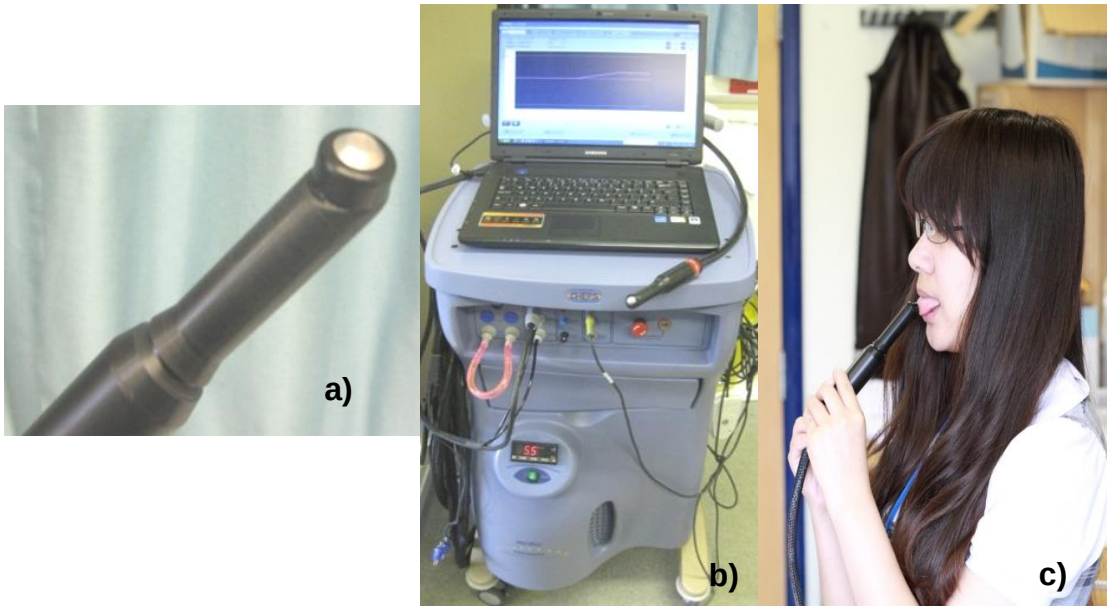

Figure 2-1: The Peltier thermode device: a) The circular intra-oral thermode (probe); b) The Pathway pain and sensory evaluation system; c) The intra-oral thermode in use.

Before testing, subjects were presented with their own reference sheet with the ratings of remembered or imagined sensations, and two further gLMS scales for each temperature trial. One was labelled ‘temperature’ and instructions were given to rate the maximum intensity of the temperature experienced. The second scale was provided to record the quality and intensity of any taste sensations perceived during temperature stimulation. Taste options listed on the second scale, were ‘no sensation’, ‘sweet’, ‘bitter’, ‘sour’, ‘salty’, ‘savoury’, ‘metallic’ and ‘others, please specify’. Subjects were told to tick the taste options and then rate the maximum intensity of the taste sensations, only if they perceived any. Subjects were clearly told that not everyone would perceive taste sensations during this procedure, but if they did, then they should record it. Warming trials always preceded cooling trails to avoid possible adaptation from the intense, sustained cold stimulation. Subjects were also told to wait until tongue temperature and sensation had returned to normal before proceeding onto the next trial.

Two temperature trials were used: a warming trial and a cooling trial. Before each temperature trial, a baseline trial was applied at body temperature (37ºC) and held for 10s. The baseline trial was performed first to allow subjects to practise reporting the perceived temperature and any other taste sensations. In the warming trial, the probe started at a temperature of 35ºC, was cooled to 15ºC and then re-warmed to 40ºC and held for 1 s. In the cooling trial the probe started at a temperature of 35ºC and was cooled to 5ºC and held for 10s. Both temperature trials are illustrated in **Figure 2-2.** The temperature ramp for all trials was 1 ºC/s.

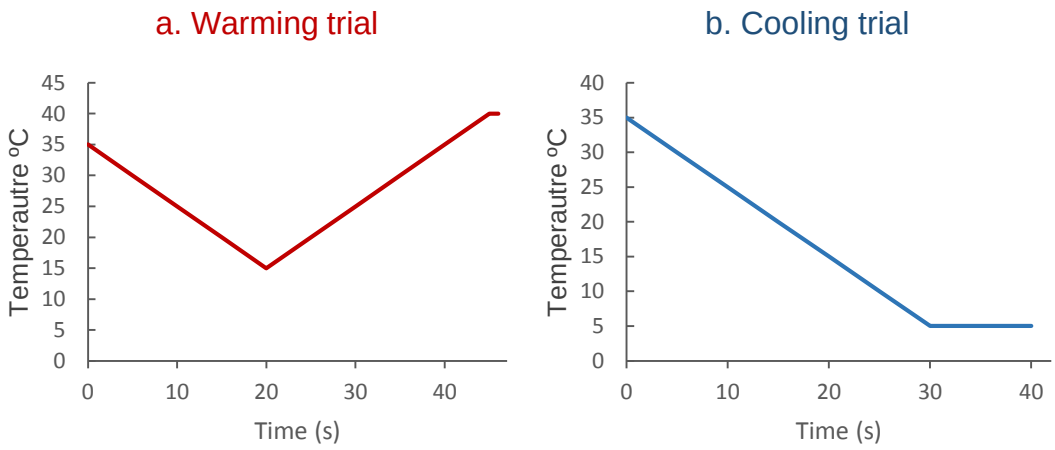

Figure 2-2: Graphic representations of the warming trial and cooling trials.

2.2.1.4. Thermal taster status categorisation

The type and number of taste sensations reported during thermal stimulation varied greatly among individuals, and in the same individual they could also vary among temperature trials and replicates. **Table 2-2** shows some examples of different subjects' responses of 'phantom taste'.

Table 2-2: Variation among 'phantom taste' experienced across different subjects

Subject	Warming Replicate 1	Warming Replicate 2	Cooling Replicate1	Cooling Replicate 2
Subject 1	Sweet	Sweet	Bitter	Bitter
Subject 2	Sweet Bitter	Sweet Bitter Sour	Sour Minty Salty	Sour Metallic Minty Salty
Subject 3	Metallic	Metallic	No taste	No taste
Subject 4	No taste	No taste	Bitter	Bitter
Subject 5	Minty	Sweet	No taste	Bitter
Subject 6	No taste	No taste	Bitter	No taste

As the mechanism behind TTS has not been well understood, this makes the

classification difficult. At this stage, only individuals who reported sensations during both replicates, and during both warming and cooling trials rated above 'weak' on the gLMS, were classified as thermal tasters (TTs) in order to be assured of their experience of this phenomenon. Thermal non-tasters (TnTs) were defined as those who did not perceive any taste sensation in any of the temperature trials. Notably, this left a group of people uncategorised (Uncat) due to inconsistencies in reporting taste sensations throughout the trials.

The criteria used in this study were slightly different from the other two researcher groups (see **Table 2-3)**. Both Green and Pickering's criteria classified subjects as TTs if they perceived 'phantom taste' from either warm or cold trial, whereas, Pickering's criteria specified the taste sensation perceived needed to be the same across two replicates.

Table 2-3: Comparison of TTS categorisation criteria used in different research groups

Thermal taster status categorisation	**TTs ('phantom taste' perceived)**	**TnTs**	**Uncat**
Yang's criteria	Both warm and cold trial Both replicates Any taste	No perceived taste throughout all temperature trials	Inconsistent 'phantom taste' responses
Pickering's criteria	Either warm or cold trial Both replicates Same taste for replicates	No perceived taste throughout all temperature trials	Inconsistent 'phantom taste' responses
Green's criteria	Either warm or cold trial Both replicates Any taste	No perceived taste throughout all temperature trials	Inconsistent 'phantom taste' responses

2.2.2. PROP responsiveness

2.2.2.1. Subjects

Subjects who previously took part in thermal taster status evaluation were invited back to measure their PROP responsiveness. Due to availability 130 of these participated (88 females, 42 males, age range 16-75 yrs).

2.2.2.2. Evaluation of perceived intensity of PROP bitterness

0.32mM PROP (Sigma Aldrich, UK) solution was prepared by dissolving PROP in water on a low heat stirring plate. Each subject was instructed to roll a saturated cotton bud, which had previously been dipped in the PROP solution (22 ± 2 ºC), across the tip of the tongue for approximately 3s. They then rated the intensity of the stimulus at its maximum using the gLMS scale. After a 5mins break, the procedure was repeated to collect duplicate ratings (Lim *et al.*, 2008).

2.2.2.3. PROP taster status categorisation

PROP taster status was defined based on mean PROP intensity ratings: PROP non-tasters (pNTs) were defined as those ratings below 'barely detectable' (1.4% on gLMS); PROP medium tasters (pMTs) were classified as those who rated above barely detectable but below moderate (17% on gLMS); and PROP super-tasters (pSTs) were those ratings above 'moderate' on the gLMS (Lim, et al., 2008).

2.2.3. Personal traits measurement

2.2.3.1. Subjects

Subjects who had been screened for both TTS and PTS were invited back to complete the personal traits questionnaires. 116 subjects completed the Food

Neophobia Scale and Food Involvement Scale questionnaires. Data collection for the Toronto Alexithymia scale and Big Five Inventory Personality Test were conducted on a different day and due to subjects' availability, 67 subjects were invited to complete the questionnaires. Within TTS phenotype, the uncategorised group (38 subjects in 116 subjects, 18 subjects in 67 subjects) was excluded for further data analysis.

2.2.3.2. Measurement of food neophobia and food involvement

The set of statements for the Food Neophobia Scale (FNS) (Pliner & Hobden, 1992) and Food Involvement Scale (FIS) (Marshall & Bell, 2003) are shown in **Table 2-4** and **Table 2-5** respectively.

Table 2-4: Statements in Food Neophobia Scale (Source: Pliner and Hobden (1992)).

No.	Food Neophobia Scale
1.	I am constantly sampling new and different foods. (R)
2.	I don't trust new foods.
3.	If I don't know what is in a food, I don't try it.
4.	I like foods from different countries. (R)
5.	Ethnic food looks too weird to eat.
6.	At dinner parties I will try a new food. (R)
7.	I am afraid to eat things I have never had before.
8.	I am very particular about the foods I will eat.
9.	I will eat almost anything. (R)
10.	I like to try new ethnic restaurants. (R)

(R) Ratings for items need to be reversed for data analysis.

In both the food neophobia and food involvement questionnaires, subjects were instructed to rate the extent of their agreement with each statement on a 7-point agreement scale moving from disagree strongly (score=1) to agree

strongly (score=7). The scores of positive statements in FNS (see (R) in **Table 2-4** and the scores of negative statements in FIS (see (R) in **Table 2-5** were reversed for further data analysis. Each individual's total scores of the FNS or FIS were calculated by summing ratings from all statements for each questionnaire, respectively. Higher FNS scores indicate greater food neophobia, i.e. they are less willing to try unfamiliar foods (Pliner & Hobden, 1992). Higher FIS scores indicate greater food involvement, i.e. they are more willing to get involved with food preparation, food displaying and setting (Bell & Marshall, 2003).

Table 2-5: Statements in food involvement scale (Source: Bell and Marshall (2003))

No.	Food Involvement Scale
1.	I don't think much about food each day. (R)
2.	Cooking or barbequing is not much fun. (R)
3.	Talking about what I ate or am going to eat is something I like to do.
4.	Compared with other daily decisions, my food choices are not very important. (R)
5.	When I travel, one of the things I anticipate most is eating the food there.
6.	I do most or all of the clean up after eating.
7.	I enjoy cooking for others and myself.
8.	When I eat out, I don't think or talk much about how the food tastes. (R)
9.	I do not like to mix or chop food. (R)
10.	I do most or all of my own food shopping.
11.	I do not wash dishes or clean the table. (R)
12.	I care whether or not a table is nicely set.

(R) Ratings for items need to be reversed for data analysis.

2.2.3.3. Measurement of personality and alexithymia

A pilot study determining emotion expression and personality traits was conducted on a small group of subjects (67 subjects) to test if any relationship

existed between personality and alexithymia and taste phenotypes. Both the Toronto Alexithymia Scale (TAS) (Bagby *et al.*, 1994) and the Big Five Inventory Personality Scale (BFI) (John & Srivastava, 1999) were used and **Table 2-6** and **Table 2-7** itemise the individual statements within each questionnaire respectively.

Table 2-6: Statements used in Toronto Alexithymia Scale (TAS).

No.	Toronto Alexithymia Scale
1.	I am often confused about what emotion I am feeling. (1)
2.	It is difficult for me to find the right words for my feelings. (2)
3.	I have physical sensations that even doctors don't understand. (1)
4.	I am able to describe my feelings easily. (2)
5.	I prefer to analyse problems rather than just describe them. (3)
6.	When I am upset, I don't know if I am sad, frightened, or angry. (1)
7.	I am often puzzled by sensations in my body. (1)
8.	I prefer to just let things happen rather than to understand why they turned out that way. (3)
9.	I have feelings that I can't quite identify. (1)
10.	Being in touch with emotions is essential. (3)
11.	I find it hard to describe how I feel about people. (2)
12.	People tell me to describe my feelings more. (2)
13.	I don't know what's going on inside me. (1)
14.	I often don't know why I am angry. (1)
15.	I prefer talking to people about their daily activities rather than their feelings. (3)
16.	I prefer to watch 'light' entertainment shows rather than psychological dramas. (3)
17.	It is difficult for me to reveal my innermost feelings, even to close friends. (2)
18.	I can feel close to someone, even in moments of silence. (3)
19.	I find examination of my feelings useful in solving personal problems. (3)
20.	Looking for hidden meanings in movies or plays distracts from their enjoyment. (3)

(1) Difficulty describing feelings subgroup; (2) Difficulty identifying feelings subgroup; items marked with (3) Externally-oriental thinking subgroup.

Table 2-7: Statements in Big five inventory personality test (BFI).

I am someone who			
1	Is talkative. (1)	23	Tends to be lazy. (R) (3)
2	Tends to find fault with others. (R) (2)	24	Is emotionally stable, not easily upset. (R) (4)
3	Does a thorough job. (3)	25	Is inventive. (5)
4	Is depressed, blue. (4)	26	Has an assertive personality. (1)
5	Is original, comes up with new ideas. (5)	27	Can be cold and aloof. (R) (2)
6	Is reserved. (R) (1)	28	Perseveres until the task is finished. (3)
7	Is helpful and unselfish with others. (2)	29	Can be moody. (4)
8	Can be somewhat careless. (R) (3)	30	Values artistic, aesthetic experiences. (5)
9	Is relaxed, handles stress well. (R) (4)	31	Is sometimes shy, inhibited. (R) (1)
10	Is curious about many different things. (5)	32	Is considerate and kind to almost everyone. (2)
11	Is full of energy. (1)	33	Does things efficiently. (3)
12	Starts quarrels with others. (R) (2)	34	Remains calm in tense situations. (R) (4)
13	Is a reliable worker. (3)	35	Prefers work that is routine. (R) (5)
14	Can be tense. (4)	36	Is outgoing, sociable. (1)
15	Is ingenious, a deep thinker. (5)	37	Is sometimes rude to others. (R) (2)
16	Generates a lot of enthusiasm. (1)	38	Makes plans and follows through with them. (3)
17	Has a forgiving nature. (2)	39	Gets nervous easily. (4)
18	Tends to be disorganised. (R) (3)	40	Likes to reflect, play with ideas. (5)
19	Worries a lot. (4)	41	Have few artistic interests. (R) (5)
20	Has an active imagination. (5)	42	Likes to cooperate with others. (2)
21	Tends to be quiet. (R) (1)	43	Is easily distracted. (R) (3)
22	Is generally trusting. (2)	44	Is sophisticated in art, music, or literature. (5)

(R) Ratings for items need to be reversed for data analysis. (1) Extraversion subgroup; (2) Agreeableness subgroup; (3) Conscientiousness subgroup; (4) Neuroticism subgroup; (5) Openness subgroup.

Subjects were instructed to rate the extent of their agreement with each item on a 5-point agreement scale, moving from disagree strongly (score=1) to agree strongly (score=5). The TAS scale has three subscales: Difficulty identifying feelings (items denoted (1)); difficulty describing feelings (items denoted (2)); and externally-oriented thinking (items denoted (3)). Higher TAS scores indicate higher possible alexithymia, i.e. they are more likely to have difficulty identifying and describing feeling and emotions in themselves.

The BFI assesses five broad domains of personality that are used to describe human personality. The factors are: extraversion (1), agreeableness (2), conscientiousness (3), neuroticism (4) and openness (5). In **Table 2-7** the numbers in brackets denote statements relating to these particular aspects. Ratings for statements marked with (R) are those which need reversing for subsequent data analysis. All questionnaire data were collected using FIZZ forms (Biosystemes, France).

2.2.4. Data Analysis

Chi-square analysis was used to examine the relationship between TTS and gender. One-way Analysis Of Variance (ANOVA), with post-hoc Tukey's test, where appropriate, was performed on 'phantom taste' and temperature intensity data of both warm and cold trials across TTS groups from the tongue tip and lateral edge, separately. One-way ANOVA, with post-hoc Tukey's test, where appropriate, was performed on pooled temperature (combined warm and cold) intensity data among TTS, replicate and location, respectively.

Chi-square analysis was performed to examine the relationship between PTS and gender. One-way ANOVA, with post-hoc Tukey's test, was performed on

pooled temperature intensity and PROP intensity among PTS, respectively. A Chi-square analysis was applied to determine if any relationship existed between TTS and PTS classification.

For personal traits, data were presented as a mean rank for each group. A non-parametric test, the Mann-Whitney U test (i.e. it is appropriate for analysing the data between two groups), was used to compare the difference between TTs and TnTs for each individual score and total scores of all four scales (FNS, FIS, TAS and BFI), as well as total subscale scores of TAS and BFI. Kruskal-Wallis tests (e.g. this is appropriate for comparing more than 2 groups), were performed on all the variables above among PTS groups. All analyses were performed using SPSS, version 21 (SPSS IBM, USA) with an α-risk of 0.05 selected for all analyses.

2.3. RESULTS

2.3.1. Thermal taster status categorisation and gender effect

Following the strict classification criteria used in this study to classify those individuals who reported taste sensations at both temperature trials, of the 204 subjects: 56 subjects (27%) were classified as TTs, and 60 subjects (30%) were classified as TnTs, while the remaining 88 subjects were uncategorised (Uncat). The proportion of TTs discovered in this study was slightly different from previous findings, which could be due to the difference in categorisation criteria used. To understand the impact of these differing criteria, further analysis was conducted to reclassify these subjects based on Pickering and Green's criteria, respectively. As illustrated in **Table 2-8**, 33% of the subjects sampled in this study would be classified TTs following Pickering's criteria,

whereas, 56% would be TTs based on Green's criteria, figures which in general agree with their previous findings where Cruz and Green (2000) revealed about 50% of their tested population were TTs, and Bajec and Pickering (2008) claimed a lower prevalence of TTs at 20%.

Table 2-8: Comparison of TTS proportion based on different categorisation criteria

Thermal taster status categorisation	**Yang's criteria**		**Pickering's criteria**		**Green's criteria**	
	n	%	n	%	n	%
TTs	56	27	67	33	115	56
Uncat	87	43	76	37	28	14
TnTs	61	30	61	30	61	30

n represents number of subjects in each group. % represents percentage within column.

Table 2-9 shows the distribution of gender across TTS groups. Results indicated that percentages of females and males are distributed equally in TTs and TnTs groups and hence Chi-square analysis demonstrated no significant correlation between thermal taster status and gender (p=0.84). Note that additional Chi-square tests also confirmed no significant correlation between gender and the two alternative TTS classification groups (Bajec & Pickering, 2008; Green & George, 2004).

Table 2-9: Gender distribution by TTS and Chi square analysis.

Thermal Taster Status	**Gender**					
	Female		**Male**		**Total**	**[a]p value**
	n	%	n	%	n	
TTs	38	29	18	25	56	
Uncat	55	42	32	45	88	0.84
TnTs	39	29	22	29	60	

[a] p value associated with Chi Square statistic. n represents number of subjects in each group. % represents percentage within column.

2.3.2. 'Phantom taste' perceived by TTs and effect of tongue location

The most common taste sensations experienced by TTs during both temperature trials at the tongue tip were metallic, bitter, sweet and sour (ranged between 8 to 30%), as shown in **Figure 2-3 (a&b)**. A few individuals also reported salty and savoury tastes. Around 14% of the participants ticked the 'others' option, and the most common sensations specified included spicy, peppery, astringent and minty. It is interesting to note that metallic taste had been reported most during both the warming and cooling trials. Metallic taste had also been reported in a recent TTS paper, but not as the most reported taste sensation (Bajec *et al.*, 2012).

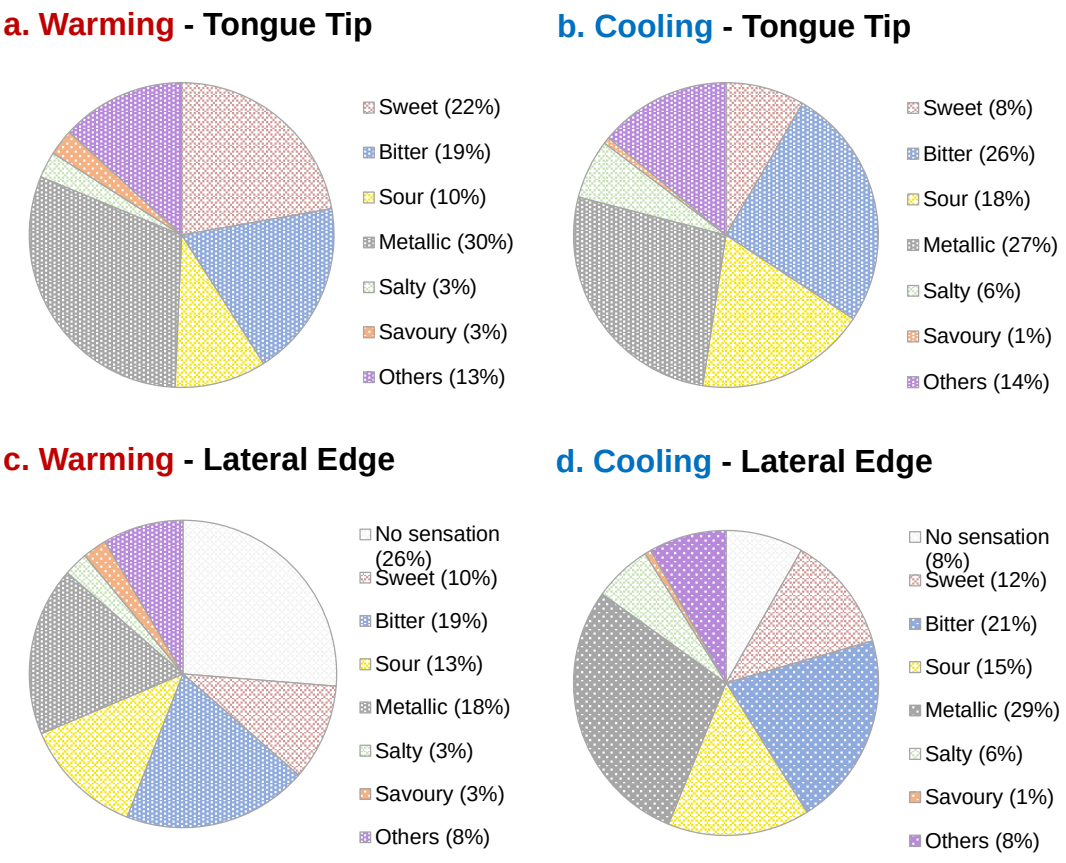

Figure 2-3: Percentage taste quality reported during both warming and cooling trials on the tip of the tongue by thermal tasters (TTs)

Furthermore, on the tongue tip, sweetness was reported 14% more frequently during the warming than the cooling trial, and both bitter and sour were reported 7 and 8% more frequently respectively during cooling stimulation. The results from the left and right edges of the tongue were combined for analysis as 'lateral edge', and interestingly, about 27% of the TTs did not perceive any tastes here during the warming trial and 8% did not perceive any tastes during the cooling trial (**Figure 2-3 (c&d)**).

The average logged perceived intensity of the taste sensations were 1.25 ± 0.35 for warming trial and 1.45 ± 0.29 for cooling trial on the tip of the tongue, which equates to around moderate for warming trials and just below strong for cooling trials on the gLMS scale (see **Figure 2-4**). The average logged taste intensity on the lateral edge of the tongue was 1.19 ± 0.32 for the warming trial and 1.28 ± 0.35 for the cooling trial (both below moderate on the gLMS), which were significantly lower than the ratings on the tongue tip (t-test, $p<0.05$), supporting previous findings that the anterior tip of the tongue is the most sensitive area for 'phantom taste' (Cruz & Green, 2000).

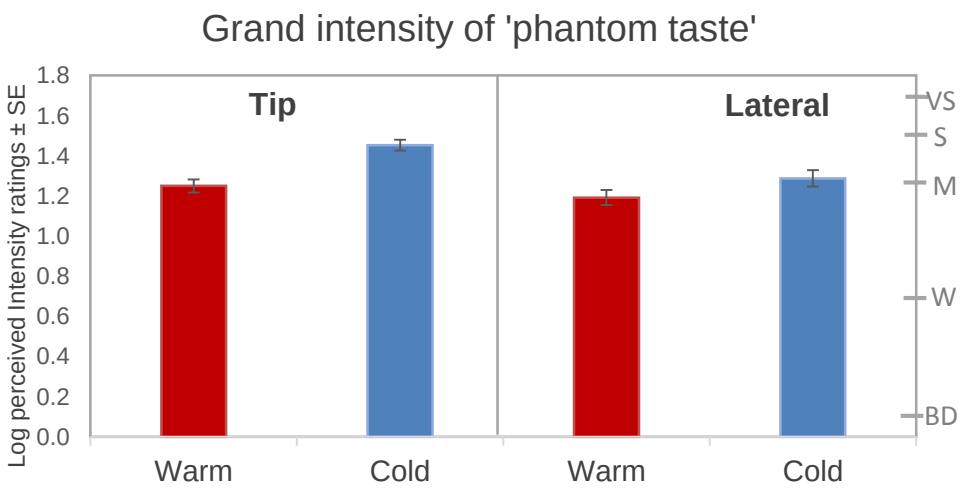

Figure 2-4: 'Phantom taste' intensity reported both warming and cooling trials on both tip and edge of the tongue by thermal tasters (TTs) (all tastes combined).

2.3.3. Thermal taster status and perceived temperature intensity

As shown in **Figure 2-5**, TTs rated warm and cold temperature intensity significantly higher than TnTs at both the tip and lateral edge of the tongue ($p<0.05$). Notably, TTs also rated the perceived temperature intensity from both warming and cooling on the tip of tongue significantly higher than the Uncat group ($p<0.05$). However, no difference was observed between the Uncat group and both the TTs and TnTs on the lateral edge of the tongue. The observation in the current study generally supports previous findings that temperature intensity ratings for TTs were significantly higher than TnTs, but previous studies have failed to observe a significant difference on the tongue tip in cooling trials, which might be because of differences in TTS classification criteria (Bajec & Pickering, 2008).

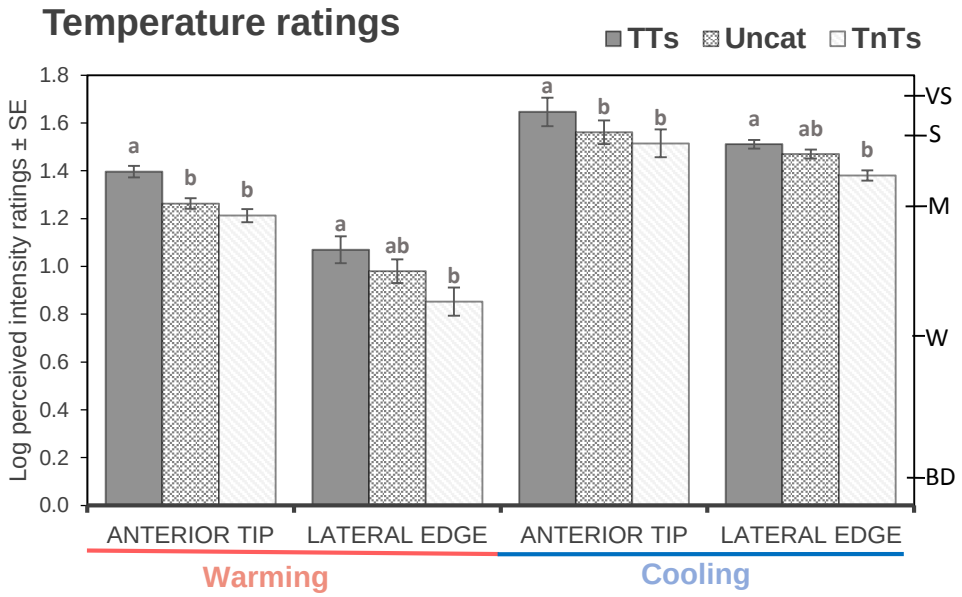

Figure 2-5: Thermal taster status on perceived temperature intensity by warming and cooling small area of tongue. Bars represent logged perceived intensity ratings ± SE. *Different letters within each variable indicate significant among TTS groups at p<0.05.*

One-way ANOVA were also performed independently to examine the effect of taster status, replicates and locations on pooled warm and cold ratings. The results are summarised in **Table 2-10**. Results revealed a significant TTS group difference on the pooled temperature intensity ($p<0.001$), further post hoc test demonstrated that TTs rated significantly more intense than Uncat and TnTs groups, additionally Uncat group rated significantly more intense than TnTs. No significant difference between replicates was observed ($p>0.05$), indicating no carry over effect and the inter-trial break was sufficient for recovery of tongue temperature. In agreement with previous findings (Bajec & Pickering, 2008; Cruz & Green, 2000), this study observed that the tongue tip was the most sensitive area for perceived temperature, having the highest perceived temperature ratings.

Table 2-10: Summary of logged temperature intensity by group, replicate and location with significance levels and post-hoc groups associated with ANOVA.

		Mean Temperature Intensity	p value
Taster Status	TTs	1.40 ± 0.38^{a}	<0.001
	Uncat	1.32 ± 0.39^{b}	
	TnTs	1.24 ± 0.42^{c}	
Replicate	Rep1	1.42 ± 0.29^{a}	0.42
	Rep2	1.44 ± 0.31^{a}	
Location	Tip	1.43 ± 0.3^{a}	<0.001
	Left Edge	1.19 ± 0.47^{b}	
	Right Edge	1.21 ± 0.46^{b}	

Data represents logged mean intensity ± standard deviation. ^{abc} different superscribe letters within each variable indicate significantly different groups.

2.3.4. Incidence of PTS and gender effect

Based on the perceived intensity of 0.32mM PROP stimulus, of the 130 subjects, 39 subjects (30%) were classified as PROP supertasters (pSTs), 64

subjects (50%) were classified as PROP medium tasters (pMTs) and 25 subjects (20%) were classified as PROP non-tasters (pNTs). No significant relationship between PTS and gender was observed (p=0.52), as illustrated in **Table 2-11**. However it was noted that males (0.99 ± 0.52) rated the intensity of PROP higher than females (0.81 ± 0.57) which is approaching the selected level of significant (p=0.08).

Table 2-11: Cross-tabulation of PTS distribution by gender and associated Chi-square analysis.

PROP Taster Status	Gender					[a]p value
	Female		Male		Total	
	n	%	n	%	n	
pSTs	25	28%	14	34%	39	0.52
pMTs	43	49%	21	51%	64	
pNTs	19	22%	6	15%	25	

[a]p value associated with Chi-square analysis. n represents number of subjects, % represents percentage within column.

2.3.5. PTS and perceived PROP intensity and temperature

By examining the effect of PTS groups on perceived PROP and temperature intensity, ANOVA revealed a significant group effect for both PROP intensity and temperature intensity ratings (both p<0.001) as shown in **Table 2-12**. pSTs rated PROP intensity significantly more intense than pMTs and pNTs, and pMTs rated significantly more intense than pNTs. Moreover, PROP tasters (pSTs and pMTs) rated temperature intensity from the Medoc thermode significantly more intense than pNTs, as reported previously (Bajec & Pickering, 2008).

Table 2-12: Summary of logged pooled temperature intensity and PROP intensity across PTS groups with significant levels and post-hoc group from ANOVA

Pooled perceived Intensity Mean			[a]p value
PROP intensity	pSTs	1.47±0.18[a]	<0.001
	pMTs	0.80±0.36[b]	
	pNTs	0.06±0.11[c]	
Temperature intensity	pSTs	1.37±0.39[a]	<0.001
	pMTs	1.34±0.40[a]	
	pNTs	1.16±0.46[b]	

Data represents logged mean ± standard deviation. [abc] different superscribe letters within each variable indicate significantly different groups. [a]p value associated with Chi square analysis

2.3.6. Relationship between TTS and PTS classification

Chi-square test revealed that there was no significant relationship between PTS and TTS classifications, as shown in **Table 2-13**, which is in accordance with Bajec and Pickering (2008) that PTS and TTS are likely to operate via different mechanisms.

Table 2-13: Distribution of PTS across TTS and associated Chi square analysis.

PROP taster status	**Thermal taster status**						**[a]p value**
	TTs		TnTs		Uncat		
	n	%	n	%	n	%	
pSTs	16	37%	12	28%	11	26%	0.35
pMTs	22	51%	23	53%	19	45%	
pNTs	5	11%	8	19%	12	29%	

[a]p value associated with Chi square analysis. n represents number of subjects. % represents percentage within column.

2.3.7. Relationship between TTS and food neophobia and food involvement

Although not significant (p=0.2), results showed that TTs had lower food neophobia mean scores than TnTs, with the same trend observed for most statements, which means TTs were tending towards lower food neophobia. Interestingly, TTs assigned significantly higher agreement to the statement of 'I am constantly sampling new and different foods' (p=0.02) than TnTs (**Figure 2-6**).

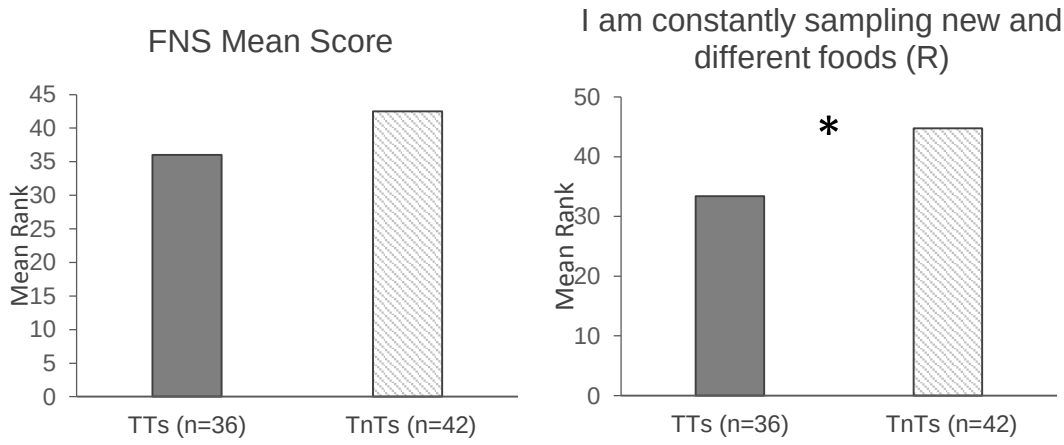

Figure 2-6: FNS mean score and example of FNS individual statement between TTs and TnTs. *Data represents mean rank of each group. * indicates significant difference at p<0.05. (R) denotes scores were reversed. Higher scores indicate greater food neophobia.*

For food involvement scores, TTs were shown to think more about food each day, feel cooking and barbecuing is more fun, and assigned a greater level of importance to their food choices, compared to TnTs (p<0.05), as shown in **Figure 2-7**. In addition, TTs self-reported to think or talk more about food while eating out, which just failed to reach significance (p=0.08). Although not significant (p=0.13), TTs have a higher FIS mean score than TnTs, and the same trend was found for most single statements. This indicates TTs self-reported to be more willing to get involved with food.

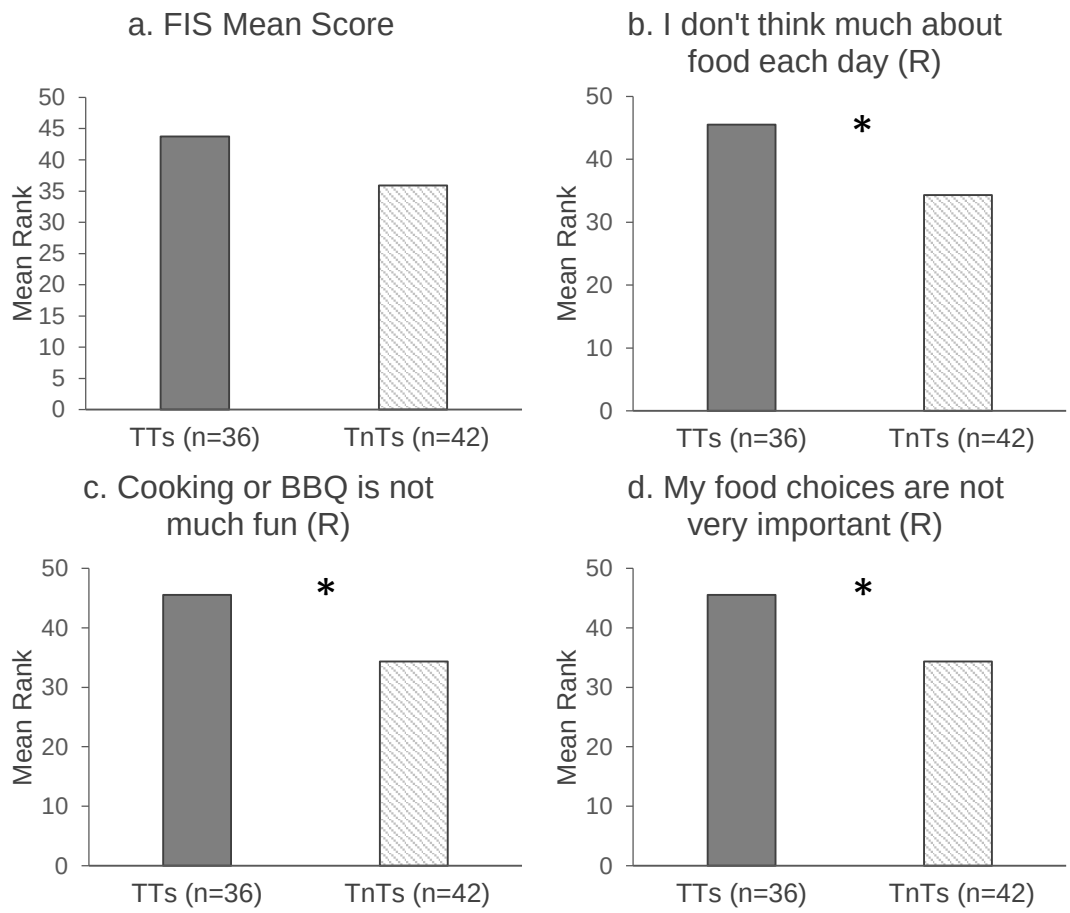

Figure 2-7: FIS scores between TTs and TnTs on a) food involvement mean score and b-d) individual statement that reach significant level. **indicates significant difference at p<0.05. (R) denotes ratings were reversed. Higher scores indicate greater food involvement.*

2.3.8. Relationship between PTS and food neophobia and food involvement

No clear trends in both the FNS and FIS single statement ratings were observed. Within each scale (either FNS or FIS), pNTs showed the highest agreement to some statements, but the lowest to others, compared to the other two PTS groups. No significant differences on FNS or FIS mean score and each individual statement score among PTS groups were observed ($p>0.05$) (**Figure 2-8)**, with one exception, pNTs were shown to be more particular about the food they eat, compared to pSTs and pMTs ($p=0.01$). Pearson's correlation coefficients further confirmed that there were no

significant correlation between FNS or FIS scores and PROP intensity ratings (r ranged from 0.009 to 0.065, p>0.05).

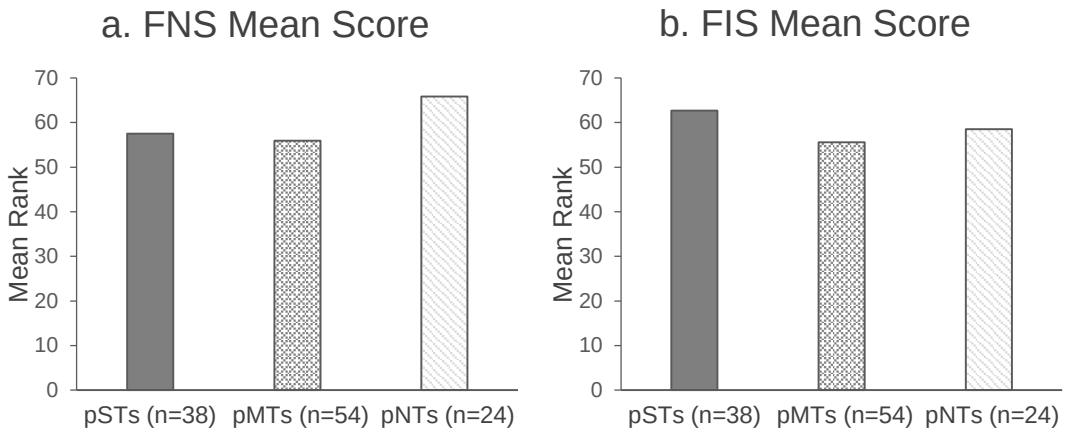

Figure 2-8: FNS and FIS mean scores across PTS groups. a) Food neophobia mean score. b) Food involvement mean score.

A Pearson's correlation coefficient was also performed to examine the association between FIS and FNS scores. A significant correlation between FNS and FIS scores was observed (r=-0.63, p=0.001), which means people who were food neophobic, were likely to have a lower anticipation of getting involved with food, which agrees with the findings of Marshall and Bell (2004).

2.3.9. Relationship between TTS and alexithymia and personality

By examining the difference between TTs and TnTs on alexithymia ratings, no significant differences on agreement ratings of either individual statement, subgroup means or total mean score were observed (p>0.05), shown in **Figure 2-9**.

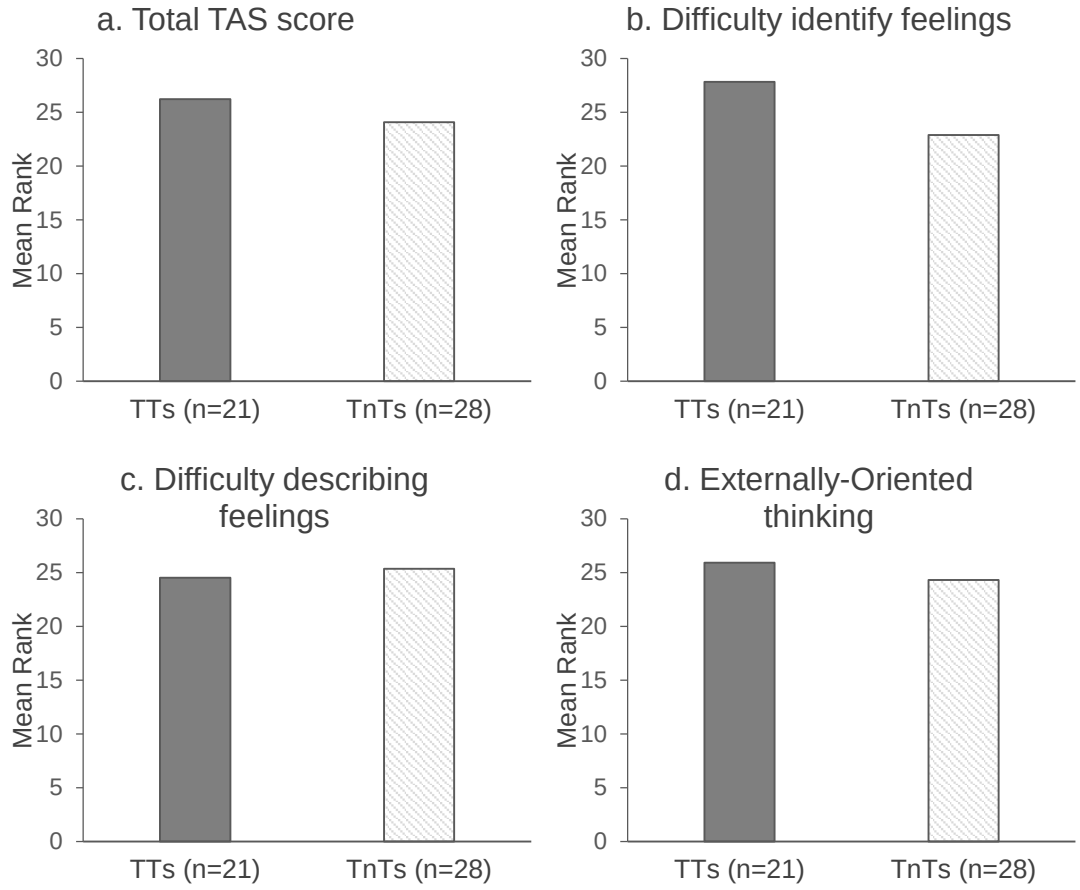

Figure 2-9: Toronto Alexithymia Scale (TAS) scores between TTs and TnTs: a) Total TAS score, b) Difficulty identifying feelings subgroup score, c) Difficulty describing feelings subgroup score, d) Externally-oriented thinking subgroup score. *Data represents mean rank of TTS group. See **Table 2-6** for items under each subgroup. Higher score indicates higher possible alexithymia.*

For the BFI test, although not significant (p=0.17), a trend was observed that TTs showed higher agreement on 'openness' dimension than TnTs, as illustrated **Figure 2-10**. TTs reported themselves as being significantly more ingenious and deep thinking (p=0.03), more inventive (p=0.02) and likely to reflect and play with ideas (p=0.03) than TnTs. They also reported themselves as being more curious about many different things (p=0.08), and were more considerate and kind to almost everyone (p=0.06), compared to TnTs, which were approached significance.

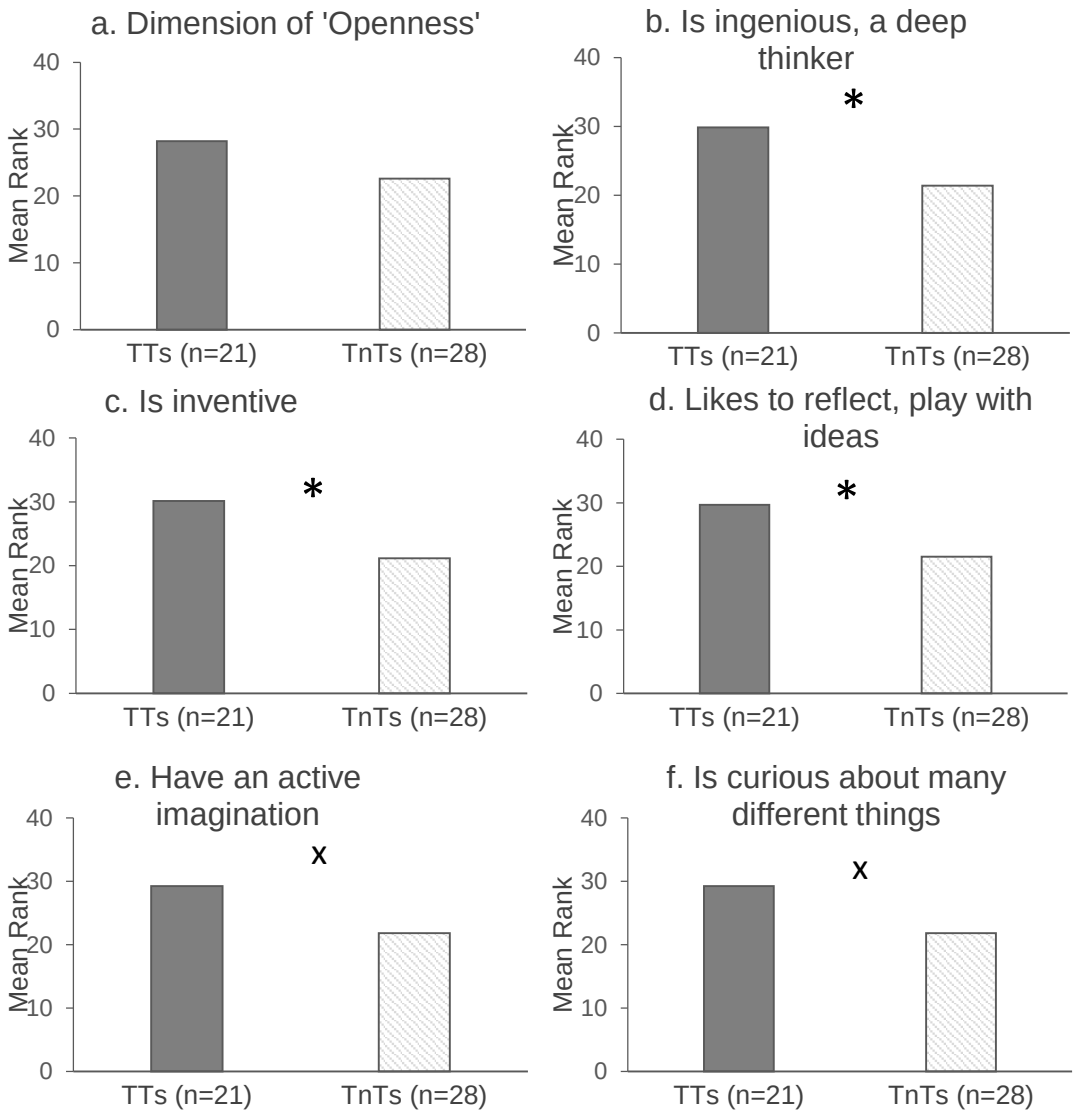

Figure 2-10: Big Five Inventory personality scores between TTs and TnTs: a) BFI openness subgroup mean score, b-f) Examples of individual BFI statement. *Data represents mean rank. *indicate significant at p<0.05, ˣindicates significant at p<0.1. See* ***Table 2-7*** *for items under openness subgroup.*

2.3.10. Relationship between PTS and alexithymia and personality

For alexithymia scores, no significant differences were observed among PTS groups for any of the individual TAS scores, as well as subgroup mean and total mean score (p>0.05) (**Figure 2-11**), with one exception: pSTs were shown to have significantly lower agreement ratings on the statement 'I can feel close

to someone, even in moments of silence' compared to pMTs and pNTs ($p<0.04$).

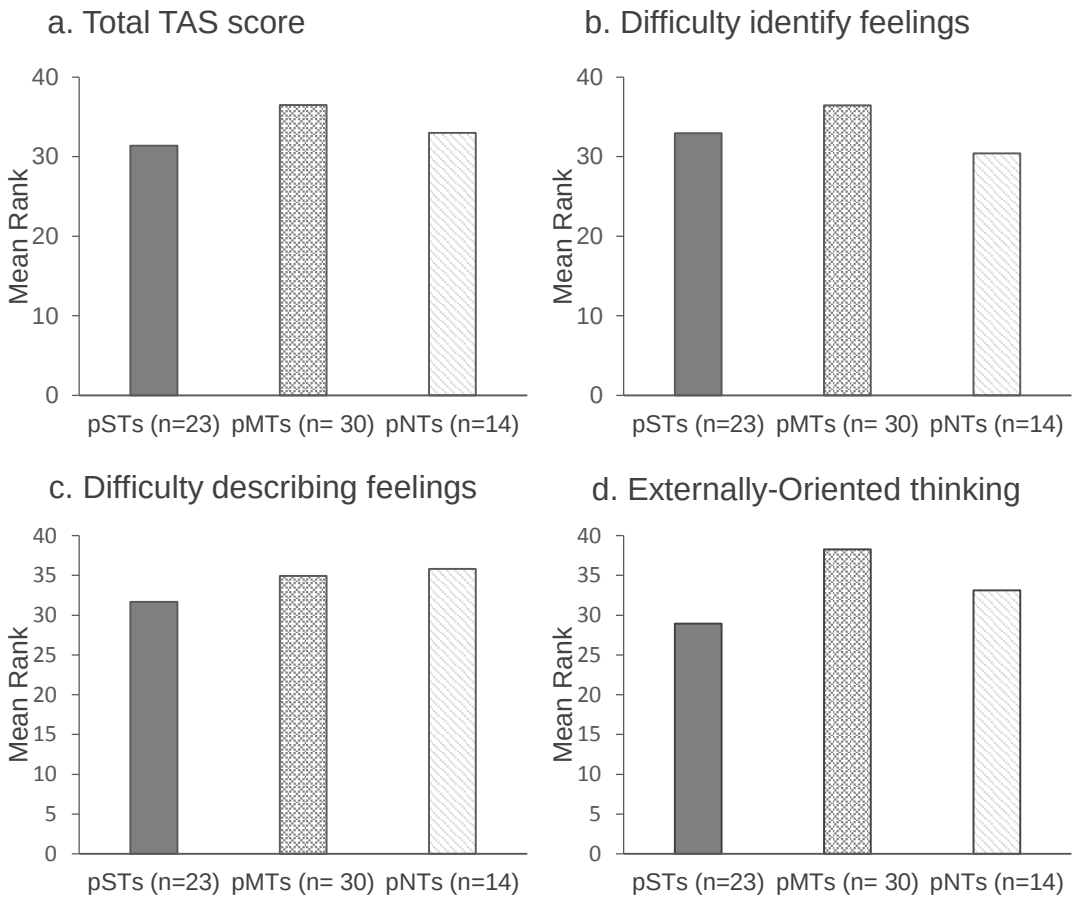

Figure 2-11: Toronto Alexithymia Scale (TAS) scores among PTS groups. a) Total TAS score, b) Difficulty identify feelings subgroup score, c) Difficulty describing feelings subgroup score, d) Externally-oriented thinking subgroup score. *See **Table 2-6** for items under each subgroup. Higher score indicates higher possible alexithymia.*

As illustrated in **Figure 2-12**, pNTs showed significantly higher conscientiousness subgroup scores than both pSTs and pMTs ($p=0.04$), suggesting that pNTs thought of themselves as more thorough, careful or vigilant. The results of Kruskal-Wallis tests revealed several individual questions were significantly different among PTS groups, such as 'does a thorough job' ($p=0.03$); 'can be somewhat careful' ($p=0.04$); 'tends to be

organised' (p=0.01); 'does things efficiently' (p=0.04), 'outgoing and sociable' (p=0.04). All of these questions showed a similar pattern with pNTs>pMTs>pSTs.

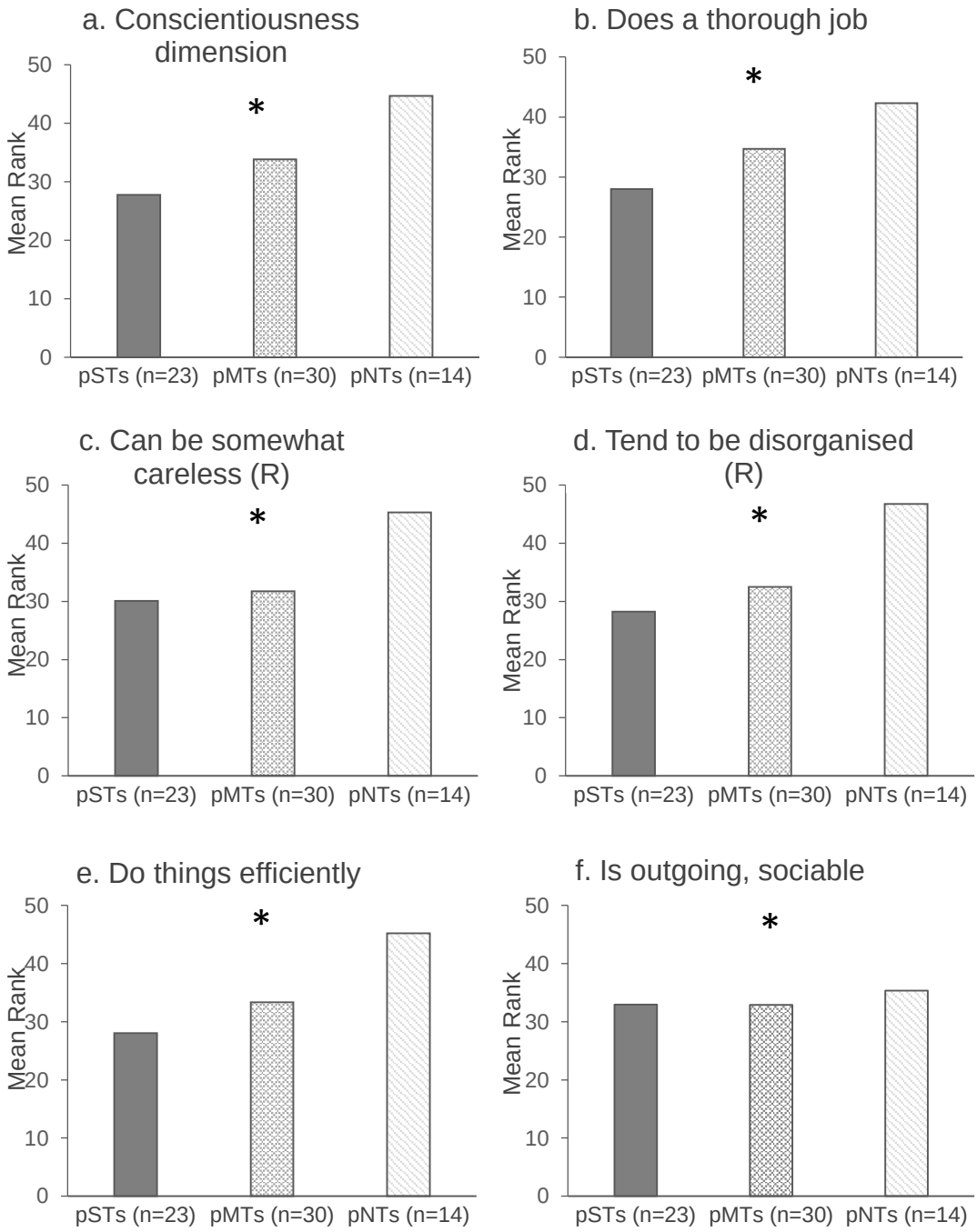

Figure 2-12: Big Five Inventory Personality scores between PROP taster status groups: a) BFI conscientiousness subgroup mean score, b-f) Examples of individual BFI statement. *Data represents mean rank of each PROP taster group. *indicates significant at p<0.05. See **Table 2-7** for items under openness subgroup.*

2.3.11. Further consideration of gender effect on personal behaviours

Further analysis was performed looking at how gender affected personal behaviours. Mann-Whitney U tests did not find any significant gender effect on total FNS or FIS scores. However, few statements were significant, for example, females showed higher agreement to the statements 'When I eat out, I think or talk about food' (p=0.01), 'At dinner parties, I will try a new food' (p=0.01), and 'I do wash dishes' (approaching significance p=0.06). For the alexithymia test, results showed that males had significantly higher ratings on the 'difficulty of describing feeling' subscale (p=0.018), indicating males had higher difficulty when describing feelings, as reported previously (Parker *et al.*, 2003). For the personality test, females had higher agreement on the overall agreeableness subgroup scores than males which was approaching significant (p=0.09). Females showed significantly higher agreement on the statements 'likes to cooperate with others' (p=0.005), 'is sometimes rude to others (R)' (p=0.012), 'is considerate and kind to almost everyone' (p=0.07), is helpful and unselfish with others' (p=0.04).

2.4. DISCUSSION

2.4.1. Incidence of TTS and different categorisation methods

As shown in **Table 2-8**, between 27% and 56% subjects were classified as TTs based on the different classifications used. In any of these cases, it is clear that TTs comprise a large proportion of the population and warrant further investigation. Further work would be needed to standardise the classification methods, so that results can be directly compared across different laboratories.

However, developing a standardised classification method can be challenging, as the data in the current study showed that large variation occurs among subjects' phantom responses. The type and number of 'phantom taste' reported could vary among individuals, and in the same individual it could also vary among different temperature trials and replicates (see **Table 2-2** for examples). So far, the mechanism behind thermal tasters has not been well understood. There is no evidence showing whether the mechanisms behind warming and cooling trials are the same or different, or if the mechanism behind different qualities of 'phantom taste' are the same or different. Thus, it is difficult to set a standardised classification method based on current knowledge on TTS. In this PhD research, subsequent studies will investigate those individuals who perceived 'phantom taste' from both warming and cooling trials (any tastes) – Yang's criteria, rather than those perceived from either warming or cooling trial – Green or Pickering's criteria, in order to be assured of their experience of this phenomenon. However, if further studies show evidence that the mechanisms behind warming and cooling trials are likely to be independent of each other, then the classification method used in the future should be closer to Green's. And if evidence was found which showed that the mechanism behind the 'phantom taste' quality is independent, then a method closer to Pickering's should be used. Thus, understanding the mechanisms behind TTS would be necessary, which in turn, might also help researchers to develop a standardised classification method.

2.4.2. 'Phantom taste' and possible mechanism behind TTS

The pooled mean intensity of 'phantom taste' reported by TTs for both temperature trials were rated above 1.2 (log value) on the scale, which is just

below moderate. It was also observed that some individuals reported that the perceived intensity of ‘phantom taste’ was more intense than the perceived intensity of temperature itself, indicating that the ‘phantom taste’ sensation is real and not just a possibility.

The present study showed that both heating and cooling most commonly evoked a metallic sensation. Metallic has also been reported by TTs in other studies, but not as one of the most frequent sensations (Bajec, et al., 2012). Previous researchers have suggested two likely mechanisms for metallic sensation: i) that it is a true gustatory mechanism evoked by electrical stimulation of taste receptors in fungiform papillae and not affected by nasal occlusion (Lawless, et al., 2005); ii) and/or that it may be multimodal involving gustatory, olfactory and trigeminal pathways (Epke, et al., 2009), particularly as a metallic sensation from ferrous sulphate has been shown to be modified by nasal occlusion (Lawless, et al., 2004). Interestingly, in the present study, temperature stimulation was applied on the anterior tip of the tongue, where fungiform papillae are housed. This raises the possibility that temperature stimulation may activate a response similar to that of an electrical current, through stimulation of gustatory pathways or trigeminal nerves resulting in perception of metallic, and potentially, other tastes. It is also possible that the increased reporting of metallic sensation in this study was due to the listing of the metallic descriptor as one of the taste options whilst previous studies required subjects to proffer descriptors themselves or had no specific metallic option (Lawless, et al., 2005).

Beyond metallic sensation, in general agreement with results from Cruz and Green (2000), sweet taste was reported more frequently during warming trials, and that bitter and sour were reported more often during cooling trials. Evidence showed TRPM5, is heat activated and highly temperature sensitive, and might be involved in 'thermal sweetness'. TRPM5 is supposed to play a key role in the perception of sweet, umami and bitter tastes (Talavera, et al., 2005, Talavera, et al., 2007). Evidence showed that increasing temperature from 15 to 35ºC markedly enhanced the gustatory nerve responses to sweet compounds in wild –type mice, whereas no such enhancement was found the the mice lacking TRPM5 gene. Other tastes such as salty or sour could not be explained by TRPM5 channel. In addition, one research has suggested that metallic sensation is unlikely to be activated by TRPM5 (Riera, et al., 2009). A recent study further proved that no significant association was found between the TRPM5 gene and thermal taster status (Bering, 2012; Bering *et al.*, 2014). It is, therefore, unlikely that TRPM5 is the only potential mechanism that contributes to the phenomenon of TTS.

In agreement with previous findings (Bajec & Pickering, 2008; Cruz & Green, 2000), the present study confirms TTs perceive the intensity of temperature as more intense, compared to TnTs. The results presented here also show that the anterior tip of the tongue is the most sensitive area for perceiving temperature and 'phantom taste'. The reason the anterior tip of the tongue is most sensitive to both thermal and 'phantom taste' may be linked to the number of fungiform papillae, as fungiform papillae are mostly located on the tongue tip (Vesnaver & Keller, 2011) and house condensed taste and

trigeminal nerve endings, which in turn, may generate more signals during thermal stimulation than the lateral edge of the tongue.

*2.4.3. PTS and TTS * PTS relationship*

As no significant differences were observed between replicates on PROP bitterness intensity, this indicates that the 5mins break used during PROP intensity measurement was sufficient for palate cleansing. The PTS group proportions reported here were 30% pSTs, 50% pMTs and 20% pNTs, which is similar to previously reported proportions (25% pSTs, 50% pMTs and 25% pNTs) (Rankin *et al.*, 2004), further suggesting that this quick and simple PROP status classification method yields similar PROP taster groups proportions as other methods. Conflicting results are seen in the literature regarding the relationship between PTS and gender. One study reported women were more likely to be supertasters (Bartoshuk *et al.*, 1994), whereas, other studies failed to find an association between PROP tasters and gender (Chang, *et al.*, 2006; Keller & Tepper, 2004). No significant relationship between PTS and gender was found in this study, and in fact, the opposite trend was observed to the findings of Bartoshuk *et al.* (1994). However, the ratio of females to males in this study was unbalanced and further study balanced for gender would be needed to further confirm this observation.

The heightened response to temperature for PROP tasters could be linked to the number of fungiform papillae, as previous research has reported PROP tasters to be associated with higher number of fungiform papilla (Bartoshuk *et al.*, 1994), which in turn, results in more condensed taste and trigeminal nerve endings, and hence, an increased trigeminal response.

In agreement with previous studies (Bajec & Pickering, 2008), there was no relationship between PTS and TTS, indicating these two taste phenotypes are likely to operate via different mechanisms. PROP responsiveness is known to be partially controlled by a bitter receptor gene - TAS2R38 (Duffy *et al.*, 2004). Evidence has shown that TTS is not associated with the TAS2R38 genotype, which further suggests these two taster statuses are independent (Bering *et al.*, 2014). However, it is important to understand the combined effects of different taste genotypes and phenotypes.

2.4.4. Relationship between taster status and personal behaviours

A consistent trend was observed that TTs were more willing to get involved with food and were less food neophobic. However, no trend was observed for both FNS and FIS scores among PTS groups. It has been suggested that higher food involvement might be associated with a greater ability to discriminate between samples, which might be linked to taste sensitivity. Bell and Marshall (2003) proposed that greater discrimination between samples might make eating experiences more interesting, and hence result in higher food involvement. However, so far, no evidence has supported this hypothesis. On the other hand, heightened taste sensitivity has been suggested to manifest in a reluctance to taste unfamiliar food (food neophobia), and evidence has shown that neophobic people tend to avoid any possible bad odour by using smaller sniff magnitudes than non-neophobic people (Dematte *et al.*, 2014). In agreement with Marshall and Bell (2004), the present study reveals a significant correlation between FNS and FIS scores, where food neophobic people are less willing to get involved with food. To the author's knowledge, there is no previous research that has specifically looked into the

relationship between taste sensitivity, food neophobia and food involvement with the same subjects. Previous studies have discovered that both thermal tasters and PROP tasters have higher sensitivity to a range of taste and trigeminal stimuli (Bajec & Pickering, 2008), so if taste sensitivity is linked to both food neophobia and food involvement, a similar effect finding with TTS and PTS would be expected. However, the data in this study showed that only TTS seems to have an effect on food behaviour. Therefore, taste sensitivity may not be the primary factor that dominates food neophobia and involvement.

The Big Five Inventory Personality test revealed that TTS were more associated with 'openness' dimension, where TTs considered themselves to be more open. Openness reflects the degree of intellectual curiosity, creativity and a preference for novelty and variety a person has. It is also described as the extent to which a person is imaginative, and depicts a personal preference for a variety of activities over a strict routine. The findings here are interesting and new. Although the reason behind this phenomenon is currently unknown, it has brought up the possibility that the higher imaginative scores might be linked to the phenomenon of 'phantom taste' perceived by TTs. However, as discussed in section 2.3.2., the 'phantom taste' reported by TTs is believed to be a true response rather than an imagined one. But it is interesting that TTs considered themselves as more imaginative and inventive, in comparison with TnTs.

Furthermore, PTS was associated with 'conscientiousness' subgroup scores, with pNTs considering themselves to be more conscientiousness than both PROP tasters. To the authors' knowledge, this was the first study that

investigated personality and taste phenotypes. Although the results of the present study are insufficient to provide any explanation behind this phenomenon, it has shed light on how taste phenotypes might be linked with personal traits. Further work with larger sample sizes would be needed to confirm these findings, and this specific relationship could be further tested to predict eating behaviour and food preference.

2.5. CONCLUSION

This study confirmed that about 27% of the population sampled in UK could consistently perceive ‘phantom’ taste sensation through both warming and cooling temperature trials. This represents a large proportion of the population and therefore warrants scientific consideration. The results indicated that thermal taster status classification can be problematic as the ‘phantom taste’ reported is not always consistent across groups and/or within individuals, and further work on a standard methodology is necessary if studies are to be comparable.

The averaged intensity of ‘phantom taste’ reported were around moderate on the gLMS scale, indicating this phenomenon is not a subtle perception. However, the mechanism behind TTS is currently not known, but it might be linked to taste and trigeminal nerves on the tongue. Further work should investigate how thermal taster status affects the sensitivity of sensory stimuli in different modalities. In general agreement with results from Cruz and Green (2000), the anterior tongue tip showed to be the most sensitive area to ‘phantom taste’ and temperature intensity. This study also confirmed that TTs perceived both warm and cold temperature more intensely than TnTs, and

PROP tasters perceived both temperature and PROP more intensely than non-tasters. No significant correlation was observed between the TTS and PTS classifications, indicating these two taste phenotypes are likely to operate via different mechanisms.

To the author's knowledge, this is the first report which explores the relationship between taste phenotypes and personal traits, and the findings here provide evidence for a link between self-reported taste phenotypes and personal traits. For example, TTs self-reported to be less food neophobic and more willing to get involved with food, compared to TnTs. Additionally, TTs considered themselves to have a more active imagination, compared to the same data collected for TnTs. Within the PTS phenotype, pNTs considered themselves to be more conscientious. This specific relationship could be further tested to predict eating behaviour and food preference. More research is now needed to ascertain why these differences in personality traits and attitudes to food should be evident between these groups. Further work on a larger sample size would be needed to confirm these findings.

The next chapter looks at the effect of TTS and PTS on the perception of a range of taste, trigeminal and olfactory stimuli at both detection threshold and suprathreshold level, as well as the interactions between TTS and PTS on these sensitivities, in order to begin to decouple the mechanism behind thermal taster status.

3. PHENOTYPIC VARIATION IN ORONASAL PERCEPTION AND THE RELATIVE EFFECTS OF PROP AND THERMAL TASTER STATUS

3.1. INTRODUCTION

3.1.1. Taste Perception from detection threshold to suprathreshold

Taste perception is shown to be an important determinant of food preference (Drewnowski *et al.*, 1999). Threshold determination and perceived intensity measurement are widely used in order to determine levels of sensitivity to different sensory stimuli. A detection threshold is defined as a concentration range below which a substance will not be detectable under any practical circumstances, and above which individuals with normal sensing would readily detect the presence of the substance (ASTM-E679, 2004). Conceptually, the detection threshold is the lowest physical energy level of a stimulus or concentration which activates the sensory receptor to generate action potential in nerve fibres that is strong enough to evoke a perception (Gutierrez & Simon, 2011). As illustrated in **Figure 3-1**, as the concentration of the substance increases, the recognition threshold level is reached, the point which the quality (i.e. sweet or bitter) of the substance can be identified. As the concentration of the substance increases further, the intensity of the substance could be determined, until the concentration increases no longer cause subsequent increases in intensity (Keast & Roper, 2007)**.**

There are many procedures available for threshold measurement such as the staircase method, signal detection theory and forced-choice methods (Lawless & Heymann, 2010b). The 3-alternative forced choice (3-AFC) with ascending concentration series described in E-679 in the ASTM standards

(ASTM-E679, 2004) is often used. Thresholds are usually considered as the point at which the probability of detection is 50%, but ASTM E-679 does not use a strict 50% estimate, it attempts to use the best estimate threshold which gives a value 'not far therefrom' (ASTM-E679, 2004). ASTM procedure E-679 offers a simple and quick procedure for data collection and calculation (Lawless & Heymann, 2010b; Peng *et al.*, 2012), hence it is popular for measuring detection thresholds for a large population.

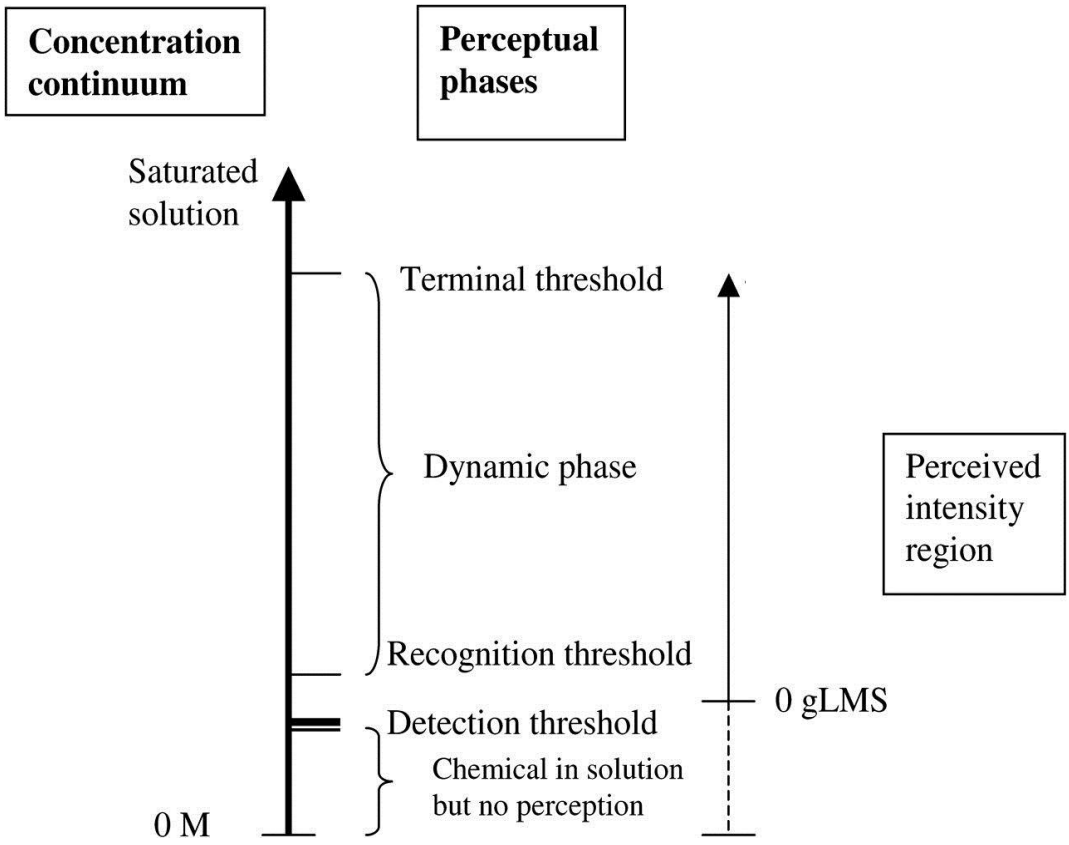

Figure 3-1: Schematic illustration of the relationship between chemical concentration, detection threshold, and suprathreshold intensity gLMS. (Source: (Keast & Roper, 2007)).

Keast and Roper (2007) have suggested that the relationship between detection threshold and suprathreshold intensity and chemical concentration is complex. They have observed a correlation between detection threshold

and perceived intensity for PROP, but no correlation was observed for caffeine. Hence, they hypothesised that if a single receptor was responsible for both the detection threshold and suprathreshold intensity, then a strong association between detection threshold and suprathreshold would be expected, such as PROP. However, if there are multiple taste mechanisms involved that are activated at varied concentrations, then there may be no associations, such as with caffeine (Keast & Roper, 2007). Additionally, Fucci *et al.* (1985) addressed the hypothesis that thresholds are associated with the sensitivity of the receptor mechanism which simply determines if there is a stimulus or not. However, suprathreshold judgments need to obtain information on the behaviour of the senses above the detection threshold level, involving determine the intensity and quality of the stimuli, which requires activity at higher cortical levels (Fucci *et al.*, 1985).

3.1.2. Individual variations in sensory perception

Oronasal sensitivity has been shown to vary greatly among individuals and is purported to affect food consumption behaviour and subsequently a range of health and disease outcomes (Stewart et al., 2010; Ullrich et al., 2004; Villarino et al., 2009). Many factors contribute to this individual variation, such as age (Heft & Robinson, 2014; Mojet *et al.*, 2003), gender (Hirokawa *et al.*, 2006; Leshem *et al.*, 2003), different genotypes (Törnwall *et al.*, 2012; Prodi *et al.*, 2004) and phenotypes (Bajec & Pickering, 2008; Green & George, 2004). Individual difference in perceiving 6-n-propylthiouracil (PROP) bitterness is the most studied source of individual variation in taste perception since Blakeslee and Fox first discovered it over eight decades ago (Blakeslee & Fox, 1932). The gene contributing to PROP perception has since been identified as

TAS2R38 (Kim *et al.*, 2003). Previous studies have also found a link between PROP taster status and increased taste perception for both detection threshold level (sucrose and quinine HCl) (Chang, *et al.*, 2006; Hong *et al.*, 2005) and suprathreshold level (sweetness of sucrose, saccharin and neohesperdin dihydrochalcone (Bartoshuk, 1979; Gent & Bartoshuk, 1983), saltiness of sodium chloride (Bartoshuk *et al.*, 1998), sourness of tartaric acid (Bajec & Pickering, 2008), and bitterness of caffeine, KCl and benzoate (Bartoshuk *et al.*, 1988)). Apart from taste perception, PROP taster status is also associated with trigeminal perception (temperature (Bajec & Pickering, 2008), lingual lingual tactile acuity (Essick *et al.*, 2003) capsaicin (Prescott & Swain-Campbell, 2000), fat discrimination ability (Tepper & Nurse, 1997) and olfactory perception (diacetyl - detection threshold) (Yackinous & Guinard, 2001), mixture of acetaldehyde, diacetyl and linalool (Pickering *et al.*, 2006)). Interestingly a greater density of fungiform papillae is associated with pSTs compared to medium and non-tasters (Bartoshuk *et al.*, 1994; Delwiche *et al.*, 2001; Essick *et al.*, 2003; Miller & Reedy, 1990), indicating pSTs have more chorda tympani and trigeminal nerve fibres. This is likely to explain the advantage of pSTs over the other two groups in terms of increased taste (Bajec & Pickering, 2008; Gent & Bartoshuk, 1983; Lim *et al.*, 2008) and trigeminal sensitivity (Essick *et al.*, 2003; Prescott & Swain-Campbell, 2000; Tepper & Nurse, 1998). Surprisingly, pSTs have also been shown to demonstrate increased perception to olfactory stimuli (Pickering *et al.*, 2006) and increased negative emotions when viewing film clips (Macht & Mueller, 2007). However, sensitivity to PROP has not always found an association with increased responsiveness to other oronasal sensory stimuli (Ly & Drewnowski,

2001; Yackinous & Guinard, 2001); and as such, other unknown factors are likely to contribute to individual variation in oronasal perception.

In 2000, Cruz and Green found a new marker of individual variation in oral sensation: thermal taste. Individuals who had the ability to perceive 'phantom' taste sensations during heating or cooling a small area of tongue were described as 'thermal tasters' (TTs). Thermal taster status also appears to have an impact on oronasal sensitivity. TTs have been reported to have a heightened response to taste stimuli (sucrose, saccharin, sodium chloride, citric acid, quinine sulphate, MSG and PROP), some trigeminal stimuli (astringent, temperature) and aroma stimuli (vanilla sensed retronasally and orthonasally) compared to Thermal non-tasters (TnTs) (Bajec & Pickering, 2008; Green *et al.*, 2005; Green & George, 2004).

Bajec and Pickering (2008) looked at the impact of both TTS and PTS on a variety of taste and trigeminal sensations. They found that PTS has a greater impact on oral responsiveness, compared to TTS. Additionally there were no significant interactions between TTS and PTS on perceived oral intensity ratings, suggesting that the impact of these two taste phenotypes on oral responsiveness are likely to operate via different mechanisms.

Both PROP taster status and thermal taster status appear to play a role in oronasal sensitivity at suprathreshold level. However, to date, there has been little research looking at detection level sensitivity, especially in relation to TTS. The study presented in this chapter aimed to:

- Determine the relationship between detection threshold sensitivity and suprathreshold sensitivity (perceived intensity ratings)

- Examine the impact of PTS and TTS on both detection and suprathreshold sensitivities across a range of gustatory, trigeminal and olfactory modalities.
- Examine the relative effect of these two phenotypes on oronasal sensitivity, in order to decouple the mechanism behind TTS.

3.2. MATERIALS AND METHODS

3.2.1. Subjects

Of the 204 subjects previously screened for thermal taster status, between 109 and 124 subjects (70 to 83 females,37 to 41 males, age range 16-75yrs) attended sessions for detection threshold study and 112 subjects (75 females and 37 males, age range 16-75yrs) were invited back to attend the suprathreshold study.

3.2.2. Detection threshold measurement

All samples were freshly prepared with Evian water (DANONE, France) on the morning of the testing day. Seven stimuli were tested – sucrose (Silverspoon, UK), sodium chloride, caffeine, N-Ethyl-2-isopropyl-5-methylcyclohexanecarboxamide (WS3), capsaicin, ethyl butyrate and isoamyl acetate (all Sigma Aldrich, UK), at concentrations listed in **Table 3-1**. ASTM standard E679 was employed (ASTM-E679, 2004) to determine individual detection thresholds. In order to obtain a testing series of nine 3-AFC presentations, a constant dilution factor (step factor) was used for each stimulus. The concentration series for each stimulus was initially established from previous literature (BS-5929-7, 1992; Devos *et al.*, 1990; Toontom *et al.*, 2001), and then modified by a pilot test in our laboratory.

Table 3-1: Series of dilutions for each stimulus used for detection threshold

	Sucrose	**Salt**	**Caffeine**	**Capsaicin**	**WS3**	**EB**	**IAA**
Step factor	1.35	1.5	1.4	1.9	1.8	2.1	2.1
Unit	mg/l	mg/l	mg/l	µg/l	µg/l	ppb	ppb
C1	630.0	78.0	30.0	1.0	91.0	2.2	1.1
C2	850.5	117.0	42.0	1.9	163.8	4.7	2.2
C3	1148.2	175.5	58.8	3.6	294.8	9.8	4.7
C4	1550.0	263.3	82.3	6.9	530.7	20.6	9.8
C5	2092.5	394.9	115.2	13.0	955.3	43.2	20.6
C6	2824.9	592.3	161.3	24.8	1719.5	90.7	43.3
C7	3813.7	888.5	225.9	47.0	3095.1	190.4	90.9
C8	5148.5	1332.7	316.2	89.4	5571.2	399.8	190.9
C9	6950.4	1999.1	442.7	169.8	10028.2	839.7	400.9

C1-C9: Concentration 1 to Concentration 9. Samples were prepared from lowest to highest concentration. Salt – Sodium chloride, EB – ethyl butyrate, IAA – Isoamyl acetate WS3 – N-Ethyl-2-isopropyl-5-methylcyclohexanecarboxamide.

Subjects attended four 1-hour sessions. Each threshold test comprised nine 3-AFC tests, presented in ascending order. According to ASTM E679 standard, subjects normally stop when respondents constantly give the correct responses; however, there was an interest from a methodological perspective in how respondents continued to respond if allowed. Hence, subjects completed the whole set of nine 3-AFC tests for each stimulus in this study. For each 3-AFC test, subjects were asked to identify which sample was different from the other two. Subjects were told to drink the sample from left to right, and were not allowed to re-taste the samples in order to avoid a variable that is left up to the individual subject and will thus differ among people (Lawless & Heymann, 2010b). The series of each subject's responses were expressed by a line of (0) for an incorrect choice or (1) for a correct choice arranged in the order of ascending concentrations. Stopping rule '3' (Peng, et al., 2012) was employed to calculate each individual's best-estimate threshold (BET), which was taken as the geometric mean of the concentration at the last

miss and the next higher concentration when three correct choices occurred in a row. If subjects missed at the highest concentration an assumption was made that the subject would answer correctly at the next concentration level (ASTM-E679, 2004), and consequently the BET was the geometric mean of the last concentration and the predicted next concentration (last concentration * step factor). Water and crackers (Carr's table water biscuits, UK) were provided as palate cleansers.

3.2.3. Suprathreshold measurement

All samples were prepared with Evian water the day prior to testing, stored in the fridge at 4°C, and brought to room temperature (22 ± 2ºC) in advance of testing. The stimuli concentrations were 0.1, 0.32 and 1M sucrose; 0.056, 0.18 and 0.56M NaCl; 5.6, 17 and 56mM citric acid (Sigma, UK); 1.8, 18 and56mM caffeine; 1.5, 9 and 22.5mM ethyl butyrate 1.8, 18 and 32μM capsaicin (Green *et al.*, 2005; Green & George, 2004). Capsaicin was first dissolved in ethanol (Fisher Scientific, UK), and then diluted with Evian Water. Samples were applied to the anterior tip of tongue via cotton buds that had been previously dipped in each testing solution. At least a 1min break was given between each stimulus, although a longer break was allowed if subjects could still perceive the previous stimulus. Palates were cleansed between stimulus using water and a cracker. All subjects repeated the gLMS scale training described in Chapter 2 (Section 2.2.1.2) before performing the suprathreshold sensitivity measurement. They were encouraged to refer back to their own reference sheet and rate the intensity of each stimulus on the computerised gLMS (Compusense Five 5.4, Canada).

3.2.4. Data analysis

Each individual BET was log transformed before further statistical analysis. Perceived intensity ratings from the suprathreshold tests were also log transformed as gLMS data is typically log-distributed.

Pearson's correlation coefficients were calculated between detection threshold and perceived intensity ratings for sucrose, salt, capsaicin and EB retro at each suprathreshold concentration to examine the relationship between detection threshold and perceived intensity.

One-way ANOVA were applied to the combined TTs and TnTs data (i.e. unclassified individuals were removed from the data set) to determine if TTS significantly affected detection thresholds for each individual stimulus. The results of perceived temperature intensity measured during initial thermal stimulation (Chapter 2) were included as part of the suprathreshold sensitivity. To determine if TTS had a significant effect on perceived intensity at suprathreshold level, a one-way ANOVA were applied to each individual stimulus across TTS groups. Further ANOVA were also applied for the combined TTs and TnTs data on global data (combination of all stimuli) and data pooled for each modality (taste, trigeminal and aroma).

One-way ANOVA, with Tukey's post hoc test, where appropriate, were also applied to determine if significant differences in detection threshold existed among PTS groups for each stimulus. One-way ANOVA, with Tukey's post hoc tests where appropriate, were used to determine if PTS had a significant effect on intensity perception of each individual stimulus.

Furthermore, a two-way ANOVA was applied to determine if interactions occurred across TTS and PTS for detection thresholds. At suprathreshold level, two-way ANOVA were performed to determine if interactions occurred across TTS and PTS for global data and data pooled for each modality (taste, trigeminal and aroma) and for each individual stimulus. Further one-way ANOVA were performed to determine the differences between TTs and TnTs of individual stimulus for each PROP taster group. All analyses were performed using SPSS, version 21 (SPSS IBM, USA). An α–risk of 0.05 was set for all statistical analyses.

3.3. RESULTS

3.3.1. Best Estimate Threshold of different stimuli

Following the ASTM E679 standard, the group BET, and the BET for each TTS and PTS respectively of each stimulus were summarised in **Table 3-2.**

Table 3-2: The group BET values for each stimulus, spit into TTS and PTS groups respectively.

Stimuli	**Unit**	**Group BET**	**TTS**		**PTS**		
			TT	TnT	ST	MT	NT
Sucrose	mg/l	3057.5	2765.4	4151.4	2927.1	3785.1	2843.6
Sodium Chloride	mg/l	458.8	473.4	397.1	443.5	590.1	462.5
Caffeine	mg/l	180.8	176.7	173	172.7	183.1	167
Capsaicin	µg/l	31.8	30.6	32.1	39.1	40.7	17.4
WS3	µg/l	1806.8	1415.5	2359.9	1197.7	2601.6	1469.9
ethyl butyrate	ppb	6.7	7.63	8.26	7.8	7.8	4.5
Isoamyl acetate	ppb	28	28.3	20.3	18.9	29.8	21.2

The group BETs for sucrose (3057.5mg/l (8.9mM)), sodium chloride (458.8mg/l (7.8mM)), caffeine (180.8mg/l (0.93mM)), capsaicin (31.8μg/l), EB (6.7ppb) and IAA (28ppb), which were close to previously reported detection threshold values: sucrose (5.5 to 20.4mM) (Gomez *et al.*, 2004; Lundgrenm *et al.*, 1976), sodium chloride (5.6 to 36mM) (Paulus & Reisch, 1980; Zaidan *et al.*, 2008), caffeine (0.35 to 0.98mM) (Paulus & Reisch, 1980), capsaicin (0.08μg/l) (Schneider *et al.*, 2014), EB (1 to 22.75 ppb) and IAA (2 to 34.2 ppb) (Fazzalari, 1978; Francis, 2013).

3.3.2. Relationship between detection threshold and suprathreshold

Pearson's correlation coefficients showed no significant correlation between detection threshold and perceived intensity ratings for any stimuli tested ($p>0.05$). **Figure 3-2** is representative of the patterns typically observed.

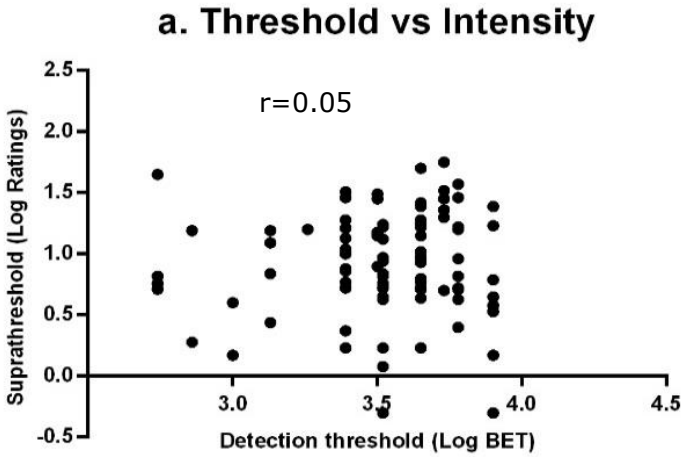

Figure 3-2: Relationship between detection threshold and suprathreshold intensity for sucrose.

3.3.3. Impact of TTS on detection threshold

Between 37 to 41 TTs and 32 to 39 TnTs participated in the detection threshold

tests. (The numbers vary as not all subjects were able to attend all sessions for a particular stimulus). One-way ANOVA revealed that TTs had a significantly lower threshold for sucrose (p=0.032). No significant difference was observed for any other oronasal stimuli at detection threshold (**Figure 3-3)**.

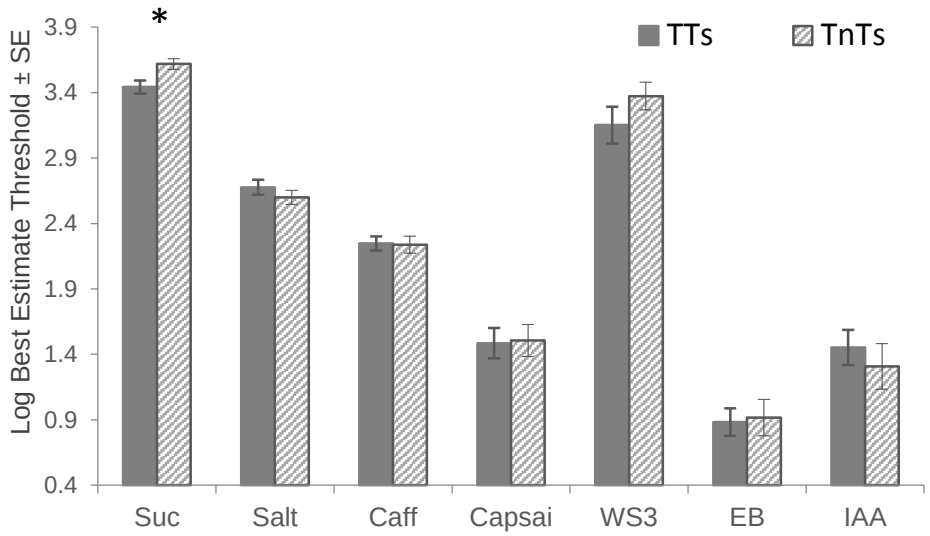

Figure 3-3: Effect of Thermal taster status (TTS) on detection threshold level oronasal sensations. *Bars represent log best estimate threshold (BET) ± Standard Error (SE). *indicates significant difference at p<0.05. (Suc – sucrose, Salt – sodium chloride, Caff – caffeine, Capsai – capsaicin, WS3 – N-Ethyl-2-isopropyl-5-methylcyclohexanecarboxamide, EB – ethyl butyrate, IAA – isoamy acetate).*

3.3.4. Impact of TTS on oronasal sensitivity at suprathreshold level

One-way ANOVA was performed on ratings of each individual stimulus in order to compare TTs and TnTs. The results showed that TTs rated both warm and cold stimuli significantly higher than TnTs (p<0.05). Data for salt (high) also approached significance (p<0.1). No significant difference was observed for any other oronasal stimuli across TTS groups. However, a trend was observed that TTs rated intensities higher than TnTs for most of the stimuli (**Figure 3-4**).

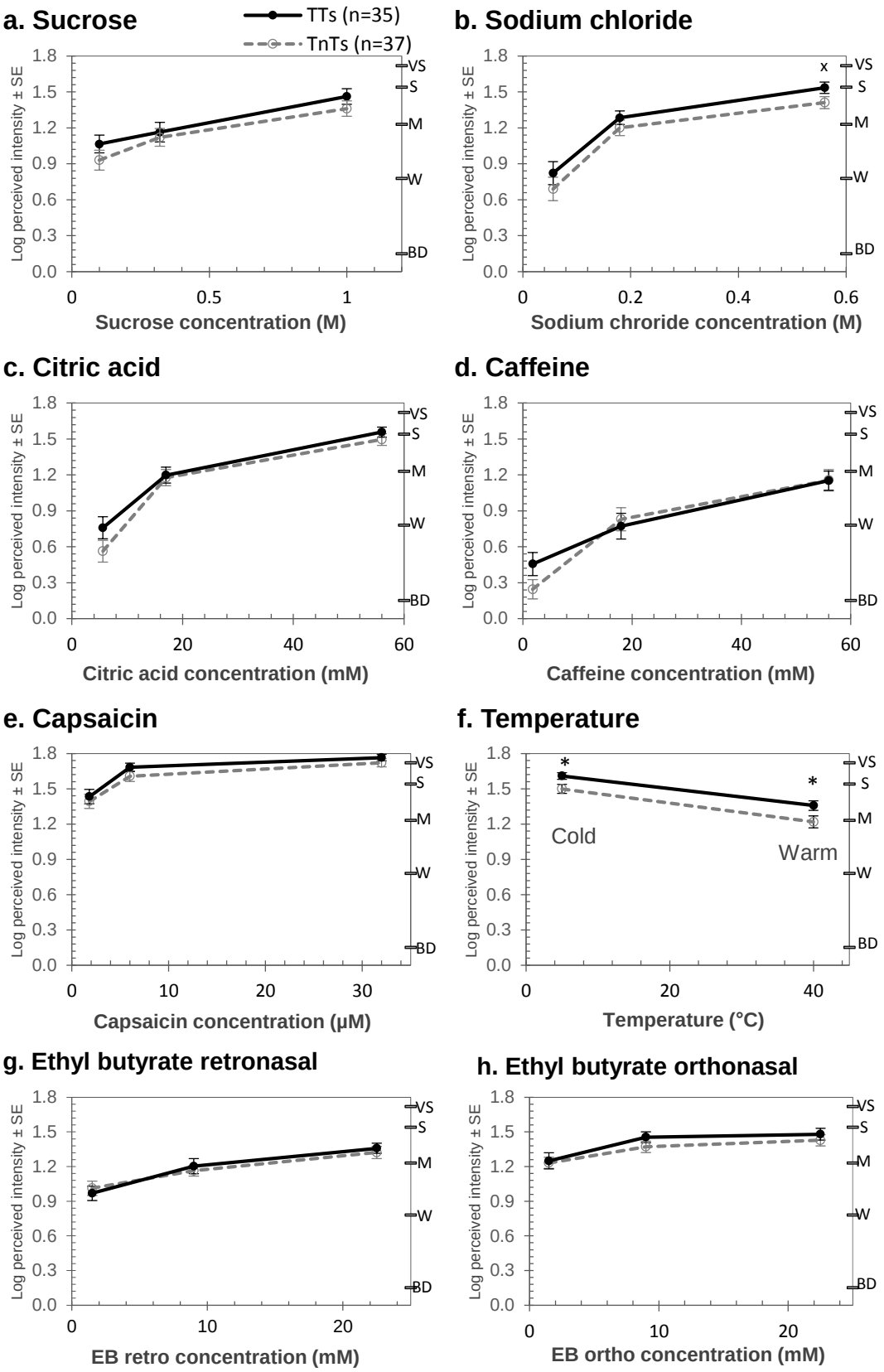

Figure 3-4: Effect of TTS on suprathreshold level oronasal sensations at different concentrations. *Data represents log mean intensity ± SE. *p<=0.05. ˣ p<0.1. (BD–barely detectable, W - weak, M - moderate, S - Strong, VS - Very strong on the gLMS scale). Three concentrations used in each graph (except temperature), from lowest to highest, labelled as L- low, M - medium, H - high in the text.*

Consequently, a further analysis was conducted by performing one-way ANOVA on global intensity ratings, and data grouped by modality. The results showed that in general TTs rated the perceived intensity significantly higher than TnTs ($p<0.05$). On the whole, TTs rated both taste ($p=0.03$) and trigeminal ($p=0.03$) modality intensities significantly higher than TnTs, however this was not the case for aroma.

3.3.5. Impact of PTS on detection threshold

Between 24-31 pSTs, 44-55 pMTs and 18-22 pSTs participated in the detection thresholds tests (Numbers vary as not all subjects were able to attend all sessions). No significant difference ($p>0.05$) was observed for any detection threshold among PTS groups (**Figure 3-5**).

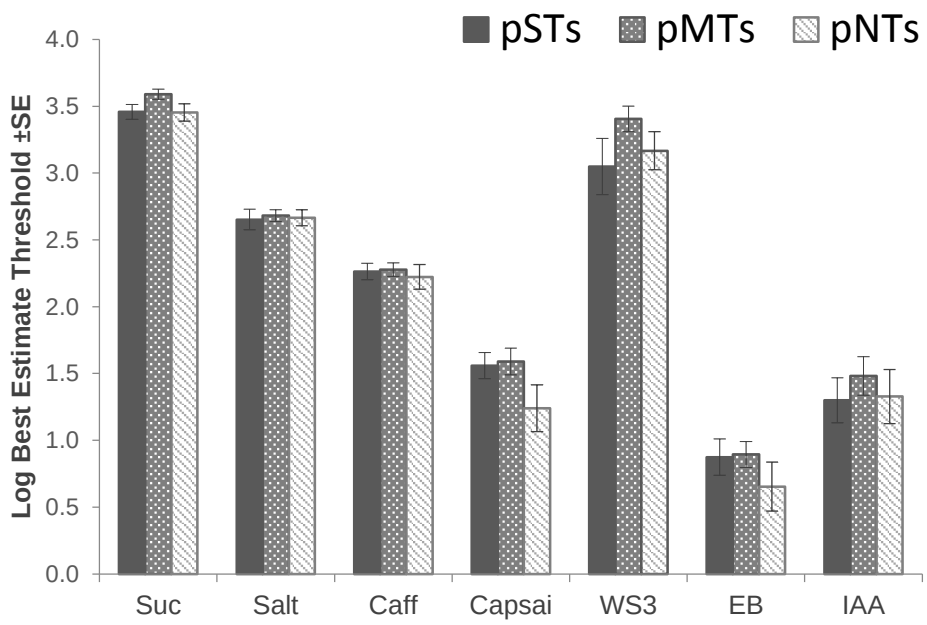

Figure 3-5: Effect of PROP taster status (PTS) on detection threshold level oronasal sensations. *Bars represent log best estimate threshold (BET) ± Standard Error (SE). (Suc Sucrose, Salt - Sodium chloride, Caff - caffeine, EB - ethyl butyrate, IAA - isoamy acetate, Capsai - Capsaicin, WS3 - N-Ethyl-2-isopropyl-5-methylcyclohexanecarboxamide).*

3.3.6. Impact of PTS on oronasal sensitivity at suprathreshold level

One-way ANOVA, followed by Tukey's post hoc tests, were performed on each individual stimulus among PTS groups with results summarised in **Figure 3-6**. The results indicated that there were significant ($p<0.05$) or approaching significance (taken as $p<0.1$) PTS group differences for all individual stimuli. pSTs rated all stimuli as significantly more intense than pNTs, except for Suc (medium) and EB ortho (high). Several additional samples were also significantly more intense by pMTs than pNTs (sucrose (low and high), salt (low, medium and high), caffeine (low), EB retro (medium and high) and EB ortho (medium)). No significant differences ($p>0.05$) in stimulus ratings were found between pSTs and pMTs.

3.3.7. Relative effects of TTS and PTS on oronasal sensitivity

Two-way ANOVA indicated no significant TTS*PTS interaction for any oronasal stimuli at detection threshold level. However, when looking at oroansal sensitivity at suprathreshold level, interestingly, two-way ANOVA, performed on global intensity ratings, revealed a significant interaction between TTS and PTS. Further two-way ANOVA on pooled data for each modality revealed significant interactions for all three modalities ($p<0.05$) (see interaction plots in **Figure 3-7**). Observations of these plots revealed a trend that pSTs who were TTs rated the intensity of all three modalities lower than pSTs who were TnTs, while the opposite trend was clearly observed for pMTs i.e. the rating of pMTS who were TTs were higher than pMTs who were TnTs. No clear trend was observed in pNT group.

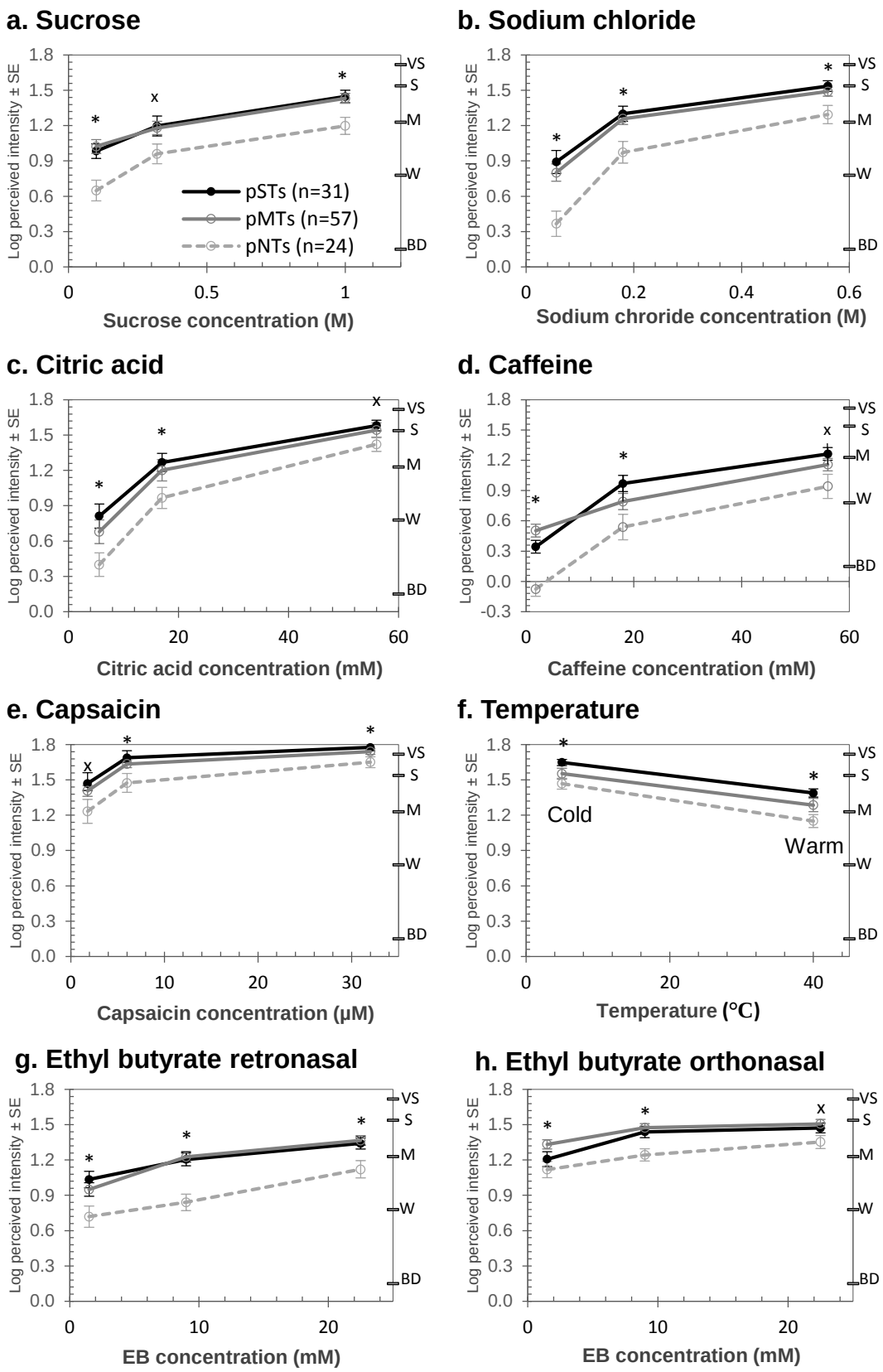

Figure 3-6: Effect of PROP Taster Status (PTS) on suprathreshold level oronasal sensations at different concentrations. *Data represent log mean intensity ± SE. *p<=0.05. [x] p<0.1. (BD - barely detectable, W - Weak, M - Moderate, S - Strong, VS - Very strong on the gLMS scale). Three concentrations used in each graph (except temperature), from lowest to highest, will label as L- low, M - medium, H - high in the text.*

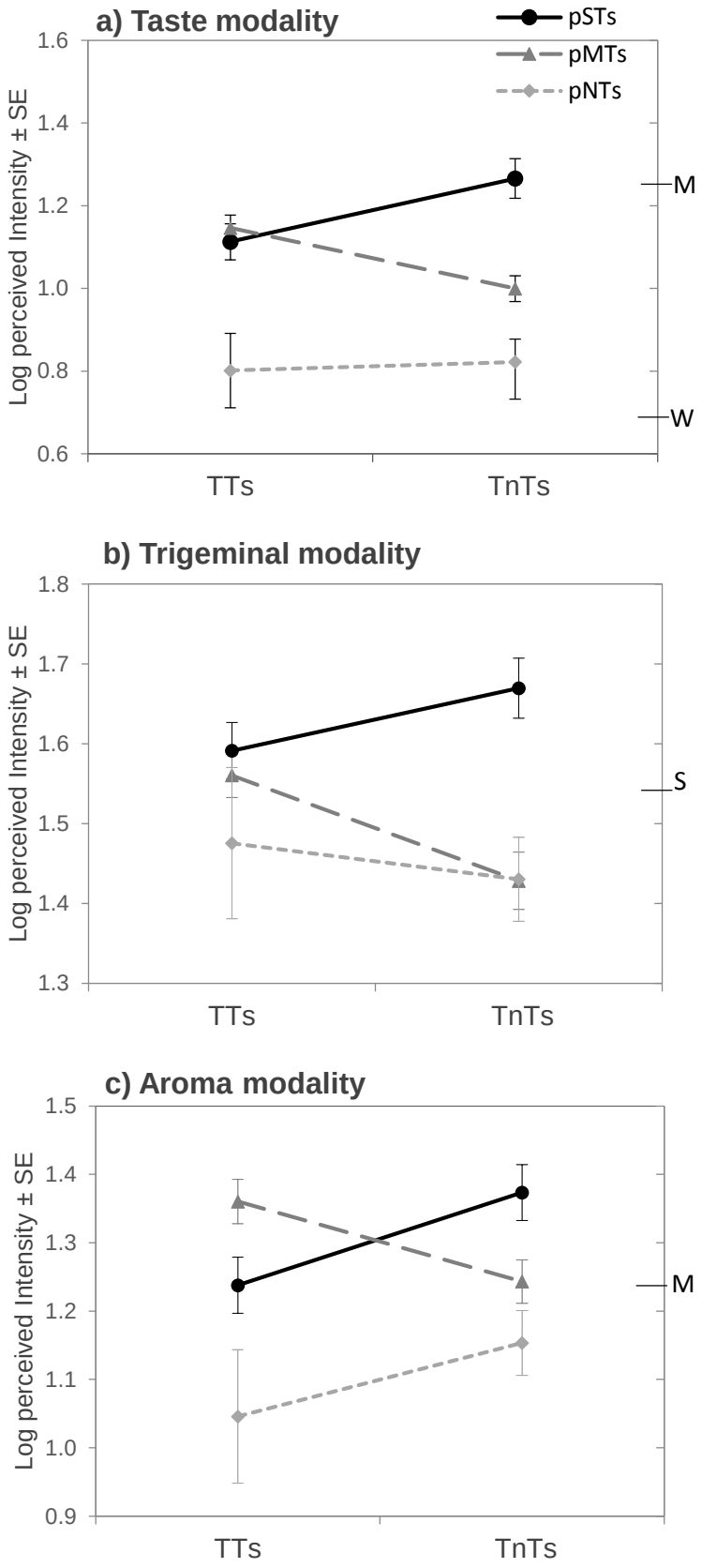

Figure 3-7: TTS*PTS Interaction plot for suprathreshold ratings on each modality: a) Taste modality, b) Trigeminal modality, c) Aroma modality. *Data represents log mean intensity ± SE. (W - Weak, M - Moderate, S - Strong on the gLMS scale).*

Although, no significant interactions were found between TTS and PTS when analysing each individual attribute, the same trend was observed and approached significance for sucrose (p=0.083) (**Figure 3-8**).

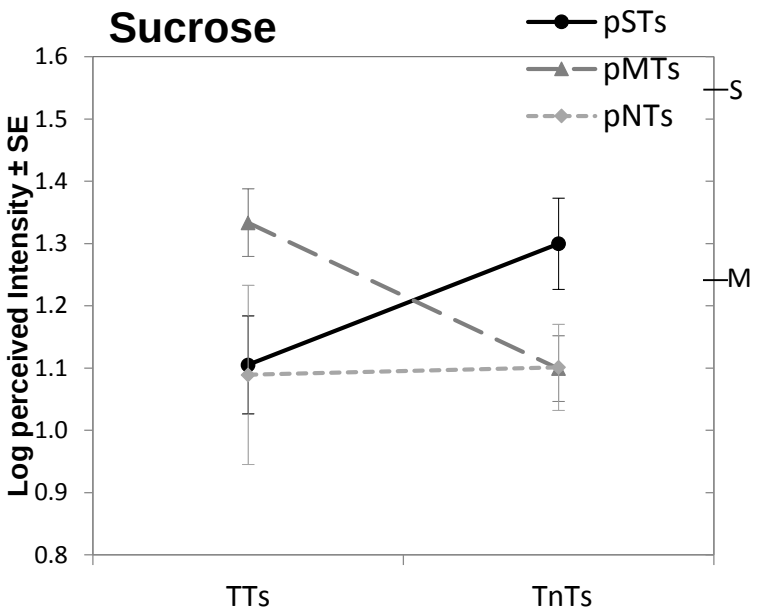

Figure 3-8: Interaction plot of TTS and PTS on suprathreshold ratings on sweet attribute (sucrose). *Data represents log mean intensity ± SE. (M - Moderate, S - Strong on the gLMS scale).*

3.3.8. Further Consideration

The above observations prompted a more in-depth analysis looking at TTS effects within each separate PTS group. Within the pSTs, no significant differences in intensity measures between TTs and TnTs were found for any oronasal stimuli, although a trend that TTs rated most oral stimuli lower than TnTs was observed. In pMTs, the trend was the opposite such that TTs rated stimulus intensities higher than TnTs. Indeed, the ratings of sucrose (low, medium and high), caffeine (low), warming and cooling were found to be significantly more intense for TTs ($p<0.05$). Results of perceived intensity for low and high concentrations of each stimulus are presented in **Figure 3-9**.

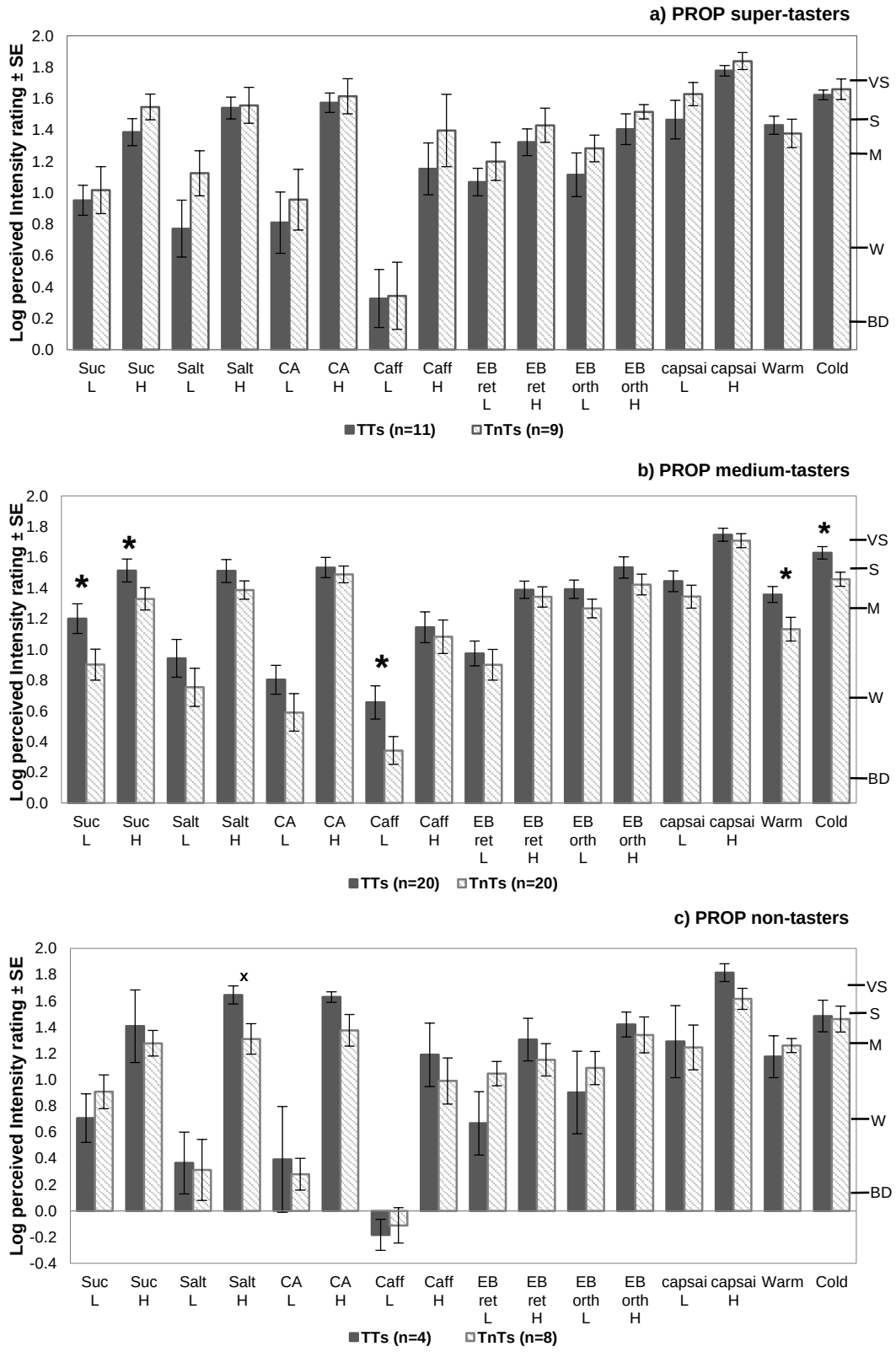

Figure 3-9: Effect of TTS on suprathreshold intensity ratings for each PTS group (a. super-tasters, b. medium-tasters and c. non-tasters). *Data represent log mean intensity ± SE. * indicates p<0.05, ^{x} p<0.1. (BD - barely detectable, W - weak, M - moderate, S - Strong, VS - Very strong on the gLMS scale). (L - low; H - high; ret - retronasal; orth - orthonasal; Suc - sucrose; Salt - sodium chloride; CA - citric acid; Caff - caffeine; EB - ethyl butyrate; Capsai - capsaicin.)*

3.4. DISCUSSION

3.4.1. BETs of tested stimuli and relationship between detection threshold and suprathreshold

In general agreement with previous studies, the BETs of most stimuli tested in the current study were not far from the detection threshold that had been previously determined.

No significant correlations ($p>0.05$) between detection threshold and suprathreshold sensitivity were observed in this study, a disassociation echoed by previous research into lingual tactile (Fucci *et al.*, 1985), PROP (Bartoshuk *et al.*, 1994) and caffeine sensitivity (Keast & Roper, 2007). Indeed, perceptual mechanisms operating at detection threshold and suprathreshold levels are likely to operate differently as there may be multiple taste transduction mechanisms that are activated at different concentrations (Keast & Roper, 2007). Detection simply involves determining if a stimulus is there or not, whereas with perception at suprathreshold levels there is a need to determine the level of intensity which involves higher central gain cortical activity (Fucci *et al.*, 1985). Furthermore, measuring the detection threshold can be challenging, as variation can exist within individuals such as personal health, mood and fatigue (Derntl *et al.*, 2013). The detection threshold itself can also be difficult to measure as by definition, it is 'the level at which detection occurs 50% of the time' (Lawless & Heymann, 2010b). In other words, at detection threshold level, a person will only be able to detect the substance half of the time, which makes the detection threshold calculation more difficult.

3.4.2. Consideration of ASTM E-679 procedure

In this study, ASTM E-679 standard was used, as it is a simple and quick procedure for measuring detection thresholds, which is crucial when collecting data from a large sample of the population. However, the ASTM E-679 detection threshold procedure used in this study has several shortcomings to note. Firstly, the ascending series may cause fatigue or sensory adaptation so that the subjects failed to detect the stimulus presentation above detection threshold level, as was observed during this study. For example, by continuing to collect data across the whole series, it was common to observe that subjects correctly identified the signal at some lower levels, but lost the signal at a higher level. Lawless (2010) argued that it is possible that a highly sensitive subject might detect the signal at lower level, but become adapted, fatigued or over-whelmed at higher level, leading to a wrong response. In addition, this study did not allow re-tasting of the samples, thus introduced a memory effect. 3-AFC tests require the relative information of each sample be stored in the memory until all samples are tasted and then to make a comparison with the memorised sensation before making a decision on the odd sample (Lau *et al.*, 2004). There are two possible theories behind the memory effect, one is memory decay, which is a result of the automatic fading of the memory trace, and the second one is memory interference, which is caused by the disruption of the memory trace by other traces (Lau *et al.*, 2004). Both memory decay and interference are shown to influence discrimination tests, and is thought to be the main factor responsible for higher sensitivity of the 2-AFC over the 3-AFC test (Lau *et al.*, 2004; Rousseau & O'Mahony, 1997; Rousseau *et al.*, 2002). Therefore, it is possible that apart from fatigue and adaptation, memory

effect could also contribute to the incorrect responses after consistent correct identification of the stimulus earlier in the concentration series. Further study should consider using 2-AFC tests in threshold analysis to minimise the memory and fatigue effect. In addition, re-testing around detection threshold level or replicate data collection should also be considered to understand the variation in of subjects' responses.

3.4.3. Effect of TTS on oronasal sensitivity and possible mechanism

No differences were found in detection threshold for any compounds investigated between TTS groups with the exception of sucrose. TTs had significantly lower sucrose thresholds than TnTs, providing further evidence that individual variation in the TRPM5 channel may play a role in observed TTS behaviours. Not finding a significant difference in detection threshold for other stimuli may be either due to the fact that a periphery factor is not the primary cause of thermal taster status, or the reliability of the detection threshold measurement method, as discussed earlier.

In agreement with previous studies (Bajec & Pickering, 2008; Green & George, 2004), a global trend was observed that TTs have a heightened response over TnTs to suprathreshold stimuli. This difference was not significant for individual attribute intensity ratings, apart from temperature. The lack of significance for most sensations could be due to a lack of power, as in this study, replicate data were not obtained. In addition, although training on using the gLMS scale was given, the narrow range covered by the stimuli (between weak '6' and very strong '54') on this absolute scale (0-100) may make subtle differences in

intensity more difficult to determine. Currently, however, no other scale is available to facilitate comparison of absolute differences in intensity.

Talavera *et al.* (2005) proposed that TRPM5 might contribute to the phenomenon of 'thermal taste'. They found that increasing temperature from 15 to 35 ℃ could markedly enhance sweet responses, however, no such increase was observed for MSG, HCl, NaCl or quinine hydrochloride. The data in this study and previous studies (Bajec & Pickering, 2008; Green & George, 2004) have observed that TTs have a global increased intensity perception of taste and trigeminal stimuli, rather than sweetness itself. Hence, the TRPM5 channel may not be the only factor that contributes to thermal taster status.

A recent fMRI study investigating cortical responses to a carbonated sweet aqueous stimulus revealed that TTs have a significant increase in several areas of the brain including the somatosensory cortex, in comparison to thermal non-tasters (Clark, 2011). The observations in this chapter found that TTs have globally significantly higher ratings at a suprathreshold level which was mirrored for both taste and trigeminal modalities and was a trend maintained for each individual attribute stimulus. Clark (2011) hypothesised that cross-wiring between the taste and trigeminal nerves at periphery in thermal tasters could cause the 'phantom taste' responses, which was actually stimulated by the trigeminal stimulus (temperature). Similarly this allows both nerves to be activated to a taste stimulus, consequently increasing intensity response and cortical activation. In the current study, no difference in aroma intensity ratings of ethyl butyrate was observed across TTS group. This further

supports the hypothesis, as the co-innervation of taste and trigeminal receptors would have restricted impact on aroma perception.

Another hypothesis is that the advantage of TTs may be happening at cortical level, arise from hyper-connection of gustatory cortex and somatosensory cortex or greater excitability in convergence of gustatory and somatosensory brain regions. The gustatory cortex lies adjacent to the somatosensory cortex (Stanfield, 2012) as shown in **Figure 3-10**, and hyper-connection or hyperactivity may occur on the joint area between gustatory and somatosensory cortices.

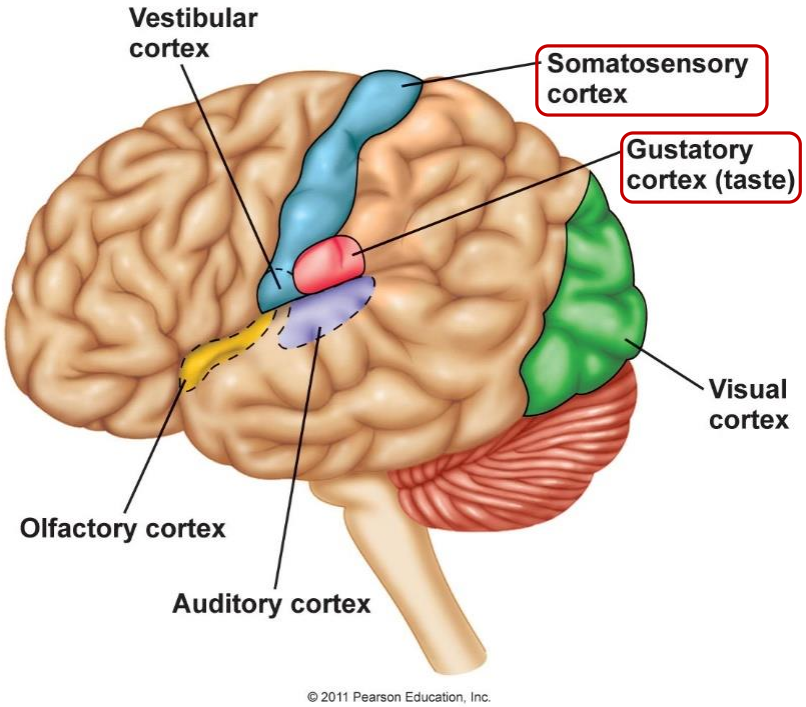

Figure 3-10: Gustatory cortex lies adjacent to somatosensory cortex (Source: Stanfield (2012))**.**

Thus, it is possible that activation of trigeminal stimuli such as temperature could induce activation of taste responses due to the increased connectivity

or hyperactivity on the adjacent regions of the gustatory and somatosensory cortex. Similarly, taste stimulus could also activate the adjacent regions of somatosensory cortex, hence increasing the intensity response (Green & George, 2004; Rouw & Scholte, 2007).

The phenomenon of 'thermal taste' could also be linked to synaesthesia. Synaesthesia is described as 'joining senses' and is a perceptual phenomenon that one sensory modality could induce experience of another sensory modality (Bargary & Mitchell, 2008). There are different types of synaesthesia, such as colour-graphemic, auditory-gustatory, colour-gustatory and so on. For example, a person with synaesthesia may taste sweetness when hearing high-pitched sounds, the expression of auditory-gustatory synaesthesia. The most popular proposed mechanism of synaesthesia is the excess connectivity between brain regions (Bargary & Mitchell, 2008). For example, colour-graphemic synaesthesia is hypothesised as cross-activation between a brain region for recognising graphs or numbers, lying adjacent to a colour-processing region, and hence the colour-grapheme synaesthesia may arise from cross-activation between these adjacent brain regions (Bargary & Mitchell, 2008; Rouw & Scholte, 2007; Wade *et al.*, 2002). Other mechanisms were also proposed to explain the phenomenon of synaesthesia, such as disinhibited feedback from a 'multisensory nexus' (Cohen Kadosh *et al.*, 2007), the hyper-binding mechanism (Esterman *et al.*, 2006). Researchers also suggest that multiple mechanisms may contribute to the phenomenon of synaesthesia, and different neural mechanisms may account for different types of synaesthesia (Hubbard, 2007). Synaesthesia is also found to be genetically associated, as synaesthesia tends to run in families (Brang &

Ramachandranm, 2011). Further research should investigate thermal taster status within family members to determine if thermal taster status, like synaesthesia is genetically associated, and also to further examine if thermal tasters are likely to be a phenomenon within synaesthesia as a result of cross-activation between taste and trigeminal processing.

Green and George (2004) reported that TTs have a higher intensity perception to olfactory stimuli (vanilla sensed both retronasally and orthonasally) and suggested that higher central gain in TTs might occur in the region of the brain where taste and aroma converge. Their work was limited to vanilla whereas here ethyl butyrate was studied. Although no difference in aroma perception between TTS groups was found here, it does not rule out the possibility that TTs may impact on aroma perception. As contradicting results have been observed on the impact of PTS groups for different aroma qualities (e.g. pSTs were more sensitive to diacetyl, but not for phenylethyl methyl ethyl carbamide (PMC)) (Yackinous & Guinard, 2001). Further studies on a wider range of aroma stimuli would be needed to further confirm if TTS have an impact on aroma perception.

3.4.4. Effect of PTS on oronasal sensitivity

This study observed that PROP tasters (both pSTs and pMTs) have increased intensity responses to taste and trigeminal stimuli, which is hypothesised to be linked with number of fungiform papillae. Previous studies suggested that pSTs tend to have higher numbers of fungiform papillae, having more taste and trigeminal nerves, and hence have a high sensitivity to oral stimuli (Bartoshuk *et al.*, 1994). The data presented in this chapter did not only

observe a difference among PTS groups on oral modality, but also observed a difference in aroma modality. Lim *et al.* (2008) have suggested that a central gain mechanism might contribute to the heightened responses of PROP tasters on sensory stimuli which would account for this observation. Recently, rs2274333 polymorphism has been found to contribute to PROP bitter tasting, as well as morphology of fungiform papillae (Melis *et al.*, 2013), therefore, gustin 232274333 genotype was speculated to be the reason behind PROP tasters' heightened sensitivity, which explores in the next chapter.

The data in this study confirmed that TTS and PTS phenotypes are independent, adding to similar evidence from Bajec and Pickering (2008) that TTS and PTS operate via different mechanisms. Recent studies have further demonstrated that there was no significant association between TTS and TAS2R38 genotype, which further suggests PROP and thermal taster status are not genetically associated (Bering, 2012; Bering *et al.*, 2014). However, interactions identified within this study indicate that their relative effects may have a significant impact on perception for certain phenotypic combinations.

A pattern was observed that TTs had, if anything, a slightly weakened response to oronasal stimuli in the pST group, but a heightened response in the pMT group, and no clear effect was seen in the pNT group. It can be hypothesised that these observations may be linked to the differing number of papillae closely linked with PTS (Miller & Reedy, 1990). pSTs tend to have very high numbers of fungiform papillae housing taste and trigeminal receptors and so have a high sensitivity to oral stimuli. The data in this study suggests that any advantage gained by also being a TT does not impact on the

perception of these already supersensitive individuals. However, for pMTs, who have moderate numbers of papillae, having TTs appears to impact on perceived intensity such that there is a considerable gain in perception compared to their TnT counterparts. For pNTs who tend to have very few fungiform papillae, the enhanced impact of TTs on oronasal sensitivity could be restricted as they have will have less gustatory and trigeminal nerve endings. This is a hypothesis and further studies are clearly needed to understand the mechanism behind the relative effects of these different phenotypes on perception.

3.5. CONCLUSION

This study revealed that the increased intensity perceived by these phenotypes at suprathreshold does not mean that they have lower detection thresholds, at least for the range of attributes tested here, and suggests different mechanisms operate at detection and suprathreshold levels.

A trend was observed, which was significant across taste and trigeminal modalities that TTs have an increased perception of oral stimuli. As no differences in perception of aroma stimuli were observed it seems more likely that the mechanism behind increased perception in TTs is either at the periphery or higher cortical level, but that several mechanisms may be involved, including cross-wiring between taste and trigeminal nerves, and hyper-connection/hyperactivity between the gustatory and somatosensory cortices. Of considerable interest were the findings relating to the relative impact of the two independent phenotypes such that it is the perception of pMTs that seems most effected by TTS. More research is required to fully

understand the reasons why but the number of fungiform papillae is one area that may be implicated. However, no fungiform papillae data was available to use in the study in the current chapter, which is one of the limitations.

Interestingly, PROP tasters have a global increased intensity perception to taste, trigeminal and olfactory modalities, which were speculated to be related to gustin rs2274333 polymorphism. The next chapter investigates the relationship between PROP taster status, TAS2R38 and gustin rs2274333 genotypes, as well as fungiform papillae density, in order to understand PROP tasters' supersensitive capability.

4. EXAMINING THE RELATIONSHIP BETWEEN TAS2R38 AND GUSTIN GENOTYPES AND FUNGIFORM PAPILLAE COUNT IN RELATION TO PROP TASTER STATUS, AND THE IMPACT OF THESES FACTORS ON ORONASAL SENSITIVITY

4.1. INTRODUCTION

The well-known variation in sensitivity to the bitter taste of phenylthiocarbamide (PTC) and 6-n-prophlthiouracil (PROP) (Bajec & Pickering, 2008; Bartoshuk, 1979; Bartoshuk *et al.*, 1988; Fox, 1932; Lim *et al.*, 2008) has been found to be partially associated with the bitter receptor gene TAS2R38, located on chromosome 7q (Kim *et al.*, 2003). There are two common haplotypes – PAV or AVI. Varying combination of these two haplotypes result in homozygotes – PAV/PAV and AVI/AVI and heterozygotes – PAV/AVI. PAV/PAV homozygotes demonstrate an increased sensitivity to PROP bitterness, AVI/AVI homozygotes are insensitive to PROP whilst PAV/AVI heterozygotes display intermediate level PROP sensitivity (Duffy *et al.*, 2004). Psychophysical methods including threshold techniques, suprathreshold techniques, and intensity comparisons between PROP and NaCl, are available to classify individuals by PROP taster status (PTS) phenotype as PROP supertasters (pSTs), medium tasters (pMTs) and non-tasters (pNTs) (Bartoshuk *et al.*, 1994; Galindo-Cuspinera *et al.*, 2009; Lim *et al.*, 2008; Tepper *et al.*, 2001; Whissellbuechy, 1990). Variation on the TAS2R38 gene has been demonstrated to explain 55 to 85% of the variations in PROP taste sensitivity (Kim *et al.*, 2003; Prodi *et al.*, 2004; Sandell & Breslin, 2006). However, overlaps between the PTS phenotype and varying TAS2R38

genotypes still occur, for example, AVI homozygotes could be phenotypically classified as PROP medium-tasters rather than non-tasters (Hayes *et al.*, 2008), implying that other factors may also contribute to the expression of this trait (Melis *et al.*, 2013). One potential factor is fungiform papillae (FP) count: previous research has indicated PROP taster status is highly positively correlated with FP count (Bartoshuk *et al.*, 1994). Hayes *et al.* (2008) have further evidenced that FP count could explain the heightened bitterness perceived from PROP in TAS2R38 homozygotes (PAV/PAV and AVI/AVI). As increased taste bud density would generally imply an increase in taste and trigeminal nerve endings, this may result in heightened responses to taste and trigeminal stimuli (Bartoshuk *et al.*, 1994; Essick *et al.*, 2003).

Studies have proved that individuals phenotypically classified as PROP supertasters (pSTs) do not only display heightened sensitivity to PROP bitterness, but also show elevated sensitivity to other tastants (Bajec & Pickering, 2008), trigeminal (Tepper & Nurse, 1997) and olfactory (Pickering *et al.*, 2006) stimuli, as also reported in the previous chapter of this thesis. So far, FP count and a central 'gain' mechanism have been proposed to explain the reason behind PROP tasters' heightened responsiveness in terms of gustatory and trigeminal sensations (Lim *et al.*, 2008). Conflicting results are however presented in the literature concerning the association between FP and PROP sensitivity, some have found an association (Bartoshuk *et al.*, 1994; Essick *et al.*, 2003; Hayes *et al.*, 2008), while a recent study failed to find such relationship (Fischer *et al.*, 2013). In addition, it has also been found that FP count remained independent of TAS2R38 genotype (Hayes *et al.*, 2008).

Recently, another factor, gustin rs2274333 genotype, was found to contribute to the PROP bitterness perception (Calo *et al.*, 2011).

Gustin, also referred to as carbonic anhydrase VI (CA6), is a zinc dependant metallo-proteinase salivary protein that secreted by parotid (Henkin *et al.*, 1975). Evidence of low levels of gustin has been implicated in disorders resulting in distorted or reduced taste and smell functions (Henkin *et al.*, 1999b), further evidencing the contribution of gustin to taste and smell functions.

The gustin gene is located on chromosome 1 (Henkin *et al.*, 1975). The relationship between gustin rs2274333 polymorphism and PROP sensitivity has been studied most over the last 5 years. It has been suggested that AA homozygotes (90Serine) exhibit full functional activity, compared to GG homozygotes (90Glycine) (Melis *et al.*, 2013). The presence of Glycine residue (G allele) may destabilise the gustin active site, reducing zinc binding, and hence affect taste sensitivity (Padiglia *et al.*, 2010). A series of studies from an Italian research group reported that gustin rs2274333 polymorphisms contribute to both PROP detection threshold and PROP intensity ratings, where gustin AA homozygotes had higher PROP sensitivity, compared to the other two gustin genotypes (AG and GG) (Calo *et al.*, 2011; Melis *et al.*, 2013; Padiglia *et al.*, 2010). The gustin rs2274333 polymorphism has also been linked with FP count and morphology. Gustin GG homozygotes were shown to have less fungiform papillae, with larger sized FP and greater variation in shape and distortion, in comparison to AA homozygotes (Melis *et al.*, 2013). The findings regarding the association between gustin (rs2274333)

polymorphism, PROP tasting and FP density, suggest gustin may be linked to PROP tasters' increased perception of oronasal sensations, as well as categorisation of phenotypic PROP taster status (PTS). The research reported in this chapter aimed to:

- Examine the relationship between PTS phenotype and TAS2R38 genotype, and PTS phenotype and gustin rs2274333 genotype.
- Examine the relationship between FP count and PTS phenotype, TAS2R38 and gustin rs2274333 genotypes.
- Confirm previous researchers' findings concerning PROP tasters' heightened sensitivity to oronasal stimuli.
- Determine the impact of TAS2R38 genotype, gustin genotype, fungiform papillae count and PROP intensity perception on oronasal sensitivity.

4.2. MATERIALS AND METHODS

Two experiments were conducted. The first experiment investigated the relationship between gustin rs2274333 genotype, TAS2R38 genotype and PTS phenotype. The second experiment evaluated the relationship between these same factors and FP count, and the effect of all these factors on oronasal sensitivity.

4.2.1. Experiment 1

4.2.1.1. Subjects

91 subjects (57 females, 34 males, age ranged 19 to 67yrs) were recruited from the consumer database at Sensory Dimensions Ltd (Nottingham, UK)

and students and staff at the University of Nottingham. The study was approved by the University of Nottingham Medical School Ethics Committee and informed written consent was obtained from all participants. A small cash incentive was given for participation in the study.

4.2.1.2. Genotyping of subjects

A kit containing buccal swab (Isohelix SK1 Buccal Swabs SK-1S) and silica gel capsule (Isohelix Dri-capsules) was provided for collecting the buccal cell samples. Subjects were asked to take the swab out from the tube, put it in their mouth, and rub firmly against the inside of their cheek. After sampling, subjects were asked to snap the shaft just above the swab head, place it into the tube provided, add a capsule inside (on the top of the swab head), and then finally seal the tube securely with the cap. Subjects were given a sticker with an ID number to stick on their buccal swab tube in order to retain anonymity when sending to the external company (LGC Genomics, Herts, UK) for genotyping. Subjects were genotyped for three single nucleotide polymorphisms (SNPs) of TAS2R38 locus at base pairs 145 (C/G), 785 (C/T), and 886 (C/T), and for gustin (CA6) polymorphism rs2274333 (A/G). The regions were amplified by PCR and sequenced. All swabs were destroyed after analysis.

4.2.1.3. PROP bitterness and PTS phenotype classification

PROP intensity data collected in the study presented in Chapter 2 was used in this investigation. Chapter 2 outlines the procedure used for its collection (Section 2.2.2.2) and the method for PROP taster status phenotype classification (Section 2.2.2.3).

4.2.1.4. Data Analyses

Cross tabulation and Chi-square tests were used to examine the relationship between the distribution of PROP taster status phenotype and both TAS2R38 genotype gustin rs2274333 genotypes. One-way ANOVA, with Tukey's post hoc tests, where appropriate, were used to determine if TAS2R38 or Gustin rs22274333 genotypes have a significant effect on PROP bitterness intensity rating. In reality, any effects of TAS2R38 and gustin rs2274333 genotypes will occur in parallel and so further one-way ANOVA, with Tukey's post hoc test was used to examine the effect of the different combinations of TAS2R38 and gustin genotypes on PROP bitterness perception.

4.2.2. Experiment 2

4.2.2.1. Subjects

Due to subject availability, of the 91 individuals who took part in the first experiment, only 49 subjects (35 females and 14 males, age ranged 19 to 67yrs) attended a second experiment.

4.2.2.2. Fungiform Papillae Measurement

A procedure for measuring fungiform papillae density was developed following that of Henkin *et al.* (1999b). Subjects were asked to extend their tongue, and a cotton bud, previously dipped in blue food colorant (Dr. Oetker, Germany) was used to stain the anterior tip of the tongue. A reinforcement ring with a 6mm^2 hole (Ryman, UK) was placed on the left side of the tongue. The reinforcement ring was used as a template to standardise a measurement area. Images were captured using a digital camera (Canon EOS 1D) with a Canon EF 24-70mm f/4L IS lens. Three to five images were taken, the clearest one

was selected and downloaded to a computer. In order to ensure FP were counted in the same area among subjects, GIPM software (version 2.8, The GIMP Development Team) was used to mark the area in which the papillae were to be counted. To do this, an outlined circle was drawn the same size as the reinforcement ring that had been placed on the tongue. This outlined circle was then placed on the left side of the middle line toward to the very tip of the tongue on the photograph. The marked circle was then zoomed in for counting FP by eye (**Figure 4-1**).

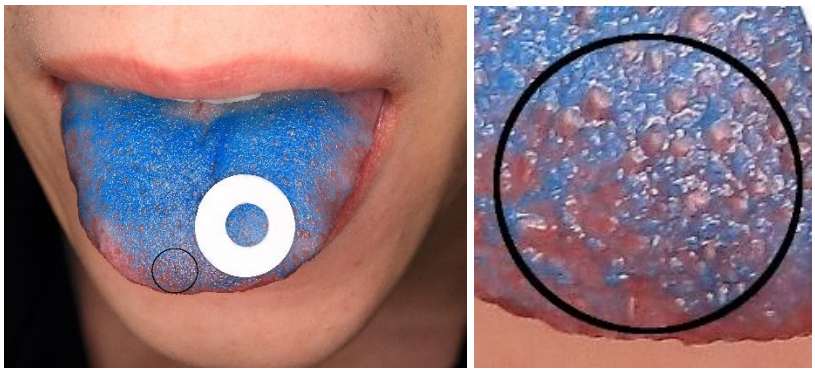

Figure 4-1: The left image shows an image of entire tongue, including the reinforcement ring as a template, the black circle is the marked circle for FP counting. The right image is the cropped and magnified area of the same image to help counting by eyes.

4.2.2.3. Intensity measurement of oronasal stimuli

Data concerning perceived intensity of oronasal stimuli that was collected eight months earlier in the study reported in Chapter 3 was used in the analysis in this chapter and is referred to as ‘replicate 1’ in this study. This was included as it provided an opportunity to investigate the consistency of intensity measurements over time. 49 subjects who attended the previous study were invited back to re-test their oronasal sensitivity to the same stimuli. Two replicates of perceived intensity were obtained on the same day following the

same procedure described in section 3.2.3, and are referred to as replicates 2 and 3 in this chapter. Two concentrations (low and high) of each stimulus were used. Thus, replicate 1 and replicates 2/3 were collected on different days, and replicate 2 and 3 were collected on the same day. In order to avoid strong carry-over effect of spiciness, capsaicin samples were always tested at the end of the session, however presentation of all other samples were randomised within each replicate. Data were collected on computerised gLMS scales in individual sensory booths (Compusense Five 5.4, Canada).

4.2.2.4. Data Analysis

One-way ANOVA, with post hoc Tukey's tests, where appropriate, were used to determine the impact of PTS phenotype, TAS2R38 and gustin rs2274333 genotypes on fungiform papillae count separately. A Pearson's correlation coefficient was also calculated to examine the relationship between FP count and perceived PROP bitterness. One-way ANOVA was also applied to examine the impact of PTS phenotype group on FP count within each TAS2R38 genotype.

For oronasal sensitivity, one-way ANOVA were performed on perceived intensity ratings of each stimulus to examine if any differences existed between replicates. The effect of PTS phenotype, TAS2R38 and gustin genotypes on perceived intensity of each taste, trigeminal and aroma stimuli were examined separately using one-way ANOVA, followed post hoc Tukey's tests, where appropriate. Pearson's correlation coefficients were calculated to examine the relationship between fungiform papillae count and perceived intensity ratings for each oronasal stimulus. In order to examine the relative

contribution of PROP intensity and FP counts together in predicting intensity ratings of taste, trigeminal and olfactory stimuli. Forward multiple linear regression was applied to predict each stimulus sensitivity using FP count and PROP intensity as predictor variables.

All analyses were performed using SPSS, version 21 (SPSS IBM, USA) with an α-risk of 0.05 set for all statistical analyses.

4.3 RESULTS

4.3.1. Experiment 1

4.3.1.1 TAS2R38 genotype with PTS phenotype

The results here demonstrated that 24% of the tested subjects were PAV/PAV homozygotes, 26% were AVI/AVI homozygotes and 39% were PAV/AVI heterozygotes, and interestingly 11% were what are referred to as rare genotypes: one PAV/AAV and one AVI/AAV were discovered in the supertaster group (pST), three AVI/AAV and one PAV/AAV were found in medium-taster group (pMT), and three AVI/AAV and one AVI/PVI were found in non-taster group (pNT).

Table 4-1 shows the cross tabulation of TAS2R38 genotype against PTS phenotype. 88.2% of the pNTs were AVI/AVI homozygotes, whereas only 20% and 4.2% of pMT and pST respectively were AVIAVI homozygotes. 50% of pSTs were PAV/PAV homozygotes compared to only 20% of pMTs. No PAV/PAV homozygotes were identified within the pNT phenotype.

Table 4-1: Cross tabulation of TAS2R38 genotype and PTS phenotype

	PTS phenotype						[a]p value
	pSTs		pMTs		pNTs		
TAS2R38 genotype	n	%	n	%	N	%	
PAV/PAV	12	50.0%	10	20.0%	0	0.0%	
PAV/AVI	11	45.8%	22	55.0%	2	11.8%	<0.001
AVI/AVI	1	4.2%	8	20.0%	15	88.2%	

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

Chi-square analysis indicated that TAS2R38 polymorphisms were significantly associated with PTS phenotype ($p<0.001$). However, it is notable that eight individuals with AVI/AVI homozygote were phenotyped pMTs, and one AVI/AVI homozygote individual was phenotyped as a pST. The data here suggest that although TAS2R38 is the dominant factor of PTS phenotype, TAS2R38 genotyping could not fully predict PTS phenotype, indicating other factors have a contribution to PROP sensitivity (Melis *et al.*, 2013). One-way ANOVA on PROP bitterness ratings further confirmed significant differences occur among TAS2R38 genotype ($p<0.001$), with those AVI/AVI genotyped giving significantly lower PROP bitterness ratings compared to the other two genotypes (PAV/PAV and PAV/AVI).

4.3.1.2 Gustin rs2274333 genotype and PTS phenotype

Table 4-2 cross tabulates gustin genotype and PTS phenotype. No significant relationship between gustin genotype and PTS phenotype was observed (Chi-square, $p=0.725$). Although not significant, a trend was observed that gustin AA homozygotes were more frequent (50 & 47.5%) in the PROP taster groups (pST and pMT), compared to the non-taster group (29.4%). One-way ANOVA did not find any significant difference in PROP intensity ratings among gustin

genotypes (p=0.3), further suggesting gustin genotype does not impact on PROP sensitivity.

Table 4-2: Genotype distribution of gustin according to PTS phenotype

	PTS phenotype						
	pSTs		pMTs		pNTs		**[a]p value**
Gustin genotype	n	%	n	%	N	%	
A:A	12	50.0%	19	47.5%	5	29.4%	
A:G	10	41.7%	18	45.0%	10	58.8%	0.725
G:G	2	8.3%	3	7.5%	2	11.8%	

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

To investigate the combined effect of TAS2R38 and gustin genotypes on PROP intensity, one-way ANOVA was performed to examine the relationship between PROP bitterness perception and combinations of TAS2R38 and gustin genotypes. **Figure 4-2** shows the average bitterness intensity perception of the different genotype combinations and significant groupings from the Tukey's post hoc test. Although, not significant, an interesting trend was observed that within TAS2R38 - AVI/AVI group, gustin AA homozygotes were shown to rate PROP bitterness higher, compared to allele G containing individuals. Unfortunately numbers of subjects were low in these groups and caution should be taken when interpreting the data, as there were only two subjects in AVI/AVI-GG group. A larger sample size is needed but the data here raise the possibility that gustin rs2274333 may play a role on PROP bitterness ratings for individuals who are AVI/AVI genotype, but may have restricted impact for those individuals containing PAV haplotype due to their supersensitivity to PROP bitterness.

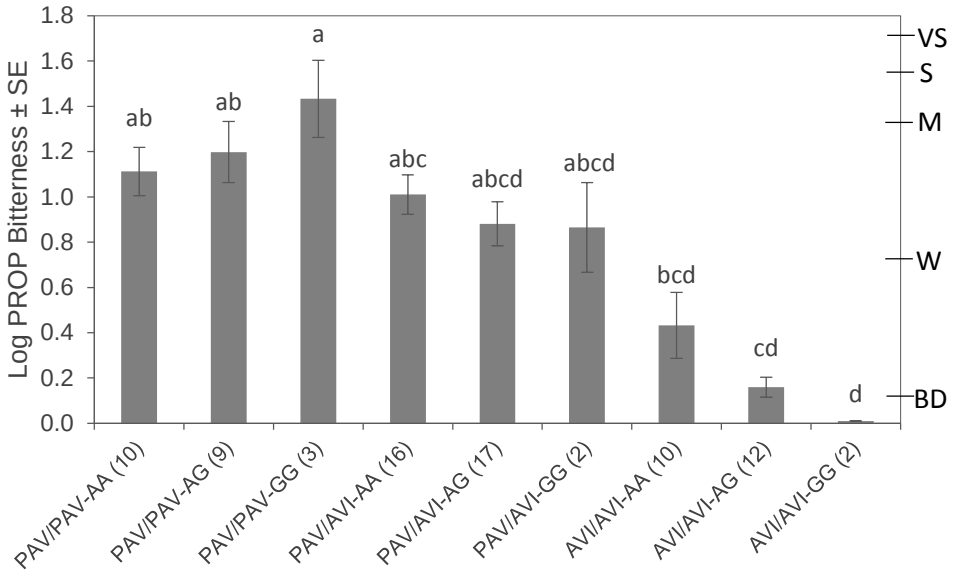

Figure 4-2: PROP Intensity among the combination of TAS2R38 and gustin rs2274333 genotypes*. Bars represent mean ± SE. Means with different letters differed at p<0.05. (BD - barely detectable, W - weak, M - moderate, S - Strong, VS - Very strong on gLMS scale). (number) shows number of subjects in each group.*

4.3.2. Experiment 2

4.3.1.3 Relationship between FP count and PTS phenotype, TAS2R38 and gustin rs2274333 genotypes

The average FP count within 6 mm^2 among these 49 subjects was 22.7, and standard deviation was 9.6, ranged from 5 to 52. Despite a trend indicating pNTs have less FP, the ANOVA showed no significant differences in papillae number among the PTS phenotype groups (p=0.6) (**Figure 4-3 (a)**). The large standard deviation indicates considerable variation in papillae number in each group. Similarly, one-way ANOVA provided no evidence of any link between FP count and either TAS2R38 genotype or gustin rs2274333 genotype (p>0.05), (**Figure 4-3 (b&c)**). However, within gustin genotype, GG homozygous had more FP, compared to the other two genotype groups, which was opposite to a trend previously reported (Melis *et al.*, 2013). It is noteworthy that there were only two subjects in the GG group, and hence a larger sample

size is needed to confirm this finding. Pearson's correlation coefficient further indicated no significant relationship between FP count and PROP intensity ratings (r=0.05, p=0.75).

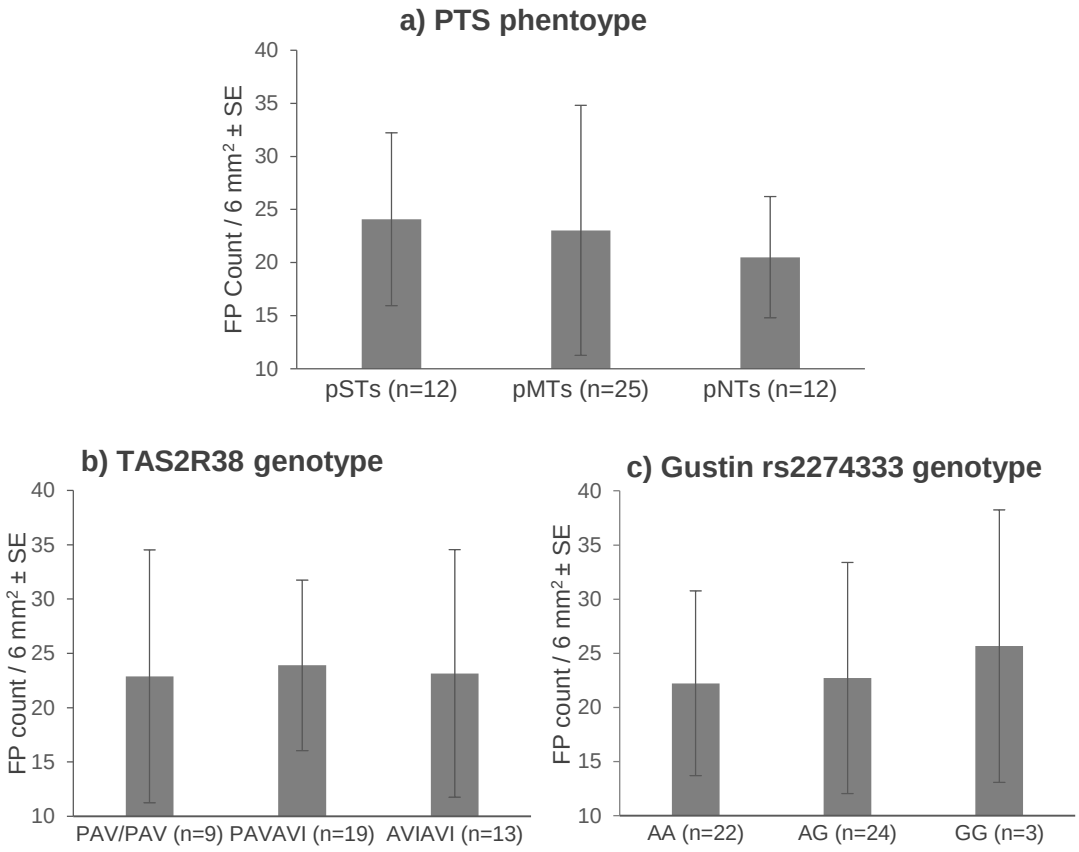

Figure 4-3: Fungiform papillae (FP) count across a) PTS phenotype, b) TAS2R38 genotype and c) Gustin rs2274333 genotype. *Bars represent mean FP count/6 mm² ± SD. n=number of subject in each group.*

4.3.2.1.1 Relationship between FP counts and PTS phenotype within each TAS2R38 genotype

A more in-depth analysis looking at FP count among PTS phenotype groups within each separate TAS2R38 genotype was performed. **Table 4-3** shows the FP counts and associated significance values from ANOVA performed for each TAS2R38 genotype. Although not significant, a trend was seen that

within the PAV/PAV group, individuals who were pST generally had more FP, compared to pMT. In other words, individuals who had less FP may have had reduced sensitivity to PROP bitterness, demoting some PAV/PAV individuals to pMT or pNT phenotypes. In the AVI/AVI group, those phenotypes as pMTs tended to have more FP and this is approaching significance (p=0.1). The increased number of FP could explain why they were phenotyped as pMTs.

Table 4-3: FP counts according to PTS phenotype and TAS2R38 genotype

Genotypic PTS TAS2R38	Phenotypic PTS	N	FP count ± SD	[a]p value
PAV/PAV	pSTs	5	26.8 ± 7.3	0.17
	pMTs	3	20.0 ± 5.6	
	pNTs	1	12	
PAV/AVI	pSTs	6	25 ± 4.7	0.78
	pMTs	12	23.4 ± 13.6	
AVI/AVI	pMTs	6	26.5 ± 8.4	0.10
	pNTs	7	20.3 ± 3.8	

[a]p value associated with one-way ANOVA analysis. N is number of subjects.

4.3.2.2. Oronasal sensitivity and the effect of PTS

Before looking at the effect of PTS phenotype on oronasal sensitivity, the data was analysed to see if ratings were consistent for each stimulus across replicates. As illustrated in **Figure 4-4**, Tukey's post hoc tests revealed that there were no significant differences between replicate 1 and replicate 2 for all stimuli tested here (p>0.05), except EB ortho H, which had reduced between days of testing. Replicates 1 and 2 were conducted on two different days (eight months apart), but the results here indicated that the suprathreshold sensitivity did not change over that time. When looking at replicate 2 and 3, which were conducted on the same day, no significant differences were

observed for the taste and aroma stimuli. A significant difference was observed however for capsaicin at low concentration. Capsaicin has strong irritation and it normally has a long term effect on the tongue. In this study, the intensity ratings of capsaicin (rep 2 & 3) were measured together at the end of the session, which may mean the tongue was desensitised, and therefore, the perceived intensity of any further capsaicin application was weakened. Further studies should consider increasing the break time and perhaps using another type of palate cleanser such as milk, to more effectively remove the sensations of capsaicin from the mouth. This data did, however, provide a good indication of consistent replicate ratings giving confidence to the next analysis concerning the effect of PTS on oronasal sensitivity.

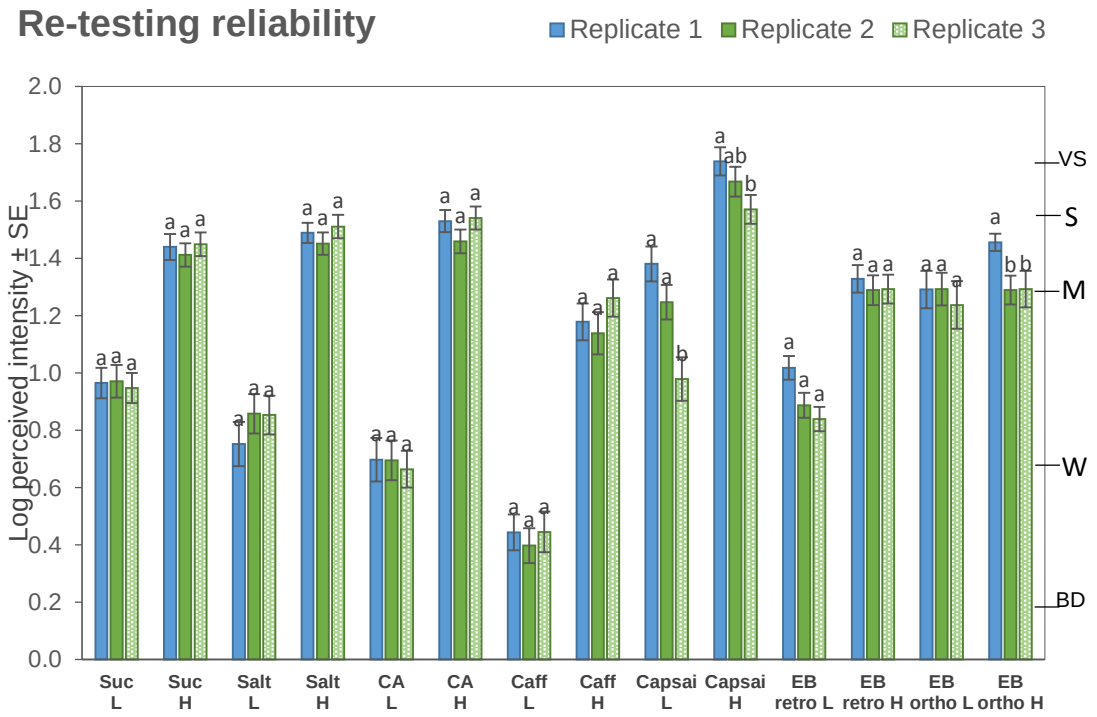

Figure 4-4: Re-testing reliability of oronasal sensitivity. Data represents log perceived intensity ± SE. *(BD - barely detectable, W - weak, M - moderate, S - Strong, on the gLMS scale), (Suc - Sucrose, Salt - Sodium chloride, CA - Citric acid, Caff - Caffeine, Capsai - Capsaicin, EB ret - Ethyl butyrate retronasally, EB ortho - Ethyl butyrate orthonasally, L - Low and H - High).*

Two-way ANOVA (PTS group and replicate) were conducted on the intensity ratings of each stimulus. Results indicated that pST and pMTs rated samples significantly higher than pNTs for most of the stimuli ($p<0.05$). Suc L, Capsaicin L & H were the exception as indicated in **Figure 4-5,** although the trend was still noticeable for Suc L. There were no significant differences between pSTs and pMTs on any of the stimuli tested here. The data for capsaicin was unexpected, as normally tasters rate such trigeminal stimuli higher. However, as described earlier, a significant difference between replicates was found for Capsaicin L & H. Hence, the lack of significance may be due to the strong carry-over effect that diminished the differences among PTS groups.

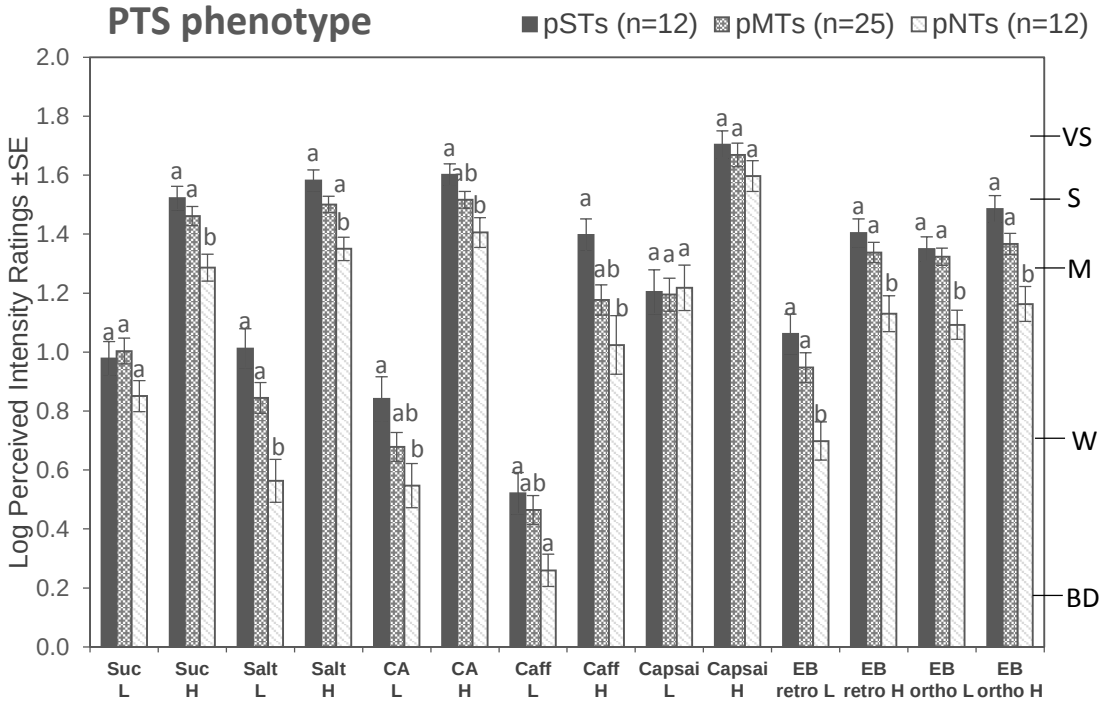

Figure 4-5: PTS phenotype with oronasal sensitivity at suprathreshold level. *Bars represent log perceived intensity ± SE. Different letters within each stimulus differed at p<0.05. (BD - barely detectable, W - weak, M - moderate, S - Strong, on the gLMS scale), (Suc - Sucrose, Salt - Sodium chloride, CA - Citric acid, Caff - Caffeine, Capsai - Capsaicin, EB ret - Ethyl butyrate retronasally, EB ortho - Ethyl butyrate orthonasally, L - Low, H - High).*

4.3.2.3. Impact of genotypes on perceived oronasal intensity

For the TAS2R38 polymorphism, one-way ANOVA revealed that there were significant group differences for Suc H, Salt H, CA H, Capsaicin L & H, EB retro H and EB ortho L & H. As indicated in **Figure 4-6 (a)**, PAV/PAV homozygotes rated the perceived intensity as significantly lower than PAV/AVI individuals for Suc H, Salt H, CA H, EB retro H and EB ortho L &H. PAV/PAV also rated significantly lower than AVI/AVI individuals for CA H, Capsai L & H. In addition, PAV/AVI individuals rated Suc H, Salt H and EB ortho L significantly higher than AVI/AVI.

In general, PAV/PAV homozygotes were shown to give the lowest intensity ratings, whereas PAV/AVI were shown to give the highest intensity ratings. PAV/PAV are genotypically thought to be the supertasters, but the data here did not find this group of people to have higher sensitivity, instead the PAV/PAV genotype group rated lowest at most of the time. The effect of PROP genotype (TAS2R38) on oronasal sensitivity did not match the effect of PTS phenotype on oronasal sensitivity, which indicates that TAS2R38 genotype does not explain PROP tasters' heightened responses to oronasal stimuli and other factors are important.

For the gustin rs2274333 genotype, no significant differences among gustin genotype on intensity measurement for any taste, trigeminal and olfactory stimuli were observed ($p>0.05$), except that gustin GG subjects rated Suc L and EB ortho L significantly higher than gustin AG group ($p<0.05$) (**Figure 4-6 (b)**).

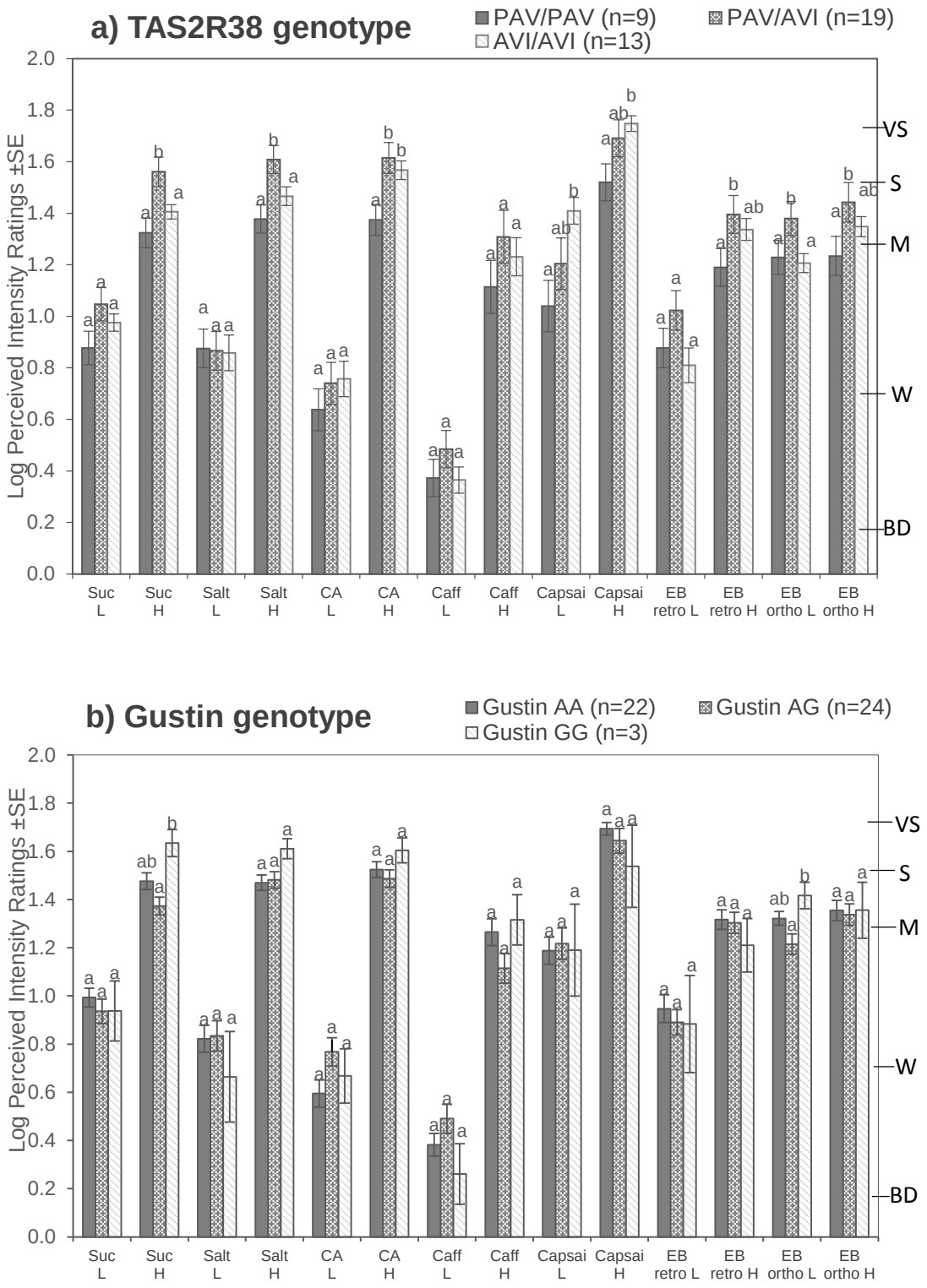

Figure 4-6: a) TAS2R38 genotype and b) Gustin genotype with oronasal sensitivity at suprathreshold level. *Bars represent log perceived intensity ± SE. Different letters within each stimulus differed at p<0.05. (BD - Barely detectable, W - Weak, M - Moderate, S - Strong, on the gLMS scale), (Suc - Sucrose, Salt - Sodium chloride, CA - Citric acid, Caff - Caffeine, Capsai - Capsaicin, EB ret - Ethyl butyrate retronasally, EB ortho - Ethyl butyrate orthonasally, L - Low, H - High).*

4.3.2.4. Impact of fungiform papillae count on oronasal sensitivity

Pearson's correlation coefficients revealed that fungiform papillae counts were significantly associated with the sensitivity of some stimuli: Sucrose L (r=0.39, p=0.006), Sucrose H (r=0.32, p=0.03), Salt H (r=0.33, p=0.02), Citric acid H (r=0.32, p=0.03), EB retro H (r=0.32, p=0.03), EB ortho L (r=0.43, p=0.002), EB ortho H (r=0.34, p=0.02), Capsaicin H(r=0.34, p=0.02), the significant scatter plots are shown in **Figure 4-7**. Although significant, the coefficients are low indicating FP has some contribution but that other factors will also contribute.

4.3.2.5. The combined impact of FP count and PROP intensity ratings together on oronasal sensitivity

Multiple linear regression was used to assess the relative contributions of PROP intensity and FP counts in predicting intensity ratings of taste, trigeminal and aroma stimuli (**Table 4-4**).

Accordingly, PROP intensity was the only significant predictor in the model for Salt L, CA L, Caff L & H and EB retro L stimuli. FP count, however, was the only significant factor that contributed to the sensitivity of Suc L and Capsai H perception. PROP intensity and FP count together significantly contributed to the perceived models for Suc H, Salt H, CA H, EB retro H and EB ortho L and H stimuli. The regression coefficients were low for all models and this indicates that these two factors do have some contributions but there must be other factors acounting for the intensity perception that were not considered in this study.

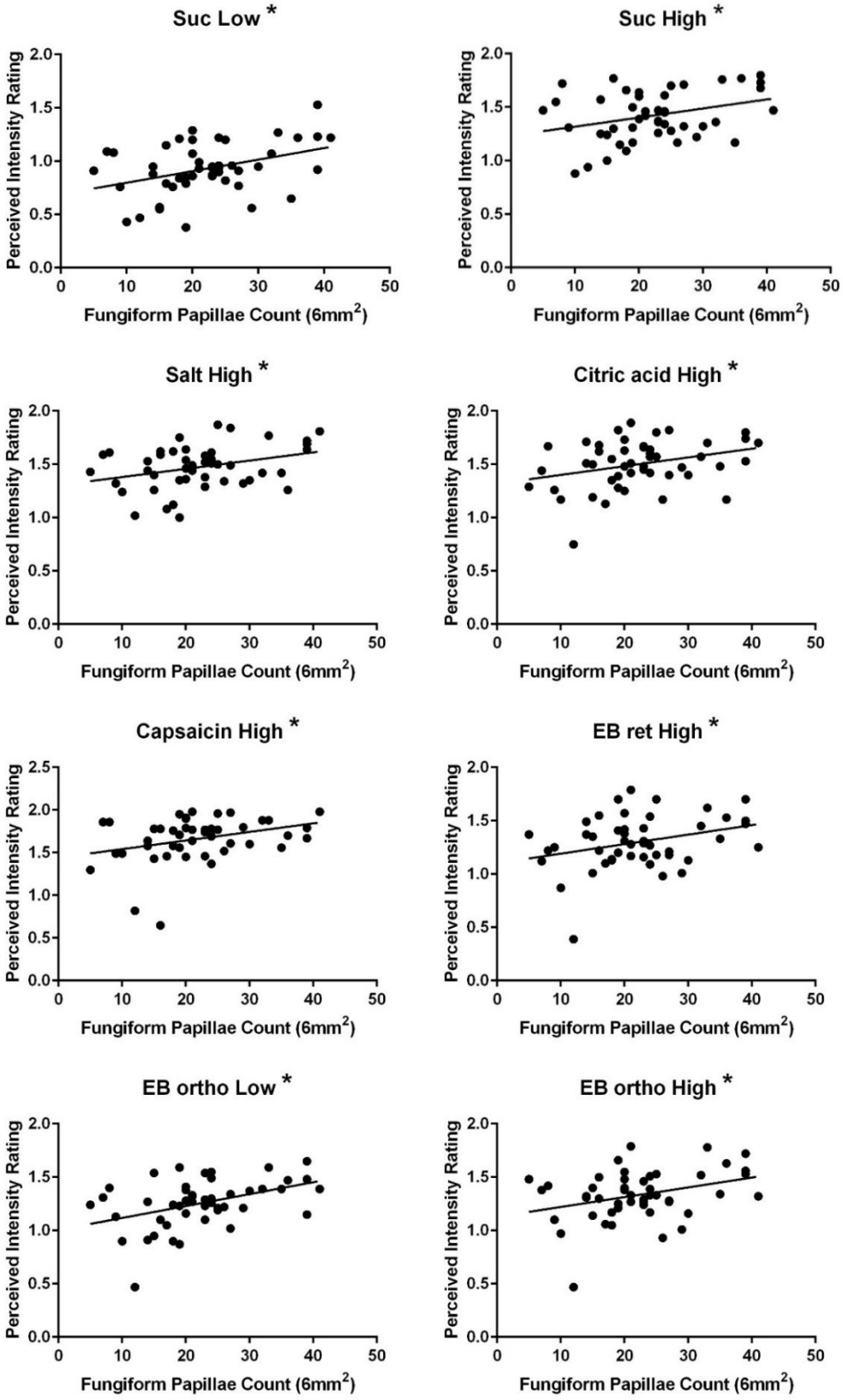

Figure 4-7: Scatter plot shows the bivariate relationship between fungiform papillae count and perceived intensity of oronasal stimulus, only significant ones are showed here. **indicates significantly association at p<0.05.*

Table 4-4: Forward multiple regression models for oronasal sensitivity with independent variables PROP intensity and FP count.

Stimuli	Significant variables in regression model	R	R^2	Adjusted R^2	p value
Sucrose Low	FP count	0.391	0.153	0.115	0.006
Sucrose High	FP count	0.423	0.179	0.143	0.033
	PROP intensity				0.046
Salt Low	PROP intensity	0.451	0.203	0.186	0.001
Salt High	PROP intensity	0.510	0.260	0.227	0.004
	FP count				0.023
Citric acid Low	PROP intensity	0.329	0.108	0.089	0.022
Citric acid High	PROP intensity	0.453	0.189	0.153	0.034
	FP count				0.032
Caffeine Low	PROP intensity	0.259	0.067	0.047	0.076
Caffeine High	PROP intensity	0.429	0.184	0.167	0.002
Capsaicin High	FP count	0.337	0.113	0.094	0.019
EB retro Low	PROP intensity	0.342	0.117	0.098	0.017
EB retro High	PROP intensity	0.342	0.117	0.098	0.027
	FP count				0.035
EB ortho Low	PROP intensity	0.570	0.325	0.295	0.004
	FP count				0.002
EB ortho High	PROP intensity	0.505	0.255	0.222	0.006
	FP count				0.021

4.4. DISCUSSION

4.4.1. PROP sensitivity modulated by TAS2R38 genotype and partly by FP count

As expected, this study confirmed that the TAS2R38 AVI/AVI genotype were shown to have the lowest PROP bitterness responsiveness. However, the

results in the current study failed to find an association between FP count and PROP bitterness intensity ratings ($p>0.05$), indicating FP count may not be the primary factor modulating PROP bitterness.

Nevertheless, the data here suggested that fungiform papillae do contribute to PTS phenotype classification. As individuals who have the AVI/AVI genotype, have two copies of recessive genes, tend to find the PROP tasteless, these individuals are generally genotypically classified as pNTs (Duffy *et al.*, 2004; Snyder, 1931). However this study revealed that some AVI/AVI individuals had been phenotypically classified as pMTs, which has also been found in a previous study (Hayes *et al.*, 2008). It is proposed that this may be due, at least in part, to fungiform papillae count. Within the AVI/AVI group, individuals with higher numbers of FP perceived higher PROP bitterness, and therefore were classified as pMT instead of pNT. A similar effect was also found in the PAV/PAV group, but not in PAV/AVI. One of the possible explanation that FP density did not impact on classification within PAV/AVI group is that the amount of PAV and AVI expression varied significantly between individuals, which is modulated by mRNA (Lipchock *et al.*, 2013). Within the PAV/AVI group, individuals can be PAV-like if they have more PAV mRNA expression or AVI-like behaviour if they have more AVI mRNA (Hayes *et al.*, 2008). The data here suggests that TAS2R38 genotype may not be the only factor controlling PROP bitterness; other factors such as FP density, variation in mRNA expression, as well as environmental factors might also contribute to the perceived PROP bitterness (Bufe *et al.*, 2005; Lipchock *et al.*, 2013).

4.4.2. Gustin rs2274333 is not associated with PTS classification and oronasal sensitivity

In contrast with three previous studies that were based on Italian cohorts (Calo *et al.*, 2011; Melis *et al.*, 2013; Padiglia *et al.*, 2010), this study provides no evidence of the link between gustin rs2274333 polymorphism and PTS phenotype. Previous studies have suggested TAS2R38 and gustin polymorphisms together better explain variation in PROP sensitivity. Previously pSTs were found to have a higher frequency of gustin AA homozygotes, whereas pNTs have a higher frequency of gustin GG homozygotes (Calo *et al.*, 2011; Melis *et al.*, 2013). This was not replicated in the UK population in this study. Interestingly, four other studies have also failed to replicate the results on American (Feeney & Hayes, 2014; Tomassini *et al.*, 2015), Canadian (Bering *et al.*, 2014) and Korean (Peres *et al.*, 2010).

Tomassini *et al.* (2015) suggested that the failure to replicate the findings among studies may be due to population-based differences in the distribution of gustin rs2274333 polymorphism genotypes. When looking at gustin GG homozygotes across different studies, the frequency of GG homozygotes varied greatly, for example, the current study revealed 9% of UK population were gustin GG homozygotes, whereas 14% to 16% were reported in the study based in US with mixed ancestry (Feeney & Hayes, 2014; Tomassini *et al.*, 2015), 20% of the Italian cohorts (Padiglia *et al.*, 2010), and 35% of the Korean cohorts (Peres *et al.*, 2010). This indicates that there are genetic differences based on ethnic origin between studies.

The Italian research group have also found that gustin polymorphism rs2274333 was associated with FP count and morphological changes (Melis

et al., 2013). They hypothesised that gustin rs2274333 polymorphism could affect an individual's PROP sensitivity by modulating the density and morphology of FP. As individuals who carried the gustin AA homozygotes in their study had higher numbers of FP (Melis *et al.*, 2013; Tomassini *et al.*, 2015), having more taste and trigeminal fibres (Whitehead *et al.*, 1985), this was hypothesised to lead to an enhanced response to oral stimuli. The findings in the current study only found that gustin rs2274333 genotype may impact on the perceived PROP bitterness of TAS2R38-AVI/AVI individuals, who had a low sensitivity to PROP bitterness. As the number of gustin GG homozygotes are low in this study, a larger sample size is needed to further confirm these findings. In addition, the results in the current study did not find any evidence of the relationship between gustin polymorphism rs2274333 and FP density, or oronasal sensitivity. A recent study agreed and did not find any evidence of a link between FP density and gustin rs2274333 polymorphism (Feeney & Hayes, 2014).

Not finding a relationship between gustin rs2274333, FP count and oronasal sensitivity does not rule out the possibility that gustin may play a role in moulding FP density and morphology, as well as oronasal sensitivity. A recent study has examined different polymorphisms within the gustin gene, and found that gustin rs3737665 and rs3765964 polymorphisms were associated with NaCl saltiness perception, and rs3737665 and rs2274327 polymorphisms were associated with KCl saltiness (Feeney & Hayes, 2014). In addition, the rs2274327 polymorphism was associated with salivary buffer capacity (Peres *et al.*, 2010), which may impact on oral sensitivity. Further work is needed to understand the relationship between a wider range of polymorphisms in the

gustin gene and FP density & morphology, as well as salivary buffer capacity in order to further investigate if gustin polymorphisms do modulate oronasal sensitivity.

4.4.3. Variables affecting oronasal sensitivity

Supporting previous findings (Bajec & Pickering, 2008; Hayes *et al.*, 2008; Henkin *et al.*, 1999b; Tepper & Nurse, 1998), the current study showed that pSTs and pMTs did not only have the ability to perceive PROP bitterness as more intensely, but also rated the perceived intensity of most taste, trigeminal and olfactory stimuli significantly higher, compared to pNTs. In agreement with Hayes *et al.* (2008), apart from PROP sensitivity, TAS2R38 (PROP genotype) could not explain PROP tasters' general heightened oronasal sensitivity,

Fungiform papillae counts were significantly associated with perceived intensity of some taste, trigeminal and aroma stimuli tested in this study. Fungiform papillae house taste receptors and are associated with trigeminal innervation, and consequently an increased number of fungiform papillae should be expected to associate with increased number of taste receptors and trigeminal nerve endings, thereby, leading to a higher sensitivity to oral stimuli (Bartoshuk *et al.*, 1994; Delwiche *et al.*, 2001). The reason why the fungiform papillae count is associated with sensitivity to olfactory stimuli is currently an unanswered question, but this study at least highlighted that factors affecting fungiform papillae density may also modulate aroma perception. More research is required to further this observed association.

The multiple regression analysis found that FP counts and PROP intensity either independently or together contributed to the predicted model of most

stimuli. However, the coefficient of determination (R^2) is low for all models (0.11 to 0.33), indicating although FP counts and PROP intensity contribute to the perceived intensity perception for some stimuli, and there are other factors that haven't been considered in this study that may also affect sensitivity.

4.4.4. Consideration of fungiform papillae measurement

Conflicting results have been obtained in the literature on the association between FP density and PROP taster status so far (Bartoshuk *et al.*, 1994; Fischer *et al.*, 2013). To date, a standardised method of measuring fungiform papillae density has not been not fully established. The most commonly used method is to count the FP numbers within a small area on the tongue tip by capturing an image using a digital camera (Fischer *et al.*, 2013; Shahbake *et al.*, 2005; Zhang *et al.*, 2009), a method also adopted in this study. The reason why the reinforcement ring was not used to calculate the FP is that asking subjects to put the ring at exactly the same location on the tongue is very challenging, hence the ring was used as a template to standardise the measuring area, then moved to the very tip of the tongue by researchers using image software. Apart from this, the method also raises a few other concerns. Firstly, the criteria that researchers use to decide whether or not the pink dot on the tongue is a FP can be subjective, and so far there is no standardised criteria to use for researchers. Secondly, variation occurs on the texture of people's tongue and in the morphology of fungiform papillae, which makes the counting process more difficult (e.g. in **Figure 4-8,** sub1 has uniform FP with little pink bumps, while sub2 has flat shaped FP and sub3 has distorted shaped FP, sub4 has rough tongue texture, and FP is hidden in the bumps). Additionally, variations also occur in the distribution of FP, for example, some

individuals have more FP on their left side rather than right side. Some individual's FP distributed evenly from anterior tip to lateral tip of the tongue, whereas some have most of their FP sited on the anterior tip of the tongue and hardly any on their lateral of the tongue.

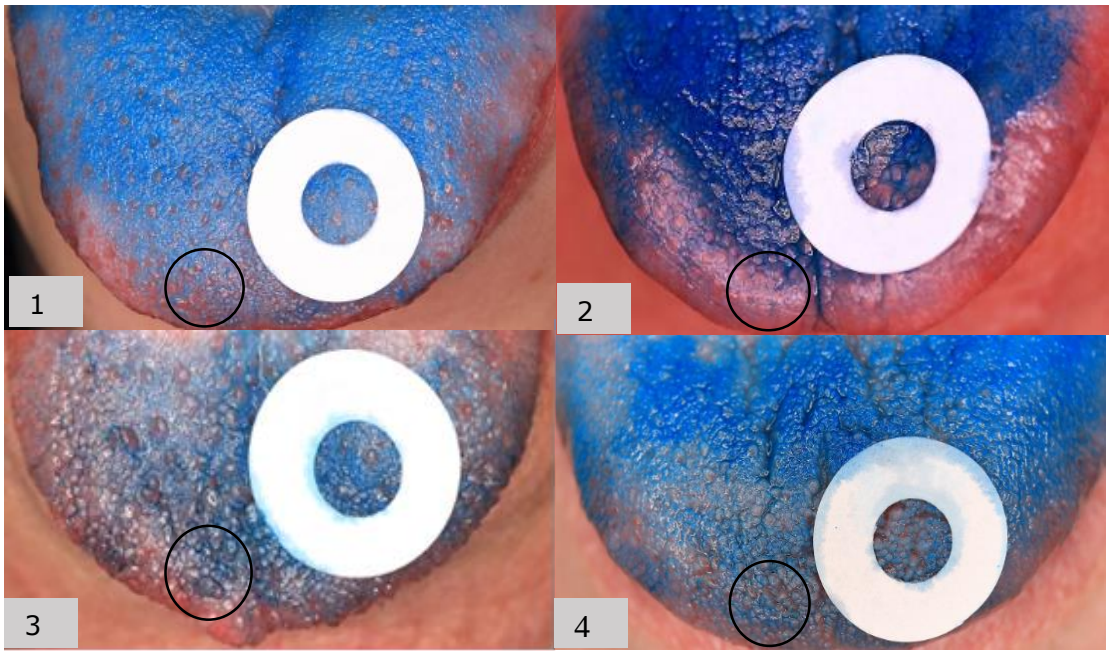

Figure 4-8: Examples of morphology of fungiform papillae among different people. *The circle indicated area that was used for counting fungiform papillae in this study.*

It is reasonable to consider that whether measuring the number of fungiform papillae within a small area on the anterior tongue tip is sufficient enough for predicting oral sensitivity. Further studies looking at fungiform papillae density on the entire tongue, rather than within a restricted area, as well as examining the morphology of the fungiform papillae, are better at interrogating the relationship between FP and other variables (e.g. PTS, oral sensitivity etc.). Moreover, standardised criteria for counting FP would be needed for enabling results to be comparable across studies. Alternatively, technology could be used such as using the contrast of colour between fungiform papillae and

tongue texture to achieve better identification of fungiform papillae (Rios *et al.*, 2012).

4.5. CONCLUSION

This study confirmed that TAS2R38 polymorphisms are a dominant factor modulating PROP peception. However, FP count also partially modulated PTS phenotype classification, as FP count offered an explanation for the misclassifications in TAS2R38 homozygotes (PAV and AVI), but not heterozygotes.

Unlike previous studies, this study did not find any relationship between gustin rs2274333 and PROP intensity, PTS phenotype, PTS genotype (TAS2R38), and oronasal sensitivity. Thus, gustin rs2274333 could not explain the heightened responsiveness of PROP tasters. Both PROP intensity and FP count were shown to contribute to the perceived oronasal intensity ratings in some extent, but the coefficients were low in all predicted modals, indicating there are other factors that may also affect oronasal sensitivities.

A limitation of this study emerged in the fact that only the rs2274333 polymorphism in gustin was examined, and other studies have now found evidence that other polymorphisms could affect taste sensitivity. Future studies are needed to look at more gustin polymorphisms to test if gustin contributes to the perceived oronasal intensity (including PROP tasting). In addition, as only 7 gustin GG individuals were found in this study, further research with a larger sample size would be needed to balance the numbers in each gustin genotype. Another limitation is that the quantification of fungiform papillae was only applied on a small area of the tongue tip, which

could be different to measuring the total number of fungiform papillae. Also, the morphology of fungiform papillae, such as the diameter of FP was not included in this study. Future studies should look at whether the total number/morphology of fungiform papillae is a better predictor of oral sensitivity.

In the current study, the oral sensitivity at the anterior tongue tip (by applying saturated cotton buds) was measured, where FP are most sited. It would be interesting to further test if PROP tasters' advantages on the anterior tongue tip would extend to the whole oral cavity by investigating whole mouth effects with real food consumption.

In the next chapter, the impact of both TTS and PTS on perceived intensity of sensory attributes in two beverages served at different temperatures under more normal consumption conditions is considered, to examine if the increased sensitivities observed on the anterior tongue of different phenotypes translates into whole mouth consumption experiences.

5. INDIVIDUAL DIFFRENCES AND THE EFFECT OF TEMPERATURE ON PERCEPTION OF WATER and A STRAWBERRY FLAVOURED DRINK

5.1. INTRODUCTION

5.1.1. Impact of temperature on sensory perception

Product temperature has long been considered to be closely related to taste perception and food acceptance. Different products have different preferred intake temperatures, for instance, ice cream is considered most pleasant when eaten cold, while French-fries taste best when consumed warm (Engelen *et al.*, 2003). It is also generally agreed that ice cream tastes sweeter when it is melted, and beer tastes more bitter at room temperature (Talavera *et al.*, 2007). Researchers have been interested in how much, and in what ways, temperature may affect taste sensations for over a century, and various studies have examined thermal effects on both detection threshold and perceived intensity for a range of tastants (Engelen *et al.*, 2003; Green & Frankmann, 1988; Prescott *et al.*, 1993; Schiffman *et al.*, 2000). Paulus and Reisch (1980) reported that the detection thresholds of sweet, salty, bitter and sour stimuli showed a U-shaped dependence with temperature, with lowest thresholds measured between 20 to 40ºC. However, the effect of temperature on suprathreshold taste sensitivity varies among concentrations and taste qualities. At low concentrations, sucrose has repeatedly been demonstrated to gain sweetness as temperature increases (Chang, *et al.*, 2006; Goldstein, 2009; Green & Frankmann, 1987). However, the temperature effect on sweet taste has been shown to progressively diminish as sucrose concentration is increased, and disappears at concentrations above 0.5M (Bartoshuk *et al.*,

1982). Temperature effects have been shown to be different even within the same taste quality. A study examining effects of both warm (50ºC) and cold (6ºC) temperatures on sweet perception with various sugars and sweeteners, revealed significant temperature effects on perceived sweetness for some saccharides, but not all (Schiffman *et al.*, 2000). Besides sweet taste, conflicting results have been reported for other tastants. Cooling the solution was reported to effectively reduce the bitterness of caffeine, but not the saltiness of sodium chloride or sourness of citric acid (Green & Frankmann, 1987). The same research suggested that the temperature of the tongue was the controlling factor that altered taste perception, rather than solution temperature. According to Green and Frankmann (1987), taste-temperature interactions are more likely to be due to a disruption of sensory transduction process than thermally induced changes in the molecular properties of the taste solution. Until now, the mechanisms underlying the effects of temperature on taste perception are unknown. However, thermal sensitive TRP channels might contribute to the phenomenon, as several members of the TRP superfamily function as thermosensors (Gardner & Johnson, 2013). Indeed, TRPM5 has been found to be associated with sweet responses and temperature changes. Evidence has shown that increasing temperature between 15 and 35ºC markedly enhances the gustatory nerve response to sweet compounds (sucrose, glucose, fructose, maltose, saccharin and SC45647) in wild-type mice but not in TRPM5 knockout mice (Talavera *et al.*, 2005), which has also been suggested to be one of the possible mechanism behind thermal taster status. This range of findings appears to point towards

multiple mechanisms contributing to the effects of temperature on taste perception.

5.1.2. Effect of individual variation in sensory perception

In recent years, researchers have started to look at individual differences in temperature perception. Thermal tasters (TTs) were found to perceive temperature intensity as more intense than thermal non-tasters (TnTs) at the anterior tip of the tongue, as well as the left and right edges (Bajec & Pickering, 2008; Cruz & Green, 2000). With PROP taster status, supertasters (pSTs) perceive higher temperature intensity compared to medium (pMTs) and non-tasters (pNTs) (Bajec & Pickering, 2008). In addition to temperature intensity, both thermal tasters and PROP tasters also have shown heightened responses to some taste, trigeminal and olfactory sensations, such as sucrose, citric acid and capsaicin (Bajec & Pickering, 2008; Green & George, 2004).

This then led to a research question concerning whether the impact of temperature on taste perception might be different within taste phenotypes (TTS or PTS). There has been little research conducted investigating taste phenotypes and the interactions between temperature and flavour perception.

As results demonstrated in Chapter 2 and 3, TTs have the ability to perceive ‘phantom taste’ from warming and cooling, as well as perceive taste and trigeminal stimuli more intensely when testing the sensitivity of the anterior tip of the tongue. Additionally, pSTs have shown heightened responsiveness to all three modality stimuli (taste, trigeminal and olfactory). Of further interest is the question that whether increased intensity observed in TTs and PROP

tasters (pST and pMT) when stimuli are applied to specific areas of the tongue still holds during whole mouth consumption of liquid samples.

Bajec and Pickering (2010) examined the influence of TTS and PTS on food liking using self-reported food preference questionnaires, and data have suggested pSTs disliked food categories of bitter, non-cruciferous vegetables and fat, compared to pNTs, whereas pMTs did not differ from both pSTs and pNTs (Bajec & Pickering, 2010).

A number of studies have reported PROP tasters have a lower preference for vegetables than pNTs, especially for cruciferous vegetables, which contain the thiourea moiety (N-C=S) that occurs in both PROP and PTC. Hence, the observed lower preference in PROP tasters has been suggested to link to stronger bitterness perception during vegetable consumption (Turnbull & Matisoo-Smith, 2002). In addition, pNTs were shown to like fatty foods more than pSTs, which was suggested to be related to fungiform papillae density as pSTs have been shown to have higher FP density than the other two groups, which in turn, could produce increased responsiveness to trigeminal sensations (Bajec & Pickering, 2010; Tepper & Nurse, 1997). For thermal taster status, that study revealed that TTs self-reported to dislike bitter, cooked fruit/vegetable and raw fruit, in comparison to TnTs (Bajec & Pickering, 2010). The same research group also investigated the connections between TTS and taste sensitivity and liking on alcoholic beverages such as wine (Pickering, Moyes*, et al.*, 2010) and beer (Pickering, Bartolini*, et al.*, 2010), but no significant differences were found across TTS groups for liking when consuming the alcoholic drink with a rinse and expectorate protocol. Until now,

no research has investigated food liking across TTS groups on non-alcoholic beverages involving real product consumption. Thus, examining if differences existed across TTS groups in terms of overall liking for a drink was of interest in this study.

5.1.3. Consideration of use if the gLMS scale

Many studies have adopted the gLMS scale for collecting perceived intensity data. The top anchor represents the 'strongest imaginable sensation of any kind', which is suggested to represent an equally intense experience for everyone, hence the top anchor was used as a standard maximum. The gLMS scale was designed to make valid across group comparisons, as well as across modality (Bartoshuk*, et al.*, 2004). The strongest imaginable sensation of any kind is commonly described as the strongest pain they had experienced or imagined happen to them such as giving birth for women; however the perceived intensity of flavour and trigeminal sensations during food and beverage consumption would be relatively weak, in comparison with the top anchor of scale. The use of the gLMS scale may, therefore, be limited in its ability to differentiate across samples or groups. It would be interesting to test if a wider physical space offered below 'very strong' on the gLMS scale (named as magnified gLMS scale (mgLMS)) could better discriminate between groups and samples.

Thus the objectives of the study reported in this chapter were to:

1) Investigate if the mgLMS allows better discrimination between samples than the gLMS.
2) Determine the impact of sample temperature on selected sensory properties of water and a strawberry flavoured drink (SFD).

3) Explore the effect of TTS on selected sensory attributes of water and SFD.

4) Explore the impact of PTS on selected sensory properties of water and SFD.

5) Investigate the relative impact of TTS and PTS on the sensory properties of water and SFD.

6) Investigate if TTS and PTS phenotypes affect overall liking of water and a SFD.

5.2. MATERIALS AND METHODS

5.2.1. Subjects

Of subjects previously screened for thermal and PROP taster status (see Chapter 2), 47 subjects (35 females, 12 males, aged between 19 and 67yrs) volunteered to participate in this study. A breakdown of the subjects participating this study according to PTS and TTS is provided in **Table 5-1**.

Table 5-1: Distribution of thermal taster status according to PROP taster status.

		PROP taster status			Total
		pSTs	pMTs	pNTs	
Thermal taster status	TTs	9	10	3	22
	TnTs	7	15	3	25
	Total	16	25	6	47

Data represents number of subjects.

5.2.2. Scale Used and training

Both the generalised labelled magnitude scale (gLMS) and a self-developed magnified labelled generalised magnitude scale (mgLMS) were used to

measure perceived intensity of sensory attributes (temperature, sweetness and strawberry), as shown in **Figure 5-1 (a&b)**. The mgLMS was developed as part of this study having considered that the gLMS might be too wide to enable differentiation across products which fall within a narrow range of intensity perception (most food fall within weak '6' and very strong '54' at the very most) on the absolute (0-100) scale. The mgLMS was designed to physically provide more space between 'no sensation' and 'very strong' on the gLMS scale without affecting the relative positioning of the category labels to that point. The position of descriptors on the mgLMS were thus: very strong (100%), strong (65%), moderate (31%), weak (11%) and barely detectable (3%). The Labelled affective magnitude (LAM) scale was used to collect overall liking (**Figure 5-1 (c)**).

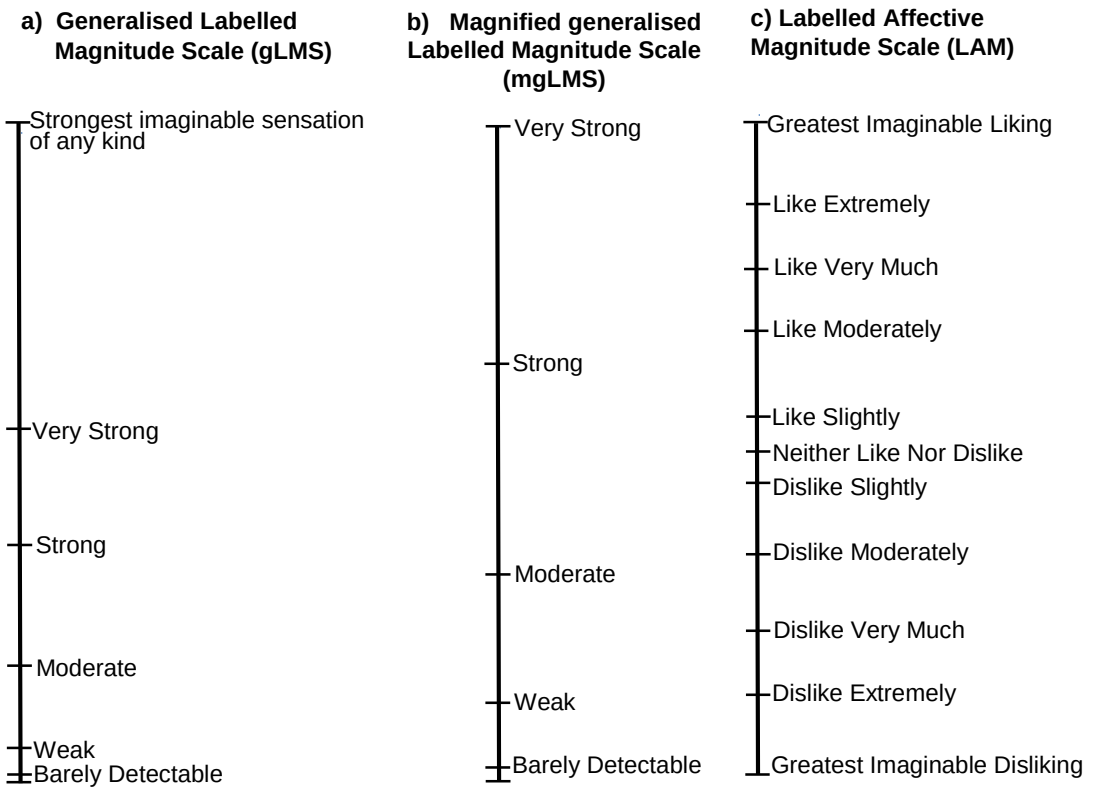

Figure 5-1: a) Generalised labelled magnitude scale (gLMS); b) Magnified generalised labelled magnitude scale (mgLMS) for perceived intensity ratings; c) Labelled affective magnitude scale (LAM) for liking rating.

Prior to data collection, all subjects repeated the training for the use of the gLMS as previously described in Chapter 2 (Section 2.2.1.2). After completing the gLMS scale training, subjects were introduced to the magnified gLMS scale (mgLMS), whereby a reference sheet for mgLMS was provided as shown in **Figure 5-2**. Subjects were told to use their imagination to envisage the circle as a magnify glass, placed on the original gLMS scale between 'no sensation' and 'very strong'. They were told the scale was designed to provide more physical space for rating their perceived intensities. After they clearly understood the nature of the mgLMS scale, they were asked to rate their 15 remembered sensations on the mgLMS scale, in comparison to their positions on the gLMS scale. They were allowed to use anywhere on the scale, even the space above the scale during this practice. They were told that during actual ratings, although only the space between 'no sensation' and 'very strong' were provided, they should always bear in mind there is still the remaining space above the scale. Subjects were provided with their own reference sheet with their 15 remembered sensations ratings (either gLMS or mgLMS) during intensity measurement, where appropriate. Subjects were encouraged to refer back to their reference sheet to enable them to make consistent judgements every time they rated perceived intensity.

5.2.3. Samples

In order to examine the effect of temperature alone on perception, a control sample of water (Evian, France) was first evaluated. This also enabled investigation of whether TTs perceived a 'phantom taste' from drinking hot and cold water. To investigate response to specific sensory properties, a second

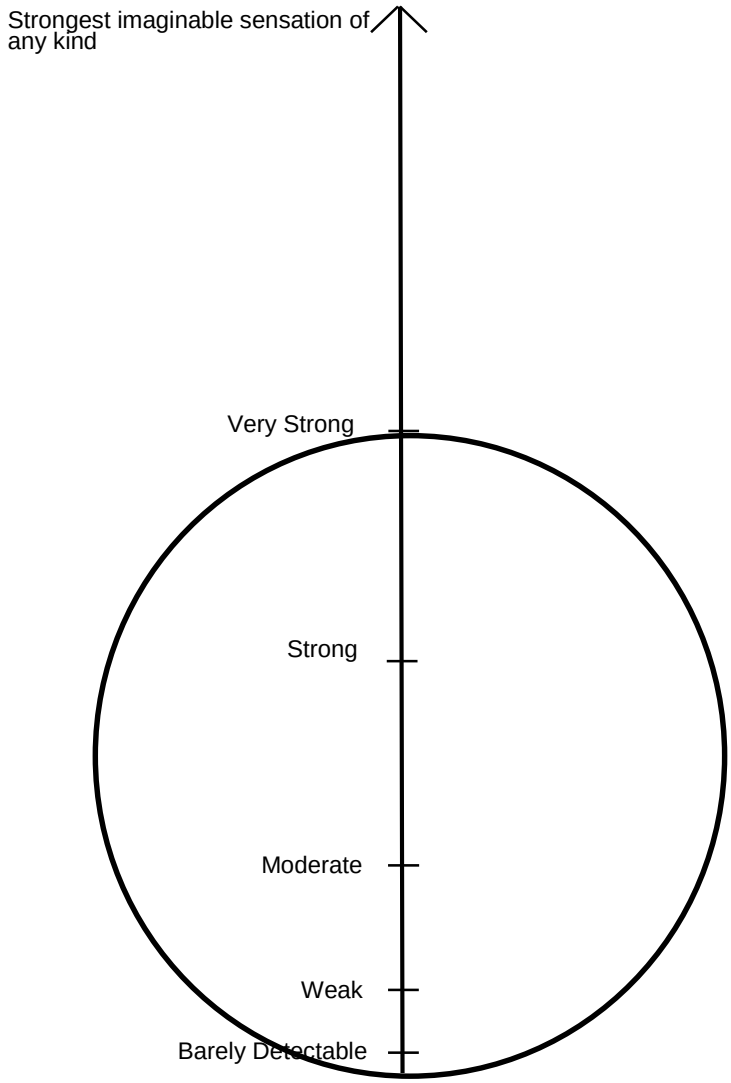

Figure 5-2: Reference sheet of magnified gLMS (mgLMS) scale.

sample, a strawberry flavoured drink (SFD) (Ribena, Suntory, UK) was chosen as it can be consumed either warm or cold. A range of four sample temperatures were selected for serving both the water and SFD samples: warm (48 ± 2ºC), ambient (20 ± 2ºC), cold (5 ± 2ºC) and frozen (-4 ± 2ºC). SFD was prepared based on the manufacturer's instructions of diluting1 part SFD with 4 parts water. Water (10ml) and SFD (20ml) were coded with 3 digit random numbers and served in polystyrene, lidded cups.

To prepare the 'warm' samples, the liquid was stored in Schott bottles after preparation and placed in a water bath at least 1 hour before serving, with the temperature set to 50ºC. A further set of samples was stored at room temperature (20 ± 2 ℃) and a third set was refrigerated at 3 ± 2ºC to create 'ambient' and 'cold' samples respectively. For the 'frozen' samples, ice cubes were made from both water and pre-diluted SFD the day before testing. These ice cubes were then crushed using a Thermomix (Thermomix, Sunningdale, UK) to make 'ice crystal drink' like samples. The samples were prepared immediately prior to serving to avoid melting. The ambient and cold samples were poured into polystyrene cups and covered with a lid before the session started, and were stored in the kitchen and fridge respectively. Warm and frozen samples were poured just before serving and covered with lids to avoid temperature loss.

5.2.4. Procedure

Each subject attended six sessions in total. The first session lasted 1.5 hours, and consisted of scale training and water sample assessment. For water assessment, there were three parts to the water evaluation: The first part was for overall liking assessment using the LAM scale, and the second part was for assessing sensory attribute intensity (temperature, sweet and strawberry) using the gLMS scale. The third part involved assessments using the mgLMS scale. Subjects were divided into two groups: Group A and Group B, the number of TTs and TnTs in each group was balanced. In addition, the order of the gLMS and mgLMS presentation were balanced, with Group A presented with the gLMS scale first and Group B with the mgLMS presented first, as summarised in **Table 5-2**. For the second and third parts, subjects were

instructed to drink the entire sample and then rate their perceived intensity of temperature, sweetness and strawberry (the same attributes as were to be measured for the SFD) on separate scales. At the end of the intensity rating of each sample, an additional question was asked 'did you perceive any other taste sensations other than sweet and strawberry?' Options were listed as 'no other sensations', 'bitter', 'sour', 'salty', 'metallic', 'spicy' and 'others, please specify'. These sensations were chosen because they are those most commonly reported by TTs during the screening sessions. Two replicates were obtained for each sample. Each replicate for each scale contained one set of four samples (warm, ambient, cold and frozen). In order to eliminate order effects, order of sample presentation was randomised within each set of four samples. A minimum inter-stimulus break of 2mins was enforced for all samples, and ambient water (20 ± 2ºC) and crackers were provided to help the temperature of oral cavity to return to normal between samples. It is worth stating that water had no added tastant or volatiles but may have elicited sensory properties. As sweet and strawberry attributes were measured for the SFD, these scales were also offered for consistency in the water trials, however, it is of course accepted that if other attributes were perceived, subjects may have dumped such perception onto the scales offered (Clark & Lawless, 1994; Hort & Hollowood, 2004).

As shown in **Figure 5-2**, the remaining five sessions (Session 2 - 6), each lasting an hour, were for SFD evaluation and assessment was divided into five blocks. The first block was for overall liking measurement, the second block was for perceived temperature intensity using gLMS, the third block was for sweet and strawberry intensity using gLMS, the fourth block was for intensity

Table 5-2: Sensory testing arrangement.

Session	Sample	Subjects	Session plan		
1	Water	Group A	LAM (Overall Liking) - 2 reps	gLMS (temperature, sweet and strawberry) - 2 reps	mLMS (temperature, sweet and strawberry) - 2 reps
		Group B	LAM (Overall Liking) - 2 reps	mLMS (temperature, sweet and strawberry) - 2 reps	gLMS (temperature, sweet and strawberry) - 2 reps
2	SFD	Group A	LAM (Overall Liking) - rep 1	gLMS (temperature) - rep 1	gLMS (sweet and strawberry) - rep1
		Group B	LAM (Overall Liking) - rep 1	mLMS (temperature) - rep1	mLMS (sweet and strawberry) - rep1
3	SFD	Group A	LAM (Overall Liking) - rep 2	mLMS (temperature) - rep1	mLMS (sweet and strawberry) - rep1
		Group B	LAM (Overall Liking) - rep 2	gLMS (temperature) - rep 1	gLMS (sweet and strawberry) - rep 1
4	SFD	Group A	LAM (Overall Liking) - rep 3	gLMS (temperature) - rep 2	gLMS (sweet and strawberry) - rep 2
		Group B	LAM (Overall Liking) - rep 3	mLMS (temperature) - rep 2	mLMS (sweet and strawberry) - rep 2
5	SFD	Group A	mLMS (temperature) - rep2	mLMS (sweet and strawberry) - rep2	gLMS (temperature) - rep 3
		Group B	gLMS (temperature) - rep 2	gLMS (sweet and strawberry) - rep2	mLMS (temperature) - rep 3
6	SFD	Group A	gLMS (sweet and strawberry) - rep 3	mLMS (temperature) - rep 3	mLMS (sweet and strawberry) - rep 3
		Group B	mLMS (sweet and strawberry) - rep 3	gLMS (temperature) - rep 3	gLMS (sweet and strawberry) - rep 3

SFD = Strawberry Flavoured Drink. Black colour represents LAM scale, Green colour represents gLMS scale, and Purple colour represents mLMS scale. Each replicate contains one set of four samples (warm, ambient, cold and frozen).

of temperature using mgLMS, and the last block was for sweet and strawberry intensity using mgLMS. Three replicates were obtained for each SFD sample. Each 1 hour session contained one replicate of three blocks. One replicate for each scale contained one set of four samples (warm, ambient, cold and frozen). Order of sample presentations was randomised within each set of four temperatures.

In the first three sessions of SFD assessment (Session 2 - 4), in order to avoid potential biasing effect of previous intensity ratings (Popper *et al.*, 2004), overall liking was always measured at the beginning. The next two blocks were either for measuring intensity of sensory attributes using gLMS or mgLMS scale, with Group A assessing using the gLMS first, and Group B assessing using the mgLMS first. The last two sessions were for sensory attribute assessment of the remaining replicates using the gLMS and mgLMS. Subjects were instructed to drink the entire 20ml samples provided each time and answer the appropriate questions on the screen. A minimum inter-stimulus break of 5mins was enforced for all samples. Subjects were provided with ambient temperature water (20 ± 2ºC) and crackers for palate cleansing and to help the temperature of oral cavity return to normal between samples.

5.2.5. Data Analysis

All data was $\log_{10}$ transformed for further data analysis. All analysis was performed using SPSS, version 21 (SPSS IBM, USA).

5.2.5.1. Comparison of gLMS and mgLMS

Before comparing ratings on the gLMS and mgLMS, the values from the mgLMS were transformed to equivalent gLMS values. Three factor ANOVA

(scale, sample type, temperature), with interactions were performed on pooled intensity ratings of each attribute (temperature, sweetness and strawberry) to determine if any significant difference or interactions on scale use existed. One-way ANOVA on each attribute intensity for each sample (water/SFD) with temperature as a factor, were then conducted to examine if the mgLMS scale gave better discrimination ability among different serving temperatures than gLMS.

Note that the above analyses revealed no effect of scale used, hence all subsequent data analysis was performed on gLMS data only.

5.2.5.2. Temperature effect on sweetness and strawberry intensity

In order to investigate the influence of sample temperature on perceived taste and strawberry intensities, one-way ANOVA with Tukey's post hoc tests, where appropriate, were performed on intensity ratings of sweet and strawberry attributes, for each beverage type (SFD and water) respectively, with serving temperature as a factor.

5.2.5.3. Effect of TTS on intensity ratings of sensory properties

For water, in order to examine the overall effect of TTS on perceived intensity responses, one-way ANOVA on pooled intensity data combining all four temperatures of each attribute, with TTS as a factor was performed. Subsequently, one-way ANOVA on each individual temperature sample for each attribute were conducted to test if differences existed on the effect of TTS at different serving temperatures. In order to examine the effect of TTS on discrimination ability between sample temperatures, the magnitude of the difference between ratings of ambient samples and samples at the other three

temperatures was calculated respectively. Rating differences of perceived temperature intensity were calculated as (Water_warm/ambient: Warm rating minus ambient rating; Water_cold/ambient: cold rating minus ambient rating; Water_frozen/ambient: frozen rating minus ambient rating). Rating differences were calculated for all three attributes respectively. One-way ANOVA on ratings differences for each attribute for water were then performed with TTS as a factor.

The data analyses indicated above for water were also performed on the SFD data across TTS groups.

5.2.5.3.1. Relationship between 'phantom' taste intensity of water and ratings of taste and trigeminal sensations.

The proposed hypothesis behind thermal taste is cross-wiring between taste and trigeminal nerves in fungiform papillae, and hence, increased co-innervation may result in higher intensities of a 'phantom taste', as well as general heightened perceptual sensitivity. In this study, although the water does not have any added tastant itself, the sweetness intensity of water sample was collected, and sweetness has been reported previously as one of the 'phantom taste' perceived by TTs from thermal stimulation. In order to examine if the intensity of a 'phantom taste' can be used as a marker of general taste and trigeminal sensitivity, Pearson's correlation coefficients were determined to examine the relationship between 'phantom' sweet taste intensity perceived from water and temperature and sweet intensities perceived from SFD within TTs and TnTs respectively.

5.2.5.4. Effect of PTS on intensity ratings of sensory properties

In order to examine the overall effect of PROP taster status on perceived intensities for the water sample, one-way ANOVA, with Tukey's post hoc tests, where appropriate, were performed on pooled data across all 4 sample temperatures of water on each attribute, with PTS as a factor. Subsequent one-way ANOVA, with post hoc tests were carried out for each serving temperature for each attribute to further test the effect of PTS at different serving temperatures. Additionally, one-way ANOVA on rating differences of each attribute were also performed.

The analyses indicated above for water were also performed on the SFD data across PTS groups.

5.2.5.5. Relative effect of TTS and PTS on sensory properties

In order to examine the relative effect of TTS and PTS on perceived intensity, two-way ANOVA, with interactions on pooled data combining all four temperatures for each beverage type (water/SFD) for each attribute were performed to test if any interactions occurred across TTS and PTS. Further two-way ANOVA, with interaction were applied on ratings of each individual sample to examine if the TTS*PTS interactions existed at different serving temperatures.

5.2.5.6. Effect of taste phenotype on overall liking

To investigate the effect of taste phenotype on overall liking, one-way ANOVA, with Tukey's post hoc tests, where appropriate, were used on pooled liking data combining all four temperatures for water and SFD respectively for each taste phenotype (TTS and PTS). Further one-way ANOVA, with Tukey's post

hoc test, where appropriate, were used on liking data (each beverage type, each temperature, and each attribute) to examine if the effect of taste phenotype on liking differed at different serving temperatures. Analyses were performed separately for TTS and PTS.

5.3. RESULTS

5.3.1. Comparison between mgLMS and gLMS scales

3-factor ANOVA indicated that scores on the gLMS were significantly higher than the scores on mgLMS for all sensory attributes (**Table 5-3**). This may be explained by a form of psychological error whereby subjects avoid using the upper end of a scale because there may be a sample that is higher in intensity than the sample that was just tested (Kemp *et al.*, 2009). 'Very strong' was physically positioned on the top of the mgLMS, hence subjects were more likely to avoid 'very strong' at the top of the mgLMS scale, whereas, 'very strong' was positioned in the middle of gLMS scale, and subjects would feel comfortable using its mid position.

Table 5-3: Mean logged intensity by sample type and temperature of each sensory attribute with associated ANOVA significance level and Tukey's post hoc groupings.

Factor		**Temperature Intensity**		**Sweet Intensity**		**Strawberry Intensity**	
		Grand Mean	p value	Grand Mean	p value	Grand Mean	p value
Scale	gLMS	1.28^{a}	0.001	0.96^{a}	0.001	0.89^{a}	0.001
	mgLMS	1.21^{b}		0.81^{b}		0.73^{b}	
Sample	Water	1.24^{a}	0.14	0.32^{a}	0.001	0.16^{a}	0.001
	SFD	1.26^{a}		1.26^{b}		1.24^{b}	
Temperature	Warm	1.26^{ab}	0.001	0.92^{a}	0.049	0.69^{a}	0.182
	Ambient	0.87^{a}		0.89^{a}		0.68^{a}	
	Cold	1.3^{ab}		0.92^{a}		0.71^{a}	
	Frozen	1.56^{c}		0.81^{b}		0.72^{a}	

Data represents log perceived intensity. ^{abc} different superscript letter denotes a significance difference within a column ($p<0.05$).

Consequently, lower ratings after transformation of ratings from mgLMS back to gLMS scale were observed. A closer inspection, looking at the trend of each subjects' ratings on both gLMS and mgLMS revealed similar trends for most subjects.

Further one-way ANOVA, with Tukey's post hoc tests among the 4 serving temperatures for each beverage type, for each attribute, revealed both scales demonstrated the same significant groups among different temperatures for all attributes examined here, as is illustrated in **Table 5-4**. This suggests that the wider range of space between 'very strong' to 'no sensation' on the mgLMS scale, did not facilitate extra discrimination between samples. The data indicates that once subjects had been well trained on the use of gLMS scale, they were able to differentiate between samples, and that the magnified gLMS did not give more discrimination among samples. Hence, in the subsequent analysis, only the data from the standard gLMS were used.

Table 5-4: Mean logged intensity of all temperatures using each scale for each sample by each sensory attribute with associated ANOVA significance level and Tukey's post hoc groupings..

Sample		Temperature Intensity		Sweet Intensity		Strawberry Intensity	
		gLMS	mgLMS	gLMS	mgLMS	gLMS	mgLMS
Water	Warm	1.25^{a}	1.23^{a}	0.41^{a}	0.14^{a}	0.24^{a}	-0.08^{a}
	Ambient	0.88^{b}	0.82^{b}	0.43^{a}	0.15^{a}	0.24^{a}	-0.08^{a}
	Cold	1.32^{a}	1.26^{a}	0.42^{a}	0.18^{a}	0.27^{a}	-0.07^{a}
	Frozen	1.61^{c}	1.51^{c}	0.53^{a}	0.30^{a}	0.50^{b}	0.26^{b}
SFD	Warm	1.31^{a}	1.23^{a}	1.38^{a}	1.31^{a}	1.34^{a}	1.26^{a}
	Ambient	0.93^{b}	0.85^{b}	1.34^{a}	1.25^{a}	1.29^{a}	1.25^{a}
	Cold	1.35^{a}	1.25^{a}	1.36^{a}	1.29^{a}	1.37^{a}	1.29^{a}
	Frozen	1.59^{c}	1.51^{c}	1.13^{b}	1.03^{b}	1.10^{b}	1.03^{b}

Data represents log perceived intensity. ^{abc}different superscript letter denote a significance difference within a column ($p<0.05$).

5.3.2. Overall effect of temperature on perceived sensory properties of water and SFD

As shown in **Figure 5-3 (a&b)**, results indicated that frozen temperature enhanced the sweet and strawberry intensities in the water sample, where strawberry attribute reached significance (p=0.001) (One-way ANOVA with Tukey's post hoc). For SFD sample, the perceived sweet and strawberry intensities of frozen sample were significantly reduced compared to the other three serving temperatures (p<0.001) (**Figure 5-3 (c&d)**). It's noteworthy that unlike the frozen temperature, cold temperature enhanced the sweet and strawberry intensities slightly.

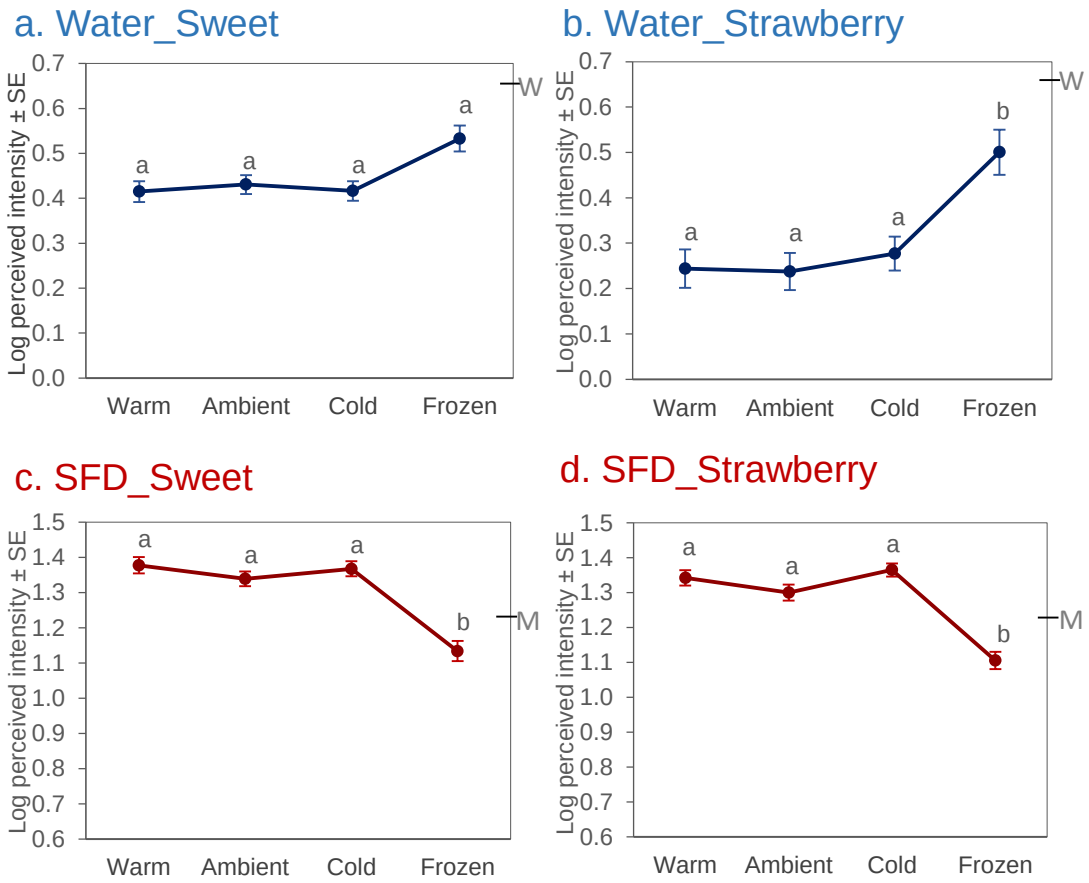

Figure 5-3: Effect of temperature on perceived sensory intensities of both beverages: a) sweetness intensity of water, b) strawberry intensity of water, c) sweetness intensity of strawberry flavoured drink (SFD), d) strawberry intensity of SFD. *Data represents logged mean ± SE. Different letter in each graph denotes significantly difference at p<0.05. (W - weak, M - moderate on the gLMS scale).*

When transforming the log value back to gLMS values (0-100), sweetness was reduced by 9.7 and strawberry was decreased by 10.5. In other words, when sample temperature was cooled down to -4℃, the perceived sweet and strawberry intensities of the SFD drink were effectively reduced by ~10%.

5.3.3. Effect of TTS on perceived sensory attribute intensity of water sample

One-way ANOVA looking at the pooled sample data indicated that the tendency for TTs to rate sweetness intensity higher was approaching significance (p=0.06) and that ratings for strawberry intensity were also significantly higher (p=0.02) than TnTs. However, no significant difference for temperature intensity was observed (p>0.1). To examine temperature measures further, one-way ANOVA were carried out looking at the data for each individual sample, as shown in **Figure 5-4**. No significant differences between TTs and TnTs were observed at any temperatures, but similar trends were observed for sweet (p value ranged 0.16 to 0.68) and strawberry intensities (p value ranged 0.09 to 0.43), with frozen_strawberry approaching significance (p=0.09).

The findings here are interesting as water itself does not have any flavour. The perceived sweetness could be explained by the fact that TTs have the ability to pick up sweetness from rapid warming or cooling during the drinking process. A closer look at the data of ‘other taste sensations’ reported at the end of intensity measurement showed that 13 out of 22 TTs reported they could perceive other taste sensation other than sweet and strawberry for more

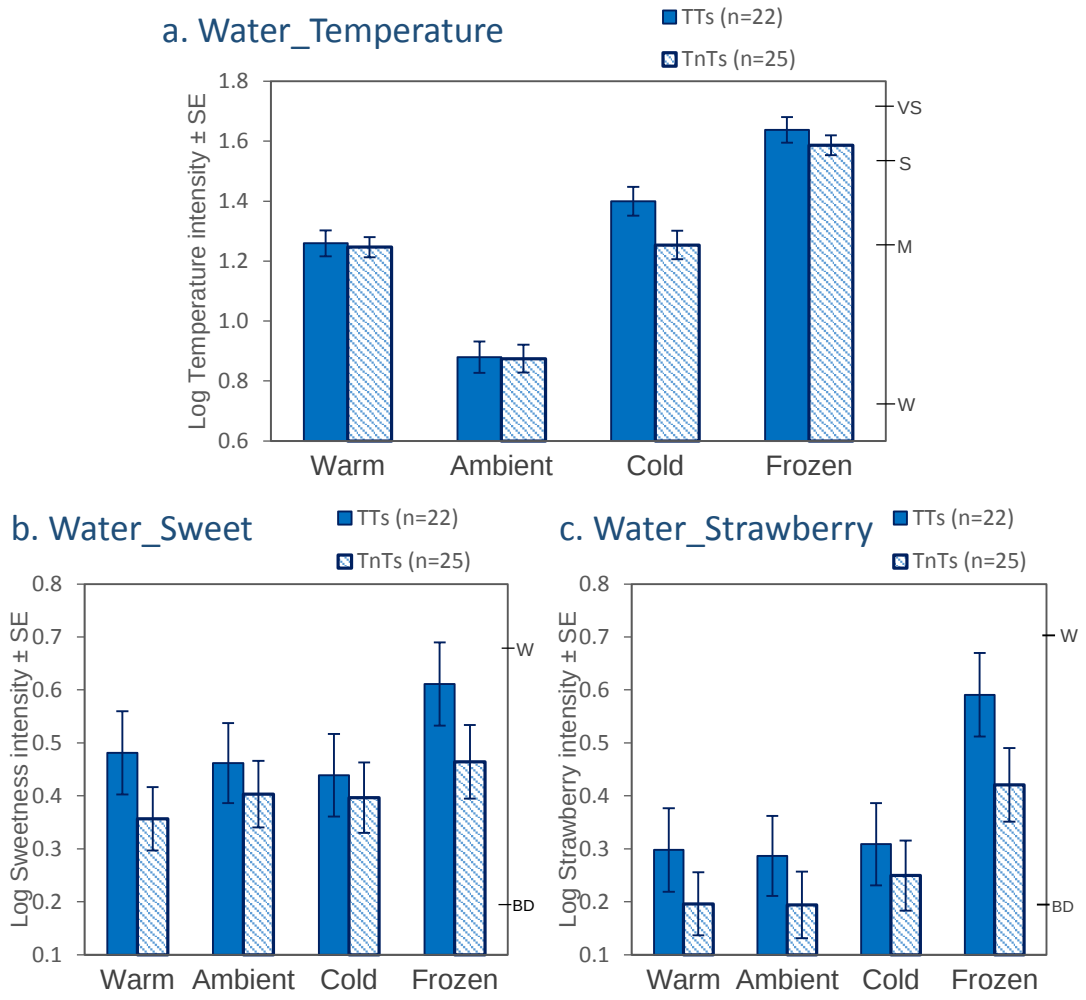

Figure 5-4: Effect of TTS on perceived intensity ratings for water at four temperatures: (a) Temperature ratings, (b) Sweetness ratings, (c) Strawberry ratings. *Data represent log mean intensity ± SE. (BD - Barely detectable, W - Weak, M - Moderate, S - Strong, VS- Very Strong on gLMS scale).*

han half of their total responses. The frequency of other tastes reported is shown in **Figure 5-5**. Other sensations reported were spicy, metallic, bitter, sour and salty. Interestingly, only one out of 25 TnTs reported they could perceive other tastes from water more than half of their total responses. The data here provides additional evidence that TTs have the ability to perceive 'phantom taste' from temperature changes of the tongue but this time induced by beverages not a thermode.

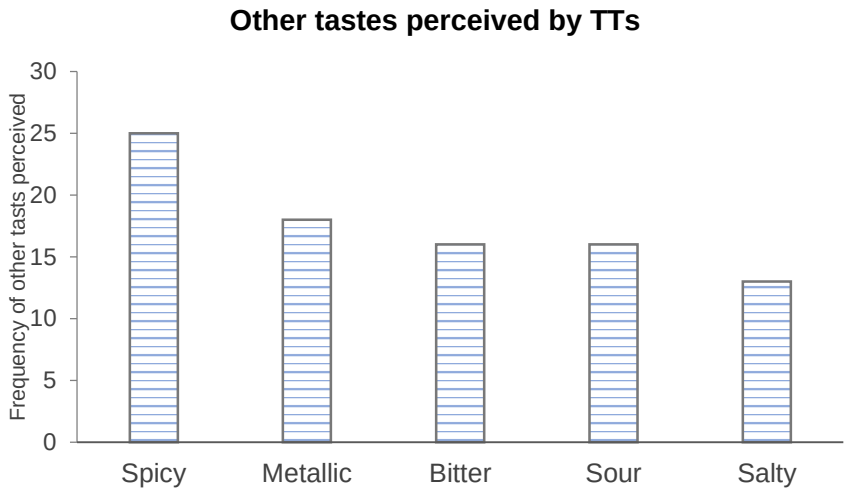

Figure 5-5: Frequency of other taste sensations elicited by water sample for TTs.

5.3.4. Effect of TTS on perceived sensory attribute intensity of SFD

One-way ANOVA on pooled data revealed that there were no significant differences between TTs and TnTs for perceived temperature, sweet and strawberry intensities. Further one-way ANOVA looking at the effect of TTS on each serving temperature confirmed these findings (see **Figure 5-6**).

Further analyses were conducted to look at discrimination ability among temperature, sweet and strawberry intensity based on rating differences between the ambient sample and samples at the other three temperatures for each attribute. Interestingly, in comparison with TnTs, TTs had greater rating differences for perceived temperature intensity. In other words, TTs were more sensitive to temperature changes when the temperature got more intense (either warmer or colder), and when comparisons of both cold/ambient and frozen/ambient reached significance ($p=0.03$), (**Figure 5-7**). No significant differences were observed for rating differences for sweet and strawberry attributes ($p>0.05$).

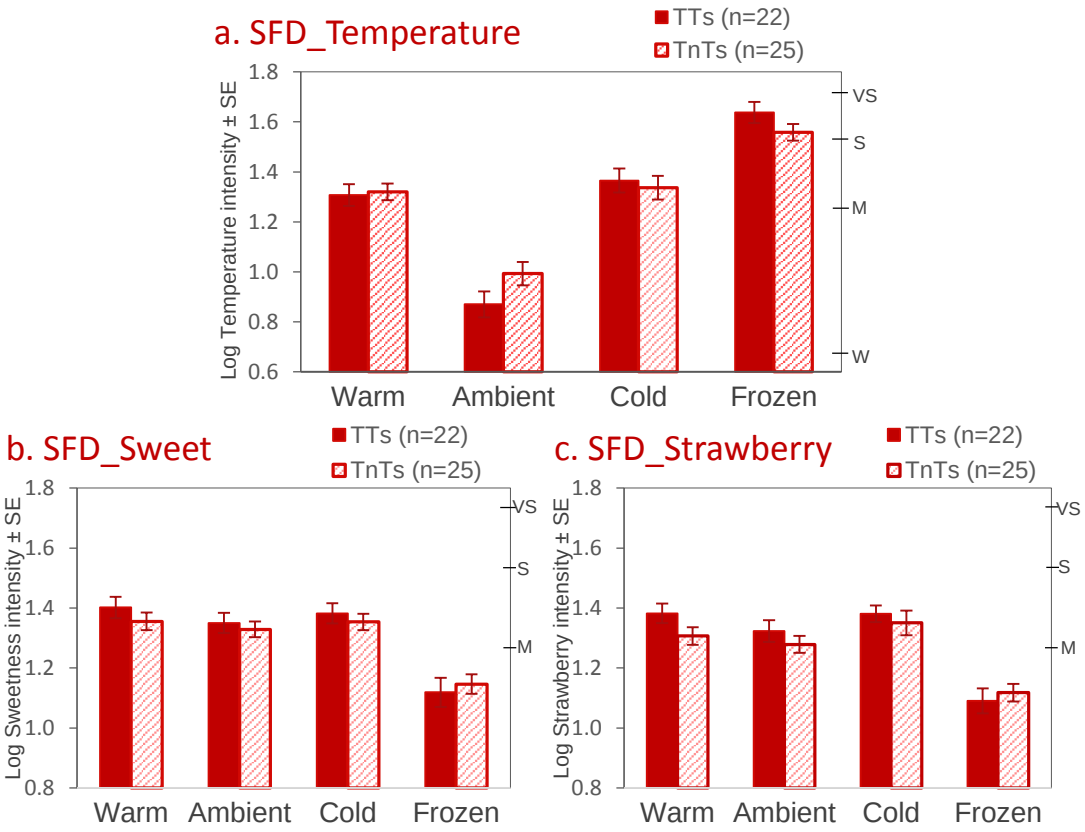

Figure 5-6: The perceived intensity ratings for SFD between TTs and TnTs: a) Temperature ratings, b) Sweetness ratings, c) Strawberry ratings. *Data represent log mean intensity ± SE. (W -Weak, M - Moderate, S - Strong, VS - Very strong on gLMS scale).*

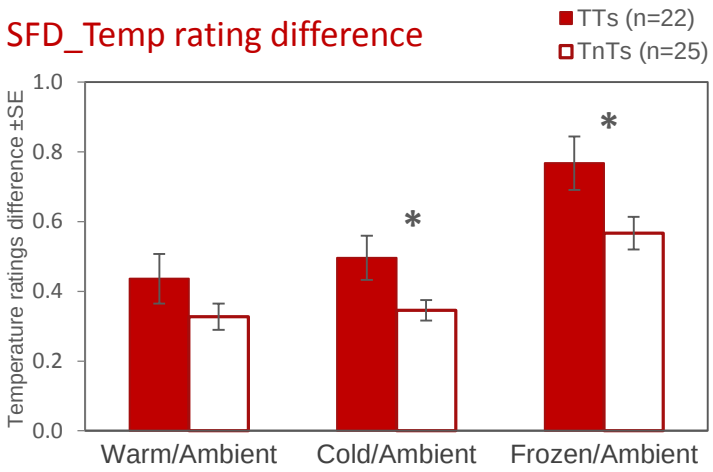

Figure 5-7: Rating difference for temperature intensity between warm and ambient, cold and ambient, and frozen and ambient. *Data represent rating differences of temperature ratings ± SE. * indicate significant different at p<0.05.*

5.3.5. Relationship between 'phantom' sweet taste intensity of water and ratings of taste and trigeminal sensations

As discussed in Chapter 3, the proposed hypothesis for TTS relates to cross-

wiring between taste and trigeminal nerves in fungiform papillae and hence it is likely that several factors (such as FP density, degree of intertwining between taste and trigeminal nerves) may contribute to taste and trigeminal sensitivity in TTs. In order to further explore this possibility, Pearson's correlation coefficients were determined to examine the relationship between the intensity ratings of an assumed 'phantom' sweet taste perceived from the water samples and the intensity of taste and trigeminal attributes perceived from SFD within TTs and TnTs respectively. As can be seen from **Figure 5-8** the intensity of phantom sweetness elicited by water was significantly correlated with temperature intensity perceived in the SFD in TTs (r=0.386, p=0.001), whereas, no significant correlation was found within TnTs group.

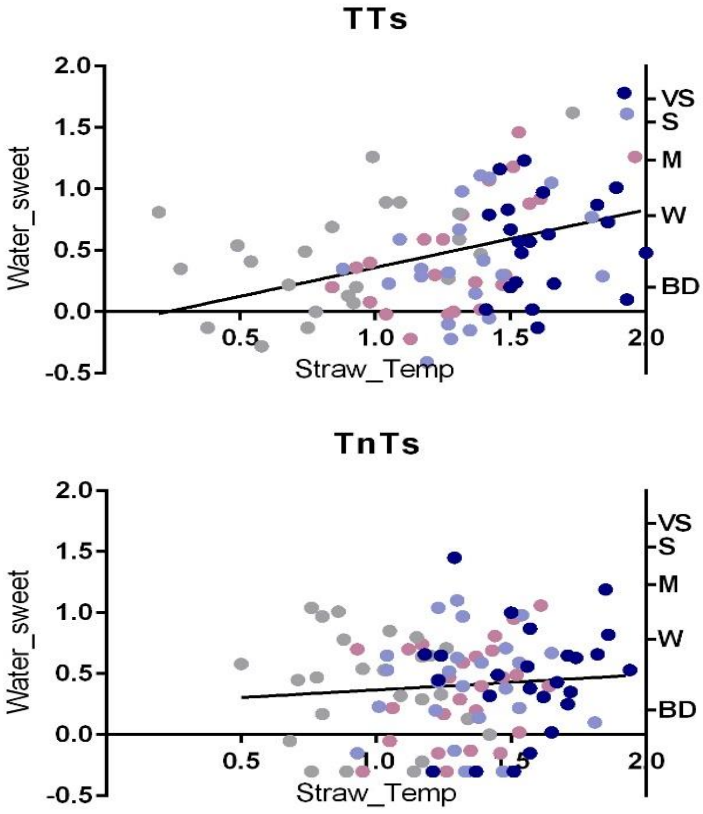

Figure 5-8: Scatter plots for 'phantom' sweet taste of water sample and temperature intensity perceived from SFD within TTs and TnTs respectively. *Pink dots – Warm sample, Grey - Ambient sample, Light blue - Cold sample, Dark blue - Frozen sample*. *(BD-Barely detectable, W - Weak, M - Moderate, S - Strong, VS - Very strong on gLMS scale).*

A closer look at the data within TTs at each serving temperature, revealed that the significant correlation was driven by measures of the warm, ambient and cold samples (r range from 0.47 to 0.63, $p<0.05$), while no significant correlation was found for the frozen sample ($r=0.2$, $p=0.3$). No significant correlation was observed between the intensity of 'phantom' sweet taste in water and sweet intensity perceived from the SFD ($p>0.05$) (**Figure 5-9**).

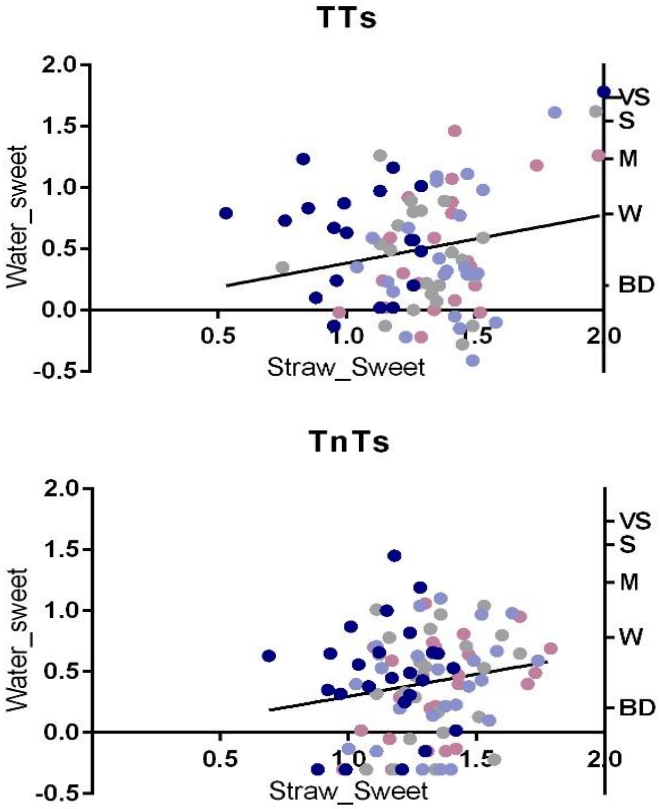

Figure 5-9: Scatter plots for 'phantom' sweet taste in water and sweet intensity perceived from SFD within TTs and TnTs respectively. *Pink dots - Warm sample, Grey - Ambient sample, Light blue - Cold sample, Dark blue - Frozen sample*. *(BD - Barely detectable, W - Weak, M - Moderate, S - Strong, VS - Very strong on gLMS scale).*

5.3.6. Effect of PTS on perceived sensory attribute intensity of water

When looking at pooled data of intensity ratings combined all temperatures of each attribute respectively, one-way ANOVA, with post hoc tests revealed that pMTs rated temperature intensity significantly more intense than pNTs

(p=0.02), but no significant difference was found between pSTs and the other two groups. No significant differences were observed for sweetness and strawberry intensities among PTS groups (p>0.05). Further one-way ANOVA looking at the effect of PTS on each temperature for each attribute, found a trend that pSTs and pMTs rated temperature intensity generally more intense than pNTs at all temperatures, and for frozen water this was close to significance (p=0.07) (**Figure 5-10 (a)**). As expected, no significant differences were observed for both sweetness and strawberry intensity ratings (p>0.05) among PTS groups at any of the temperatures (**Figure 5-10 (b&c)**).

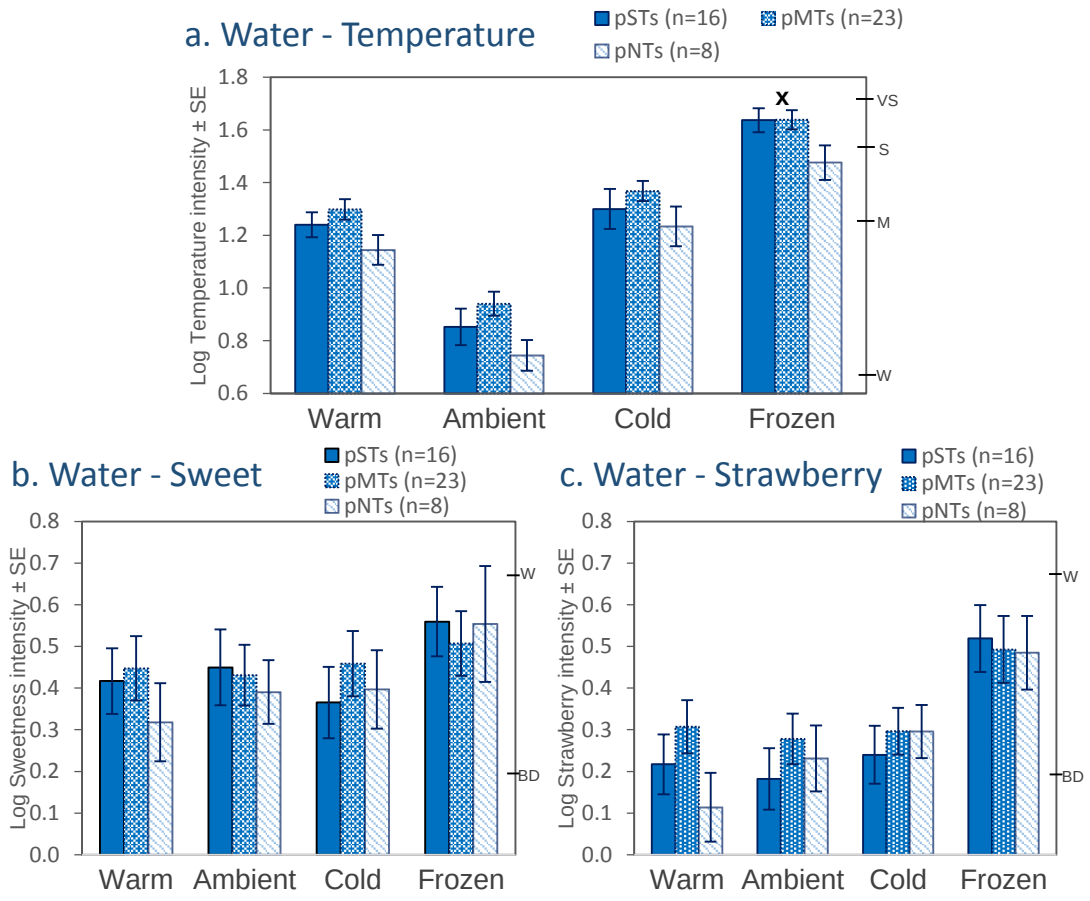

Figure 5-10: Effect of PTS for perceived intensity ratings: a) Temperature ratings; b) Sweetness ratings; c) Strawberry ratings on the gLMS scale. *Data represent log mean intensity ± SE. (BD - Barely detectable, W - Weak, M - Moderate, S - Strong, VS - Very strong on the gLMS scale).* ^{X} *approaching significance at p=0.07.*

5.3.7. Effect of PTS on perceived sensory attribute intensity of SFD

One-way ANOVA, followed post hoc tests on pooled data of intensity ratings (all four temperatures combined) for each attribute, revealed that pSTs and pMTs rated all three attributes (temperature, sweet, strawberry) significantly higher than pNTs ($p<0.001$).

Further analysis using one-way ANOVA, with post hoc tests, where appropriate, looking at each individual serving temperature revealed that pMTs rated temperature intensity for ambient ($p=0.01$) and frozen ($p=0.001$) as significantly more intense than pNTs. In addition, pSTs rated temperature significantly more intense than pNTs for frozen samples. For sweet intensity, pMTs were found to rate sweetness significantly higher than pNTs at ambient temperature ($p=0.04$), and no difference was observed between pSTs and the other two groups. For strawberry intensity, pMTs rated higher than pNTs at warm ($p=0.04$), ambient ($p=0.01$) and frozen ($p=0.02$) temperatures, and two additional samples (ambient and frozen) also rated significantly higher for pSTs than pNTs.

Figure 5-11 shows the ratings and post hoc groupings among PTS groups for temperature (a), sweet (b) and strawberry (c) attributes. As reported earlier, frozen temperature ($-4 \pm 2^{o}C$) could effectively reduce the perceived intensities of sweetness and strawberry for SFD. This study observed that PROP tasters perceived higher temperature intensities than pNTs, however, the increased coldness perceived by PROP tasters did not further reduce perceived sweet and strawberry intensities.

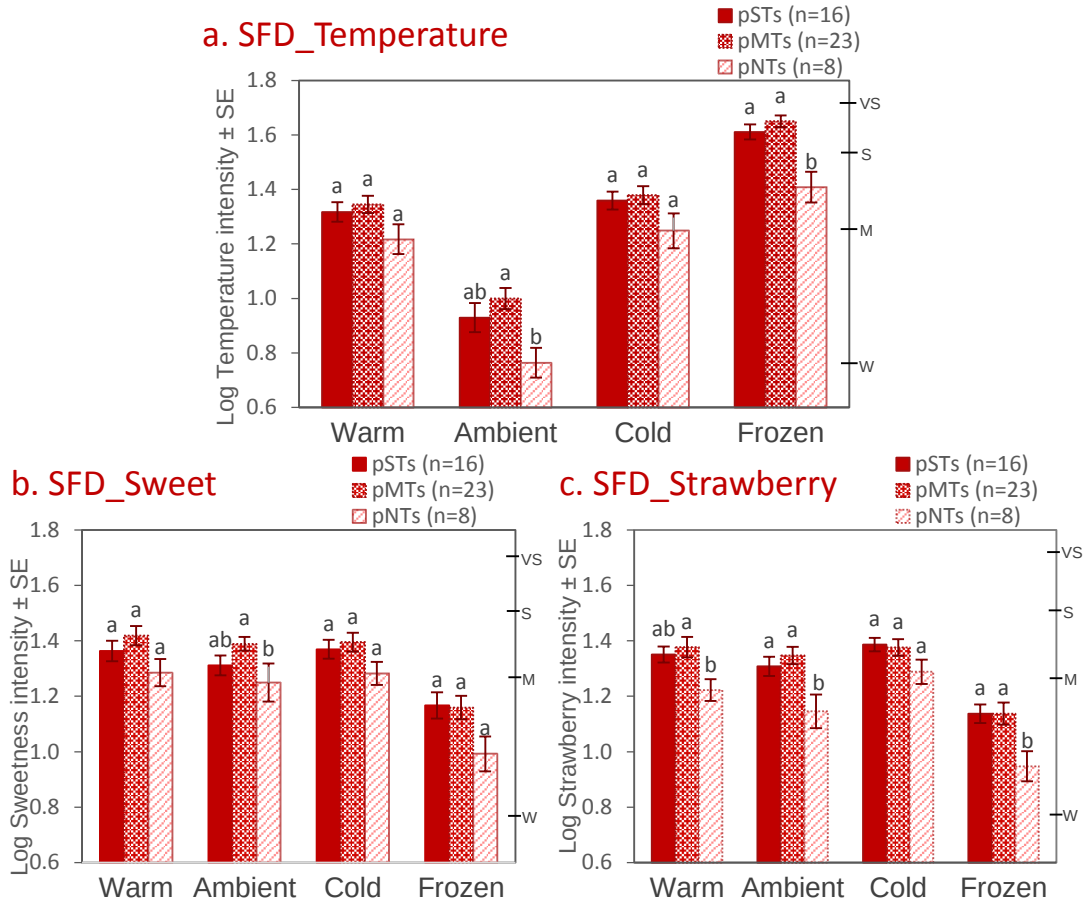

Figure 5-11: Effect of PTS on perceived intensity ratings for SFD at warm, ambient, cold and frozen temperatures: a) Temperature ratings; b) Sweetness ratings; c) Strawberry ratings on gLMS scale. *Data represent log mean intensity ± SE. (W - Weak, M - Moderate, S - Strong, VS - Very Strong on the gLMS scale). Different letters within each individual serving temperature denotes significantly difference at p<0.05.*

5.3.8. Relative impact of PTS and TTS on perceived sensory attribute intensity

Two-way ANOVA (factors = TTS and PTS), with interactions were performed on pooled data combining all four serving temperature together for each product category and each sensory attribute. Results indicated that no significant interactions were found between TTS and PTS on perceived temperature intensity for either water or SFD ($p>0.05$). However, significant interactions for sweet and strawberry intensities of water and strawberry intensity of SFD were observed ($p<0.05$). **Figure 5-12 (a&b)** show the

interaction plots for the water, with interactions occurring because the TTs who were pNTs did not rate sweetness and strawberry intensity higher than the TnTs. It should also be noted that as the numbers within in each subgroup were small (e.g. 3 TTs and 3 TnTs who were also pNTs), caution must be taken in interpreting these results. When looking at the sweet and strawberry intensities perceived from SFD, as indicated in **Figure 5-12 (c&d)**, a trend was found that pMTs who were TTs rated both strawberry intensity higher than pMTs who were TnTs, whereas an opposite trend was observed in pST and no clear trend was observed in pNT group.

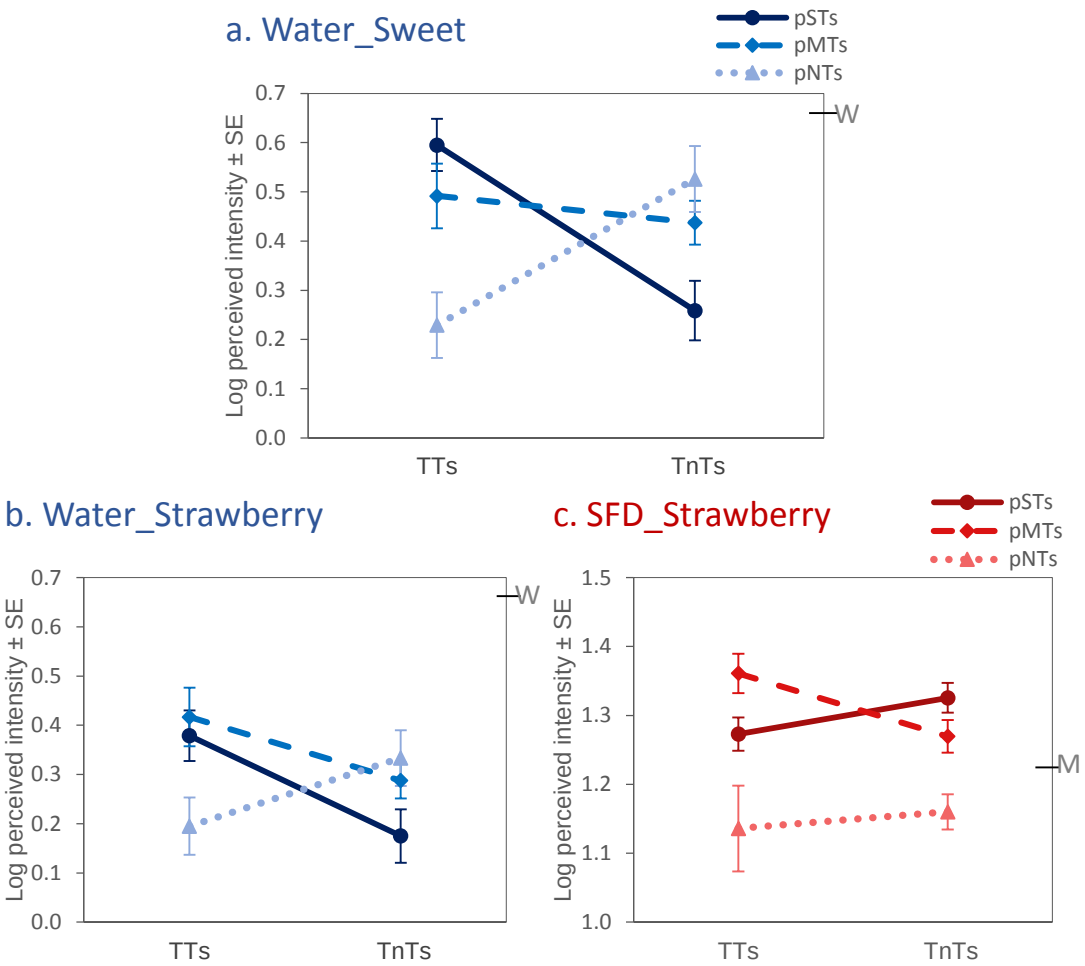

Figure 5-12: Interaction plot of TTS and PTS on pooled logged perceived intensity ratings: a) Sweet intensity of water, b) Strawberry intensity of water, c) Strawberry intensity of SFD. *Data represent log mean intensity ± SE. (W - Weak, M - Moderate).*

5.3.9. Effect of individual variation on overall liking

For TTS taste phenotype, one-way ANOVA on pooled overall liking data did not find any difference concerning water. However, for SFD, TnTs showed significantly greater liking scores compared to TTs. Further one-way ANOVA on each individual serving temperature confirmed no significant difference for water at any of the temperatures (**Figure 5-13 (a)**). However, interestingly TnTs showed significantly greater liking scores than TTs for SFD samples ($p<0.05$) at warm, cold and frozen temperatures. In other words, as the temperature got more intense (either warmer or colder), TTs liked the SFD sample significantly less than TnTs ($p<0.05$) (**Figure 5-13 (b)**).

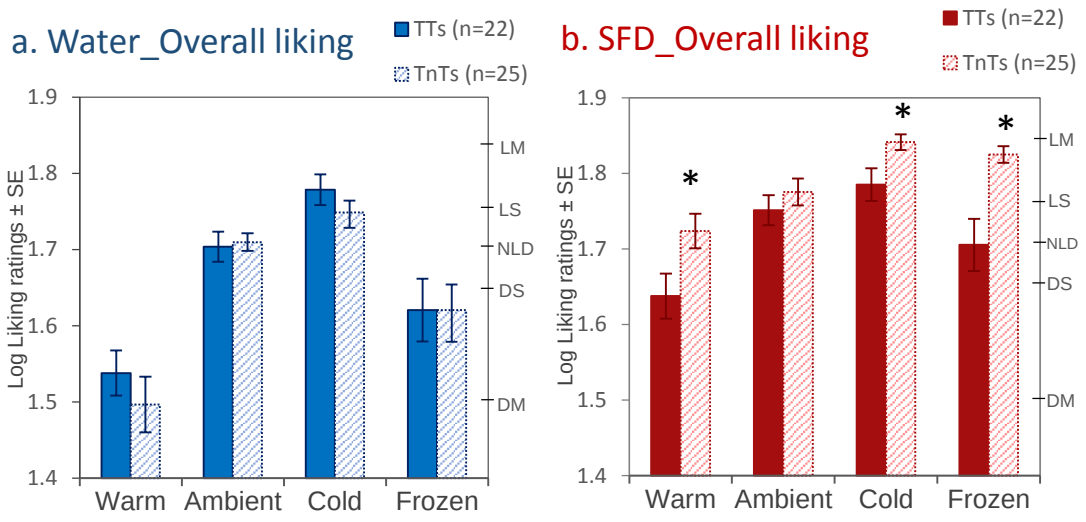

Figure 5-13: Effect of TTS on overall liking scores of water and strawberry drink at four temperatures. *Data represent log mean liking ± SE. (LM - Like moderately, LS - Like slightly, NLD - Neither like nor dislike, DS - Dislike slightly, DM - Dislike moderately on the LAM scale).*indicates significant difference at p<0.05.*

For PROP taster status, one-way ANOVA on pooled overall liking data revealed no significant differences among PTS groups for both water and SFD beverages ($p>0.05$). Further ANOVA on each individual sample confirmed

these findings (**Figure 5-14**), with one exception, where pNTs liked cold water significantly more than pSTs (p=0.04).

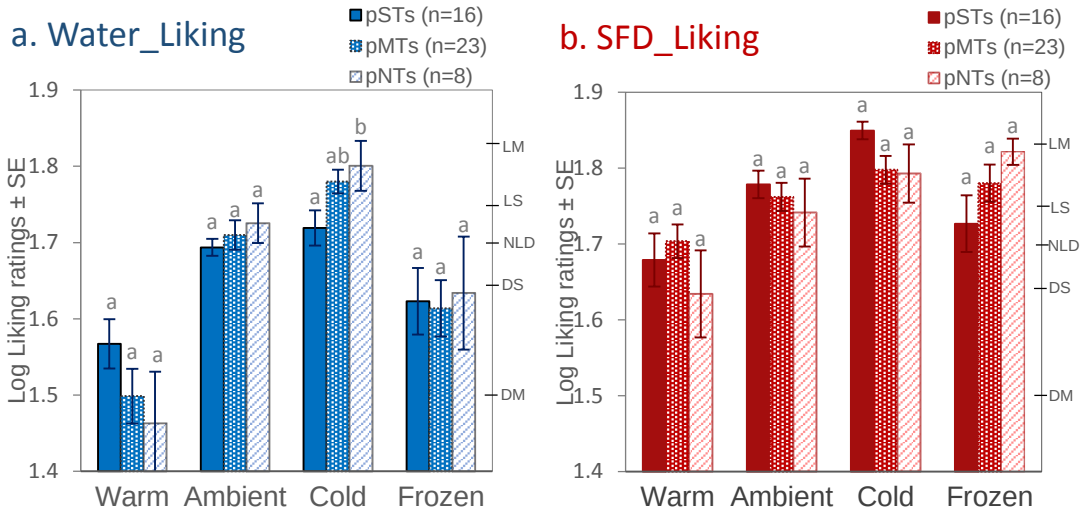

Figure 5-14: Effect of PTS on liking scores of SFD and water at four temperatures. *Data represent log mean liking ± SE. (LM - Like moderately, LS - Like slightly, NLD - Neither like nor dislike, DS - Dislike slightly, DM - Dislike moderately on the LAM scale). Different letters within each group indicate significantly difference at p<0.05.*

5.4. DISCUSSION

5.4.1. Comparison between gLMS and mLMS scales

The results in Chapter 3 demonstrated a general trend that TTs rated higher than TnTs for oral sensations, but with a lack of significance for some individual samples. It was originally speculated that failure to reach significance might be due to the narrowed space between 'no sensation' to 'very strong'. Currently however, no other scale is available to facilitate comparison of absolute differences in intensity. Hence, the magnified gLMS was developed in our laboratory, stretching the area from 'no sensation' to 'very strong' to offer more space for intensity ratings. However, the results for both the gLMS and mgLMS scales yielded the same significant different groups between samples, suggesting the mgLMS did not give any extra

discrimination between samples. The findings here are novel and it further confirms that gLMS is a reliable scale to use.

5.4.2. Overall effect of temperature on perceived sensory attribute intensities of SFD

This study observed that reducing the temperature of samples below freezing significantly decreased the perceived sweet and strawberry intensities of the SFD, but both that less dramatic differences in temperature (warm and cold) showed negligible impact, partially agreeing with previous findings that cooling could reduce perceived sweetness (Green & Frankmann, 1987). Finding a significant reduction for strawberry intensity at frozen temperature could be explained by the fact that sweetness has been indicated as a key driver for strawberry flavour intensity (Hort & Hollowood, 2004; Pfeiffer *et al.*, 2006), and reducing sweet intensity could consequently reduce perceived strawberry intensity. It may also be related to the rate of flavour release, which has been closely associated with temperature, with lower temperature inhibit flavour release, and therefore result in the decreased perceived intensity of strawberry flavour.

For sweetness intensity, finding an impact for the frozen temperature but not cold temperature, could be due to the fact that frozen sample could more effectively cool the tongue. Green and Frankmann (1988) found that cooling the tongue reduced the sweetness more than cooling the solution, as the temperature and taste interaction may have had a larger impact on the transduction pathway, rather than thermally induced changes in the molecular properties of the solution. Furthermore, it has been shown that the impact of temperature on sweetness varies with concentrations, and the temperature

effect diminishes with progressively higher concentrations and finally becomes negligible at about 0.5M (sucrose solution) (Bartoshuk *et al.*, 1982), suggesting the effect of temperature would be restricted to high levels of sweetness (Green & Frankmann, 1988). In this study, the sugar content of the SFD was higher than 0.5M which may explain the absence of an association between temperature and perceived sweetness ratings for warm and cold temperatures. However, a significant reduction in sweet ratings when sample temperature dropped to -4ºC was observed, suggesting that extreme temperatures may have an impact on sweet intensity. The lack of association between temperature and sweetness perception above 0.5M sugar content in the previous study could be due to the fact that they did not test extreme cold temperature such as -4ºC, and in fact, the lowest temperature tested was 4ºC which was a similar intensity to the cold sample used in this study.

The mechanism behind the interactions between temperature and sweet perception is currently unclear. Many factors could contribute to the interactions between temperature and taste, such as the interaction between tastants with their receptors, the process of transmitter release, and thermal modulation of the signalling pathways (Talavera *et al.*, 2007). A previous study reported that TRPM5 is a temperature sensitive cation channel, and increasing the temperature from 15ºC to 35ºC has been clearly shown to increase chorda tympani response to a variety of sugars (Talavera *et al.*, 2005). This might provide an explanation for the previous finding of increased sweet taste associated with increased temperature at a sucrose concentration below 0.5M (Bartoshuk *et al.*, 1982). Further studies will have to test the impact of temperature across a wider temperature range as well as a wider range of

sweet concentrations to fully understand how temperature impacts sweetness perception.

5.4.3. Effect of TTS on perceived sensory attributes intensity

The current study observed that TTs perceived sweetness and some other taste sensations during consumption of water served at different temperatures. This is consistent with the results in Chapter 2, as well as other TTS studies (Bajec & Pickering, 2008; Cruz & Green, 2000). The findings in this study further confirm that TTs have the ability to perceive taste sensations from temperature changes on the tongue elicited by drinking hot or cold water, not simply isolated temperature applications such as that achieved via a thermode device.

This study did not observe any significant difference between TTs and TnTs in perceived temperature, sweetness and strawberry intensities for the SFD. The findings here contradict previous findings, as both Bajec and Pickering (2008) and the data in Chapter 2 reported TTs perceived temperature intensity as more intense compared to TnTs on both the tip of the tongue and lateral edges of the tongue. However, the current study observed that TTs were more sensitive to temperature changes (rating differences between ambient sample and samples at other temperatures) rather than perceived temperature intensity itself for SFD. A couple of studies have also reported that TTs could perceive stronger sweet intensity evoked by sucrose solution (Bajec & Pickering, 2008; Green & George, 2004). However, conflicting results on sweet intensity were obtained for more complex food products such as beer and wine, where TTs only rated the sweetness of a few samples significantly

higher than TnTs, with most of the samples rated at a similar level of sweetness (Pickering, Bartolini*, et al.*, 2010; Pickering, Moyes*, et al.*, 2010). Interestingly, TTs were also shown to rate the intensity of vanilla flavour as more intense, compared to TnTs (Green & George, 2004). However, the results in Chapter 3 and in this chapter did not provide any evidence to indicate that TTs have increased aroma perception, in comparison with TnTs.

It is possible that the lack of significant differences between TTs and TnTs in this study compared to others might be due to the differences in classification methods used by different research groups, as discussed previously (Bajec & Pickering, 2008; Green & George, 2004). Another possible reason could be that most of the previous studies used simple food systems, such as sucrose solution, vanilla or Suc/Van solutions, however, when it investigating more complex food products such as strawberry flavoured drink, beer and wine, the TTs advantage on perceived intensities might be reduced (Pickering, Bartolini*, et al.*, 2010; Pickering, Moyes*, et al.*, 2010). In addition, the lack of difference between TTs and TnTs could also be because of high level of sweetness in the SFD or temperature involvement (-4 to 48 ℃) in this study. Both would increase the background noise, and so could restrict the effect of TTS due to the saturation of the receptors. One other TTS study also failed to find any difference for taste stimuli including sucrose served at both 5 and 35 ℃ (Mony *et al.*, 2013).

Data reported in Chapter 3 had revealed TTs had an overall advantage regarding perceived intensity of taste and trigeminal stimuli over TnTs, however, the current study failed to replicate similar findings. The contradicting

results could be due to the differences in protocol used. In Chapter 3, a cotton bud swabbing method was used, which measured the sensitivity on the anterior tip of the tongue, whereas this study required participants to consume the entire sample with swallowing and measured the sensitivity from stimulation of the of the whole oral cavity. As discussed in Chapter 3, the proposed hypothesis related to TTS is co-innervation of taste and trigeminal nerves in fungiform papillae. It is likely that the mechanism of TTS might occur within fungiform papillae, which distributed most at the anterior tip of the tongue. Hence, the impact of TTS on perceived taste and trigeminal intensities might be diminished within whole mouth consumption.

Interestingly, this study found that the intensity of 'phantom' sweet taste in water was significantly associated with the temperature intensity in the SFD in TTs, but not in TnTs. The findings here provide further evidence for the proposed hypothesis of co-innervation of taste and trigeminal nerves in TTs. It was speculated that taste and trigeminal nerve are intertwining would be the dominant factor for perceiving 'phantom taste', and consequently FP density and degree of intertwining may contribute to the variation in perceived 'phantom taste' intensity. As a higher number of FP and a higher level of intertwining could produce more signals during either taste or trigeminal stimulation, this in turn could cause a heightening of response to 'phantom taste, as well as heightening taste and trigeminal responsiveness. The findings suggest that in TTs, 'phantom taste' intensity could be used as a marker of general temperature sensitivity, but not for general sweetness sensitivity. The disassociation between 'phantom taste' intensity and sweet intensity observed in SFD in this study could be due to various reasons including the high level

of sweetness (a similar effect was found for frozen temperature vs 'phantom taste' intensity), and the validity of 'phantom taste' intensity (some may rate sweetness itself on the scale, whereas others may rate whatever taste they perceive on the scale via attribute dumping). The potential association between 'phantom taste' and general temperature intensity deserves further attention, as it may provide insight into the variation within TTs group and help to understand the mechanism behind TTS.

5.4.4. The effect of PTS on perceived sensory attribute intensities

In general agreement with previous studies (Bajec & Pickering, 2008; Hayes *et al.*, 2008), pSTs and pMTs tended to rate the perceived intensity of trigeminal (temperature) and taste (sweetness) higher than pNTs for the SFD. The results in Chapter 3 indicated there were advantages for PROP tasters (pSTs and pMTs) in sensitivity on the anterior tip of tongue, and the current results further confirmed that the observed advantages of PROP tasters extended to the whole oral cavity. The findings suggest that PROP taster status might not be only associated with fungiform papillae density, but also associated with papillae that surround the whole oral cavity, or it is a stronger effect within the fungiform papillae.

In this study, the perceived intensities of three attributes were measured, which covered all three modalities: trigeminal (temperature), taste (sweet) and aroma (strawberry). Results presented here did not only demonstrate PROP tasters have heightened intensity responses to trigeminal and taste modalities, but also for aroma modality. The heightened response to aroma could be due to cross modal interactions between aroma and sweetness, as sucrose has

been shown to be a key driver of fruit flavour intensity (Hort & Hollowood, 2004; Prescott, 1999). However, it does not rule out the possibility that PROP tasters do have an increased intensity response to aroma sensations, indeed Chapter 3 discovered that PROP tasters have a higher responsiveness to ethyl butyrate sensed both retronasally and orthonasally in the absence of sweetness.

5.4.5. Relative impact of TTS and PTS on perceived sensory attribute intensity

No significant interactions were observed regarding temperature intensity for both water and SFD, which contradicts the findings in Chapter 3, where data showed that within the pMT group, TTs rated higher than TnTs on perceived trigeminal intensity. It is possible that these interactions may have been weakened when stimuli were applied to the whole oral cavity compared to specific application on the anterior part of the tongue where fungiform papillae are located.

Significant interactions were observed for both sweet and strawberry attributes in the water sample. Water itself does not have any tastant and volatile, but TTs may have perceived some tastes from consuming water at different temperatures. However, although significant interaction existed, the level of perception was so low (between barely detectable and week on the scale), they are not so important. When the sample has tastant and volatile (SFD), the TTS*PTS interaction was found to be significant for the strawberry attribute. Within the pMT group, TTs rated higher than TnTs, while an opposite trend was observed in pSTs and no clear trend was seen for pNTs. The latter findings agree with the results in Chapter 3 that the sensitivity of pMTs was

most affected by TTs. Although no significant TTS impact was found on aroma perception, the data in both Chapter 3 and the present chapter found an interaction of TTS*PTS on aroma attribute. In future studies, a much larger sample size and a wider range of aroma stimuli would be needed to further confirm these findings, in order to understand the mechanism behind the relative effects of these two phenotypes on aroma perception.

5.4.6. Relationship between taste phenotypes on overall liking

This study revealed that when temperature got more intense (either warmer or colder), TTs liked the SFD less than TnTs, however, no differences were observed for the water sample. The reason behind why TTs liked SFD less, especially when the temperature got more intense (warmer or colder) is currently unknown, but it might be linked with TTs who are generally more sensitive to temperature changes. Temperature changes of the tongue could evoke taste sensations in TTs, some of the taste sensations might be unpleasant such as bitter, metallic and spicy, which had been reported in both TTS screening sessions and consuming warm and cold water, therefore, resulting in a lower overall liking of the sample. However, currently no evidence could support this hypothesis. Further research with a larger sample size would be necessary to conclude the extent to how thermal taster status phenotype influences beverage liking and ultimately consumer behaviour.

For PROP taster status, no differences on overall liking scores among PTS groups were observed for both the water and SFD. As reported earlier, significant differences for perceived intensity were observed among PTS groups, but not for TTS groups. Thereby, the data here suggested that

variations in oronasal intensity perception did not translate into overall liking, which agrees with previous studies (Pickering, Bartolini*, et al.*, 2010; Pickering, Moyes*, et al.*, 2010). Some studies revealed that sensitivity for PROP is related to food intake and preference for particular foods with a strong bitter taste, such as broccoli and alcohol (Duffy *et al.*, 2004; Thomas *et al.*, 2014), as well as fatty food products (Eldeghaidy *et al.*, 2011; Tepper & Nurse, 1998). However, the samples used in this study do not contain bitter or fat elements that may have induced negative emotions, and so may diminish the difference among PTS groups on their overall liking. However, even for bitter-related products, not all studies have found a clear association (Pickering, Bartolini*, et al.*, 2010; Pickering, Moyes*, et al.*, 2010), indicating other factors such as physiological and environmental factors contribute to food and beverage preference and eating behaviour.

5.5. CONCLUSION

Contrary to the original hypothesis, the data here suggests that the mgLMS did not give better discrimination than the gLMS, but does highlight scale end effects. It has also shown that once subjects were well trained on the gLMS scale, they were able to differentiate between samples.

The data in the current study confirms that reducing the temperature below freezing could effectively reduce the perceived sweetness of the SFD, but enhance the sweetness of water. Further work should investigate the impact of a wider range of temperatures across a wider range of sweet concentrations to better understand temperature-taste interactions.

The study of the two beverages revealed that TTs have the ability to perceive 'phantom taste' from drinking hot and cold liquids and that TTs are more sensitive to temperature changes rather than the perceived intensity per se, in comparison with TnTs. In addition, the significant correlation between the 'phantom' sweet taste of water and temperature intensity perceived from the SFD adds weight to the proposed hypothesis of co-innervation of taste and trigeminal nerves in TTs. FP density and degree of intertwining were speculated to contribute to both 'phantom taste' intensity and general taste and trigeminal sensitivity. However, more research would be needed to confirm this hypothesis.

PROP tasters' heightened intensity (taste, trigeminal and aroma) perception was found to exist on the anterior tip of the tongue (Chapter 3 results) and here was shown to extend to the whole oral cavity, whereas, the TTs' advantage on perceived intensity (taste and trigeminal) on the anterior tip tongue was diminished when measuring the whole oral cavity. The data here suggests that the mechanism behind TTS is likely to occur in fungiform papillae, which are distributed mostly on the anterior tip of the tongue. PTS is more likely to be related to the papillae that surround the whole cavity or it is a stronger effect within the fungiform papillae, as well as factors that contribute to aroma perception. The findings of the relative impact of TTS and PTS further suggest that the perception of pMTs seems most effected by TTS not only for taste attribute (sweet), but also for the aroma attribute (strawberry). Further research would be needed to investigate the effect of TTS and relative effect of TTS*PTS on aroma perception.

This study observed that when the sample temperature got warmer or colder, TTs liked the SFD significantly less than TnTs, which might be explained by TTs' sensitivity to increased temperature changes. In addition, no difference in liking data was observed among PTS groups. Thus, the findings here suggest that differences in oronasal sensations do not transform to specific overall liking for water and SFD.

Overall, the results in the current study, and in particular those indicating 'phantom taste' intensity as a marker for the perceived temperature intensity for SFD in TTs, adds more evidence to the proposed hypothesis of taste and trigeminal integration in TTs. Consequently it is possible to hypothesise that greater taste-trigeminal intertwining would potentially induce an increased activation in cortical areas of the brain involved in gustation and somatosensory processing. In recent years, neuroimaging techniques have become popular, as they can provide information on neural and cortical responses. In the next chapter, the fMRI technique was applied to look at the relationship between sensory and cortical responses across TTS group in relation to sensory perception, in order to further investigate the proposed hypothesis behind TTS.

6. INVESTIGATING SENSORY AND CORTICAL RESPONSES ACROSS THERMAL TASTER PHENOTYPE

6.1. INTRODUCTION

Functional magnetic resonance imaging (fMRI) is a tool used by numbers of scientists to investigate the brain functions underlying psychological tasks (Aue *et al.*, 2009). Therefore, fMRI offers an opportunity to investigate how the brains of different TTS groups respond to sensory stimuli particularly when combined with collection of behavioural sensory data. This chapter describes a multi-school project in which the author collaborated with Dr Sue Francis at the SPMMRC. Dr Sally Eldeghaidy facilitated stimuli delivery set up, and Dr Turki Abualait was responsible for collecting and processing the fMRI data which was then given to the author for evaluation alongside sensory data.

6.1.1. Principle of fMRI and BOLD Contrast

Conventional MRI uses powerful magnets and radio waves to safely produce images of the brain or other structures inside the body and is extensively used for diagnosis. MRI normally produces higher resolution spatial maps, and differentiates soft tissues very well (Cammoun *et al.*, 1985; Hornak, 1998). The contrast within images result mainly from variations in the density of water within tissues and in the manner in which water interacts with macromolecules (Gore, 2003). The fMRI method investigates brain function in *vivo*. It typically has a coarser resolution and is not used for anatomical detail and diagnosis. Instead, it measures the activity of large populations of neurons in the brain (Logothetis & Pfeuffer, 2004). When the body is performing a particular task

(looking at changing colour, experiencing a taste stimulus etc.), certain parts of the brain becomes activated and neurons start exchanging information. This process is an energy-requiring process, because of this, areas in the brain where this transfer occurs has a greater demand for energy and therefore results in an increased demand for oxygen. To meet the increased metabolic demand, neuronal activation is accompanied by increased local blood flow (Astolfi *et al.*, 2004), as illustrated in **Figure 6-1**.

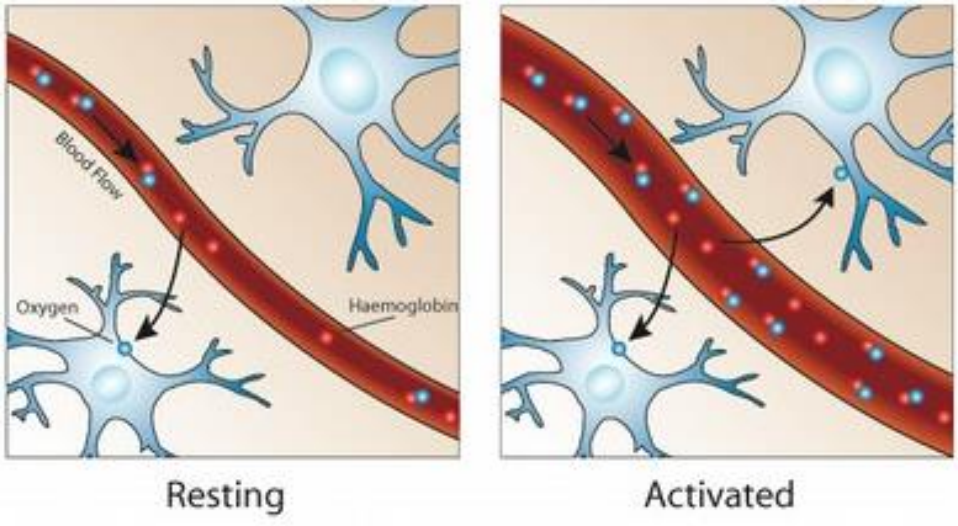

Figure 6-1: Comparison of haemoglobin in blood cell on resting state (left) and activated state (right) (Source: http://www.fmrib.ox.ac.uk/research/education).

Oxygen is delivered to neurons by haemoglobin in capillary red blood cells. Haemoglobin is diamagnetic when oxygenated but paramagnetic when deoxygenated. This difference in magnetic properties leads to small differences in the MR signal of blood depending on the degree of oxygenation. Since blood oxygenation varies according to the level of neural activity, these differences can be used to detect brain activity, and is called blood oxygenation level dependent (BOLD) imaging (Demitri, 2007).

The haemodynamic response function (HRF) is the time course of the BOLD signal change associated with neural activity. After stimulus activation, there is a momentary decrease in blood oxygenation immediately after neural activity increases, known as the 'initial dip'. This is followed by a period where the blood flow increases, and approximately 6s later, the positive BOLD signal peaks and in a further 8 to 20s time it returns to baseline, this is often accompanied by a 'post-stimulus undershoot' (Afonso, 2007; Kornak *et al.*, 2011), as shown in **Figure 6-2**. Modelling the hemodynamic response function is essential to explore the relationship between the experimental stimulus and the fMRI signal. It is crucial to design the fMRI experiment with an accurate estimated HRF that reflects the way the brain respond to stimulus, ensuring that the follow-up statistical inference is valid.

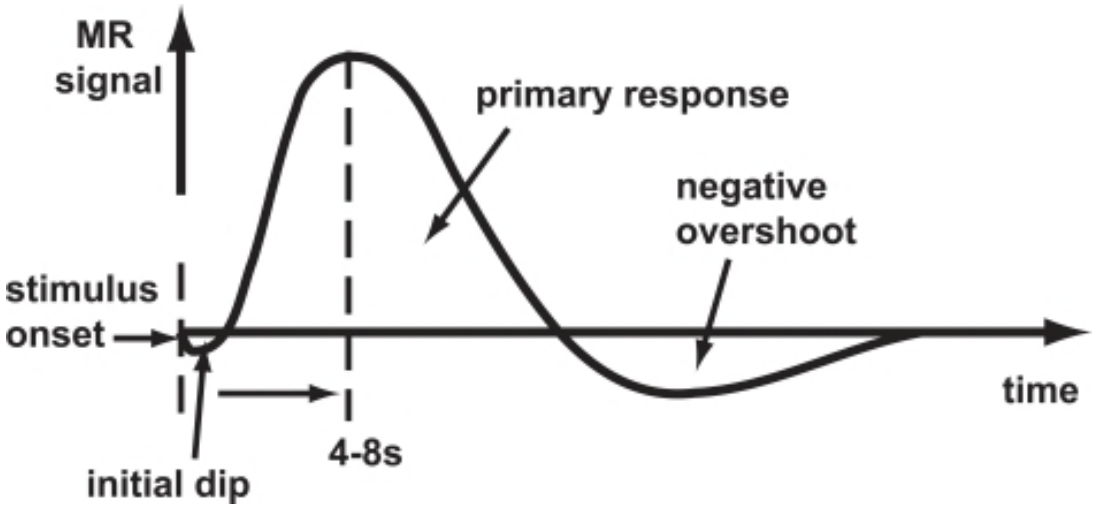

Figure 6-2: Schematic representation of the haemodynamic response function (HRF) to a period of neuronal stimulation (Source: Kornak *et al.* (2011).

The fMRI techniques were widely used by neuroscientist to localise the neural activity that correlates to sensory, motor and cognitive process, and to

examine the detailed response profile across tasks for known Regions of Interests (ROIs). The General Linear Model (GLM) is the most standard statistical analysis procedure used to analyse fMRI data. In GLM, the parametric test of T-statistic or F-statistic, where appropriate, are normally carried out. The GLM beta values or Z scores, derived from t-statistics, represent BOLD signal changes (cortical activation) of specific brain regions (ROIs) (Brett, 2013). In many psychological studies, beta values or Z scores are further used to test the association between behavioural data and cortical responses (De Araujo & Rolls, 2004; Eldeghaidy *et al.*, 2011).

6.1.2. Sensory Cortex

The brain has three main parts: cerebrum, cerebellum and brain stem. The cerebrum is the largest area of the brain and controls all higher mental functions, such as thinking and memory. The cerebrum contains four lobes, frontal, parietal, occipital and temporal lobes (Clarke, 1994), as illustrated in **Figure 6-3**. The frontal lobe plays an integral role in memory formation, emotions, decision making and personality. The parietal lobe plays a major role in senses and integrates sensation, spatial awareness and perception. The occipital lobe has primary function in the processing, integration and interpretation of vision and visual stimuli. Finally the temporal lobe play an integral role in hearing, organisation/comprehension of language, as well as information retrieval. The cerebellum, also called ‘little brain’, coordinates voluntary movements such as posture, balance, coordination, and speech etc. The brainstem is the region of the brain that connects the cerebrum with the spinal cord, its job is to pass signals between the cerebral cortex and the rest of the body (Hines, 2013).

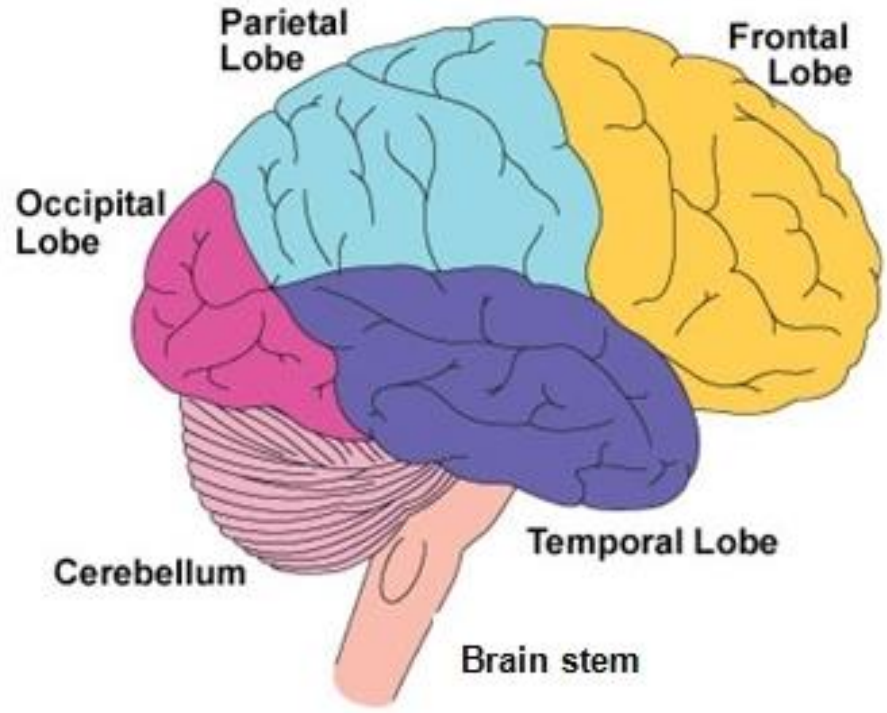

Figure 6-3: The structure of cerebrum: frontal, parietal, occipital and temporal lobes (Source: http://www.headway.ie/information/abi/introduction-to-the-brain/).

The sensory cortex refers to the visual cortex on the occipital lobes, the auditory cortex on the temporal lobes, the primary olfactory cortex on the uncus of the piriform region of the temporal lobes, the gustatory cortex on the insula lobe, and the primary somatosensory cortex on the anterior parietal lobes (Berk, 2012).

Taste information projects from the tongue, conveyed through the cranial nerves X, IX and VII to rostral division of the nucleus of the solitary tract (NTS). Taste neurons in the NTS project to the ventral posterior nucleus of the thalamus, followed by the primary taste cortex including anterior insula and frontal operculum area, and finally to the orbitofrontal cortex (OFC), lateral

hypothalamus, amygdala, striatum and anterior cingulate cortex (ACC), also known as secondary taste cortex (Buck & Bargmann, 2013; Rolls, 2012), see **Figure 6-4** for details of gustatory pathway. The thalamus sorts and directs sensory inputs to areas of the cerebral cortex, and nearly all sensory impulses travel through the thalamus. The primary gustatory cortex is shown to represent the identity and intensity of taste, whereas OFC and ACC represents the reward value of taste, which correlates with subjective pleasantness of taste (Grabenhorst & Rolls, 2008; Grabenhorst *et al.*, 2008; Rolls, 2012). Published evidence showed that both OFC and amygdala not only respond to affective pleasant taste such as the taste of glucose, but also respond to aversive taste stimuli such as NaCl (O'Doherty *et al.*, 2001). The OFC is involved in the processing of temperature, touch, smell and taste, and is suggested to be an integration area (Critchley & Rolls, 1996).

When aroma (volatiles) arrive at the nasal cavity either retronasally or orthonasally, the aroma bind to specific sites on the olfactory receptors. The olfactory receptors then send electric signals, which are transmitted in glomeruli in olfactory bulb – a brain structure directly above the nasal cavity and below the frontal lobe. The information perceived from the olfactory bulb is then directly sent to the olfactory cortex (piriform) and amygdala, and further projects to the OFC area (**Figure 6-4**) (Buck & Bargmann, 2013). The piriform tends to represent the identity and intensity of aroma, and OFC and ACC represents the reward value of aroma (Grabenhorst *et al.*, 2007). Studies have showed that an independent presentation of tastant or odorant produces overlapping activation in regions of the insula, the OFC, amygdala and ACC (de Araujo *et al.*, 2003; Small *et al.*, 1997; Small *et al.*, 1999; Zald *et al.*, 1998).

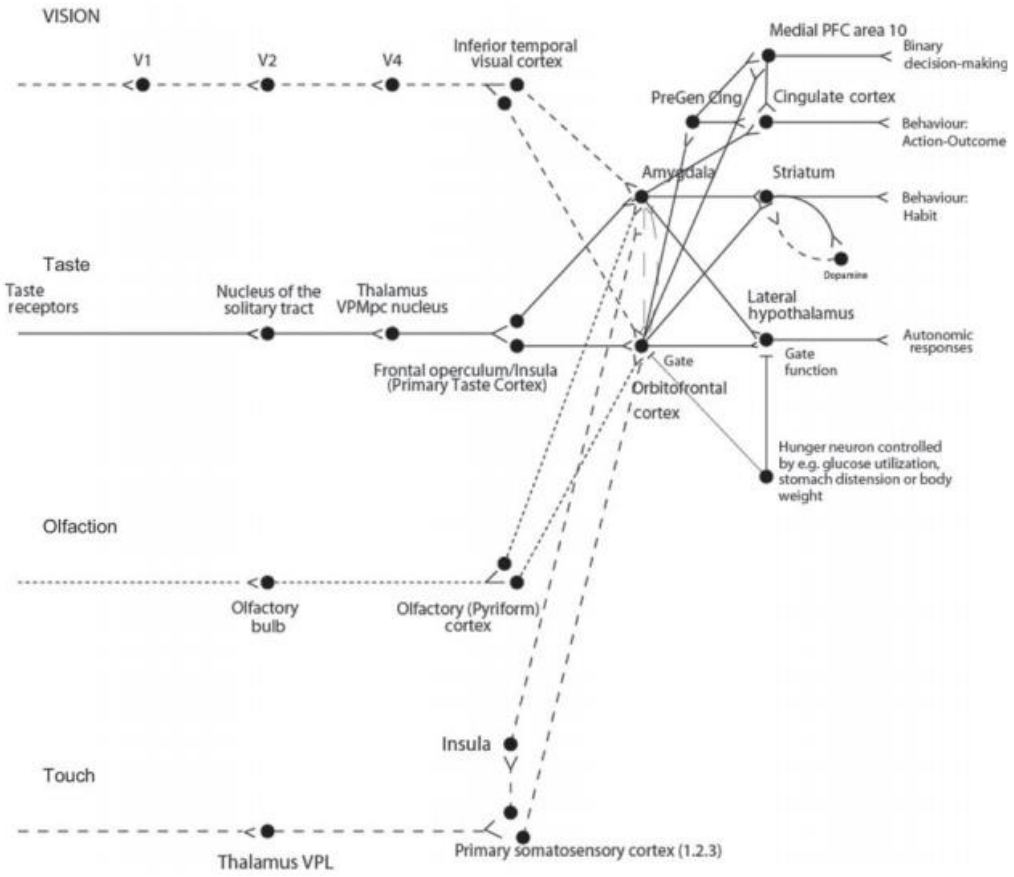

Figure 6-4: Schematic diagram showing the gustatory, olfactory, visual and somatosensory pathways (Source: Rolls (2012)).

The trigeminal nerves surrounded in the oral cavity receive temperature information from food. The information is sent to the ventral posterior medial (VPM) of the thalamus, then project mainly to primary somatosensory cortex (SI). This then distributed from the SI to secondary somatosensory cortex (SII) which further sends projections to amygdala, hippocampus and OFC (**Figure 6-4**) (Engelen, 2012; Rolls, 2012). A few studies have researched cortical responses to oral texture and oral temperature in the human sensory pathways and the brain (De Araujo & Rolls, 2004). De Araujo and Rolls (2004) found that the primary taste cortex, and mid-insula were also activated by a tasteless and odourless thickening agent, carboxymethyl cellulose, representing food

texture. An fMRI study investigating cortical activation of oral fats has shown that fat stimuli could activate SI, SII, right ACC, right mid-insula, anterior insula and bilateral frontal operculum (Eldeghaidy *et al.*, 2011). The pleasantness and reward value of fat texture was correlated with activations in mid-OFC and ACC. The lateral hypothalamus and amygdala were more activated by high-fat stimuli versus low-fat stimuli (Grabenhorst *et al.*, 2010). Rolls (2010) reported that intra-oral thermal stimulation showed activations in the insula taste cortex, a part of the somatosensory cortex, the OFC, the ACC, and the ventral stream. The activation in mid-OFC and pregenual cingulate cortex (PCC) were correlated with the pleasantness of fat texture (Kadohisa *et al.*, 2004; Rolls, 2010).

6.1.3. Effect of taste phenotypes on cortical activation

As discussed in previous chapters, both PROP and thermal taster status showed an impact of population differences in oronasal perception. To date, only two studies have examined the effect of taste phenotypes on cortical responses, both studies were conducted in our laboratory. The first study examined the effect of PROP taster status on perception of fat emulsions. A strong correlation on self-reported preference with cortical responses in both SI, SII and mid-insula, and anterior insula, as well as amygdala and OFC cortices was found. These support previous findings of PROP tasters having higher lingual spatial tactile acuity as a consequence of the co-innervation of fungiform papillae by trigeminal nerves (Eldeghaidy *et al.*, 2011; Essick *et al.*, 2003). The second study examined the effect of both PROP and thermal taster status using model drink systems with 3 carbonation levels (No CO_2, low CO_2, and high CO_2) (Clark, 2011). Clark (2011) found that PROP tasters have a

higher general activation strength than pNTs for all three samples. For thermal taster status, TT group showed an overall higher cortical activation in the somatosensory cortex (SI, SII) and ACC than TnT group. The behaviour data also revealed that TTs have a better CO_2 discrimination ability and preferred samples with no CO_2, which was suggested as a result of increased cortical activation in TTs.

In Chapter 5, frozen temperature (-4 ± 2℃) was found to dramatically decreased the sweetness intensity of strawberry flavoured drink, whereas coldness (4 ± 2℃) slightly enhanced sweetness. In addition, Chapter 3 observed that TTs were more sensitive to taste (e.g. sweet, bitter, etc.) and trigeminal sensations (e.g. temperature, capsaicin) than TnTs. So far, the mechanism behind TTS is not well understood, Green and George (2004) suggested that the TTs mechanism could be from periphery to cortical responses. This study aims to gain some further knowledge in order to decouple the mechanism behind TTS by utilising fMRI imaging alongside behavioural sensory data. In this chapter, the objectives of the study in this chapter were to:

- Examine if the thermal taster status phenomenon is consistent over time.
- Determine if perceived intensity and cortical response to a taste and an aroma stimulus differ between TTs and TnTs.
- Investigate the impact of sample temperature on sweetness perception and cortical response across TTS.
- Examine if intensity of ‘phantom taste’ is associated with cortical responses in TTs.

6.2. METHOD DEVELOPMENT

The author's roles in this multi-school project were: subject recruitment, subject screening, thermal taster status re-testing, PROP taster status testing, stimuli preparation, sensory data collection, analysis and interpretation for intensity measurement, explanation of the fMRI activity to subjects before scanning, and setting up the EMG for each subject to monitor swallowing. In addition, the author also supported the fMRI scanning by helping to set up the delivery tube for each subject, ensuring the stimuli temperature were correct and topping up the stimuli when needed.

6.2.1. Subjects

This study was approved by the University of Nottingham Medical School ethics committee and informed consent was obtained from all subjects. A disturbance allowance was paid to participants. 24 subjects were selected from volunteers who have been previously screened for their TTS and PTS in previous chapters, and passed an fMRI safety questionnaire to provide a balance of 12 TTs (6 females, 6 males, age range 20 to 59yrs) and 12 TnTs (8 females, 4 males, age range 21 to 57yrs), all of whom were PROP tasters. All subjects were chosen to be PROP tasters to reduce the variability in brain responses that have previously been shown between PROP tasters and non-tasters (Clark, 2011; Eldeghaidy *et al.*, 2011). Subjects were asked to have a light breakfast on the morning of scanning, and were restricted from eating or drinking any strong flavoured food 2 hours before the study. The whole session lasted a maximum of 2 hours including re-testing thermal taster status,

explanation and scanning, subjects were in the scanner for approximately 1hr including set-up.

6.2.2. fMRI stimuli and delivery system

All samples were prepared fresh on the morning of scanning with Evian Water (Evian, France). Stimuli were sucrose (3%) (Silver Spoon, UK) at both cold (5 ± 2°C) and ambient (20 ± 2°C) temperature, ethyl butyrate (200ppm) (Sigma, UK) and a mixture of sucrose and ethyl butyrate (3% sucrose +200ppm EB) both at ambient temperature (20 ± 2°C), named SUC_{COLD}, $SUC_{AMBIENT}$, $EB_{AMBIENT}$, $SUC/EB_{AMBIENT}$, respectively. The cold samples were refrigerated for at least 1 hour to reach 5°C then stored in the refrigerator to maintain the cold temperature. The stimuli were chosen based on the findings from previous chapters. Results from previous studies showed that TTs had a lower sucrose threshold and perceived the sweetness of sucrose higher than TnTs. EB was the aroma used in previous studies and although no differences were found concerning aroma perception across TTS groups. This project provided an opportunity to further test the effect of TTS on aroma perception in cortical activations. Finally this study enabled further understanding of temperature effects. TTs were shown to have an increased sensitivity to temperature compared to TnTs, hence both cold and ambient sucrose sample were included for comparison in this study.

Delivery of stimuli was performed using an automated spray delivery system. Solutions were delivered via nozzles placed in the subject's mouth. Subjects were instructed to hold the nozzles in the middle of their mouth to receive the solution. The delivery system was placed outside the scanner room and was

controlled by Presentation software trigged by the scanner, shown in **Figure 6-5**. To maintain the coldness of SUC_{COLD}, the bottle of SUC_{COLD} was surrounded with an ice pack. In addition, specially made ice cubes (made from 3% sucrose solution) were added into the bottle frequently to ensure the temperature remained cold. The residue liquid in the tube was flushed away between each subject to eliminate the non-cold solution.

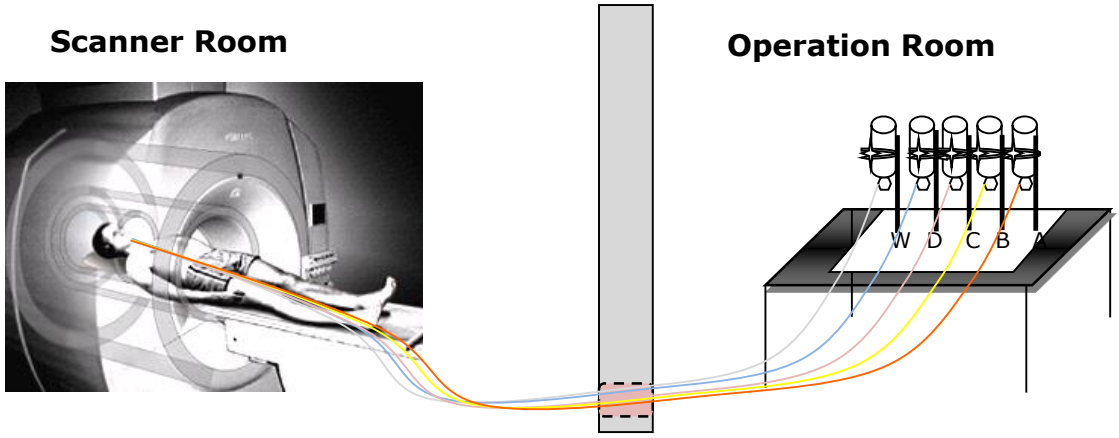

Figure 6-5: Stimuli Delivery System: Delivery of stimuli was controlled by presentation software. *(A - SUC_{COLD}, B - $SUC_{AMBIENT}$, C - $EB_{AMBIENT}$, D - $SUC/EB_{AMBIENT}$, W - $WATER_{AMBIENT}$).*

6.2.3. fMRI paradigm

One cycle of the fMRI paradigm is shown schematically in **Figure 6-6**. In each cycle, 3ml of the sample was delivered over a 3s period. Subjects were instructed by a visual cue on the screen (Blue Cross) to swallow. 10s following the stimulus delivery, a mouth rinse (3ml of water) was given to cleanse the oral cavity.

After the mouth wash, subjects were asked to rate the fruitiness and sweetness intensity of the sample on an adapted version of the gLMS scale

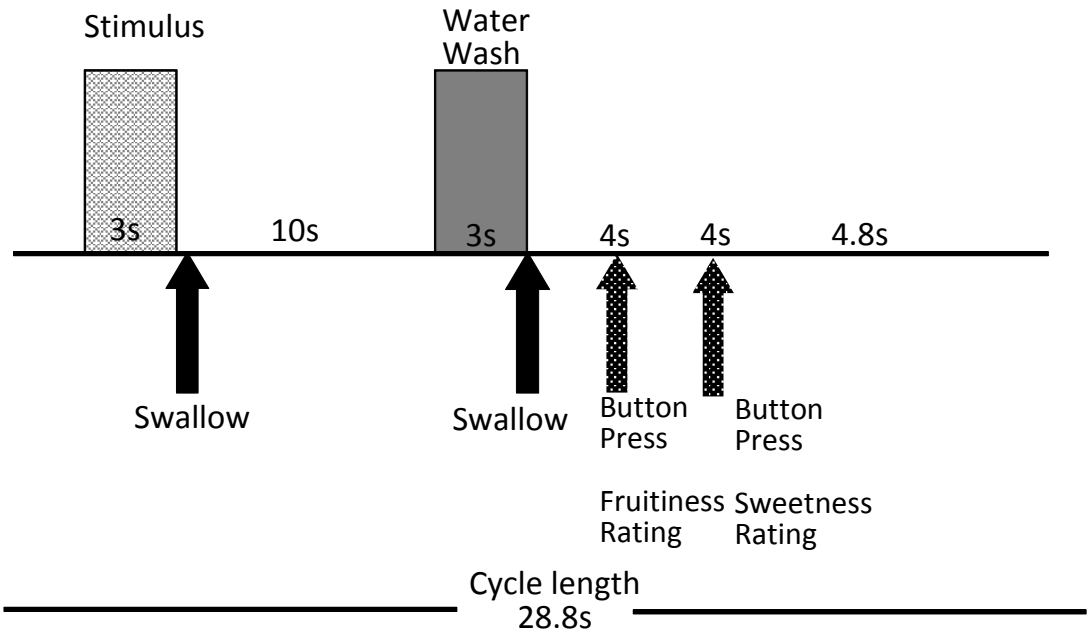

Figure 6-6: One cycle of the fMRI paradigm.

shown on the screen (see **Figure 6-7 (a)**). An adapted version of the gLMS scale was developed as it was not possible at the time for subjects to use a mouse inside the scanner for rating on the gLMS scale. Prior to the scanning,each subject was given a 'Button Press' to hold in their right hand (**Figure 6-7 (b)**) and was instructed on how to use the 'Button Press' to rate on the scale. When the scale appeared on the screen, they were instructed to press the button immediately. They were informed that no press corresponded to no sensation (0 on gLMS); 1 corresponded to weak (equivalent to 6 on gLMS); 2 corresponded to moderate (equivalent to 17 on gLMS); 3 corresponded to strong (equivalent to 35 on gLMS); 4 corresponded to very strong (equivalent to 53 on gLMS). There was a delay of 4.8s before repeating the entire cycle, the time of the entire cycle was 28.8s. For each subject, 72 cycles were performed in 3 blocks (6 of each stimulus were randomised in

each block). Each block took approximately 12mins to complete, between each block the wellness of the subject was checked by the operator. Subjects were able to stop the scanning procedure if they felt uncomfortable inside the scanner by pressing the emergency button, which was held in their left hand.

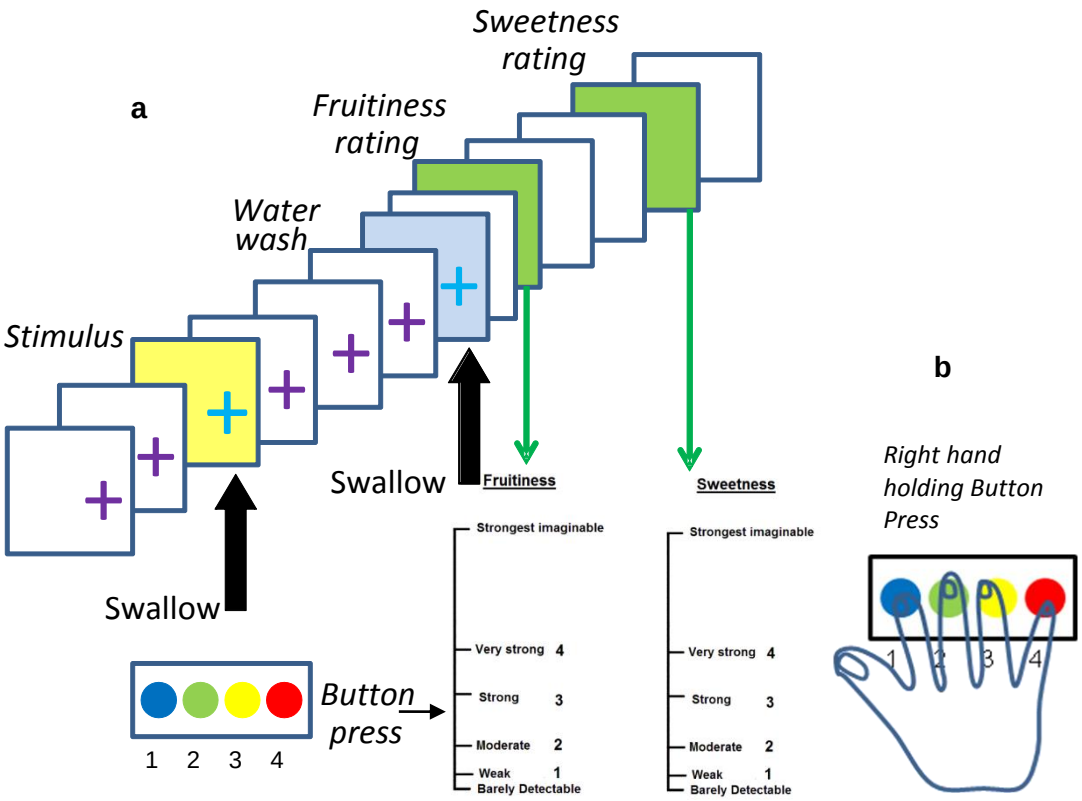

Figure 6-7: a) Visual cue on the screen, b) Button Press was held in right hand. *Blue cross indicates swallowing immediately. When the gLMS scales appears on the screen, they need to rate the intensity of fruitiness or sweetness, respectively using the button press holding in their right hand.*

6.2.4. fMRI data acquisition and processing

The fMRI data acquisition and processing was performed by researchers at the SPMMRC. Data acquisition and processing are summarised in **Figure 6-8**.

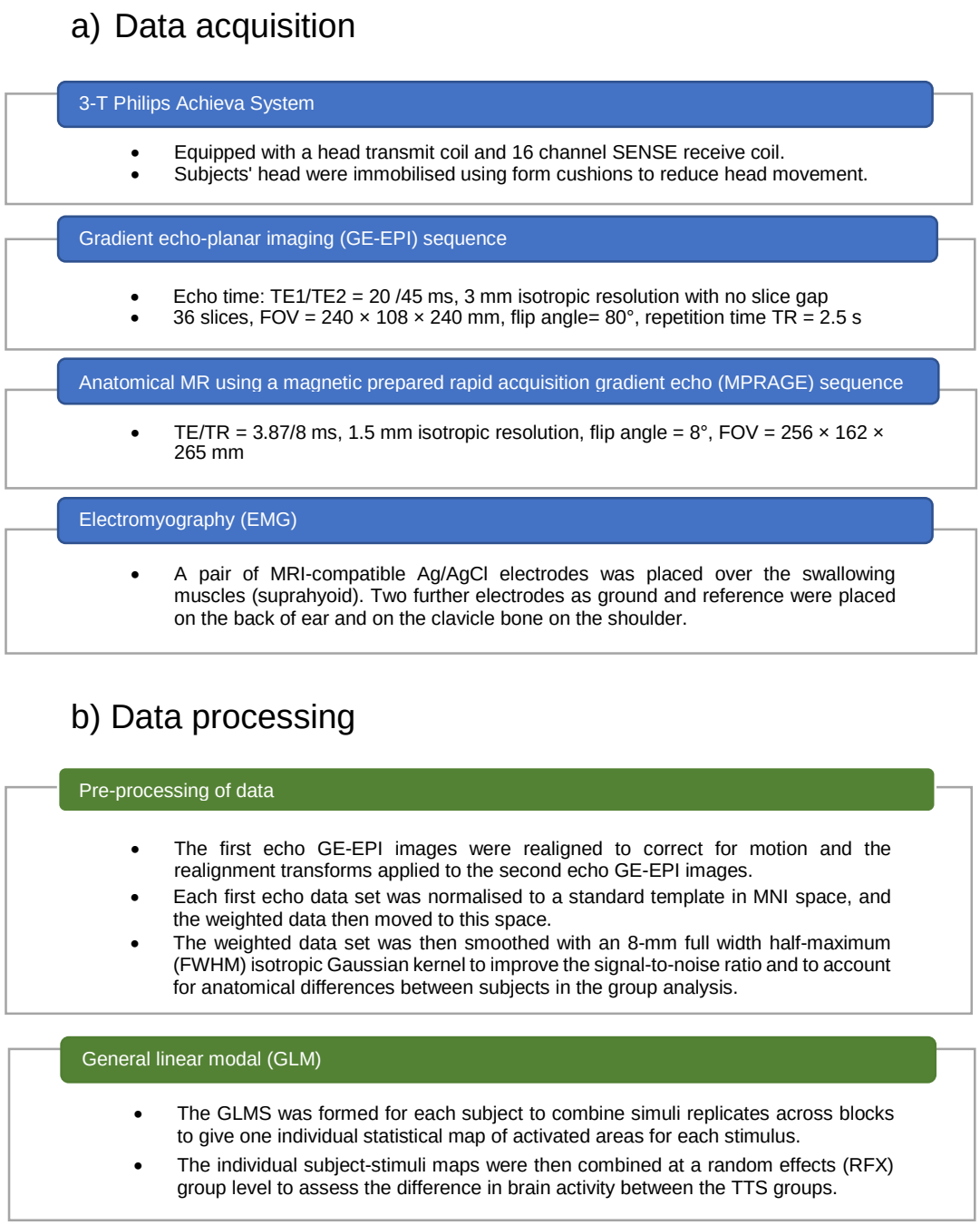

Figure 6-8: Details and steps of processing fMRI data: a) Data acquisition and b) Data processing of fMRI data.

The areas of the brain associated with sensory perception, known as Regions of Interests (ROIs) were defined by colleagues in the SPMMRC from previous fMRI taste studies (Eldeghaidy *et al.*, 2011) (**Table 6-1**). Following data

processing, the beta values associated with each ROI for each stimulus of each individual was provided for further data analysis and interpretation.

Table 6-1: *A priori* chosen ROIs to assess beta values in response to stimuli for each taster status group, along with the definition of how these ROIs were generated.

***A priori* region**	**Mask**
S1(mouth)	8mm sphere centred at 60, -6, 20
SII	Brodmann area (BA) 43 dilated by 1
Anterior insula	8m sphere centred at 40, 10, -2;
Mid-insula	8m sphere centred at 40, 0, 0
Posterior insula	8mm sphere, dilated by 1 centred at 44, -32, 12
Thalamus	determined anatomically (from AAL atlas)
ACC	14mm sphere, dilated by 1 and centred at 2, -10, 56
Amygdala	determined anatomically (from AAL atlas)
Lateral OFC	8mm sphere centred at 28, 30, -10
Medial OFC	8mm sphere centred at 6, 44, -2.

6.2.5. Data analysis

The consistency of thermal taster status phenotyping with time (one year apart) was first evaluated. To examine if any differences exist on the pooled temperature (both warm and cold) and 'phantom taste' intensity ratings over two visits within each TTS group, one-way ANOVA was used respectively.

The button press data were transformed to the equivalent log ratings on the gLMS scale (e.g. 0=-0.03, 1=0.67, 2=1.23, 3=1.54, 4=1.73) for further analysis. One-way ANOVA with Tukey's post-hoc tests, where appropriate, were applied on each attribute (sweetness and fruitiness) among all four samples (SUC_{COLD}, $SUC_{AMBIENT}$, $EB_{AMBIENT}$, $SUC/EB_{AMBIENT}$). One-way ANOVA were

then used to compare TTs and TnTs on perceived intensity ratings (sweetness and fruitiness respectively) for each sample (SUC_{COLD}, $SUC_{AMBIENT}$, $EB_{AMBIENT}$, $SUC/EB_{AMBIENT}$). Furthermore, a t-test was applied to sweetness intensity ratings between SUC_{COLD} and $SUC_{AMBIENT}$ within each TTS group to examine if sample temperature had any effect on perception.

For fMRI data, the beta values for each ROI for each stimulus and each individual was extracted by colleagues in the SPMMRC to enable further data analysis. One-way ANOVA, with Tukey's post hoc tests where appropriate, were performed on beta values across the four samples at each brain ROI, to determine if differences existed in cortical responses to different stimuli. To examine the effect of TTS groups on cortical responses, one-way ANOVA were performed on the pooled beta values for all four samples at each ROI. To further investigate if there were any differences caused by any particular stimulus, one-way ANOVA on the beta values at each ROI for each stimulus were applied across TTS groups.

To test if differences were present between TTs and TnTs in terms of temperature effect (cold) on cortical response, the difference in cortical response to SUC_{COLD} and $SUC_{AMBIENT}$ (SUC_{COLD}>$SUC_{AMBIENT}$) of each ROI was calculated. One-way ANOVA were then performed on the difference in cortical responses to SUC_{COLD} and $SUC_{AMBIENT}$ among TTS groups.

An overall heightened cortical response was found for TTs compared to TnTs. To investigate if the intensity of 'phantom taste' perceived by TTs was associated with any ROI cortical responses, Pearson's correlation coefficients were calculated to examine the relationship between perceived 'phantom taste'

intensity (warm and cold trial respectively) and cortical response to all four stimuli combined at each ROI. To further test if the association was driven by the cold temperature sample, Pearson's correlation coefficients were also calculated between cold 'phantom taste' intensity and cortical response to SUC_{COLD} at each ROI.

6.3. RESULTS

6.3.1. Reliability of thermal taster status phenotype

One of the 24 subjects was re-classified, due to the results from the second evaluation. The subject was previously identified as a TT, but was unable to perceive 'phantom taste' on her second visit, and was therefore re-classified as a TnT. To balance the number of participants in each TTS group, a randomly selected TnT along with the aforementioned subject were removed prior to further analysis. No significant differences in perceived temperature and 'phantom taste' intensities were observed between these two visits within each TTS group (see **Figure 6-9**). The data suggests that TTS phenotype, perceived temperature and 'phantom taste' intensity did not change over time for most subjects.

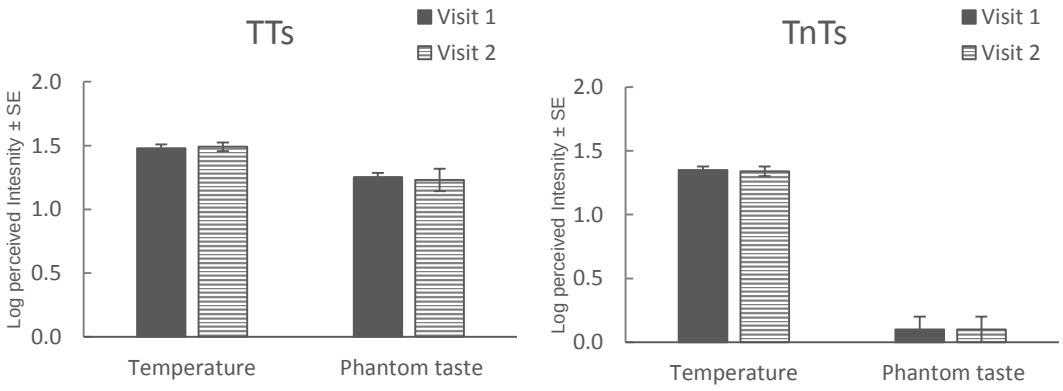

Figure 6-9: Log perceived intensity of temperature and 'phantom taste' between visit 1 and 2 within each TTS group.

6.3.2. Perceived sensory attribute intensity

Across all individuals, one-way ANOVA among the four samples revealed significant differences for both sweet and fruity intensity ratings ($p<0.001$). Post-hoc tests showed that for the sweet intensity rating, $SUC/EB_{AMBIENT}$ was rated significantly higher than $SUC_{AMBIENT}$ and $EB_{AMBIENT}$. $EB_{AMBIENT}$ was rated higher than the other three samples. Additionally, SUC_{COLD} did not differ from $SUC_{AMBIENT}$ or $SUC/EB_{AMBIENT}$. For fruitiness intensity, the $SUC/EB_{AMBIENT}$ sample was rated significantly higher than the other three samples and $EB_{AMBIENT}$ was rated significantly lower. No significant difference was observed between SUC_{COLD} and $SUC_{AMBIENT}$, as indicated in **Figure 6-10**. The data here suggests that the combination of SUC/EB enhances both sweet and strawberry intensity.

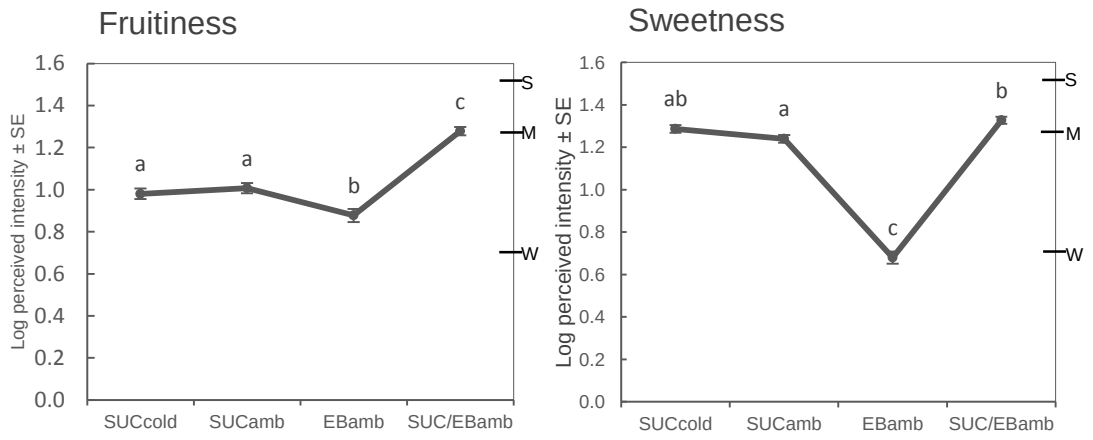

Figure 6-10: Log perceived sweetness and fruitiness among SUC_{COLD}, $SUC_{AMBIENT}$, $EB_{AMBIENT}$ and $SUC/EB_{AMBIENT}$. *Different letters within each graph indicate significant different at $p<0.05$.*

6.3.3. Perceived Intensity of sensory attributes among TTS group

One-way ANOVA indicated that TTs rated sweetness of SUC_{COLD} as significantly higher ($p=0.02$) than TnTs. Ratings for $SUC/EB_{AMBIENT}$ were also higher and this approached significance ($p=0.07$). No significant differences in

sweetness ratings were observed for $SUC_{AMBIENT}$ and $EB_{AMBIENT}$ ($p>0.05$) between TTs and TnTs. For fruitiness, TTs tended to rate $EB_{AMBIENT}$ higher than TnTs and this approached significance ($p=0.07$), whereas, no significant differences were observed for the other three stimuli. The t-test applied to examine the temperature effect (cold) on the perceived sweet intensity of sucrose (SUC_{COLD} vs $SUC_{AMBIENT}$) within each TTS group revealed that at a cold temperature perceived sweetness was enhanced in TTs ($p=0.06$), whereas no temperature effect was found in TnTs group. ($p= 0.4$) (**Figure 6-11**).

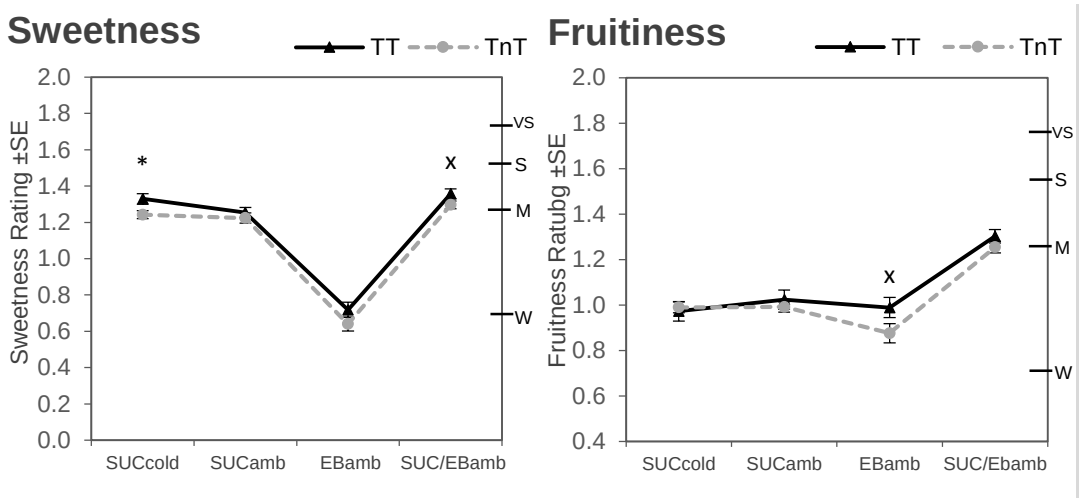

Figure 6-11: Sweetness and fruitiness intensity ratings between TTs and TnTs perceived inside scanner. *Data represents mean intensity scores ± SE. Means that differ at significance level of p<0.05, p<0.1 are indicated by *, ˣ, respectively.*

6.3.4. Cortical activation among four stimuli

Figure 6-12 shows the cortical activation in beta values in response to all four individual stimuli (SUC_{COLD}, $SUC_{AMBIENT}$, $EB_{AMBIENT}$ and $SUC/EB_{AMBIENT}$). One-way ANOVA revealed significant differences among the four stimuli for most ROIs ($p<0.05$), with the exception of the piriform. Post-hoc tests showed that the SUC_{COLD} were generally found to induce higher cortical activation than the

$SUC_{AMBIENT}$, with ACC, SI, SII and mid-insula reaching significance. In addition, SUC_{COLD} induced significantly higher activation than $EB_{AMBIENT}$ and $SUC/EB_{AMBIENT}$ for most ROIs, except for the thalamus and piriform. $SUC_{AMBIENT}$ induced significantly higher activation than $EB_{AMBIENT}$ and $SUC/EB_{AMBIENT}$ in the anterior insula, ACC, SI, SII, and mid-insula. Surprisingly, no significant difference was found for activation strength in any ROI between $EB_{AMBIENT}$ and $SUC/EB_{AMBIENT}$.

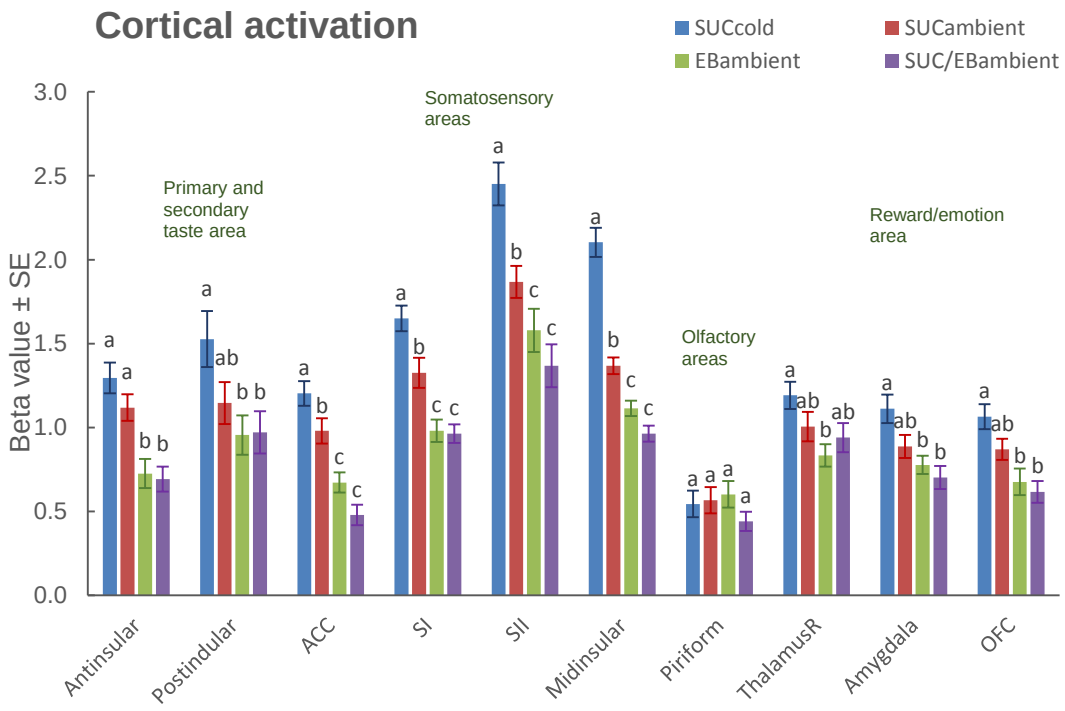

Figure 6-12: Cortical activation in response to SUC_{COLD}, $SUC_{AMBIENT}$, $EB_{AMBIENT}$ and $SUC/EB_{AMBIENT}$. *Data represents beta value ± SE. Different letters within each brain region indicate significant difference at p<0.05.*

6.3.5. Main effect of TTs and TnTs on cortical activation

One-way ANOVA on pooled data (SUC_{COLD}, $SUC_{AMBIENT}$, $EB_{AMBIENT}$, $SUC/EB_{AMBIENT}$ combined), revealed that TTs had a generally higher cortical activation than TnTs, and that some specific ROIs (SI, SII and mid-insula)

approached significance ($p<0.1$). To assess whether the heightened cortical response was driven by one stimulus, further one-way ANOVA comparing TTs and TnTs for each stimulus were applied. **Figure 6-13** shows that the difference was driven by SUC_{COLD}. For this comparison, a significant increase in the beta values of SI, SII and mid-insula was found for TTs compared to TnTs ($p<0.001$). Although the same trend was found for most ROIs, the other three stimuli were not significantly different between TTs and TnTs. **Figure 6-14** shows the cortical map highlighting the differences between TTs and TnTs for SUC_{COLD}.

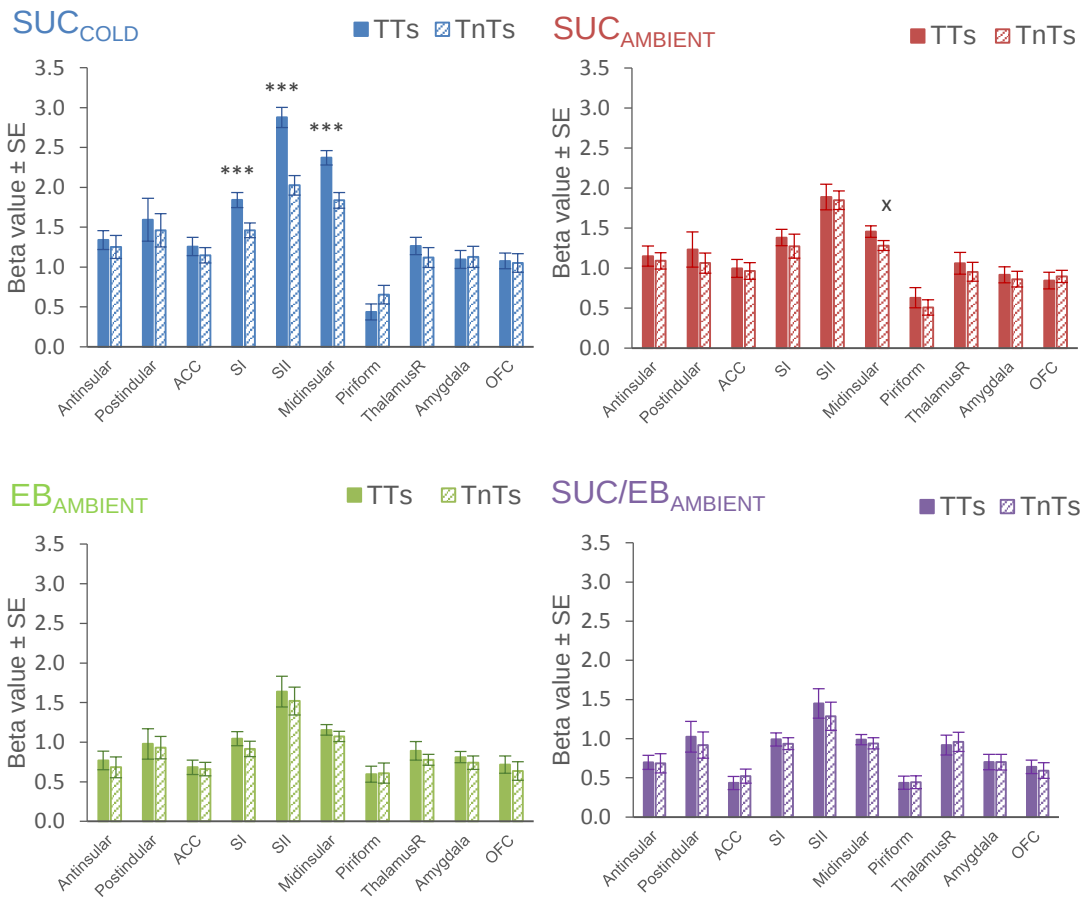

Figure 6-13: Cortical activation between TTs and TnTs for SUC_{COLD}, $SUC_{AMBIENT}$, $EB_{AMBIENT}$, $SUC/EB_{AMBIENT}$. *Data represents beta value ± SE.* ^{x} indicates significantly different at $p<0.05$, ***indicates significantly different at $p<0.001$.

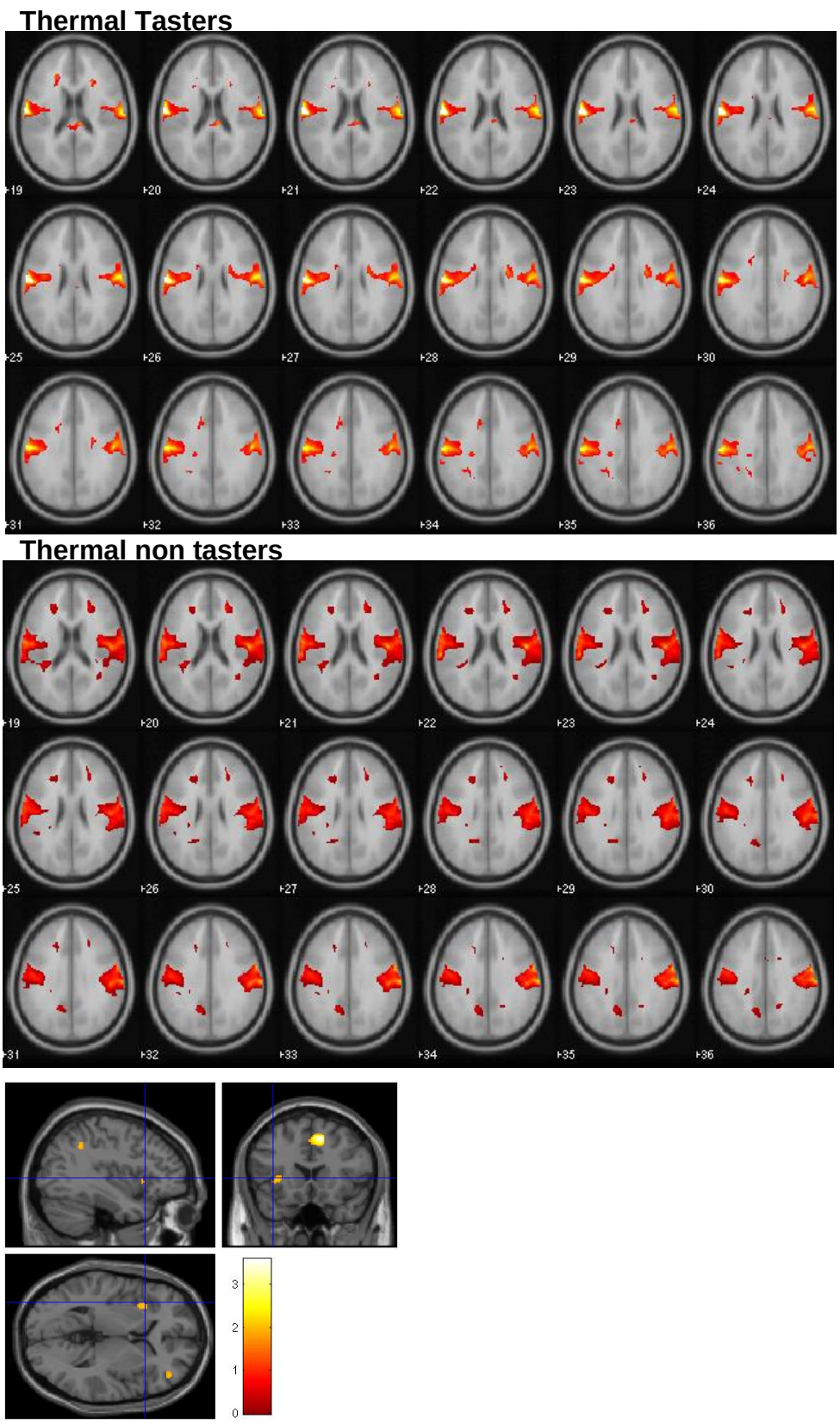

Figure 6-14: Cortical map between TTs and TnTs for SUC_{COLD}. *Colour scale indicates the degree of significance of each pixel (Higher significance of difference of activation in white pixel and poorer activation in dark red pixel). Figure courtesy of SPMMRC.*

6.3.6. Comparison between SUC_{COLD} and $SUC_{AMBIENT}$ among TTS groups

Figure 6-15 highlights that TTs produce higher activation than TnTs when comparing the responses of SUC_{COLD} and $SUC_{AMBIENT}$ (SUC_{COLD} > $SUC_{AMBIENT}$). One-way ANOVA revealed a significant increase in beta value in mid-insula (0.008), and SII (0.001) for TTs compared to TnTs. This suggests the cortical response is enhanced at cold temperature in TTs compared to TnTs.

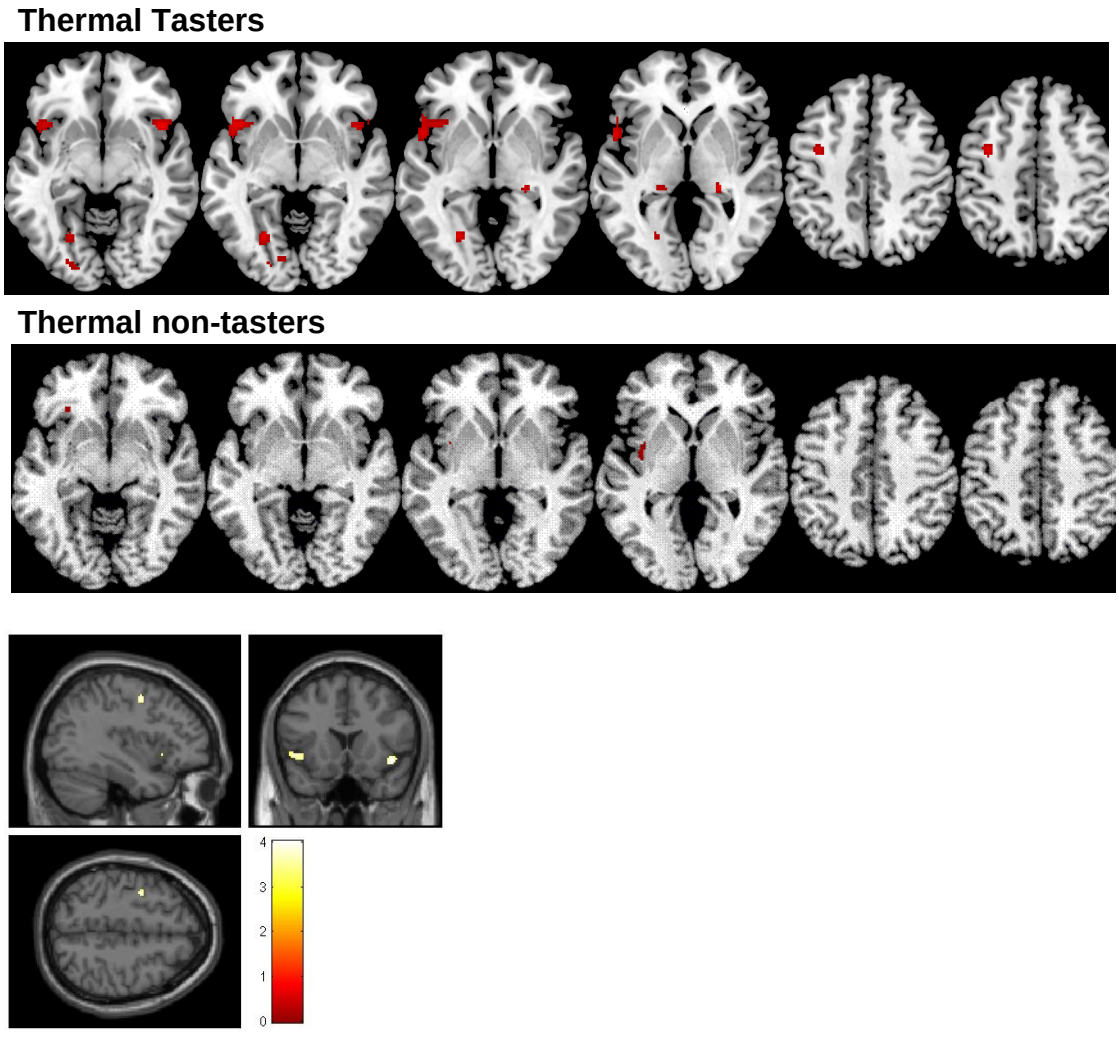

Figure 6-15: Difference in response in TTS group to the sucrose at cold and ambient temperature (SUC_{COLD}>$SUC_{AMBIENT}$). *Colour scale indicates the degree of significance of each pixel (Higher significance of difference of activation in white pixel and poorer activation in dark red pixel). Figure courtesy of SPMMRC.*

6.3.7. Relationship between 'phantom taste' intensity and brain activation within TTs

As discussed previously in Chapter 5, 'phantom taste' intensity may be a marker of sensitivity to oral sensations. One possible mechanism is the co-innervation of taste and trigeminal nerves in TTs, the degree of intertwining and number of taste buds contribute to the intensity of 'phantom taste'. It is interesting to test if the intensity of 'phantom taste' was associated with cortical activation in ROIs. In order to examine this relationship, Pearson's correlation coefficients were calculated between 'phantom taste' intensity (warm and cold trial, respectively) and pooled cortical activations for all four stimuli combined. Results revealed that the 'phantom taste' intensity perceived from the cooling trial was significantly correlated with the post-insula ($r=0.654$, $p=0.03$) and SI ($r=0.682$, $p=0.02$) (**Figure 6-16)**. No significant association was found between 'phantom taste' intensity from the warming trial and cortical activations in any ROIs, this could be due to the fact no warm stimulus was included in this study.

Further analysis investigating the relationship between 'phantom taste' intensity (cooling) and cortical response to each stimulus (SUC_{COLD}, $SUC_{AMBIENT}$, $EB_{AMBIENT}$, $SUC/EB_{AMBIENT}$) was performed to determine if the correlation was driven by any particular stimuli. An association approaching significance was found between 'phantom taste' intensity (cold trial) and beta values in post-insula area ($r=0.55$, $p=0.08$) induced by SUC_{COLD}, further suggesting that the post-insula might contribute to 'phantom taste' responses perceived in TTs.

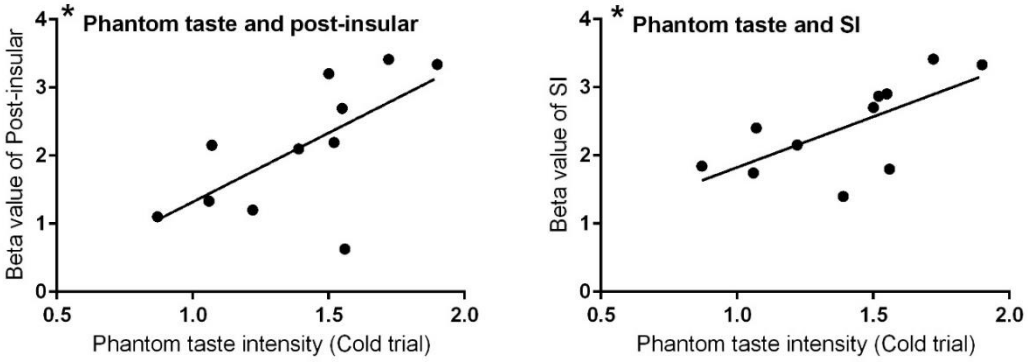

Figure 6-16: Scatter plot between 'phantom taste' intensity (Cold trial) and beta value of post-insula and SI. *The beta value shows pooled data of all four stimuli stimulation. *indicate significant correlation at p<0.05.*

6.4. DISCUSSION

6.4.1. Reliability of TTS phenotype

This is the first study to investigate and subsequently demonstrate that TTS phenotype are consistent over time (measures were one year apart) and the consistency of perceived temperature and 'phantom taste' intensity. This adds to the evidence concerning the existence of this phenotype. In addition, it also suggested that the mechanism behind TTS is constant and was not altered over time.

6.4.2. Comparison between behaviour and cortical activation among four stimuli

The results in the current study revealed that SUC_{COLD} induced increased cortical activations in gustatory and somatosensory cortices compared to $SUC_{AMBIENT}$, but not in the primary olfactory cortex (piriform). The increase in gustatory and somatosensory cortices may explain the slightly heightened perception of sweetness perceived from SUC_{COLD} compared to $SUC_{AMBIENT}$, and the slightly enhanced the sweetness of cold SFD observed in Chapter 5. Perceived intensity ratings showed that SUC/EB combination enhanced the

intensity of sweet and fruity, compared to SUC or EB presented alone. However, this trend was not observed in the cortical activations, in fact, $SUC/EB_{AMBIENT}$ and $EB_{AMBIENT}$ induce lower cortical activations than SUC_{COLD}. Previous studies showed super-additive effects for the mixture of sucrose + Vanilla (retronasally) in ACC, dorsal insula, anterior ventral insula extending into the OFC, frontal operculum, ventral lateral prefrontal cortex, and posterior parietal cortex (Small *et al.*, 2004), when comparing the activation of SUC/Vanilla and sum of SUC and Vanilla (SUC/Vanilla – (SUC + Vanilla)). In two other studies, using similar stimuli to this current study, one revealed a significant enhancement on the left anterior OFC for the stimulation of SUC/Strawberry (retronasally) (de Araujo *et al.*, 2003), whilst the other observed deactivation for SUC/Strawberry (orthonaslly) (Small *et al.*, 1997) relative to unimodal presentation of taste and smell stimulation. The previous findings together with the current study suggested that flavour processing is complex and may not be represented by simple convergence of each stimulus. In addition, the differences in sensory perception are not necessarily reflected at cortical level.

6.4.3. Effect of thermal taster status on cortical activation

This present study found an overall heightened activation for TTs compared to TnTs, which was mainly driven by one stimulus (SUC_{COLD}). Compared to TnTs, TTs had a significantly higher activation in the somatosensory regions (SI, SII and mid-insula) for SUC_{COLD}. This seems to relate to measures of temperature intensity, as TTs were shown to rate the temperature intensity perceived from both warming and cooling as significantly more intense

(Chapter 2), as well as being more sensitive to temperature changes (Chapter 5) than TnTs.

The only previous TTS study conducted by Clark (2011) examined the effect of TTS on cortical responses to 3 levels of CO_2, and found that TTs showed significantly higher activations in SI, SII and ACC to TnTs. Interestingly, Clark (2011) found that TTs could correctly discriminate the high CO_2 sample more times than TnTs, which also appeared to shape preference, as the high CO_2 sample was liked least and no CO_2 sample was more preferred amongst TTs. In agreement with Clark (2011), the findings here suggest that the differences in cortical response to trigeminal stimuli between TTs and TnTs seem to occur in the somatosensory area, which further supports the proposed hypotheses in TTs of intertwined taste and trigeminal nerves in fungiform papillae. However, it could also support the hypotheses of a central 'gain' mechanism (hyper-connection of greater excitability in convergence of gustatory and somatosensory brain regions) (Green & George, 2004).

As indicated in **Figure 6-4**, the insula, ACC and thalamus were involved in transmission of taste information. This study observed a trend, although not significant, that TTs also have a higher activation in the anterior insula, post-insula, ACC and thalamus than TnTs with SUC_{COLD} stimulation. It is therefore likely that the enhancement of the perceived sweetness in SUC_{COLD} compared to $SUC_{AMBIENT}$ in TTs is a direct result of the increased cortical activation in gustatory and somatosensory cortices in TTs.

Additionally, no differences were observed in the olfactory cortex (piriform) across TTS groups, contributing to the hypothesis of co-innervation of taste

and trigeminal nerves. Because the co-innervation of taste and trigeminal nerves would have restricted an effect on aroma perception. Although no difference was observed in the olfactory cortex between TTs and TnTs, there is a difference in perceived fruity intensity for $EB_{AMBIENT}$, with TnTs rating lower than TTs. It is possible that other ROIs that were not included in this study may contribute to this phenomenon or that perception is more related to the combination of all ROIs. The fruity data suggests that differences in cortical activation are not always related to perceived differences in perception and further work is necessary to discover what increase in cortical response may cause an increase in perception.

This study also revealed that the 'phantom taste' intensity perceived from the cooling trail was significantly correlated with cortical activation in SI and post-insula. This suggests that post-insula and SI may contribute to the processing of the 'phantom taste' responses perceived in TTs. One of the limitations of this study is that temperature stimulus alone was not included. This study examined the temperature effect on sucrose solution, and hence, the activation of brain regions could not be differentiated between temperature stimulus and sucrose stimulus. Further studies examining the effect of TTS on cortical activation of temperature stimulation alone would be needed to further confirm if the post-insula and SI are correlated with 'phantom taste' responses perceived from coldness. Further studies should also investigate the temperature effect of both warming and cooling, in order to define if the 'phantom taste' evoked from warming and cooling correlate with cortical activation in different ROIs. This would contribute to the current knowledge of

the fundamental mechanisms of TTS (e.g. if 'phantom taste' perceived for warming or cooling are independent).

6.5. CONCLUSION

This is the first study to test the reliability of TTS phenotype classification over time, and interestingly the TTS phenotype was found to be generally consistent. Although the SUC/EB combination significantly enhanced the intensity of sweetness and fruitiness compared to SUC or EB presented alone. At cortical level, this was not the case, the $SUC/EB_{AMBIENT}$ did not show any additive effect over SUC or EB alone. This indicates that flavour processing is complex and the differences in sensory perception may not be clearly reflected in differences in cortical activation. More studies are needed to further investigate the relationship between perception and cortical activation.

TTs were found to respond to taste and trigeminal stimuli more intensely than TnTs (Chapter 3). It was interesting to test if the difference in perception across TTS groups was reflected at the cortical level. This is the first study providing the opportunity to investigate the difference in cortical activation of taste (sucrose) and aroma (EB) stimuli, as well as examining the temperature effect on cortical activation across TTS groups. The cortical activations in TTs were generally heightened to all stimuli combined, compared to TnTs, and the difference was suggested to be driven by SUC_{COLD}. In detail, TTs showed significantly higher cortical activation than TnTs in regions of the somatosensory cortex (SI, SII and mid-insula) to SUC_{COLD}, which is a direct reflection of behavioural data: the sweetness perceived from sucrose was enhanced by cold temperature in TTs, whereas no enhancement was found in

TnTs. The increased cortical activation in somatosensory cortex could also be a direct reflection of the findings that TTs are more sensitive to temperature than TnTs (as reported in previous chapters).

In addition, no differences in cortical activation in the olfactory cortex across TTS groups were observed. The lack of differences in the olfactory cortex (piriform) and the differences on somatosensory cortex contribute to the hypothesis of co-innervation of taste and trigeminal nerves in TTs, as co-innervation between taste and trigeminal nerves would have little impact on olfactory perception. Interestingly, novel findings here are also the significant correlation between ‘phantom taste’ intensity perceived from cooling train and the cortical activation in the brain regions of SI and post-insula in TTs. This finding provide plausible evidence that SI and post-insula may contribute to the perceived intensity of ‘phantom taste’. A limitation of this study was that temperature alone was not included, causing a lack of evidence on the relationship between ‘phantom taste’ evoked from temperature and their associated cortical activations in TTs. Further studies investigating the cortical response to ‘phantom taste’ perceived from both warming and cooling are needed in order to decouple the mechanism behind TTs. In addion, tests to understand if the mechanisms behind warming and cooling trials are independent would also be of interest.

7. GENERAL DISCUSSION AND FUTURE WORK

7.1. INTRODUCTION AND OBJECTIVES

A large body of research has been conducted to understand the impact of PTS in taste perception and how is linked to food preference and food intake (Drewnowski *et al.*, 1997; Gent & Bartoshuk, 1983). However, there has been far less research looking at the newly discovered taste phenotype - thermal taster status (TTS). Only nine published papers from three labs have investigated thermal taster status and its impact on sensory perception and food liking, and the mechanism behind TTS remains unknown. So far, few hypotheses have been proposed to explain the phenomenon of TT: The first hypothesis is that TRPM5 might contribute to the phenomenon of 'phantom taste', as Increasing the temperature from 15 to 35 ℃ was thought to enhance sweetness perception in wild mice compared to TRPM5 knockout mice, however, no such increase was observed for other stimuli (e.g. MSG, HCl or NaCl) (Talavera *et al.*, 2007; Talavera *et al.*, 2005). The second hypothesis is cross-wiring between taste and trigeminal nerves in fungiform papillae may be the reason behind 'phantom taste' responses and their heightened intensity responses (Clark, 2011). The third hypothesis is that increased intensity perception in TTs is happening at a higher level and that, a central gain might contribute to the mechanism behind TT (Green & George, 2004).

The overall aim of this research was to: 1) examine the incidence of TTS in the UK population and also attempt to understand the relationship between personal traits and taste phenotypes; 2) investigate taste phenotypes, specifically TTS and PTS, and the relative effect of TTS and PTS across a

range of gustatory, trigeminal and olfactory modalities, in order to decouple the mechanism behind TTS and PTS; 3) investigate the relationship between PTS phenotypes and genotypes, specifically TAS2R38 and gustin rs2274333 as well as FP counts and oronasal sensitivity; 4) working with colleagues at the SPMMRC provided the opportunity to examine the cortical activation to sensory stimuli across TTS, to add to evidence concerning the mechanism involved in TTS.

7.2. MAIN FINDINGS

7.2.1. Incidence and classification of TTS

This study found that approximately 27% of the population sampled in the UK were TTs, who could consistently perceive 'phantom taste' by both warming and cooling. Previous studies have reported that the proportion of TTs was between 20 to 50% (Bajec & Pickering, 2008; Green & George, 2004). The differences in the proportion of TTs were speculated to be due to the classification method which varied among the studies. The results presented in Chapter 2 highlighted the challenges of classification, as the 'phantom taste' responses were inconsistent for some individuals. However, it is difficult to standardise the classification method, as the mechanism behind TTS is not fully understood.

7.2.2. Link between taste phenotypes and personal traits

Few studies have investigated the relationship between PTS, food preference and intake (Dinehart *et al.*, 2006; Duffy, 2004; Keller *et al.*, 2002; Tepper & Nurse, 1998). The novel study presented in Chapter 2 looked at the link between PTS and TTS as well as self-reported personal traits. Interestingly,

TTs self-reported to be less food neophobic and more willing to get involve with food than TnTs. In addition, both taste phenotypes (TTS and PTS) were found to be associated with personality. TTs considered themselves to have a more active imagination than TnTs, whereas pNTs thought themselves to be more conscientious than PROP tasters.

7.2.3. The impact of TTS on oronasal sensitivity

Taste, trigeminal and aroma intensities on the anterior tip of the tongue (rolling saturated cotton buds) were collected from each subject described in Chapter 3. In general agreement with previous studies (Bajec & Pickering, 2008; Green & George, 2004) TTs were found to have a heightened intensity perception compared to taste and trigeminal stimuli than TnTs, but no difference was found for aroma stimuli. A lack of the difference in aroma perception between TTs and TnTs, contribute to the proposed hypotheses of the co-innervation of taste and trigeminal regions at the periphery or cortical level. As the co-innervation of taste and trigeminal nerves would have a restricted impact on aroma perception.

In comparison to the results in Chapter 3, the study described in Chapter 5 continued to examine the sensitivity within the whole oral cavity. The impact of TTS on oronasal sensitivity diminished when measured the whole oral cavity, thus suggesting the mechanism behind TTS is likely to occur in fungiform papillae.

The data also highlighted for the first time that TTs could perceive ‘phantom taste’ not only from a thermode, but also from drinking hot and cold liquid. In addition, TTs were more sensitive to temperature changes than TnTs.

The most interesting finding was that ‘phantom taste’ intensity from water was positively correlated with temperature intensity of SFD in TTs, indicating that ‘phantom taste’ intensity may be a marker of general temperature intensity. If cross-wiring of taste and trigeminal nerves is the mechanism behind TTs, then a higher degree of intertwining and number of fungiform papillae (normally indicating more taste and trigeminal nerves) could boost signals during either taste or trigeminal stimulation, which could cause a heightened responses to ‘phantom taste’, as well as an overall heightened taste and trigeminal responsiveness. However, as due to the small sample size of this study, further work with a larger number of subjects is required to confirm these findings.

7.2.4. The impact of PTS on oronasal sensitivity

For PROP taster status, pSTs and pMTs rated the perceived intensity of oronasal sensations more intense than pNTs either measuring the sensitivity of the anterior tongue tip or the whole oral cavity. The data suggests PTS is more likely to be related to the papillae that surrounds the whole oral cavity or is a stronger effect within the papillae.

7.2.5. The relative effect of TTS and PTS on oronasal sensitivity

No significant correlation was found between TTS and PTS classification, indicating that these two taste phenotypes are likely to operate via different mechanisms. This is the first study to investigate the combined impact of both taste phenotypes on oronasal sensitivity. Interestingly, the results in Chapter 3 and 5 found some interactions between TTS and PTS in perceived intensity, with pMTs most affected by TTS. The reason behind these findings is unknown,

but it may be linked to fungiform papillae, as there may be considerable gain in pMT group.

7.2.6. Impact of TAS2R38, Gustin rs2274333 and FP counts on oronasal sensitivity

The results in Chapter 4 further proved that the dominant gene, TAS2R38, modulating PROP perception, could not explain PROP tasters' heightened sensitivity to other oronasal stimuli. In addition, gustin rs2274333 polymorphism did not link to FP counts or oronasal sensitivity (including PROP). The linear regression model using PROP intensity and FP counts further revealed that both PROP intensity and FP count contributed to oronasal sensitivity, but the coefficients were low in all predicted models, indicating there are other factors that may also affect oronasal sensitivities.

7.2.7. Cortical responses to sensory stimuli across TTS group

In collaboration with SPMMRC, this study investigated the cortical activation to taste and aroma stimuli, as well as temperature effects across TTS groups. Interestingly, the cortical activation in the somatosensory areas of TTs were found to be significantly increased when comparing SUC_{COLD} and $SUC_{AMBIENT}$, compared to TnTs. The evidence of the enhanced cortical activation in TTs could be a direct reflection on the enhancement of sweetness by cold temperature in TTs, whereas no such enhancement was found in TnTs. It may also be a direct reflection of temperature findings observed previously, where TTs rated the temperature intensity on the tongue tip to be significantly more intense (Chapter 3), and TTs were also more sensitive to temperature changes (Chapter 5).

Interestingly, the ‘phantom taste’ intensity perceived from the cooling trial was significantly correlated with the pooled activation of the ROI regions of post-insula and SI in TTs, which raised the possibility that the SI and post-insula brain areas may be involved in the ‘phantom taste’ phenomenon.

7.3. MAIN CONCLUSIONS

This study continues to highlight the considerable incidence of thermal tasters in the population and the range of phantom taste, indicating that the phenomenon of TTs warrants scientific consideration. In addition to understand the mechanism behind TTS, more work is also needed to develop a standardised classification method, once the mechanism behind TTS is better understood.

This is the first study providing evidence linking taste phenotypes and self-reported personal traits. Although more research is required to confirm if taste phenotypes could be used as a marker of food behaviour, such findings could further inform the understanding of food choice behaviour models. This specific relationship could be further tested to predict food preference and food behaviour. But more research is now needed to ascertain why this would be the case.

This research confirmed that TTs have an overall increased intensity perception of taste and trigeminal stimuli (including temperature) when measuring at the anterior tip of the tongue, but not for aroma. Interestingly, TTs showed to have a greater sensitivity to temperature changes than TnTs in SFD when measuring the sensitivity of the whole oral cavity. However the heightened intensity response in TTs was diminished when extends from

anterior tip to the whole oral cavity. Such findings now contribute to the proposed hypothesis of cross-wiring between taste and trigeminal nerves in fungiform papillae, with effects more prominent on the tip of the tongue compared to stimulation of the whole oral cavity. This is the first study that observed variation within TTs concerning ‘phantom taste’ intensity compares with the intensity data from the SFD, interestingly found that ‘phantom taste’ intensity may be a predictor of perceived temperature intensity perception. This further contributes to the hypothesis of cross-wiring between taste and trigeminal nerves in fungiform papillae in TTs. As FP number and degree of intertwining were speculated to modulate both perceived intensity of ‘phantom taste’ and oral sensitivity.

No significant correlation was found between TTS and PTS classification, indicating these two taste phenotypes are likely to operate via different mechanism. Interestingly, pMTs were found to be most affected by TTS, as pMTs who were TTs, they can be upgraded to similar level of sensitivity as pSTs. The reason why pMTs were most affected by TTS is unknown, but FP counts were speculated to be one of the reason.

PROP tasters’ heightened oronasal intensity perception was found to exist on the anterior tip of the tongue and this extends to the whole oral cavity. This study found that both TAS2R38 and gustin rs2274333 genotypes could not explain the increased sensitivity of PROP tasters. Although both FP counts and PROP intensity together could predict the intensity of some stimuli, the coefficients were low in all regression models, indicating other factors may also contribute to oronasal sensitivity that have not been considered in this study.

The findings on investigating the cortical activation to sensory stimuli using fMRI were interesting and novel. It confirmed that TTs have greater cortical activation in the somatosensory cortex than TnTs, especially when consuming SUC_{COLD}. It provided evidence that the difference between TTs and TnTs are likely to happen during the processing of trigeminal activation. The findings support either periphery or cortical hypotheses: co-innervation of taste and trigeminal nerves in fungiform papillae or hyper connection/excitability of gustatory and somatosensory cortices. More research is now needed to pinpoint at which level of the co-innervation of taste and trigeminal nerves is happening.

7.4. IMPLICATIONS

7.4.1. Understanding consumer behaviour

A key novel finding in this work is that TTs do not only have the ability to pick up 'phantom taste' from a thermode, but also from drinking hot or cold beverages. This information is typically useful for industries producing products with extreme temperatures, as it may provide an explanation for some odd cases of customer complaint regarding metallic taste from cold water or beer consumption and variation in consumer responses.

7.4.2. Developing personalised foods and beverages

This study has advanced the current understanding concerning how taste genotype and phenotype, as well as combinations of different phenotypes can affect oronasal sensitivity. Considering the implications found here, it could aid food manufactures to reformulate or develop new products for different

genotypic and phenotypic groups to meet individual sensory demands (Brookman, 2013).

7.4.3. Understadning sensory panels' sensitivity

It is useful to be aware of a sensory panel's taste phenotype as it may help to explain some of the disagreement amongst panellist data. It is also possible to create a sensory panel of thermal tasters, which may help increase sensitivity and reduce the variability.

7.4.4. Possible marker of food preference and food choice behaviour

The novel findings here provide evidence of a link between taste phenotypes and self-reported personal traits, indicating that taste phenotypes may be a predictor of personal traits.

The data in this study also showed that TTs have a lower preference to strawberry flavoured drink when consuming at extreme temperatures than TnTs. This provides preliminary evidence that temperature may affect food preference across TTS groups, especially for food and beverages served at extreme temperatures such as pizza, beer and ice cream. The results also demonstrated that TTs were more sensitive to temperature changes than TnTs, thus TnTs compared to TTs may have greater tolerances to varying serving temperatures.

Fundamental research into individual variation in sensory perception is not limited to researchers, but also extends to the wider public. Understanding individual variation in sensory perception will help to further understand

consumer food choice behaviour and ultimately links to feelings of health and wellness.

7.5. FURTHER WORK

Suggestions for further research have been made throughout this thesis, and are summarised below:

7.5.1. Decouple the mechanism behind TTS

Further work is needed to test at what temperatures ‘phantom taste’ were evoked, and if different temperatures evoke different ‘phantom taste’. This may provide information on associations between ‘phantom taste’ and some temperature sensitive transduction pathways, and therefore contributing to identify the mechanisms behind TTS. In addition, the fMRI technique could also be used to help identify the brain regions that are activated during the process of perceiving ‘phantom taste’ in TTs to further elucidate the mechanism behind TTS. Once the mechanism is better understood, further work should be conducted to develop a standardised TTS classification method to enable valid comparison between studies.

Thermal taster status was speculated to be linked to synaesthesia, and this is known to run in families. Hence, it would be interesting to evaluate if TTS is happening in families as well, which may help to determine if TTS is under genetic controlled and if it is indeed part of the synaesthesia family.

7.5.2. Further understand the relationship between taste phenotypes and personal traits

This study provided preliminary evidence for the link between TTS, food behaviour and personality traits. Further studies examining the relationship between taste phenotypes and more food choice behaviour questionnaires such as the Food Choice Questionnaire (Steptoe *et al.*, 1995), Health and Taste Attitudes (Roininen *et al.*, 1999), Three-Factor Eating Questionnaire (Stunkard & Messick, 1985), Affect Intensity Questionnaire (Larsen *et al.*, 1986) are needed to determine if taste phenotypes can be used as a marker of food choice behaviour. More research is also needed to ascertain why this would be the case.

7.5.3. Understand the relationship between taste phenotypes and food preference and preferred serving temperature

Further investigations into how taste phenotypes impact food preference using both Food Preference Questionnaires (Meiselman & Waterman, 1978) and real food consumption are needed to determine the impact of taste phenotypes on food preference. In particular, the impact of TTS on liking of food products that require serving at hot or cold temperatures, such as coffee, beer and ice cream should be explored.

Interestingly, TTs have demonstrated a greater sensitivity to temperature and temperature changes than TnTs in this research project, hence it is possible that TnTs may be better at tolerating extreme temperatures. Further research should investigate the above hypothesis, in addition to testing the preferred serving temperature of hot or cold food and beverages across TTS groups.

For example, at what temperature do TTs prefer to drink their tea, would that be different from TnTs?

7.5.4. Further investigate the relative effect of TTS and PTS

More research is needed to further investigate the impact of TTS and PTS on olfactory perception, in order to confirm the impact of taste phenotypes. In addition, further studies with larger sample sizes on a wider range of taste, trigeminal and olfactory stimuli are needed to confirm the findings observed in this study where pMTs are most affected by TTS, compared to pSTs and pNTs. Further studies should also examine the relative effect of TTS and PTS on fungiform papillae counts and morphology, in order to understand such interactions.

7.5.5. Fungiform papillae measurement

This study highlighted that the measurement of fungiform papillae can be problematic. Further works developing algorithms for detection and quantification of fungiform papillae in tongue images programmed using Matlab (Rios *et al.*, 2012) is essential, in order to obtain reliable FP data. In addition, morphology measurements of the fungiform papillae should also be considered in further sensory studies.

7.5.6. Further investigate if gustin polymorphisms contribute to oronasal sensitivity

Although gustin rs2274333 genotype was not associated with PROP perception in this study, it could not rule out the possibility that gustin gene may contribute to PROP perception as well as oronasal sensitivity. Further investigation is necessary to understand the relationship between a wider

range of polymorphisms in the gustin gene, FP density, morphology, and oronasal sensitivity, in order to further investigate the role of gustin polymorphisms in sensory perception.

Many studies concerning PROP taster status have been conducted, but researchers also need to look at thermal taster status, as this study emphasises that TTS also affects sensory perception. This study has provided new information about the cortical activation across TTS groups, supporting the hypothesis that mechanism behind TTs is likely to result from of taste and trigeminal processing. This thesis has advanced our understanding of how combinations of different phenotypes affect oronasal sensitivity and provide preliminary evidence that additional taste phenotype (TTS) may be a marker of food behaviour. More studies are now required to elucidate the mechanism behind TTS, and the impact of taste phenotypes (PTS and TTS) on food behaviour.

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	
Phone number	Weight	Height if applicable

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?
   (These stop working near MR Scanners) 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 ?
    (e.g. Do you have a fever, cardiovascular disease, hypertension, diabetes or cerebrovascular disease?) Y/N
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: **Scanner Operator Only:**	**Date:**

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SYMPOSIA PRESENTATIONS:

Poster: 5th Eurosense Conference – A Sense of Inspiration (2012).

Poster: PFSG Conference – A Sense of Change (2012).

Poster: 1st Nursten Flavour Symposium (2013).

Oral Poster: PFSG Conference: Fast forward (2013).

Oral: Early Career Researcher Event (2013).

Poster: 10th Pangborn Sensory Science Symposium (2013).

Oral: 2nd Nursten Flavour Symposium (2014). The best oral presentation awarded.

Oral: SenseAsia Symposium (2014).

Poster: Eurosense Conference (2014). Successful applicant of SSG's travel award (2014).

Oral + Poster: SSG Conference – Putting sensory in Context (2014). The best poster awarded.

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