Neural and Psychological Mechanisms of Oral Sensation

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List of abbreviations

Glossophyngeal nerve	IX^{th}
Trigeminal nerve	V^{th}
Fungiform Papillae	FP
carbon dioxide	CO_2
Phenylthiocarbamide.	P.T.C.
6-n-propylthiouracil	PROP
Facial nerve	VII^{th}
aluminium potassium sulphate	Alum
Copper Sulphate	$CuSO_4$
Labelled Magnitude Scale	LMS
General Somatic Afferent	GSA
Brachial Efferent	BE
Slowly Adapting Type 1	SAI
Slowly Adapting Type 2	SAII
Rapidly Adapting Type 1	RAI
Central Nervous System	CNS
Cold Detection Threshold	CDT
Warm Detection Threshold	WDT
Cold Pain Threshold	CPT
Hot Pain Threshold	HPT
Quantitative Sensory Testing	QST
Visual Analogue Scale	VAS
Acute Tryptophan Depletion	ATD
Tryptophan	TRP
Serotonin	5-HT
C-Tactile Afferents	CT
Tryptophan depletion	TRP-
McGill Pain Questionnaire	MPQ
Touch Experiences and Attitudes Questionnaire	TEAQ

Abstract

This thesis set out to explore oral sensory processing. Oral sensory processing extends beyond taste perception, the nerves that innervate the mouth and carry taste information to the brain also carry chemosensations, thermal sensations and somatosensations. While a great deal is understood about oral chemo and thermal perception, this thesis focuses on the not fully recognised oral somatosensory processes. A substantial amount of movement occurs within the mouth, from movement while speaking to chewing food. As food moves around the mouth, different oral receptors are activated and the consumption experience changes.

Taste perception varies between individuals in a way that has led to the identification of the taster status genetic polymorphism of taster status where three taster groups (hyper-taster, taster, tolerant taster) with differing sensitivity to bitter tastes were identified. This sensitivity is further represented in anatomical differences with differing densities of fungiform papillae on the tongue.

Using psychophysical methods and the taster status phenotype, this thesis examined if different regions of the tongue and mouth experienced different chemostimulant intensity and if dynamic touch changed the intensity perception of chemostimulants in chapter 4. This identified that different regions of the oral cavity experience chemostimulant intensity differently with the tip of the tongue being the most sensitive and the vermillion of the lower lip the least sensitive to sensation. Furthermore, whilst there was no main effect of touch on sensation intensity an interaction between touch type, taster status and oral locations was found when using 10-ppm capsaicin and Sichuan pepper. A dynamic touch on the lip with mint oil was also considered more intense than a static touch.

Chapter 5 investigated the possibility that C tactile (CT) afferents were present in the lower lip, the structure of the lip skin widely suggests that CTs are not present but their regular use in the affective behaviour of lip-to-lip contact between individuals suggests otherwise. By applying the standardised psychophysical stroking approach to the lip, cheek and mucosa the classic psychophysical inverted U associated with CT like behavioural responses to touch

was found on the cheek where CTs are known to be present as well as on the lower lip. This CT like response on the lip warrants further detailed investigation.

Serotonin (5-HT) is widely associated with hedonic experiences and reduced 5-HT levels are a linked with depression and anhedonia. 5-HT is also a candidate neurotransmitter associated with taste transduction. Chapter 6 describes an acute tryptophan depletion (ATD) study that examined the peripheral and central effect of reduced 5-HT levels on taste perception. The primary findings highlight that tryptophan levels do not effect sweet, sour, salt and bitter taste detection ability. A significant difference in bitter taste intensity and pleasantness was identified with tryptophan depletion increasing the taste intensity and decreasing bitter pleasantness at suprathreshold concentration. An effect of taster status was identified in bitter intensity ratings with tolerant-tasters reporting a greater intensity of sensation in the tryptophan depletion session than in the control.

During the course of the experimental phase of this thesis, it became clear that describing oral sensations was a difficult task. When asked to describe how sensations felt within their mouth in chapter 4, participants were unable to find words to describe sensations. Therefore, the final study in chapter 7 describes the development of a candidate oral lexicon to aid in describing mouth feel and oral sensations highlighting that the approach to lexicon development previously used to develop the McGill Pain Questionnaire and the Touch Perception Task can successfully be applied to the development of an Oral Lexicon.

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Chapter 1 : Literature Review

Abstract

This chapter begins by setting the scene of taste and flavour (section 1.1) and then proceeds to briefly explore the multisensory interactions from all of the senses that make up flavour perception (section 1.2). The next section explains the discovery of taster status (section 1.3) and the differences in oral sensation between the taster status groups. Furthermore, the larger impacts of taster status on lifestyle behaviours and choices are briefly explained (section 1.4). The chapter ends with an outline of the thesis structure, aims and hypotheses (section 1.5).

1.1 Introduction

Humans are complex beings. Each person lives in an individual sensory world that is shaped "by a combination of anatomy, medical history, genetics, culture, and life experience" (Stuckey, 2012, p29). Combined, these factors serve to influence a person's experience of almost everything they perceive.

The sense of taste is a highly complex modality that is not a monosensory perception. When eating and drinking you experience a range of sensory inputs from the food that add to its taste and influences flavour perception. The gustatory experience is a combination of the olfactory, visual, oral-somatosensory, auditory, and trigeminal cues (see Delwiche, 2004; Spence, 2002; Stillman, 2002).

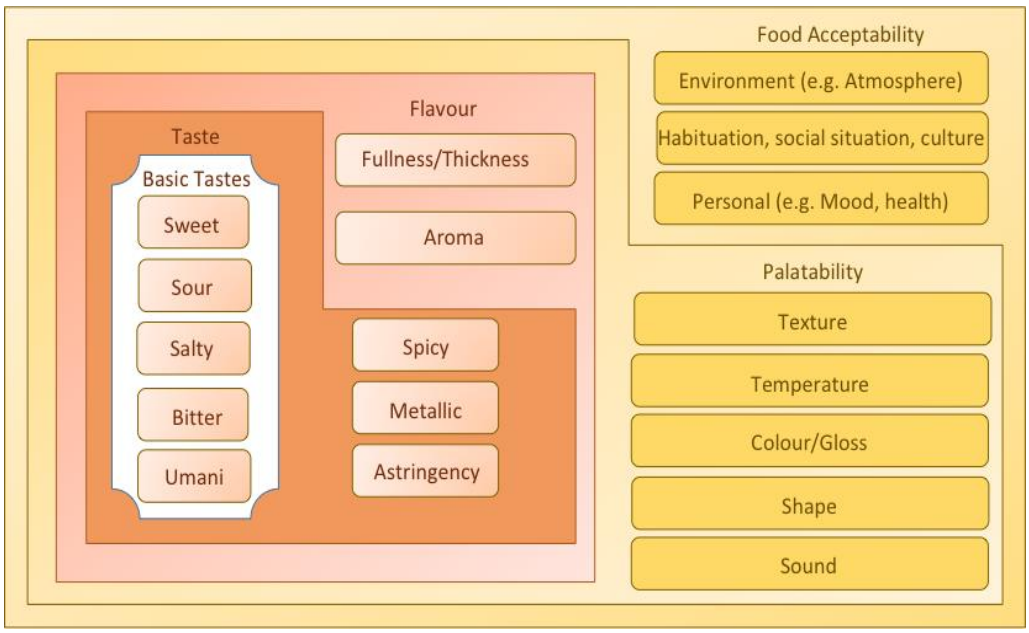


Figure 1.1 The components that contribute to the overall experience of foods consumed. At the top of the scale are the five basic tastes that people experience along with the sensations often induced from consuming foods. Combining the olfactory senses along with the taste provides food flavour. Other factors have been found to contribute to the overall palatability of foods and when that is all accounted for the external environment influences the acceptability of foods (adapted from Umami Information Centre).

It has been established that food aroma (Dalton, Doolittle, Nagata & Breslin, 2000) appearance (Spence, 2015c), what it sounds like when consumed (Spence, 2012, 2015a), how it feels in the mouth (Breslin, 2013), the temperature (Green,

1984), and finally trigeminal chemosensory sensations such as pain, irritation and touch (Auvray & Spence, 2008; Essick, Chopra, Guest, & McGlone, 2003; Spence, 2015b) the combination of these systems when unifying during the eating process is considered 'taste sensations' or flavour (Abdi, 2002; Prescott, 1999; Small & Prescott, 2005). The perception of flavour is possibly the most multisensory experience of everyday life. Although flavour perception comes from a combination of multisensory perceptions it is possible to distinguish and separate out these modalities experimentally (see Figure 1.1).

1.2 Multisensory perception

Abdi (2002) reasoned that although the gustatory, olfactory, and trigeminal systems are obviously anatomically separate and have separate functions, they are not cognitively independent. Numerous researchers have explored the interactions between these senses and their impact on flavour (e.g. Prescott, 2015; Spence, 2015a, b, c; see Figure 1.2).

When the senses are taken separately, four of them (touch, vision, audition and olfaction) function in diverse behavioural contexts but the sense of taste evolved to regulate and drive feeding behaviours (Yarmolinsky, Zuker & Ryba, 2009). The taste of a food informs us about the potential toxicity and nutrient content of the things we select to ingest and helps us make informed decisions as to the safety of and consumption value of foods (Breslin, 2013). Anatomically this makes sense, as the head is primarily innervated by the facial and trigeminal cranial nerves. One of the functions of the trigeminal system is protect the organism from the effects of harmful substances. This is indicated by it stimulating sweating, tears and running noses along with expressing pain sensations (Abdi, 2002).

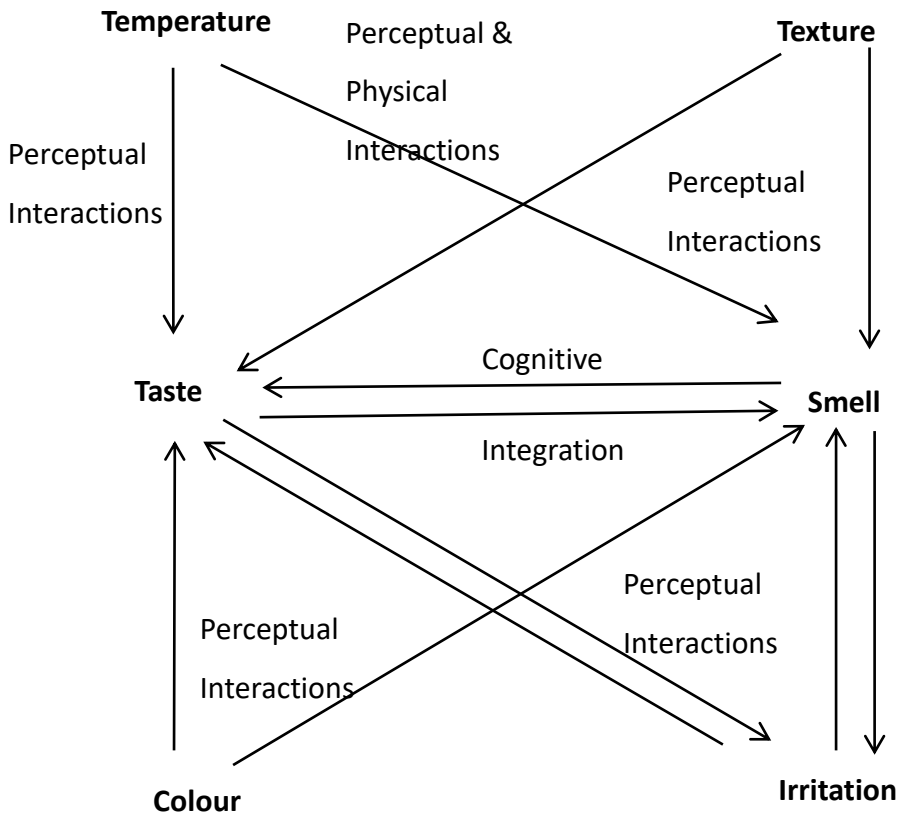


Figure 1.2 Summary of the perceptual interactions involved during ingestion. The arrow direction indicates the modality that has been demonstrated in research to interact with another modality (adapted from Delwiche, 2004).

1.2.1. Olfaction

While it is often assumed that flavour perception comes from the sense of taste, olfaction provides the majority of information contributing to flavour perception (see Spence, Smith & Auvray, 2014). It has been suggested that olfaction could have as much as an 80-90% influence over food flavour perception (Stuckey, 2012). When smell is combined with taste, it has been found to enhance the perceived flavour. A seminal research study conducted by Dalton, Doolittle, Nagata and Breslin, (2000) highlights the importance of olfaction clearly. Participants were given bottles of odours and had to determine which contained benzaldehyde (an almond-cherry like scent). When participants had a solution of saccharin (a solution that possesses no taste or smell) the scent from the benzaldehyde was perceived as being significantly more intense than the baseline condition in which water or a monosodium glutamate (MSG) solution was held in the mouth (see Spence 2015b for a review). This is a culture specific finding found

only in western participants. Japanese participants demonstrated a perceptual enhancement with the MSG condition over the saccharin (Dalton, Doolittle, Nagata & Breslin, 2000). These findings, when taken together, suggests that our brains bind the combinations of smell and taste associated with our common cuisine as in Japanese cuisine, it is common for the almond flavour to be combined with savoury tastes whereas in western cuisine it is combined with sweet tastes in desserts. This learning has been seen to take place in utero with neonates whose mothers regularly consumed anise-flavoured food during pregnancy were more likely to orient to the scent after birth (Schaal, Marlier & Soussignan, 2000).

The link between olfaction and feeding is so strong that evidence suggests premature new-born babies switch from tube feeding to oral feeding faster when the transition was combined with olfactory stimulation. Odour stimulated premature babies were discharged from hospital an average of 3.4 days earlier than those who were not odour stimulated (Cao Van, Guinand, Damis, Mansbach, Poncet & Hummel, *et al.,* 2018).

Stevenson, Prescott, and Boakes (1999) paired two taste solutions (sucrose and citric acid) with 20 different odours. Odours, which had a strong learned association sweetness (e.g. caramel), enhanced a rating of sweet tastes and suppressed sour ratings. This demonstrates the importance of learning and memory on taste perception. Other specific taste-smell interactions have been identified with sweet taste enhancement from the addition of a strawberry odour and salt taste enhancement by soy sauce odour (Djordjevic, Zatorre, & Jones-Gotman, 2004).

1.2.2. Somatosensation

The mouth is a highly sensitive organ as it is one of the most densely innervated (Mountcastle, 1974 as cited in Engelen & van der Bilt, 2008). Though most research has focused on the mouth's chemosensory role, taste research has begun to emphasize the interactive roles of taste, temperature and touch in oral sensory processing. There is anatomical evidence the nerves from the Glossopharyngeal (IX^{th}) innervate the circumvallate papillae and surround and

penetrate vallate taste buds but also that somatosensory ending of the trigeminal (V^{th}) innervates the fungiform papillae (FP) to the extent that somatosensory innervation rivals or surpasses the gustatory innervation (Whitehead, Ganchrow, Ganchrow, & Yao, 1999). Foods and beverages that we consume stimulate multiple receptors in the V^{th} with tactile sensations like particle size, texture and creaminess stimulating mechanoreceptors, temperature of foods and beverages stimulating thermoreceptors and irritants stimulating nociceptors (Duffy, 2007).

The sensations of taste can be localized to a specific area within the mouth through touch. Todrank and Bartoshuk (1991) swept tastants across the tongue in a semi-circular motion from the side, across the tip of the tongue and around to the other side. This represented a change in FP density starting with a lower density on the side, the greatest density is found on the tongue tip and returning to a lower density on the side. Participants were asked to judge the taste intensity as they swept it across the tongue tip in an arch. Lowest intensity ratings were found at the start of the arch than when the bud reached the tip of the tongue. Importantly the intensity ratings remained at an increased level when the arch was completed on the other side of the tongue indicating that perception of taste is generalized across the area that receives tactile stimulation (see Green 2002).

Of particular interest within oral sensory perception is the attraction of carbonated beverages. When carbon dioxide (CO_2) is applied to the skin, it excites nociceptive fibres (Steen, Reeh, Anton & Handwerker, 1992). Sensations elicited from consumption of carbonated beverages in the mouth is an often sought after and pleasurable sensation despite the sensation being irritating and sometimes painful. It is often debated if the sensation is mechanical in origin from the CO_2 bubbles bursting and stimulating oral mechanoreceptors or chemogenic due to the formation of carbonic acid in the mucosa, which then stimulates polymodal nociceptors of the oral cavity (Dessirier, Simons, O'Mahony & Carstens, 2001). The primary evidence that carbonation is not simply a mechanical sensation is that tingle induced by consumption of carbonated water persists after it has been expectorated (Green, 1992a).

Furthermore, a phenomenon called 'the champagne blues' occurs in mountaineers taking the carbonic anhydrase inhibitor acetazolamide to combat

mountain sickness. When later consuming carbonated beverages, mountaineers report a lack of tingle from the bubbles and that beer tastes like dishwater (Graber & Kelleher, 1988). This mean that carbonic anhydrase inhibitor acetazolamide not only alters the tingle experience but also the overall taste experienced.

1.2.3. Audition

When it comes to the important senses associated with food perceptions, audition tends to come at the bottom of the list (Spence, 2015b). Yet, research has identified that auditory cues do have an important role in the perception of our foods including attributes like how crispy, crunchy and crackly something is or how carbonated it feels and even how creamy the foods is perceived as being (see Spence, 2015b for review)

By modifying the sounds associated with mastication it is possible to dramatically change our experience of foods within the mouth. Zampini and Spence (2004) demonstrated that by varying loudness and frequency composition of auditory feedback that is usually generated when eating specific food products could alter the perception of crispness and freshness. Variation was around 15% with crispness and freshness being considered higher when the auditory input was increased. This finding is reflected in further research by Woods, Poliakoff, Lloyd, Kuenzel, Hodon & Gonda *et al.,* (2011) who conducted two experiments with three varying sound volumes and asked participants to rate foods on the saltiness, sweetness and liking or crunchiness and liking. They found that sweetness and saltiness ratings were lower when accompanied with loud noise than quiet sounds but crunchiness was the opposite, when the noise was loud, the food was rated crunchier. This suggests that sounds can suppress basic taste perception when the taste is unrelated to sounds or can enhance the experience when the food property uses auditory channels like crunching sounds.

Recently a similar approach was undertaken with moist crisp apples. The key difference in this study was that rather than increasing the sounds heard they decreased sounds heard when consuming apples and found that crispness was

significantly reduced when the sounds were lowered (Dematte, Pojer, Endrizzi, Corollaro, Betta & Aprea *et al.,* 2014).

1.2.4 Vision

A growing body of research suggests our experience of taste and flavour is largely determined by our expectations prior to consumption (Spence, Levitan, Shankar & Zampini, 2010). The most common finding being that changing the hue of a drink changes the perceived flavour. Dubose, Cardello and Maller (1980) demonstrated that a participant's ability to identify the correct flavour of a beverage was significantly decreased when it was inappropriately coloured. For example, 26% of participants reported the drink to be lime-flavoured when it was coloured green to a no lime flavour response when the drink was red. Other studies showed that if a drink was coloured orange participants would perceive it as tasting of orange, if it were green tasting of lime even where the drink was actually cherry-flavoured (see Zampini, Sanabria, Phillips & Spence, 2007; Zampini, Wantling, Phillips & Spence, 2008). Beverage colour has been shown in psychophysical studies to deliver an increase in taste perception, specifically as much as a 10% increase in perceived sweetness (Clydesdale, Gover, & Fugardi, 1992)

The strength of visual influence on taste experience has been found to extend to the colour of the plates and cutlery used. A spicy bean curd given on a red plate was perceived as significantly spicier than when on a white or green plate (Tu, Yang & Ma, 2016). Even the shape, size, weight and colour of the cutlery used when consuming foods has been shown to influence the taste. The taste of yoghurt was perceived as denser and thought to be more expensive when consumed from a lighter, plastic spoon. Taste was also affected by the colour of the cutlery but that also depended on the colour of the food. Finally, food is rated as being saltiest when it was consumed from the knife rather than other cutlery or a toothpick (Harrar & Spence, 2013).

1.3 Taster Status

There is a large variability in the population in how individuals perceive taste. Earliest research indicating this variability dates back to the late 1800's where Bailey and Nichols (1888) explored the perception of five different tastes (bitter, sweet, acid, alkaline and saline). Participants in their study were presented with successive serial dilutions of the five tastes and pure water then tasked with separating out the different tastes. Solutions that were unrecognizable were classed as water. In this simple early study, bitter tastes were identified as more clearly identified than the other tastes with the sensitivity order found as bitter, acid, salt, sugar and then alkali. Finally, they also identified that women were better able to correctly detect the tastes than men. This was true for all tastants with the exception of salt taste where no gender difference of perception was found (Bailey & Nichols, 1888).

Generally, people like sweet tastes and dislike bitter tastes but not all bitter tastes are unpleasant. It is estimated that up to 70 million cups of coffee are consumed daily in the UK (Howie, 2012). Bitter tasting compounds are also often used to enhance or suppress sweet and sour tastes, for example, chefs often recommend putting a small bit of dark chocolate into a chili to enhance the taste and occasionally chocolatiers recommend a dash of chili power in a hot chocolate for the same reason.

Compounds that are perceived as bitter do not share a similar chemical structure but small changes to it can covert the bitter taste to an intensely sweet one (Drewnowski, 2001). No matter how structurally diverse the bitter compounds are, they all elicit a single bitter taste. This suggests that more than one mechanism is responsible for the perception of and transduction of bitter taste (Drewnowski, 2001).

The effects of this bitter perception differences were first established in 1931 by chemist Arthur Fox when he was preparing phenylthiocarbamide (P.T.C) and some dust particles dispersed into the air, whilst Fox tasted nothing, his colleague, Dr. C. R. Noller, commented on how bitter the atmosphere air tasted (Fox, 1932). Blakeslee and Fox (1932) teamed up to research this taste

phenomenon at a conference for the American Association for the Advancement of Science. They invited visitors to their exhibit, asked them to take a plastic capsule containing enough P.T.C crystals to test themselves and family back home should they wish, and then asked them to vote on a voting machine as to whether the substance was tasteless, bitter, sour or some other taste. By the end of the five-and-a-half-day event, 2550 total votes were cast, 28% reported it to be tasteless, 65.5% voted for bitter, 2.3% for sour and 4.2% said they perceived another taste (Blakeslee & Fox, 1932). They noted that some visitors to their exhibit tasted nothing and demonstrated it by eating a large portion of the capsules content without hesitation while this elicited a response claiming they must be abnormal from those who could taste the bitter taste. Two thirds of the participants who engaged in the study were P.T.C tasters, meaning that one third of the 2550 participants could not taste the bitter compound (Blakeslee & Fox, 1932). This bimodal bitter taste distribution was the first taste polymorphism identified in humans and lead to the group designations of 'tasters' for those who could detect bitter tastes and 'taste blind' for those less sensitive (Hall, Bartoshuk, Cain & Stevens, 1975).

Where Blakeslee and Fox (1932) identified a bimodal taster status within the population as people who could or could not taste P.T.C, advances in the research methodology allowed for research in the area of taste phenotypes to develop and grow through adaptation of the classic sensory threshold testing methods (Harris & Kalmus, 1949). This new method allowed the identification that substances of a similar chemical composition to P.T.C were highly correlated with the thresholds of P.T.C and was able to distinguish between 'tasters' and 'non-tasters'. The substance that was identified as most reliably able to distinguish the 'tasters' from the 'taste blind' in line with P.T.C was 6-*n*-propylthiouracil (PROP). PROP and P.T.C are members of a class of bitter-tasting compounds known as thioureas. Though all bitter tasting compounds do not have the same chemical structure, these two compounds both contain the chemical moiety N-C=S which is responsible for the bitter taste they elicit (Zhao, Kirkmeyer & Tepper, 2003; see Figure 1.3). PROP became the standard taster test stimulus as it lacks the sulphurous odour that P.T.C

possesses and as it is a medication used in the treatment of Graves ' disease safety limits can be assessed and set (Lawless, 1980).

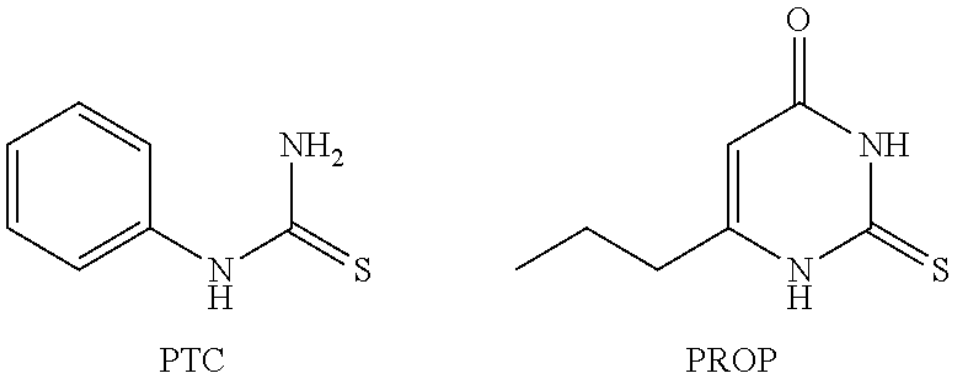


Figure 1.3 The chemical structures of P.T.C and Prop. They are the commonly used thioureas for assessment of taster status and have similar chemical moiety N-C=S which is responsible for the bitter taste (http://patentimages.storage.googleapis.com/US20120058965A1/US20120058965A1-20120308-C00001.png)

Blakeslee and Fox (1932) bimodal model in later years was found to be further subdivided between those who perceived saturated PROP concentrations as moderately bitter and those to whom it was extremely bitter (Bartoshuk, 1993). These new subdivisions were termed as 'non-tasters', 'tasters' and 'supertasters' with the latter being identified by the perceived intensity sensation elicited by PROP (Bartoshuk, Duffy & Miller, 1994). Supertasters are defined as a subgroup of people who report an intense bitter sensation from P.T.C and PROP (Bartoshuk, Duffy & Miller, 1994). Studies have shown that approximately 25% of the population cannot detect the bitter sensation of PROP and these are classed as 'non-tasters'. Of the remaining population, the 25% who detect the bitter sensation as the most intense and aversive sensation are classed as 'supertasters'. The remaining 50% that can detect the bitter sensation do so at less intensity than reported by supertasters and these are termed either 'tasters' or 'medium tasters' (Catanzaro, Chesbro & Velkey, 2013). Multiple research studies conducted by different researchers over the last two decades have led to strong support for the existence of supertasters (Bufe, Breslin, Kuhn, Reed, Tharp & Slack *et al.,* 2005; Drewnowski, Henderson, & Barratt-Fornell, 1998; Essick, Chopra, Guest, & McGlone, 2003; Hayes, Bartoshuk, Kidd, & Duffy, 2008; Lim, Urban, & Green, 2008; Yackinous & Guinard, 2001).

Since the discovery of the supertaster, the terms for the subgroups as coined by Bartoshuk (1993) have become the standard language recognised by science and popular culture. This acclaim has not prevented people from questioning the use of the current terms however. The terms could be considered misleading as 'supertaster' implies an adventurous eater who enjoys strong flavours which research indicates to be untrue, they possess a preference for blander food tastes and it has been suggested that the term 'hyper-taster' be used instead (Stuckey, 2012). At the other end of the taste spectrum Stuckey (2012) suggests that 'non-taster' should be replaced by the term 'tolerant-taster' because they do taste flavours but possess a much higher threshold for bitter and sweet detection along with being more adventurous eaters finding enjoyment in strongly flavoured foods whilst being less picky. These statements are true and are more accurate in the way they describe and explain the different tasters; due to this the terms super-taster, taster and non-taster will be replaced with hyper-taster, taster and tolerant-taster respectively as suggested by Stuckey (2012) during the remainder of this thesis.

There are several differences between the taster groups. Compared with tolerant-tasters, hyper-tasters perceive a greater intensity to sweet, sour, salty, bitter (Prutkin, Fast, Lucchina & Bartoshuk, 1999), are better able to establish the fat content of substances and different liking levels of fat content (Yackinous & Guinard, 2001) which transfers into heightened tactile sensations from high-fat salad dressings (Tepper & Nurse, 1997). Hyper-tasters also perceive more chemesthetic sensations from carbonated drinks, alcohol, ginger, black pepper and chili peppers (Prescott & Swain-Campbell, 2000). These differences translate into differing lifestyle choices such as vegetable consumption, tobacco and alcohol use (Fischer, Griffin & Kaplan, 1963) and other associated health risks with interactions between ageing and the genetic variation in taste perception and its effects on dietary behaviours have begun. There are also significant differences across the taster groups and lingual somatosensory functions and perceptions (Essick, Chopra, Guest & McGlone, 2003) all of which will be addressed separately. Duffy (2007) hypothesizes that hyper-tasters are also more likely to suffer with greater potential

to experience oral pain particularly in some conditions that affect oral health such as during cancer treatments.

1.3.1 Taster Status and Bitter Tastes

Sensory researchers and neuroscientist agree that there are five basic tastes (sweet, sour, salty, bitter and umami). This means that any taste for which there is a receptor on our tongue for is included in the basic tastes. Each of these tastes are mediated by separate classes of receptor cells that respond to a single taste quality (see Anatomy section 2.4.4 pg 74 for further taste transduction details). Exploration of the brains coding of taste in the primary taste cortex has demonstrated topographic segregation in the functional architecture of the gustatory cortex with each taste being represented in its own separate cortical field, revealing the existence of a gustotopic map in the brain (Chen, Gabito, Peng, Ryba & Zuker, 2011).

Tater status is assessed through an individual's sensitivity to bitter tastes, specifically P.T.C. or PROP (see Methodology section 3.3 pg 92 for further information on taste test), however taster status has been associated with enhancing the perception of other bitter compounds that are found in ordinary foods, most notably caffeine and saccharin, which is perceived as bitter by some taster individuals (Bartoshuk, 1979, 1993; Gent & Bartoshuk, 1983). Early studies identified that PROP tasters reported more dislikes of common foods, such as cabbage, Brussels sprouts, rhubarb, beer and coffee than tolerant-tasters (Akella, Henderson & Drewnowski, 1997; Fischer, Griffin, England & Garn, 1961;) but there are often inconsistent results within the research (see Drewnowski & Rock, 1995 for review). Sweet, sour, salt and bitter tastes have all been positively correlated to PROP sensitivity (Bartoshuk, Duffy & Miller, 1994).

1.3.2 Taster Status and Salt Tastes

In the 1990's it was made clear that to some individuals concentrated PROP tasted about as intense as 1M salt (NaCl), while others considered the NaCl more intense (Bartoshuk, 1993). Furthermore, the perceived intensity of NaCl and PROP

were found to be positively correlated with each other, meaning that the stronger the perception of PROP the stronger the perception of NaCl (Bartoshuk, Duffy, Lucchina, Prutkin & Fast, 1998). The individuals who experiences strongest sensations of PROP and NaCl were classed as hyper-tasters. This led to the assumption that NaCl would be a good standard for PROP studies based on magnitude matching study using tones as the standard (Marks, Stevens, Bartoshuk, Gent, Rifkin & Stone, 1988).

Even with this assumption and use of NaCl as a standard there are disagreements within the literature about the role taster status plays in NaCl intensity. For example, whilst running their study assessing taster status with PROP and the NaCl standard, Zhao, Kirkmeyer, and Tepper (2003) identified hyper-tasters gave significantly higher intensity ratings to NaCl than tasters. Conversely, Schifferstein and Frijter (1991) found that the two taster groups (tasters and tolerant-tasters) when assessed using P.T.C did not differ in their perception of NaCl. Furthermore, NaCl detection thresholds were found to be related to the number and density of FP with greater NaCl intensities experienced in participants with a greater density of FP (Doty, Bagla, Morgenson, & Mirza, 2001).

Studies suggest that a person's history of sodium consumption has an impact on preference for, future consumption of NaCl (Stein, Cowart, Epstein, Pilot, Laskin & Beauchamp, 1996; Pittman & Contreras 2002). This suggests that the heritability of salty taste perception is difficult to detect or that the genetic contributions to NaCl taste variability are too low to assess (Wise, Hansen, Reed & Breslin, 2007). Given the links between the bitter taste perception and NaCl use as a standard it is surprising that a genetic heritability is hard to find as taster status has strong genetic links.

1.3.3 Taster Status and Sour

Sour taste detection serves as part of the body's nutritional gatekeeper by detecting unripe fruits and spoiled foods in order to avoid acid induced tissue damage (Lindemann, 2001). Only a few studies have explored individual

differences in sour perception with it being noted that a preference for strong tasting foods was found in those with higher PROP taste thresholds, meaning that those who were more likely tolerant-tasters preferred strong tasting foods (Glanville & Kaplan, 1965).

This link was experimentally made many years later when the taster groups were asked to discriminate variations in sweet, sour and bitterness within two foods and beverages. Hyper-tasters were found to be able to discriminate smaller variations in taste concentrations than tolerant-tasters, particularly in the bitter and sours tastes (Prescott, Soo, Campbell & Roberts, 2004). A second experiment conducted by Prescott, Soo, Campbell and Roberts (2004) where participants rated the sourness, sweetness and carbonated irritation in sparkling fruit drinks found that the ratings of sourness were higher in tasters (combined hyper-taster and taster group) and lowest in tolerant-tasters. A further study conducted by Lee, Prescott and Kim (2008) agrees that when the sour and NaCl levels are altered in foods a combined taster group is more sensitive to the variations than tolerant-tasters and tasters are also more likely to reject an increased sour tasting orange juice and a less salty beef soup than a tolerant is. The findings were reportedly less clear when the taster statuses were divided into the commonly used three groups (Lee, Prescott & Kim, 2008).

1.3.4 Taster Status and Sweet

Sweet tastes are generally classified as a liked stimulus (Yeomans, Tepper, Rietzschel & Prescott, 2007) but there is considerable variation in the hedonic responses to sucrose. Most participants demonstrate an increase liking with increases in sucrose concentrations but there is a significant minority that find with increases in sweet concentrations there is a decrease in liking, occasionally showing a peak liking at a very low concentration (Drewnowski, Henderson, Shore & Barratt-Fornell, 1997; Looy, Callaghan, & Weingarten, 1992).

It has been suggested that the degree to which participants like or dislike sweet tastes may be related to their taster status. This idea stems from findings that PROP taster's rate the sweetness of sucrose as more intense than tolerant-

tasters (Bartoshuk, 1978; Gent & Bartoshuk, 1983). This effect is especially seen at high concentrations (Lucchina, Curtis, Putnam, Drewnowski, Prutkin & Bartoshuk, 1998; Ko, Hoffman, Lucchina, Snyder, Weiffenbach, & Bartoshuk, 2000). Looy and Weingarten (1992) found that in both children and adults PROP sensitivity was predictive of pleasantness responses. Those that were sweet likers were usually tolerant-tasters and those that were sweet dislikers were usually tasters. Yeomans, Tepper, Rietzschel & Prescott (2007) established in their study that 67% of hyper-tasters were sweet dislikers compared to only 12% of tolerant-tasters.

This taster status and sweet liking interaction is reflected in food choices with hyper-tasters tending to show less liking for foods with a high sweet content just as they do for bitter tasting foods (Duffy & Bartoshuk, 2000; Looy & Weingarten, 1992) and tolerant-tasters have been reported to consume more sweet foods than PROP tasters (Duffy, Peterson, Dinehart & Bartoshuk, 2003).

Yet all of these reported findings are controversial as Drewnowski, Henderson, Shore and Barratt-Fornell (1998) failed to link taster status to sweet intensity or pleasantness ratings so this link These differences between tasters and tolerant-tasters are small and inconsistently observed in studies (Schiffman, Crofton & Beeker, 1985).

1.3.5 Taster Status and Umami

The relationship between umami tastes and PROP taster status have widely been explored. A study with Filipino adults suggested that sweet, salty, umami and bitter recognition thresholds negatively correlated with PROP status (Villarino, Fernandez, Alday & Cubelo, 2009). The umami taste has been considered to be comparable to sweet taste in that it signifies the presence of essential nutrients which are calorie-rich at the same time (Frank, Hettinger, & Mott, 1992; Kim, Breslin, Reed, & Drayna, 2004). According to Kim, Breslin, Reed, and Drayna (2004) though sweet and umami tastes differ perceptually they are related phylogenetically. The receptors for umami and sweetener have been found to be 50% identical (DuBois, 2004) and sharing a common subunit receptor (Li, Staszewski, Xu, Durick, Zoller & Adler, 2002). This suggests that

the relationship between umami and taster status may be similar to that of sweet tastes and taster status.

1.3.6 Taster Status and Fat Perception

Chemoreception of dietary fat is largely attributed to activation of the somatosensory system, which carries information regarding the textural properties of fat (Mela, 1988). The most salient cue for fat perception lies in its texture and mouth feel (Rolls, Critchley, Browning, Hernadi & Lenard, 1999) with some research suggesting that fatty acids do not elicit taste qualities like that of sweet, sour, salty, bitter and umami but rather appears to define a detection threshold (Stewart, Feinle-Bisset, Golding, Delahunty, Clifton & Keast, 2010). Essick, Chopra, Guest and McGlone (2003) established the hyper-tasters are also hyper-feelers as they are significantly better able to identify objects with the tip of the tongue than tasters and tolerant tasters. This touch detection ability is expressed anatomically with hyper-tasters possessing a greater density of FP and as such a greater level of innervation from the V^{th} nerve, which is a mechano, thermal, pain and chemoreceptive nerve.

Taster status and oral sensory perception has been found to be different between the taster subgroups and perception of fat content in food products. This includes such things as high fat content dairy products (Duffy, Bartoshuk, Lucchina, Snyder and Tym, 1996; Kirkmeyer & Tepper, 2003), thickeners (Prutkin, Fast, Lucchina, & Bartoshuk, 1999) and salad dressings (Tepper & Nurse, 1997; Hayes & Duffy, 2007) with hyper-tasters found to dislike high calorie and fatty foods (Tepper & Nurse, 1997).

Duffy, Bartoshuk, Lucchina, Snyder and Tym (1996) used a series of milks that contained increasing amounts of fat to establish any differences between the taster subgroups. Hyper-tasters were able to perceive greater creaminess in the milk drinks as fat content increased. This is supported by findings from Tepper and Nurse (1998) who showed that tasters and hyper-tasters could discriminate high fat salad dressings from low fat ones, something the tolerant-tasters were unable to do but the tolerant-tasters liked the sampled salad dressing more than the PROP tasters. This is a finding that is reflected across taster statuses with tolerant-tasters

showing increased preference for high-fat and strong-tasting foods (Akella, Herderon & Drewnewski, 1997; Choi, & Chan, 2014; Dinehart, Hayes, Bartoshuk, Lanier & Duffy 2006; Drewnowski, Henderson & Shore, 1997; Drewnowski, Hernderson, Hann, Berg & Ruffin, 2000; Duffy, Davidson, Kidd, Kidd, Speed & Pakstis et al., 2004; Hayes & Duffy, 2008; Keller, Steinmann, Nurse & Tepper, 2002; Tepper & Nurse, 1998; Tepper, White, Koelliker, Lanzara, d'Adamo, & Gasparini, 2009). Even questionnaire data agrees with the laboratory findings, based on answers from a preference questionnaire for 82 foods and beverages that individuals who reported being more sensitive to PROP demonstrated lower preferences for high fat foods (Duffy, Bartoshuk, Lucchina, Snyder & Tym, 1996). Duffy, Bartoshuk, Lucchina, Snyder and Tym (1996) speculatively explain that those who are more sensitive to PROP may possess increased fat sensitivity due to the increased V^{th} innervation in the oral cavity.

There is discrepancy in the research however, with several studies not finding a relationship between PROP taster status and creaminess or fattiness rating. Catanzaro, Chesbro and Velkey (2013) sampled 139 college undergraduate students and examined enjoyment ratings of 12 foods and beverages through a questionnaire-based survey. Analysis of variance found no significant differences between the taster groups in ratings of how much they liked Brussel sprouts, raw broccoli, cabbage, spinach, crushed red pepper, jalapeños, creamy salad dressing, mayonnaise, red wine, or black coffee. There was a negative correlation between PROP status and chili peppers and dark chocolate (Catanzaro, Chesbro & Velkey, 2013). Other studies that failed to find relationships with taster status and fat perception include Yackinous and Guinard (2001) who found, that though participants in their study were able to accurately assess the fat content of the fat containing foods, the differences between taster status's ability was only present in the chocolate drink but not in the other fatty food products presented.

1.3.7 Taster Status and Anatomy

Another way that taster status is often assessed in through anatomical differences, specifically examination of the density of FP on the tongue. The

density of FP on the tongue varies across with taster groups with hyper-taster possessing a greater number of them than tolerant-tasters (Bartoshuk, Duffy & Miller 1994). Taste pores are located on the FP so this variation in FP density reflects as a variation in taste buds, which could explain some of the variation in taste perception between the taster groups (Miller, 1988)

In a highly influential study, Bartoshuk, Duffy and Miller (1994) suggests that individuals who are hyper-tasters possess a greater number of FP and as such, more taste pores than tolerant-tasters. Due to the volume research, hyper-taster status has become synonymous with a high density of FP (Delwiche, Buletic & Breslin, 2001; Duffy, Hayes, Davidson, Kidd & Bartoshuk., 2010; Essick, Chopra, Guest & McGlone., 2003; Hayes & Keast, 2011; Prutkin, Fast, Lucchina & Bartoshuk, 1999; Yakinous & Guinard, 2001). Most recently Walliczek-Dworschak, Schöps, Feron, Brignot, Hahner & Hummel, (2017) identified a positive association between FP density and taste perception. For further explanation of the anatomy of taster status see Chapter Two: Oral Anatomy section 2.5 pg 79.

1.3.8 Taster Status and Sensation

The notion of being a hyper-taster originally referred to heightened bitter sensitivity but has since been generalized to include the influence taster status has on other tastes and somatosensory stimuli (Bartoshuk, Duffy, Luchina, Prutkin & Fast 1998; Prescott & Swain-Campbell 2000; Hayes & Duffy 2007; Hayes & Keast 2011). Psychophysics has long been applied to the study of oral perception. There is a growing evidence that suggests amongst the differences between the taster groups is differences in lingual somatosensory perception. Yackinous and Guinard (2001) assessed the sensitivity of the tongue with the psychophysical method of Von Frey filaments. Von Frey Filaments allow the assessment of the mechanical sensitivity of trigemino-vascular sensory neurons. Participants in this study were tested using two Von Frey filaments (no. 2.36 & no. 2.44) across four sections of the tongue. Differences in lingual tactile sensitivity among the taster groups were seen with hyper-tasters demonstrating the highest sensitivity to the lower weight Von Frey (no.236) in the median section of the tongue. The heavier weighted filament

(no. 2.44) did not discriminate between the different tongue regions or taster status. They did not see differences in tactile sensitivity between taster groups and the front of the tongue indicating the taster groups has equal sensitivity in the front of the tongue (Yackinous & Guinard, 2001).

An alternative method for assessing oral tactile perception is through lingual spatial tactile acuity on the tip of the tongue. This was assessed by Essick, Chopra, Guest and McGlone, (2003) with embossed precision-milled Teflon strips placed on the tip of the tongue and participants had to identify the letter that was embossed on them (see Figure 1.4). The letters presented to participants increased or decreased in size depending on if the participant identified the correct letter. Essick, Chopra, Guest and McGlone, (2003) established that hyper-tasters were 25% more tactually acute than tasters and more than twice as acute as their tolerant-taster counterparts. When the same approach was applied to assess the lingual tactile acuity of children compared to their mothers, Lukasewycz and Mennella (2012) found that children were just as able to complete the task effectively as their mothers and that the children took less time identifying each letter stimulus.

Figure 1.4 Lingual tactile acuity embossed precision-milled Teflon strips placed on the tip of the tongue (Essick, Chopra, Guest & McGlone, 2003).

There is limited research in the area of tactile somatosensation and taster status. The majority of evidence and support for differences in somatosensation comes from studies that foods or chemical to provide stimulation.

1.3.8.1 Chemosensation

Chemosensation relates to the somatosensory responses to chemical irritants that cause a sensation of burning, cooling or tingling (Alimohammadi & Silver, 2002). Oral irritants from substances found in food such as capsaicin in chilies and piperine from black pepper are recognized for the illusory heat sensation

they invoke when consumed. It has been found that the human response to these irritants varies considerably (Cliff & Green, 1996; Craft & Porreca, 1992;McBurney, Balaban, Popp & Rosenkranz, 2001; Prescott, 1999).

Genetic factors have been found to be a major influence on the liking of spicy foods and oral pungency, actually accounting for 18-58% of the variation in one study on adult Finnish twins (Törnwall, Silventoinen, Kaprio, & Tuorila, 2012). Taster status is strongly genetically determined and PROP tasting ability appears to have some relationship with it. The underlying reasons for the variation in chemosensory perception are still unknown there is some suggest that these individual differences in perception vary with the levels of sensitivity to PROP (Duffy, 2007).

The majority of chemosensory perception is mediated by the trigeminal nerve (V^{th}), one of the 12 cranial nerves. In fact, as much as 75% of FP innervation comes from the V^{th}, which responds to pain, touch and thermal stimulation. The remaining 25% comes from the chorda tympani (Farbman & Hellekant, 1978; Silver & Finger, 1991 as cited in Prutkin, Duffy, Etter, Fast, Gardner & Lucchina *et al.,* 2000). One study established that within the rat FP and taste buds there were as many as three times the number of Vth fibres than facial nerve (VII^{th}) fibres (Farbman & Hellekant, 1978). This co-innervation means that FP are not solely taste sensory organs but also organs for perception (Lawless & Stevens, 1988).

With the duel innervation of FP, irritants like capsaicin produce a greater burning sensation in hyper-tasters than tolerant-tasters (Karrer & Bartoshuk, 1991; Prutkin, Fast, Lucchina & Bartoshuk, 1999).

1.3.8.2 Capsaicin

When exploring relationships between taster status and chemo-sensation the primary chemo-stimulant used in the research is capsaicin, the main pungent ingredient in chili peppers, and despite the burning sensation experienced from consuming it most individuals get pleasure from the experience. It is most commonly used to explore oral chemosensory perception and the trigeminal nerve (Green & Schullery, 2003). This is because it triggers a response from the capsaicin-sensitive receptor TRPV1. The first identified link between FP densities, PROP taster

status and the burn from capsaicin was found by Karrer and Bartoshuk (1991). Karrer and Bartoshuk (1991) used a series of concentrations of capsaicin from 0.1ppm to 100ppm to examine desensitization. Participants rated the intensity of sensation elicited and then how long it took the sensation to build and dissipate. 100ppm capsaicin continued to build over 7 minutes where 10ppm concentrations intensity did not build over time, it remained consistent. The 100ppm concentration slowly decreased in intensity over a 15-minute period, but it remained higher than the 10ppm intensity rating that appears to decrease at a lower rate. When compared with taster status tolerant-tasters rated the burn created by capsaicin as significantly less intense than hyper-taster (Karrer & Bartoshuk, 1991). The research is unable to come to a uniform consensus in regards to the association between taster status and capsaicin intensity with some research finding associations (Karrer & Bartoshuk, 1991; Bartoshuk, Duffy & Miller, 1994) and others not. Tepper and Nurse (1997) did find associations between PROP taster status and the perceived burn from capsaicin though not at all concentrations of capsaicin. More recently Spinelli, De Toffoli, Dinnella, Laureati, Pagliarini & Bendini *et al.,* (2018) related PROP responsiveness with burning intensity ratings.

In contrast, McBurney, Balaban, Popp & Rosenkranz (2001) reported no difference between PROP tasters and non-tasters and reported intensity of the burning sensation. Even more recently, failure to identify relationship between taster status and oral pungency was found by Törnwall, Silventoinen, Kaprio and Turila (2012). In their study with 300+ participants consisting of monozygotic and dizygotic twins and some twin individuals without their co-twins, all participants underwent a taster status test and rated the pleasantness and intensity of strawberry flavoured jelly spiked with capsaicin in comparison to un-spiked jelly. Pleasantness of spicy foods and oral pungency caused by spices were also collected via questionnaires. The participants were grouped based on their pleasantness rating of the capsaicin-spiked jelly as non-liker, medium-likers and likers. Those who were non-likers rated the intensity of the capsaicin spiked jelly as more intense and that spicy foods and spices, be they mild, strong or highly strong as less pleasant than likers. The overall findings in relation to taster statuses found no

differences between the taster groups and the pungency ratings of the non-likers and likers or in the sensory test and questionnaires. Genetic factors only accounted for between 18-58% of the variance in pleasantness (Törnwall, Silventoinen, Kaprio & Turila, 2012).

Some studies have found that capsaicin produces a bitter taste (Green & Schullery, 2003) in some individuals that is more pronounced when perceived at the back of the tongue than on the front (Green & Hayes, 2003). Lim and Green (2007) found in their study that the bitter taste of quinine sulphate (QSO_4) and the burning sensation elicited by capsaicin can be perceptually similar and, under some conditions, confusable. There are possible explanations for capsaicin's ability to induce bitter sensations in some individuals could just be simple judgments of similarity or confusion with the poorly bitter stimuli or that the burning and bitter are inherently perceptually similar (Lim & Green, 2007).

Kalantzis, Robinson and Loescher (2007) demonstrated in their study that regular consumers of spicy foods experienced a greater difference in perception of warm detection thresholds than people who didn't. Research conducted in a more naturalistic environment indicates that super-taster status is associated with a lower preference for spicy foods (Tepper, White, Koelliker, Lanzaro, d'Adamo & Gasparini, 2009). One factor that could account for variation in spicy food preference and differential levels of intensity perception is regularity of spice consumption. Ludy and Mattes (2012) compared regular consumers of spicy food with individuals who do not regularly consume spicy foods and found that PROP intensity ratings could not predict spicy food consumption. They did however find that early childhood exposure to spicy foods did predict consumption. This is supported by research that suggest individuals adapt to the sensation elicited by capsaicin with regular consumption of spicy stimuli considering the burn to be less intense than non-users (Cowart, 1987; Lawless, Rozin, and Shenker, 1985; Karrer & Bartoshuk, 1991; Stevenson & Prescott, 1994; Prescott & Stevenson, 1995; Stevenson & Yeomans, 1993; Tepper & Nurse, 1997; Bartoshuk 2000; Yoshioka, Doucet, Drapeau, Dionne & Tremblay, 2001). Possibly explaining the differences seen with the taster status population and inconsistent findings in chemo-sensation research with capsaicin.

1.3.8.3 Astringency

Astringency is a sensation described by oral dryness and feelings similar to a dry, puckering sensation within the mouth (Gawel, 1998) and is often elicited by foods that contain high concentrations of polyphenol and tannis often found in tea and red wine (Schobel, Radtke, Kyereme, Wollmann, Cichy, Obst, et al., 2014). Research in rodents indicates that astringency activates the chorda tympani taste nerve as well as the glossopharyngeal (Schiffman, Suggs, Sostman & Simon, 1992) implying that astringency could be both a taste sensation (Schobel, Radtke, Kyereme, Wollmann, Cichy, Obst, et al., 2014) and a somatosensory sensation (Breslin, Gilmore, Beuchamp, & Green, 1993; Green, 1993a; Lim & Lawless, 2005).

To test if an astringent sensation was a taste or somatosensory sensation Breslin, Gilmore, Beuchamp and Green, (1993) applied the astringent producing substance aluminium potassium sulphate (ALUM) to the surface of the mouth, between the upper lip and gum. This location is a non-gustatory surface and has no taste buds. They demonstrated that the astringent sensation was perceivable at this location so gustatory input is unnecessary for the production of the astringent sensation (Breslin, Gilmore, Beuchamp, & Green, 1993). This is finding supported by Lim and Lawless (2005) with the astringent sensation being generated by copper sulphate ($CuSO_4$) in the same location. Breslin, Gilmore, Beuchamp, and Green, (1993) reported that over trials the participants reported an increase in astringency but hypothesize this could be due to the cumulative removal of salivary lubricants through the repeated stimulus applications. This has led to research examining the effect of repeated exposure to astringent sensations.

Des Gachones, Mura, Speziale, Favreau, Dubreuil, and Breslin (2012) examined the prolonged perceptual effects of astringent substances over 80 sips of an astringent liquid. They found that weak astringent sensation would become strong over repeated sampling. When they added other oral sensations between astringent sips they found that as the astringent sensation was introduced the less able individuals were able to perceive fattiness than a control participant that was using drinking water rather than an astringent inducing drink. Interestingly, they also found that the group of participants, which had an astringent drink but not a

fatty drink, experienced a greater growth in astringent sensation over multiple sips than the group that had the additional fatty food (des Gachones, Mura, Speziale, Favreau, Dubreuil, & Breslin 2012). This would imply that fat could play a role in reducing astringent build up within the oral cavity.

The majority of evidence for the tactile nature of astringency comes from the characteristic differences between astringency and the five gustatory sensations. This key characteristic difference is that where other tastants decrease in intensity or flavour over repeated ingestion astringent sensations clearly increase with repeated exposure and as such could not be a gustatory sensation (Green, 1993a). In contradiction to this argument is the findings of Lyman and Green (1990) who established through a sip and spit comparison that the astringent sensation increased significantly over time but bitterness also increased over time and bitter is considered one of the five basic tastes. When sweetness was added to the experiment in combination with the other stimuli both the bitter sensation and dryness from the tannic acid was reduced (Lyman & Green, 1990).

Early research failed to establish a relationship between taster status and perception of astringent sensation. When examining the interaction between astringency and sweetness in red wine it was found that as sweetness increased, astringency decreased (Ishikawa & Noble, 1995) supporting the findings of Lyman and Green (1990) but that taster status had no impact of the perception of astringency or sweetness (Ishikawa & Noble, 1995). In a similar study done by producing astringency and bitter with grape seeds they also found that taster status has no impact on the perception of the sensations (Smith, June & Noble, 1996). These findings could be due to the method used to assess taster status by their threshold sensitivity rather than with a labelled magnitude scale (LMS).

Studies conducted using the LMS suggests differently. Contrary to previous findings when the LMS was used to rate the bitterness, astringency and acidity of three red wines all were correlated with individual PROP taster status. This found that tolerant-tasters gave significantly lower intensity ratings than hyper-tasters for all three factors examined (Pickering, Simunkowa & DiBattista, 2004).

1.4 Taster Status, Lifestyle Choices and Health Implications

Similarly, to taster status links with different tastes and sensations there is substantial literature that relates PROP sensitivity to lifestyles behaviours and choices such as dietary preferences, control of food intake and risks of obesity and alcoholism (Anliker, Bartoshuk, Ferris, & Hooks 1991; Looy & Weingarten 1992; Pelchat & Danowski 1992; Drewnowski & Rock 1995; Hong, Chung, Kim, Chung, Lee, & Kho 2005; Shafaie, Koelliker, Hoffman, & Tepper, 2013).

By the nature of what PROP sensitivity is, taster status has been linked to differing eating behaviours, preference for particular foods and lifestyle choices and body weight. Variation in oral sensation can influence behaviours when it comes to food and beverage preference and consumption. Given that people eat what they like and avoid foods they do not like, it is unsurprising that research has indicated that hyper-tasters are less inclined to consume cruciferous vegetables (Drewnowski, Henderson & Shore, 1997) and avoiding food and beverages that have a strong bitter taste such as broccoli, turnips and alcohol (Duffy & Bartoshuk, 2000; Tepper & Nurse, 1997). The impact of orosensory variation and its role in food and beverage preference and intake with the inclusion of phenotypic markers have expanded the knowledge related to chronic disease risk and susceptibility (Duffy, 2007).

People whom are hyper-tasters were also found to taste vegetables as most bitter and least sweet (Dinehart, Hayes, Bartoshuk, Lanier & Duffy, 2006). PROP sensitivity explained most variability in vegetable preference and intake via only the vegetable bitterness but quinine explained variability in vegetable preference and intake via vegetable bitterness and sweetness (Dinehart, Hayes, Bartoshuk, Lanier & Duffy, 2006).

1.4.1 Obesity

Various studies have explored the relationship between taste perception, taster status and body mass index (BMI). Early studies failed to find links between obese and normal weight individuals and their sweet taste detection ability

(Grinker, Hursch & Smith, 1972; Thompson, Moskowitz & Campbell, 1977). Using a generalised labelled magnitude scale (gLMS) a negative association was found between salt, sweet, umami and fatty tastes (Bartoshuk, Duffy, Hayes, Moskowitz & Snyder, 2006; Sartor, Donaldson, Markland, Loveday, Jackson & Kubis, 2011). Not all studies exploring this relationship fail to identify an impact of obesity on taste perception. A comparison of taste thresholds and hedonics for four basic taste modalities of sweet, sour, salty and bitter were made between lean and obese individuals. Obese participants were found to have lower thresholds the lean participants for sweet and salt tastes indicating a higher sensitivity in obese individuals. Intensity ratings for the lower concentrations of sweet, salty and sour were also found to be higher in obese individuals indication over all that the being overweight impacts on the perception of the tastes (Hardikar, Höchenberger, Villringer & Ohla, 2017).

Tepper, Neilland, Ullrich, Koelliker and Belzer (2011) investigated food energy intake and its interaction with taster status by measuring the calorie intake of a control meal compared to the three different buffet lunches. Averaging the energy across the buffet lunches found that tolerant-tasters consumed more energy from the buffet meal than the hyper-tasters but not more fat containing foods. This indicates the tolerant-tasters are more vulnerable to negative dietary exposure than hyper-tasters (Tepper, Neilland, Ullrich, Koelliker & Belzer, 2011).

1.4.2 Oral sensation, vegetable intake and cancer risk

Intake of vegetables are known to be beneficial for general health but there is growing suggestion that vegetable intake potentially has a role in cancer risk. Diets that are rich in fruit and vegetables have been linked to lower rates of coronary heart disease and cancer (Steinmetz & Potter, 1996).

Cancer research has tried to find ways of helping and protecting the individual while they go through chemotherapy. Specifically, mechanisms of cancer chemo-prevention has focused on benefits of the biological activity of the compounds found in cruciferous and leafy vegetables, citrus fruit, green tea and red wine (Chung, Wong, Wei, Huang & Lin, 1998; Rhodes, 1996). These compounds are

called phytochemicals or phytonutrients and have been found to possess chemo-preventive properties (Drewnowski & Gomez-Carneros, 2000) meaning that they reverse, suppress, or prevent the development of cancer.

Studies on the benefits of phytonutrients and health often fail to consider the bitter taste of the vegetables that contain them. Cancer research proposes that heightened bitterness might be a positive feature of the vegetable by allowing consumers to select broccoli sprouts with the highest glucosinolate content that is reflected in the stronger bitter taste (Duffy, Davidson, Kidd, Kidd, Speed & Pakstis et al., 2004; Green & Hayes, 2004).

In terms of taster status this could imply that hyper-tasters are more likely to develop cancers, as they are less likely to consume vegetables that contain the high levels of phytonutrients. One study by Basson, Bartoshuk, DiChello, Panzini, Weiffenbach and Duffy (2005) found that there was a possible increased risk of colon cancer in men who are hyper-tasters.

1.4.3 Taster status and alcohol

Studies have found that taster status is related to the pleasantness and unpleasantness of the sensations elicited from alcoholic beverages. Hyper-tasters often report that alcoholic drinks are more irritating and bitter than Tolerant-tasters (Duffy, Peterson & Bartoshuk., 2004; Prescott & Swain- Campbell, 2000; Intranuovo & Powers, 1998; Pickering, Simunkova & DiBattista, 2004). Tolerant-tasters perceive scotch as less bitter and more sweet than hyper-tasters (Lanier, Hayes & Duffy, 2005).

Intranuovo and Powers (1998) assessed 100 participants liking and disliking for two beers with ratings on an LMS. They found that hyper-tasters reported consuming significantly less beer than tolerant-tasters during their first year of regular drinking but no differences between the groups in current drinking levels (Intranuovo & Powers, 1998). This could be due to the bitterness and irritation perceived by hyper-tasters from alcohol taking longer to come to tolerate the sensations elicited. There is evidence that young adult who taste PROP as more bitter consume less beer (Guinard, Zoumas-Morse, Dietz, Goldberg,

Holz, Heck, & Amoros, 1996). Duffy, Peterson, and Bartoshuk (2004) found that PROP hyper-tasters consume alcohol less frequently than the tolerant-tasters.

Recently Yang, Dorado, Chaya and Hort (2018) explored the hedonic and emotional responses of the taster groups to beer. They identified that PROP taster status had an influence over the liking level of alcoholic beverages with hyper-tasters having a higher level of liking than tolerant-tasters. They further found that when using the beer emotion lexicon hyper-tasters scored higher in the positive descriptive words of excited and content than the tolerant-tasters (Yang, Dorado, Chaya & Hort, 2018).

As with all research in taster status there is contention in regards to the relationship between taster status and alcohol intake. Mattes and DiMeglio (2001) found that 50 participants who were light but regular alcohol consumers had no effect for tater status. In fact, ethanol use was not associated with gender or dietary characteristics (Mattes & DiMeglio, 2001). Research indicates that non-tasters are at an increased risk of alcoholism (Duffy, Davidson, Kidd, Kidd, Speed & Pakstis et al., 2004) with some studies conducted with alcoholics finding an excess of tolerant-tasters among the alcoholic participants (DiCarlo & Powers, 1998). Studies of family alcoholism history Pelchat and Danowski (1992) found that weather or not the children of alcoholics were significantly more likely to be tolerant-tasters than the children of non-alcoholic but others were unable to show a relationship between taster status and parental history of alcohol misuse (Kranzler, Skipsey & Modesto-Lowe., 1998). This familial relationship to alcoholism however could be questioned given what is known about the genetic influences on taster status. As there is potential that an individual's taster status is genetically passed on by their parents then the passing on of the behaviour could be more influenced by the environment than the taster status.

1.5 Thesis Structure

The overall aim of this thesis is to explore oral sensory perception. In order to achieve this, it is important to first understand the anatomy and neuroanatomy of the oral cavity, a highly complex anatomical structure with intricate innervation. When one nerve that supplies the mouth is damaged, the entire perception within the oral cavity changes.

The taste phenotype outlined above allows a simple population segmentation to explore oral perceptual differences. From the literature review outlined in this Chapter it is clear that there are many factors involved in oral sensory perception and that the experience is not solely based on the flavour or taste of something. The taster group in which a person belongs has clear lifestyle and health implications so further understanding of the influences and underlying mechanisms that impact on or are altered because of taster status is important for longer term wellbeing. Tolerant-tasters who are less susceptible to the bitter taste of alcohol so appear more likely to become alcoholics, yet hyper-tasters who are very sensitive to the bitter taste are less likely to consume vegetables due to bitter tastes than tolerant-tasters and vegetables are essential for a healthy life. This means that there are different benefits and risks to each of the taste phenotypes.

The role of somatosensation in oral perception is unclear, the nerves that supply the mouth and the FP carry more than taste but also touch, chemo-sensations, thermal sensations and pain to the brain for processing. Yet there remain inconsistencies and questions through the oral perception field.

1.5.1 Study 1: The role of somatosensation and taster status in oral chemosensory perception

Chapter 4 aimed to examine the differences in chemosensory perception between the taster groups, different regions of the oral cavity and the role of somatosensation on intensity perception.

(**1**) By using capsaicin, menthol, aluminium potassium sulphate (Alum), Sichuan pepper and mint oil to generate different somatosensations, alterations in perceived sensation intensity between hyper-taster, tasters and tolerant-tasters

were assessed with the expectation that hyper-tasters would perceive the sensation as more intense than both tasters and tolerant-tasters reflecting the majority of published research. (**2**) With what is known about anatomical differences between the taster groups five different locations were targeted within the mouth to assess the potential for different innervation and receptor quantities influence on sensations. Finally, (**3**) touch has been implicated in increasing astringent sensation but has not been explored for its impact on other chemo-stimulants. This was done in Chapter Four by having participants rub the stimuli treated surface against another oral surface, creating a naturalist oral tactile sensation or by having them do nothing once the stimuli were applied permitted the examination of touches role in oral chemosensory perception.

1.5.2 Study 2: Are lips a social organ?

Given the literatures indication that taster status reflects a greater density of FP and as such innervation, and that hyper-taster possess better discriminative touch abilities with the tongue than tolerant-tasters Chapter Five aimed to further explore the role that touch has in oral perception.

Some interesting interactions between touch and oral locations were identified in the previous study, particularly that a dynamic touch to the vermillion of the lips increased the perceived intensity of several stimulants, particularly that of mint oil. This led to the hypothesis that C tactile (CTs) afferents, a specific class of mechanosensitive afferents, that respond to a slow gentle touch, may be present in the lips.

1.5.3 Study 3: Acute Tryptophan Depletion: Exploring serotonins role in taste perception

Multiple neurotransmitters have been implicated in the transduction of taste. By utilizing an acute tryptophan depletion (ATD) experiment in Chapter Six, taste detection, intensity and pleasantness were assessed. Previous research has indicated that by administering an acute dose of serotonin taste detection abilities increase, however, they all fail to account for participant taster status.

1.5.4 Study 4: The candidate oral lexicon

When individuals were asked to describe the sensation's experienced whilst participating in Study 1 struggled to find words. In fact, the only available tools for describing oral sensations are designed by specialised panels for specific products, often requiring specialised training before they can be used. When faced with similar problems in assessing pain experience Melzack (1975) developed a lexicon that could be used by clinicians that explored the sensory, emotional and overall pain experience.

This developmental protocol was later applied to the development of a touch lexicon. The aim of study 4 in Chapter Seven was to test if the procedure for lexicon development that was successfully developed by Melzack (1975) and applied to the development a touch lexicon (Guest, Dessirier, Mahrabyan, McGlone, Essick & Gescheider *et al.,* 2011) could be applied to the successful development of an oral lexicon.

Chapter 2 : Oral Anatomy

Abstract

This chapter outlines the basic oral anatomy that needs to be considered throughout the processes of oral sensory assessment. It addresses the larger structure of the mouth and the mucosal surface structure and oral innervation. After providing an overview of the essential receptors and transient receptor potential (TRP) channels the chapter concludes by exploring the anatomical differences of the taster groups and the genetics behind these differences.

2.1 Introduction

The sense of taste is one of the major protective evolutionary mechanisms that animals possess. The taste of a food informs us about the toxicity and nutrient content of the foods we ingest and helps us make informed decisions as to their safety and consumption value (Breslin, 2013). Even with this clearly important role in survival and relationship with our other senses the variability and evolutionary mechanisms and benefits behind variation in taste sensitivity across populations remains unclear (Hayes & Keast, 2011). Research aimed at understanding mechanisms behind flavour perception is for the most part relatively recent (Small & Prescott, 2005).

Flavour perception is a complex mechanism that is not a unisensory but comes from a range of sensory inputs. Research has found that inputs from the olfactory (Dalton, Doolittle, Nagata & Breslin, 2000), visual (Spence, 2015c), auditory (Spence, 2012; 2015a) and somatosensory systems (Auvray & Spence, 2008; Breslin, 2013; Spence, 2015b) combine to provide what we consider taste sensation (see section 1.2 pg 23 for information on the multisensory aspects of flavour perception).

There is large variability in the population in how individuals perceive taste. Earliest research indicating this variability dates back the late 1800's (Bailey & Nichols, 1888) and since then psychologists and neuroscientists have been fascinated in the reasons why. With the discovery of subgroups of tasters within the population by Blakeslee and Fox (1932) and the addition of a third taster group by Bartoshuk (1993) allows a phenotype to be used to assess differences and establish why there is such variability within the population (see section 1.3 pg 29 for further information on taster groups).

To understand variability in taste and oral sensation a knowledgeable understanding of oral anatomy is required. The oral cavity is a distinct anatomical region, differing from other bodily surfaces and with a highly complex structure it is one of the most densely innervated parts of the body. Research in taste variability led to the hypothesis that taste perception was a simple case of Mendelian recessive genetics however, over time genetic research has identified the possibility

that more than 25 different genes are involved in taste perception (Hayes, Bartoshuk, Kidd & Duffy, 2008). The combination of genetic adaptations influences an individual's taster status and manifest in physiological differences.

The mouth experiences a large amount of trauma on a daily basis from the teeth, prostheses, foods and beverages, foreign objects of therapeutic and nontherapeutic varieties, chemical agents, extreme fluctuations in temperatures, hydration levels and diverse microbial flora (Hand & Frank, 2014). This means that the oral mucosa must be highly resilient

2.2 Oral Sensory Anatomy

2.2.1 The Lips

The lips are the portal to the oral cavity and a tactile sensory organ of exquisite sensitivity. They can even be considered erogenous zones due to their role in kissing and acts of intimacy between individuals. The lips possess many nerve endings and react as part of the tactile senses. They are also highly sensitive to both warming and cooling (Manrique & Zald, 2006). This high sensitivity helps to explain why the mouth plays such an important sensory role for babies and toddlers suckling behaviours and exploring the unknown world around them.

Glabrous skin possess a thick superficial layer skin made of keratin which is not innervated. The epidermis under it is living and is structured in a geometric manner so that the papillae of epidermal-dermal junction are more frequent in the ridges. These papillae house the Meissner corpuscles. Hairy skin does not have such deep organisation with hair associated with muscular and sensory fibres that innervate the hair follicle (see Figure 2.1; Hayward, 2018).

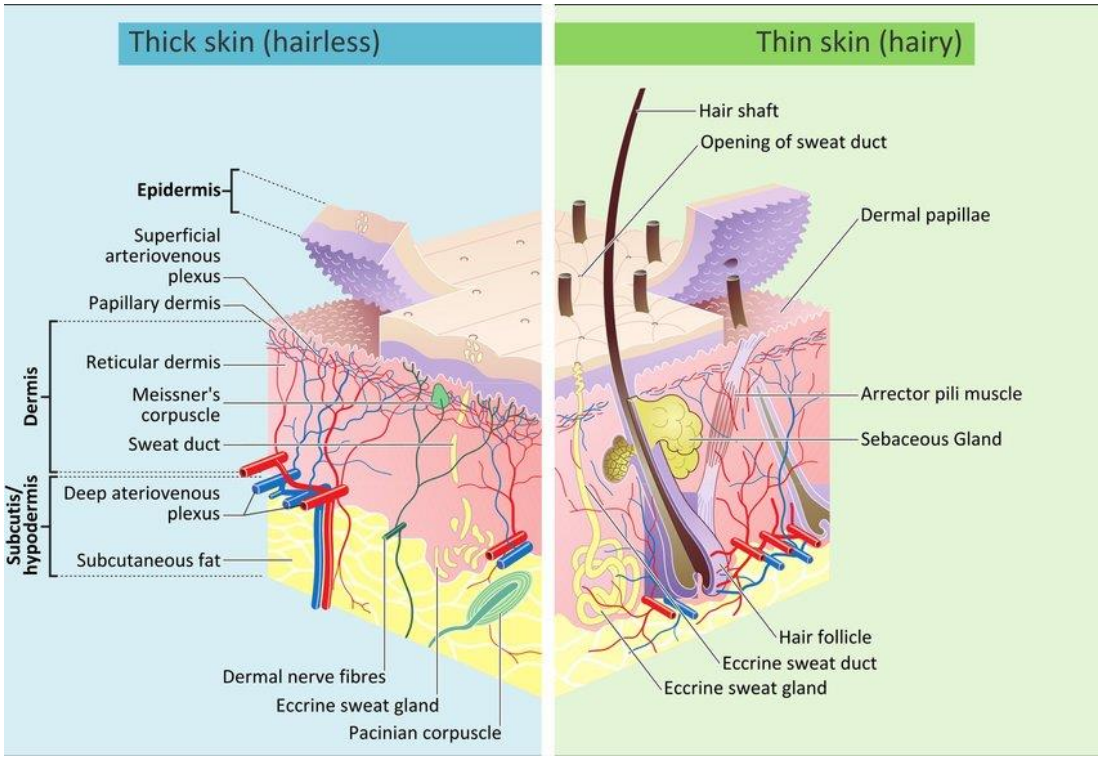


Figure 2.1: Diagram of the similarities and differences between the thick glabrous skin (**A**) of the palm and the thin hairy skin (**B**) (Photo by M.Komorniczak under CC BY 2.0; Barrios Muriel, 2017).

Receptor types are similar in both types of skin but their distribution, organisation and biomechanical properties vary greatly. The largest receptor in the skin is the Paciani corpuscle. It is found in the subcutaneous tissues and its density is moderate with a cadaver study indicating there are approximately 300 in the whole hand (Stark, Carlstedt, Hallin & Risling, 1998). All sensory receptors, whether it's a chemoreceptor, photoreceptor, thermoreceptor or mechanoreceptor are set to respond to a certain class of stimuli. The Pacian corpuscle is not an exception to this and its very specific role in the skin is vibration detection (Hayward, 2018).

Classification of lip skin is a complex process, it has similarities to the mucocutaneous skin of the oral cavity but it is thought to be neuro-anatomically more similar to the glabrous skin of the palm than the hairy skin of the torso.

The lips are structured by three anatomical subdivisions; two external and therefore dry subdivisions and 1 internal and therefore wet due to possessing a mucosal lining. The upper lip is termed Labium superius oris and the lower lip is the Labium inferius oris with the vermilion of the lip being the highly vascular borders

where the lip meets the skin of the face. Unlike the skin of the face that consists of up to 16 cellular layers, the skin of the lips is comparatively thin with between only 3 and 5 cellular layers. This thinness is what gives rise to the lip colouring against pale skin as the blood vessels that supply this region are visible under the surface and explains the blue colouring due to reduced blood supply in cold weather conditions (Hand & Frank, 2014). The skin of the lip borders the interior mucous membrane of the inside of the mouth called the labial mucosa. The labial mucosa is the internal wet subdivision of the lips. It contains prominent vascular markings and has a rich complement of submucosal minor salivary glands which provide secretions to the mucosal surface providing lubrication for the soft tissues and teeth in order to provide protection and comfort (Hand & Frank, 2014).

The lack of sweat and protective bodily oils means that the skin of the lip dries out considerably faster than other epidermal locations and is why lips become chapped more easily. The oral mucosa is reported to be more permeable to water than the skin but also that the floor of the mouth is significantly more permeable than other regions (Squier & Hall, 1985). The water retaining functions of the vermillion lip border have been found to be significantly lower than the water retaining capacity of the facial skin. This can be assessed by measuring the high frequency conductance of the skin with the lip possessing a lower conductance level than the cheek indicating a poor capacity for holding water (Kikuchi, Kobayashi, Le Fur, Tschachler & Tagami, 2002).

Although the lip skin is more similar to glabrous skin and is highly sensitive to stimulation like the fingertip, it also possessed similarities to hairy skin such as not being as thick as the glabrous skin. The classification of lip skin type remains unclear but with further understanding of the responses the lip has to stimuli and how those responses compare to other regions of the body may allow a widely agreed classification decision to be made.

2.2.2 Oral Mucosa

Oral mucosa is classed as a specialised epithelium and begins at the junction between the dry vermilion border of the lip and the moist labial mucosa. It is a wet,

soft tissue membrane that lines internal body spaces. There are three layers to the oral mucosa: the surface epithelium, supporting lamina propria which consists of a layer of loose connective tissue (papillary layer) just below the layer of epithelium and a deep layer of dense irregular connective tissue (reticular layer) and finally the underlying submucosa which is also constructed of dense irregular connective tissue. This thick deep submucosal layer often contains minor salivary glands and can contain adipose tissue in some locations. In areas of the oral cavity where the submucosa is absent the mucosa connects to the either muscle or bone by the lamina propria (Fehrenbach & Popowics, 2015).

2.2.3 Oral Mucosal Structure

The general structure of the oral mucosa is stratified squamous epithelium. It consists of squamous (flattened and scale-like) epithelial cells that arranged in layers upon a basal membrane. It is a highly organised and semipermeable ectodermal tissue that varies in thickness and keratinization of the surface depending on oral location and function. To maintain structural integrity, the various layers adhere to each other and one base layer connects to the basal membrane. These cells are tightly packed and have no intercellular spaces so are well suited to locations that are subjected to constant abrasion, such as within the oral cavity. This is because layers can be sequentially cast off and replaced before the basal membrane becomes exposed. Due to the constant abrasion that the mucosal surface experiences on the daily basis there is rapid turnover and replenishment of cells every 9 to 15 days (Yee, Li, Redding, Iwatsuki, Margolskee & Jiang, 2013) such structures also form the outermost later of skin and lining of the oesophagus, vagina, palm and sole of the foot.

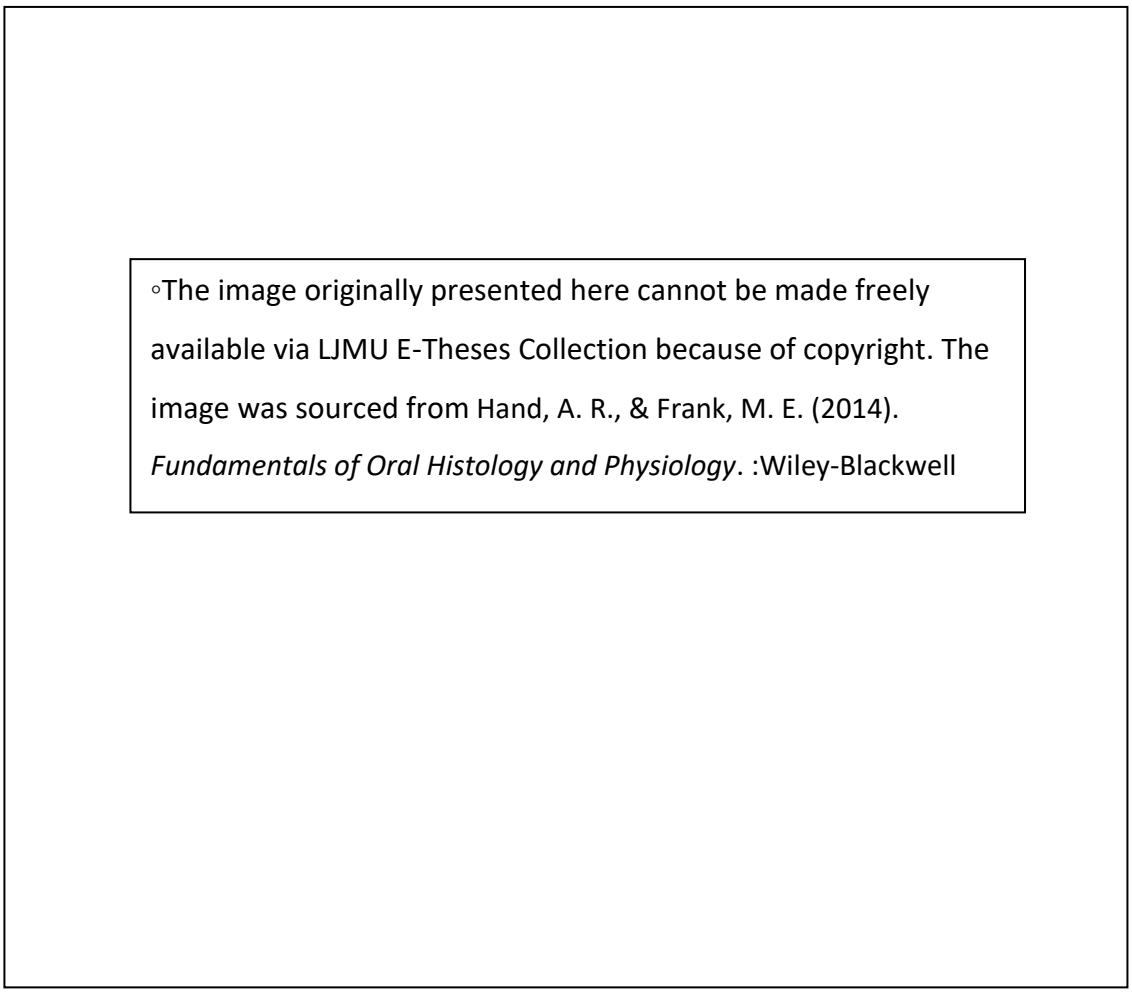


Figure 2.2 Light micrograph showing the layers and the components of oral mucosa. A submucosa is not present in all regions of the oral cavity (Hand & Frank, 2014).

The basal-most surface of oral mucosa is arranged in rippling projections called rete pegs. These are part of the mechanisms of attachment of oral mucosa to the basement membrane. The main function of the basement membrane is to separate it from several layers of underlying stromal connective tissues. The rete pegs interlock with the papillary lamina propria.

The lamina propria is the superior and widest layer of stromal connective tissues. The stromal connective tissue can be subdivided into two layers; the superficial papillary layer and deeper reticular layer. Within the oral cavity, making a distinction between these two sub-layers can be a difficult task. The papillary layer is a relatively loose segment of the lamina propria that lies immediately below the epithelium. It is a thin, fibro-collagenous tissue stroma which contains vascular channels, elastic fibres, fibroblasts and peripheral nerves (Hand & Frank, 2014).

Blood vessels and minor salivary glands located in the lamina propria and submucosa are innervated by efferent autonomic nerve fibres in the papillary layers.

The second sub-layer of the lamina propria is the reticular layer. It gets its name from the lattice-like network structure it possesses from its layers of collagen and elastic fibres being woven together. In contrast to the papillary layer the collagen bundles of the reticular layer are generally denser and more concentrated than the loose collagen fibres of the papillary layer (see Figure 2.2; Hand & Frank, 2014).

2.2.4 Types of Oral Mucosa

Oral mucosa can be subdivided into three basic types:

1) Moveable mucosa (or lining mucosa).
2) Masticatory mucosa.
3) Specialized mucosa.

2.2.4.1. Moveable Mucosa (or Lining Mucosa)

Most surfaces within the oral cavity are lined with movable mucosa. It is noted for its softer surface texture, ability to stretch and be compressed, having a moist surface and cushioning the structures that underlie it (see Figure 2.3). It has a non-keratinized stratified squamous epithelium with short and broad rete pegs and connective tissue papillae. This type of mucosa is found in oral locations where the mucous membrane is pliable and not attached to underlying bone, particularly the labial and buccal surfaces and their contiguous vestibular and alveolar mucosae, on the soft palate, uvula and tonsils, lateral surfaces and ventral surfaces of the tongue and the floor of the mouth. Movable mucosa is generally less subject to the frictional tearing and shearing during mastication, however the labial and buccal mucosa are often exposed to trauma from the teeth and chemical agents resulting in frequently stressing the mucosal resiliency (Fehrenbach & Popowics, 2015; Hand & Frank, 2014).

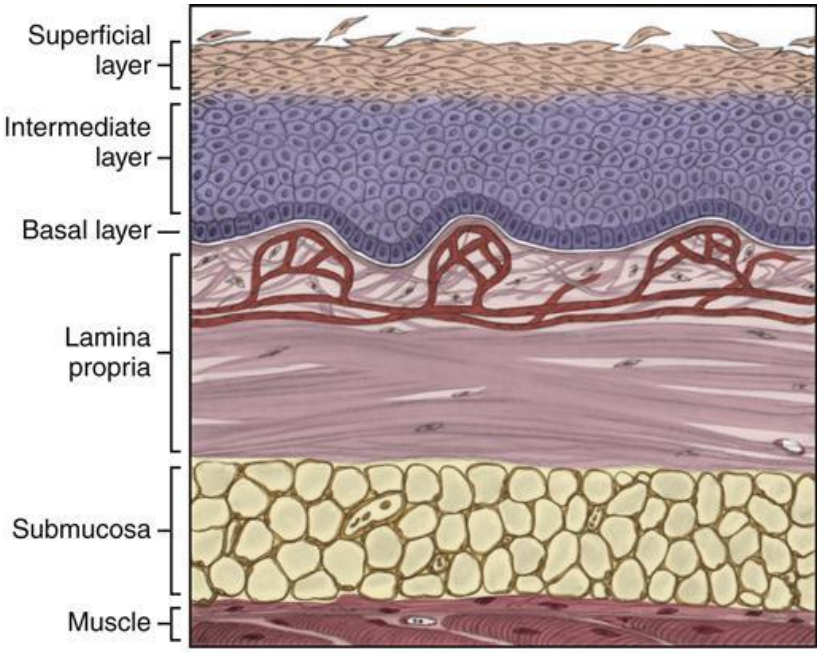


Figure 2.3 Histological features of movable mucosa composed of nonkeratinized stratified squamous epithelium, overlaying the lamina propria. A submucosal layer is usually present overlaying muscles (Fehrenbach & Popowics, 2015).

2.2.4.2 Masticatory Mucosa

There are several key differences between masticatory and moveable mucosa. Masticatory mucosa is immobile, thinner, firmer and bound down to the underlying alveolar bone. It also has a stratified squamous epithelium with longer and more numerous rete pegs (Fehrenbach & Popowics, 2015). This type of mucosa is associated with orthokeratinized stratified squamous epithelium (see Figure 2.4 A) as well as parakeratinized stratified squamous epithelium (see Figure 2.4B).

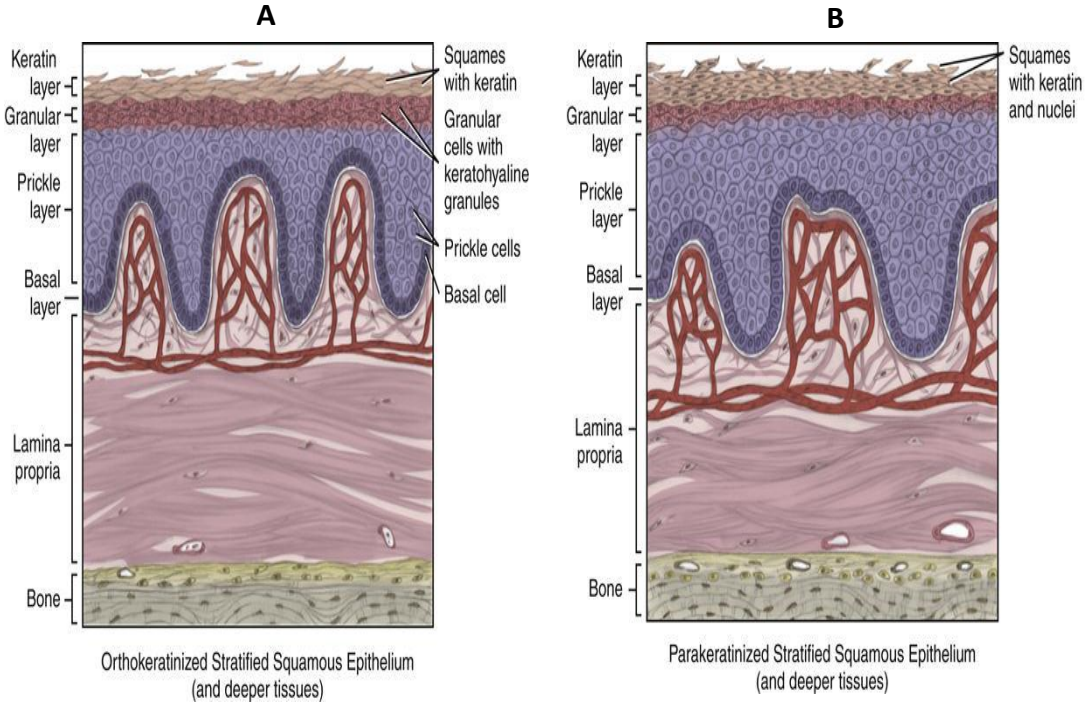


Figure 2.4 Features of the two types of masticatory mucosa. (**A**) Features of masticatory mucosa composed of orthokeratinized stratified squamous epithelium overlying lamina propria. The cells in the keratin layer have lost their nuclei and are filled with keratin. (**B**) Structure of parakeratinized stratified squamous epithelium overlying lamina propria. The cells in the keratin layer have retained their nuclei and are filled with keratin. In both a deeper, thin submucosa layer may or may not be present, and may overlay bone (Fehrenbach & Popowics, 2015).

The border between the epithelium and lamina propria in masticatory mucosa is highly interlocked by numerous pronounced rete ridges and connective tissue papillae (Fehrenbach & Popowics, 2015). It is this that gives it a firm base. The submucosal layer is either extremely thin in this mucosa or absent. Masticatory mucosa is primarily found in gingiva and hard palate tissues. The gingival mucosa does not have a submucosal layer but other hard palate locations that possess masticatory mucosa do have a submucosal layer and can be subdivided into 2 distinguishable regions based on their submucosal contents. The first region, often termed the fatty region, is comprised of the palatal zone lying lateral and anterior to the midline palatal raphe. This region contains an abundance of adipose tissue (Hand & Frank, 2014). The second region, termed the glandular region, contains an

abundance of submucosal glands and is located laterally and posteriorly to the fatty region, lying between the gingiva and palatal raphe.

2.2.4.3 Specialised Mucosa

The mucosa found on the dorsal tongue surface is termed as specialised as it contains four distinct surface projections called the lingual papillae but is functionally masticatory mucosa (Hand & Frank, 2014). These specialised projections play significant roles in oral sensory and taste perception with three of the four lingual papillae carrying taste receptors or taste buds. Each of the lingual papillae will be addressed separately.

2.2.4.3.1 Filiform Papillae

Of the four specialised papillae, filiform are the most abundant. They cover most of the anterior two thirds of the dorsal tongue and are responsible for the light pink or white colouring often observed. They are hair-like in appearance and possess a relatively rough and abrasive texture. When looking at the tongue they appear as rows of keratinized chevron-like extensions that point in a posterior direction towards the oesophagus due to the role they play in the process of chewing and preparing food for swallowing. Importantly these are the papillae that do not contain taste receptors thus playing no part in taste perception (Hand & Frank, 2014).

2.2.4.3.2 Foliate Papillae

These papillae are located bilaterally on the far posterolateral surfaces of the tongue. Though they can be difficult to see with the naked eye they display as a small cluster of slightly raised pink to orange parallel ridges that are separated with grooves. These papillae have a redder appearance than the rest of the tongue due to the skin/surface being thin in this location (Miller & Bartoshuk, 1991 as cited in Bartoshuk, 1993). The epithelium that lines the ridges is punctuated with taste buds of which there are numerous. The receptive endings of the taste buds on these papillae open into the grooves separating the papillae providing them with a large

receptive field. This allows for prolonged contact with chemical substances introduced into the mouth enhancing their ability to stimulate taste signals (Hand & Frank, 2014). It is thought that the foliate papilla contains hundreds of taste buds (Buck & Bargman, 2000).

2.2.4.3.3 Circumvallate (vallate) Papillae

Circumvallate papillae are the largest of the papillae and present in the fewest numbers. They are located on the posterior third of the dorsal tongue surface and on average an individual will only have 12 or fewer of them. They are lined in two obliquely oriented rows that form a V-shape. Circumvallate papillae are recognisable by their reddish/orange colouring and as round, slightly raised keratinized surfaced nodules that are each encircled by a trough. It is the epithelium lined; non-keratinized troughs trough's that the taste receptors associated with this papilla are located. Of special note with these papillae is that the bases of the troughs have exits from the excretory ducts of underlying serous-secreting minor salivary gland the von Ebner's (lingual serous) gland. Ingested food and substances enter the troughs, are dissolved by the secretions and bathe the taste receptors in the chemical reactants. This functions to enhance the mechanisms that underlay taste perception (Hand & Frank, 2014).

2.2.4.3.4 Fungiform Papillae (FP)

There are considerably fewer fungiform than filiform papillae but they are scattered amongst each other. Upon examination of the tongue, they appear as a single small, smooth-surfaced and round mushroom like projections on the surface of the tongue. They are distributed most densely on the tip and anterior portion of the dorsal tongue. They are of a pink or reddish colour due to surface keratin and richly vascular connective tissue core (Hand & Frank, 2014). Unlike the filiform papillae, these do each possess one to five taste buds on their superior surface (Batoshuk, 1993; Buck & Bargman, 2000).

2.3 Innervation

In 1664 the publication *Cerebri Anatome,* written by physician Thomas Willis, classified the cranial nerves. Willis's obsession with the brain was partly due to his attempts to understand the soul based on brain investigation. Through the addition to human dissection to that of animal dissections that were regularly conducted at that time Willis was able to add great details to the understanding of the brain and nervous system (Harley, 1994). Willis also spent substantial amount of time adding case histories from his living patients to his anatomical and experimental philosophy (O'Connor, 2003). His classifications of the cranial nerves were used for 100 years and the first six nerves are still classified as Willis' originally termed them (Pickover, 2013).

Cranial nerves are special nerves as unlike others that emerge from the spinal cord, the cranial nerves connect directly to the brain. Humans have 12 pairs of cranial nerves that both enter and exit the cranium through foramina or fissure in its floor or walls (Mahadevan, 2012).

Table 2.1 The twelve cranial nerves and which sensations they code from the locations they innervate.

Nerve	
(I) Olfactory	Sensations of smell from the nose
(II) Optic	Sensations of vision from the eye
(III) Oculomotor	Eye-movement control
(IV) Trochlear	Eye-movement control
(V) Trigeminal	Sensations from face (including nose, lower eye lids and lips) and sinuses; anterior two-thirds of the tongue, teeth and oral mucosa membranes; chewing muscle control
(VI) Abducens	Eye-movement control
(VII) Facial	Sensations of taste from the anterior of the tongue; facial and neck muscle control
(VIII) Auditory-Vestibular	Sensations of hearing and balance
(IX) Glossopharyngeal	Sensations of taste from the posterior of the tongue; neck muscle control
(X) Vagus	Interface with the heart, lungs, intestines, larynx and other organs
(XI) Spinal Accessory	Neck muscle control
(XII) Hypoglossal	Tongue muscle control

Cranial nerves I and II are different from the other 10 pairs in that they do not start in the brain stem, this leads them to not be considered true nerves but rather as fibre tracts from the forebrain (Mahadevan, 2012). Cranial nerve X is also different to the other nerves as unlike the others, it is not confined to innervating only the head neck but extends beyond them to the thorax and abdomen (Mehadevan, 2012).

The 12 pairs of cranial nerves can be grouped based on functionality, so those that are purely of a sensory nature are nerve I, II and VIII. Those that are

purely motor in nature are the III, IV, VI, XI and XII and those that provide input to the brain of both motor and sensory information are the V, VII, IX and X.

What makes innervation of the face and oral cavity unique is that it is entirely innervated by mixed sensory cranial nerves, primarily by 2 nerves, the V^{th} and the VII^{th}. The V^{th} nerve is a sensory nerve and innervates the face, sinuses and teeth. It has three divisions: The Ophthalmic n. (V^{th}_1), Maxillary n. (V^{th}_2) and Mandibular n. (V^{th}_3). The tongue is innervated by three cranial nerves: the facial nerve (VII^{th}), the glossopharyngeal nerve (IX^{th}) and the trigeminal nerve (V^{th}). The V^{th} cranial nerve carries thermal, touch and pain sensations from the anterior two-thirds of the tongue, the IX^{th} carries taste, thermal, touch and pain sensations from the foliate and circumvallate papillae on the posterior one-third of the tongue and the chorda tympani, a branch of the VIIth carries taste sensation from the FP (Bartoshuk, 1993; Whitehead, Ganchrow, Ganchrow & Yao, 1999; Green, Alvarez-Reeves, George & Akirav, 2005).

2.3.1 Trigeminal Nerve (V^{th})

The trigeminal nerve (V^{th}) is the largest of the cranial nerves and is named due to the three principal divisions that it is composed of and can literally be translated meaning the three twins because the V^{th} nerve branches into 3 sensory subdivisions providing the general sensory innervation to the oral cavity; the Ophthalmic (V^{th}_1), Maxillary (V^{th}_2) and Mandibular (V^{th}_3) (see Figure 2.5). These major divisions divide further as the nerve traces its path through the head. The nerve begins from the anterolateral aspect of the pons and contains two roots; one that is a large sensory root and the other is a slender motor root (Mahadevan, 2012). The nerves path, including both sensory and motor roots, runs through the head as follows: forwards and upwards crossing the upper border of the petrous

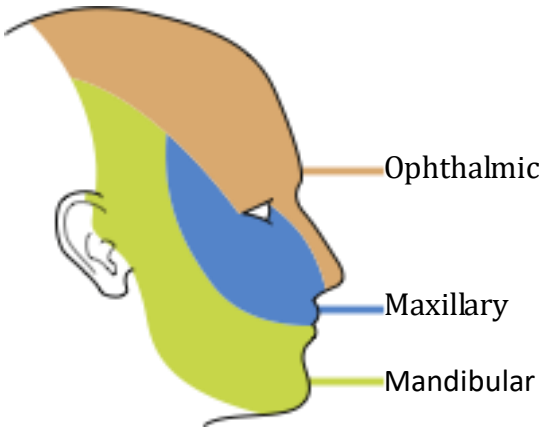


Figure 2.5 The head locations that branches of the V^{th} innervate (Craven, 2010).

temporal bone before entering the middle cranial fossa. From here the sensory root expands into the trigeminal ganglion which contains cell bodies for the sensory neurons, and it is here that the three divisions of the sensory nerve emerge. The motor root runs separately beneath the ganglion before joining the mandibular division within the foramen ovale. It is important to note that the motor root shares no fibres with the ophthalmic or maxillary division of the Vth. It carries both general somatic afferent (GSA) and branchial efferent (BE) fibres. GSA fibres generally function in the perception of touch, pain and temperature and for the trigeminal nerve provide the sensory input from the face, anterior half of the scalp, the oral and nasal cavity mucous membranes and the paranasal sinuses, the nasopharynx, some part of the ear and external acoustic meatus, part of the tympanic membrane, the orbital contents and conjunctiva and dura mater in the anterior and middle cranial fossae (Craven, 2010). The BE fibres are motor innervation to the skeletal muscles and innervate the muscles used for mastication.

Table 2.2 V^{th} nerve and its divisions, branches and sub-branches.

Cranial Nerve	Nerve Division	Nerve Branch	Sub-Branch
Trigeminal Nerve	Ophthalmic Nerve	Lacrimal Nerve	
		Frontal Nerve	Supraorbital Nerve Supratrochlear Nerve
		Nasociliary Nerve	Anterior ethmoidal Nerve Posterior ethmoidal Nerve Infrotrochlear Nerve Ciliary Nerve
	Maxillary Nerve	Infraorbital Nerve	
		Zygomatic Nerve	
	Mandibular Nerve	Buccal Nerve	
		Auriculotemporal Nerve	
		Inferior Alveolar Nerve	Mylohyoid Nerve Mental Nerve
		Lingual Nerve	

2.3.1.1 The Ophthalmic Division (V^{th}_1)

- This is the smallest division of the V^{th}
- This branch is purely a sensory branch containing only afferent fibres. It innervates the forehead, eyes, nose and the mucous membranes of the frontal sinus and nasal vestibule.

2.3.1.2 The Maxillary Division (V^{th}_2)

- This branch is also purely a sensory branch as it only consists of afferent nerve fibres. It innervates the middle of the head and face including the upper lip, upper cheeks, upper teeth and upper jaw and the roof of the mouth to the palatopharyngeal arch.

2.3.1.3 The Mandibular Division (V^{th}_3)

- Unlike the V^{th}_1 and V^{th}_2 this branch contains both afferent and efferent nerve fibres. The efferent fibres innervate the muscles associated with mastication, the tensor veli palatini which tenses the soft palate of the mouth, the digastric muscles which lowers the mandible and the tensor tympani (Hand & Frank, 2014). The afferent fibres have a wide distribution within the oral cavity, innervating the skin and mucous membrane of the inner cheek via the buccal nerve branch, the gums and teeth via the inferior alveolar nerve and the mucous membrane of the lower lip and chin via the mental nerve. Finally, the lingual nerve branch of the V^{th}_3 is the largest branch of the division. It passes along the side of the tongue and supplies sensory innervation to the anterior two-thirds of the tongue, mouth floor and lingual gum. The lingual nerve also joins the chorda tympani branch of the facial nerve (VII_{th}) that carries the parasympathetic fibres to the sublingual salivary glands and taste fibres from the anterior two-thirds of the tongue (Craven, 2010).

2.3.2 Facial Nerve (VII)

This is the seventh cranial nerve. The afferent components of the facial nerve consist of GSA fibres and special afferent (SA) fibres that are involved in smell, taste, vision and hearing perception along with balance. It is a mixed nerve as it is composed of a combination of sensory, motor and parasympathetic secretomotor fibres (Mahadevan, 2012). The motor fibres innervate the ipsilateral muscles of facial expression, the stapedius muscle that is located in the middle ear

and the occipito-frontalis muscle in the scalp. The sensory fibres are distributed ipsilaterally and extend to the taste buds located on the FP on the anterior two-thirds of the tongue (Mahadevan, 2012).

2.3.3 Glossopharyngeal Nerve (IXth)

The glossopharyngeal nerve (IXth) has both sensory and motor fibres. The sensory fibres innervate the posterior third of the tongue and the oropharynx wall. It sends special sensation perception from receptors in the walls of the sinus, chemoreceptors in the carotid body and gustatory receptors located on the circumvallate papillae. The motor innervation from the IXth serves the stylpharyngeus muscle and parasympathetic secretomotor innervation of the parotid salivary gland (Mahadevan, 2012).

2.3.4 Vagus nerve (Xth)

The Vagus nerve (Xth) is the most extensive nerve; it has the widest distribution of all the cranial nerves. It also has sensory and motor nerve fibres. The motor fibres innervate the pharyngeal musculature and some muscles of the larynx. The sensory fibres are part of the oropharynx, laryngopharynx and the interior of the larynx. Parasympathetic fibres associated with this nerve extend down to viscera of the thoracic and abdomen (Mahadevan, 2012).

Vagal afferents innervate the gastrointestinal tract, pancreas, and liver and vagal efferents combined with the sympathetic nervous system (SNS) and hormonal mechanisms together determine the rate of nutrient absorption, partitioning, storage, and mobilization (Berthoud, 2008). Furthermore, there is some suggestion that the vagus nerve regulates eating behaviour and body weight. Studies where a blockade or transection of the nerve has occurred have reported individuals suffering dramatic weight loss (Camilleri, Toouli, Herrera, Kulseng, & Kow, *et al*., 2008; Sarr, Billington, Brancatisano, Brancatisano, Toouli, & Kow, *et al.,* 2012) but when stimulated with norepinephrine, it drives excessive eating in satiated rats (Sawchenko, Gold, & Leibowitz 1981).

2.4 Chemoreception and Perception

Chemosensation describes sensations that occur as a result of chemically induced activation of receptors associated with senses other than olfaction and gustation. This means that it triggers somatosensations like pain, touch and temperature. The functioning of all cells within the body is sensitive to temperature fluctuations as the rate of chemical reactions depends on temperature.

2.4.1 Nociceptors

Pain perception serves as an important protective function for human and animal alike. The perception of pain is mediated by nociceptors, which respond to stimuli that have the potential to cause damage through extremes of pressure, temperature or burning from chemical substances (Gardner, Martin & Jessell, 2000).

2.4.2 Mechanoreceptors

These receptors respond to tactile stimuli like pressure and tapping. Within the lip and oral cavity there are 3 subgroups of mechanoreceptors that respond to specific types of tactile stimulation:

1. Slowly adapting type 1 (SA I)

- These respond to pressure stimulus and possess a small but well-defined receptive field (Trulsson & Essick, 1997).

2. Slowly adapting type 2 (SA II)

- These respond to the tactile sensations associated with the stretch of the skin and have a large, less-well defined receptive field (Trulsson & Essick, 1997).

3. Rapidly adapting type 1 (RA I)

- These mechanoreceptors respond to tapping sensations and possess a small but well-defined receptive field (Trulsson & Essick, 1997).
- Detects changes and respond only to application and removal of a stimulus (Trulsson & Essick, 1997)

The majority of the mechanoreceptive afferents found in the facial skin are SA I and the primary mechanoreceptive input on the transitional zone of the lip and the oral mucosa is slowly adapting (Johansson, Trulsson, Olsson & Westberg, 1988). The tongue is primarily associated with rapidly adapting mechanoreceptors. An additional deep tongue receptor has been found in the tongue that possesses a large receptive field and a high force threshold. Trulsson and Essick (1997) stipulate that these are most likely muscles spindles located deep within the muscles of the tongue.

Further exploration of the low-threshold mechanoreceptive afferents in the mucosa with microneurography established that those innervating the mucosa of the lower lip have properties similar to those innervating the skin and other parts of the human body (Trulsson & Johansson, 2002). Hair follicle afferents and rapidly adapting type 2 afferents (Pacinian-corpuscles) were not identified as present in the mucosa (Bukowska, Essick & Trulsson, 2010) supporting other psychophysical studies on the mechanoreceptive innervation of the face and mouth indicating that Pacinian-corpuscles are generally absent in the orofacial region (Johansson, Trulsson, Olsson & Wessberg, 1988; Trulsson & Essick 1997; Trulsson & Johansson 2002). This results in the orofacial region being insensitive to high frequency vibrations and mechanical transients which are the sensations the Pacinian corpuscles are most responsive to.

2.4.3 Thermoreceptors

Thermal sensations result from difference in temperature between the object touching the body and the temperature of the skin. Neurons that are exceptionally sensitive to temperature are thermoreceptors. They possess a specific membrane mechanism that responds to temperature stimuli. Studies indicate that sensitivity to hot and cold are not uniformly spread, with some locations being sensitive to one or the other (Jones, 2009) and there exist regions on the body that lay between regions that are highly sensitive which are relatively insensitive to the temperature change (Jones, 2009). Taken together this indicates that separate receptors must code for the different stimuli. The specific

temperature sensitivity that a neuron possess is dependent on the type of ion channel that neuron expresses (Bear, Connors & Paradiso, 2007). Specifically, in the face, eyes, nose and mouth this is done through activation of transient receptor potentials (TRP) channels.

2.4.4 Taste transduction and TRP Channels

Taste particles are detected by taste cells that are clustered in the taste buds of the tongue, palate, pharynx, epiglottis and the upper third of the oesophagus. Taste buds are primarily located in the papillae of the tongue.

Figure 2.6 Each taste bud contains 50-150 taste cells. Taste cells extend from the base of the taste bad up to the taste pore where the microvilli of taste cells make contact with the tastant which is dissolved in saliva and taste pore mucus. Tight junctions between the cells prevent tastants from accessing the basolateral region. Taste cells have a short-lived life and are replaced from the stem cells at the base of the taste bud. The three cell types in each taste bud (light, dark and intermediate cells) may represent the different stages of cell life. Taste stimuli detected at the apical microvilli induce action potentials that trigger the release of neurotransmitters at the synapses formed at the base of the taste cell with gustatory fibres transmitting the signals to the brain (Buck & Bargman, 2000).

Four morphologically distinct cell types have been found in the taste bud: basal cells, dark cells, light cells, and intermediate cells (Azzali, 1997; Buck & Bargaman, 2000; see Image 6). The basal cells are small round cells located at the base of the taste bud. They are thought to be the stem cells from which the other cells are derived (Buck & Bargman, 2000). The basal cells play no role in taste transmission (Delay, Roper & Kinnamon, 1986; Roper, 1989). The three remaining cell types are all referred to as taste cells, they are elongated cells that stretch from the epithelial opening of the taste bud to its base. Taste stimuli that is detected by the pore induce action potentials that trigger the release of neurotransmitters at the synapses formed at the base of the taste cell with gustatory fibres transmitting the signals to the brain (Buck & Bargman, 2000; see Figure 2.6).

At least two pathways are available to convey the information from the taste bud to the central nervouse system (CNS). The first is the secretion of ATP from receptor cells which may pass directly to the afferent nerve fibres expressing P2X receptors (Finger, Danilova, Barrows, Bartel, Vigers & Stone, *et al.,* 2005; Huang, Maruyama, Dvoryanchikov, Pereira, Chaudhari & Roper, 2007). The second is a parallel pathway involving the presynaptic cells. Huang, Maruyama, Dvoryanchikov, Pereira, Chaudhari and Roper (2007) found that mutant mice lacking the $P2X_2/P2X_3$ receptors showed a reduced response to sweet, bitter and umami tastants. This reduction however was not present in the sour taste. This taste transduction follows the first pathway using the receptor cells.

The second pathway is highlight in Huang, Chen, Hoon, Chandrashekar, Guo, Trankner, Ryba & Zuker (2006) when they genetically ablated taste cells that sense sour. The other tastes were unaffected. Tomchik, Berg, Kim, Chaudhari and Roper (2007) conducted a study that indicated sour sensitive cells are the presynaptic (type 3) cells therefore it is most likely that Huang, Chen, Hoon, Chandrashekar, Gue & Tränkner *et al.,* (2006) ablated those (see Figure 2.7).

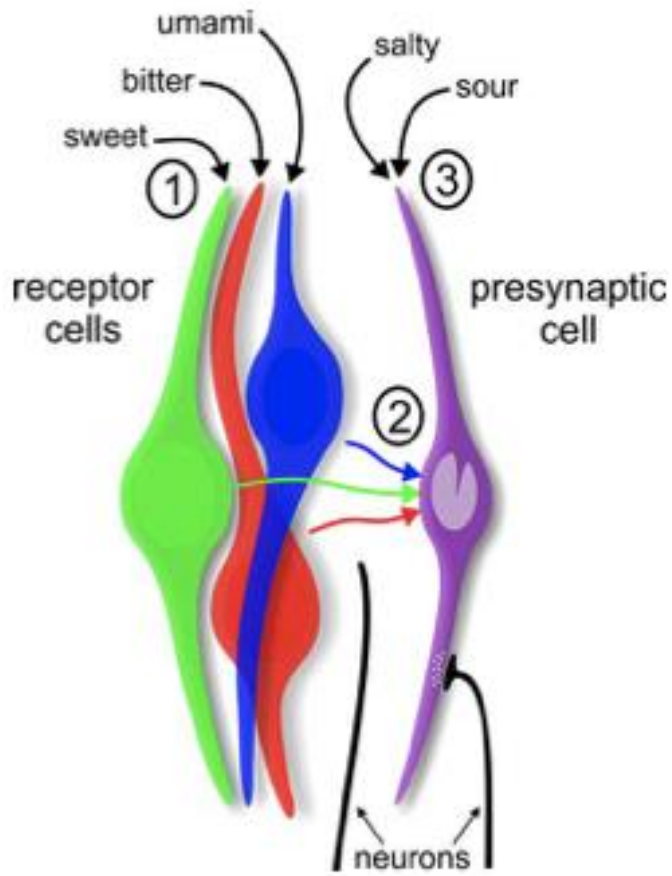


Figure 2.7 Schematic of gustatory processing taken from Tomchik, Berg, Kim, Chaudhari and Roper (2007; CC BY 4.0). 1) Represents receptor cells (type 2; Clapp, Yang, Stoick, Kinnamon & Kinnamon, 2004; DeFazio, Dvoryanchikov, Maruyama, Kim, Pereira, Roper & Chaudhari 2006) are tuned for sweet, bitter and umami tastes but rarely salt and sour (Tomchik, Berg, Kim, Chaudhari & Roper 2007) and as such are often considered specialist cells (Roper & Chaudhari, 2017). 2) It is thought that the signals from the receptor cells converge on the intermediate (type 3) presynaptic cells and release ATP which stimulates with the intermediate presynaptic cells (Roper, 2006; Huang, Maruyama, Lu, Pereira, Plonsky, Baur, Wu, & Roper, 2005; Huang, Dando & Roper, 2009). 3) Presynaptic cells are widely responsive to sweet, bitter and umami tastes but also high concentration of salt and sour tastes (Huang, Maruyama, Dvoryanchikov, Pereira, Chaudhari and Roper, 2007; Huang, Maruyama & Roper 2008). After stimulation the intermediate presynaptic cells release 5-HT (Huang, Maruyama, Dvoryanchikov, Pereira, Chaudhari & Roper, 2007).

Multiple neurotransmitters are expressed from the presynaptic cells, including an iso-form of glutamic acid decarboxylase, GAD1 (also called GAD67), and a biosynthetic enzyme for GABA (DeFazio, Dvoryanchikov, Maruyama, Kim, Pereira, Roper, & Chaudhari, 2006). Presynaptic cells were also found to release 5-HT in response to taste stimulation meaning the presynaptic cells may use

monoamines as neurotransmitters (Huang, Maruyama, Lu, Pereira, Plonsky, Baur, Wu, & Roper, 2005; Huang, Maruyama, Dvoryanchikov, Pereira, Chaudhari and Roper, 2007).

Of the important roles that TRP channels have, one of the most critical is the response to all sensory stimuli including light, sound, touch, temperature and chemical. They respond by allowing certain molecules to enter cells and alter the membrane potential with specific channels allowing specific elements through, either Sodium (Na^+), Potassium (K^+) and/or Calcium (Ca^{++}). There are two groups of TRP families and for the subgroups of TRP channels, thermoreceptors are part of three subfamilies in group 1:

1) Transient Receptor Potentials Vanilloid (TRPV)

2) Transient Receptor Potentials Melastatin (TRPM)

3) Transient Receptor Potentials Ankyrin (TRPA)

The TRPV receptors are warm receptors, they respond to temperatures between 25°C and 45°C. There are four different TRPV receptors, TRPV1 are non-selective cation channels activated by capsaicin and a noxious heat of 42°C or more. TRPV3 and TRPV4 are both warm receptors responding to temperatures between 27°C and 38°C. The TRPV3 receptors are thought to be associated with TRPV1 and may modulate its responses. Unlike the TRPV1 which are responsive to capsaicin the TRPV3 receptors are capsaicin insensitive (Smith, Gunthorpe, Kelsell, Hayes, Reilly & Facer *et al.,* 2002).

TRPM8 receptors are cool receptors and are often classed as a cold and menthol receptor because they activate to both ambient temperatures of approximately 26°C and cooling chemical agents like menthol (Peier, Moqrich, Hergarden, Reeve & Andersson *et al.,* 2002).

Temperatures above the 45°C of the TRPV responses and below the 12°C of the TRPM responses are thought to be mediated by TRPA receptors, which become active in extreme cold temperatures but also respond to cinnamon, mustard oil and hydrogen peroxide, leading to speculation they are polymodal nociceptors (Hand & Frank, 2014). At moderate skin temperatures both receptors types could be active but both cold and warm receptors will stop firing altogether as the temperature extends into damaging ranges leaving the nociceptive receptors to respond to

either freezing or burning pain rather than the temperature change (Gardner, Martin & Jessell, 2000). The rate of response from the receptors is proportional to the rate of temperature change and the degree at which the temperature is changing. The ability to perceive the temperature change depends upon how large the area of fibre activation is.

TRP channels are found on epithelial and mucosal trigeminal free nerve endings and they mediate the perception of both heat from chillies and cool from menthol. It remains unknown if the sensation of astringency, which is described as a tight or puckering sensation, is mediated via TRP channels (Hand & Frank, 2014). For more detailed reviews on TRP channel and taste transduction see Clapham, Runnels, & Strübing, (2001); Lindemann (2001).

2.5 Taster Status and Anatomy

One of the many ways research has attempted to explain variations in taster status is through anatomical differences, specifically examination of the density of FP on the tongue and thus taste bud density explains taste perception variation. Taste bud densities were found to vary by 100-fold and the FP densities on which the taste pores are located therefore, vary greatly (Miller, 1988).

When the human tongue is stained with 0.5% methylene blue (or blue food colouring has been used as an alternative) the filiform papillae take up the stain but the FP do not. This leaves the taste pores ringed, visible and countable (Miller & Reedy, 1990). FP densities on the tip of the tongue have been found to vary greatly across studies with some research recording densities in 22-74 (papillae/cm^2) (Miller & Reedy, 1990), 33-156 (papillae/cm^2) (Bartoshuk, Duffy & Miller, 1994), 33-184 (papillae/cm^2) (Essick, Chopra, Guest & McGlone, 2003) and one study had a mean FP range of 0-212.2 (papillae/cm^2) (Fischer, Cruickshanks, Schubert, Pinto, Klein & Pankratz *et al.,* 2013). The variation in range of FP densities between these studies could be due to methodological differences, Miller and Reedy (1990) used a square area of 1cm^2, a method replicated by Essick, Chopra, Guest and McGlone (2003), Bartoshuk, Duffy and Miller (1994) used a 3x3mm diameter square where as Fischer, Cruickshanks, Schubert, Pinto, Klein & Pankratz *et al.,* (2013) chose to use a

different method of a 6mm diameter circle. A further possible explanation for the vast range in mean FP densities in Fischer, Cruickshanks, Schubert, Pinto, Klein & Pankratz *et al.,* (2013) could be due to a them possessing a considerable larger sample size than the other studies and a reasonably even distribution of genders.

In a highly influential study, Bartoshuk Duffy and Miller, (1994) suggests that individuals who are hyper-tasters possess a greater number of FP and as such more taste pores than tolerant-tasters (Delwiche, Buletic & Breslin, 2001; Duffy, Hayes, Davidson, Kidd & Bartoshuk 2010; Essick, Chopra, Guest & McGlone, 2003; Hayes & Keast, 2011; Prutkin, Fast, Lucchina & Bartoshuk, 1999; Yakinous & Guinard, 2001). Due to the volume research hyper-taster status has become synonymous with a high density of FP (Hayes & Keast, 2011).

This synonymous relationship is clearly demonstrated by Essick, Chopra, Guest and McGlone (2003) who examined the FP density of participants in their study. On examination of 83 participants tongues they found significant differences in the FP density of hyper-tasters and tolerant-tasters (see Figure 2.8).

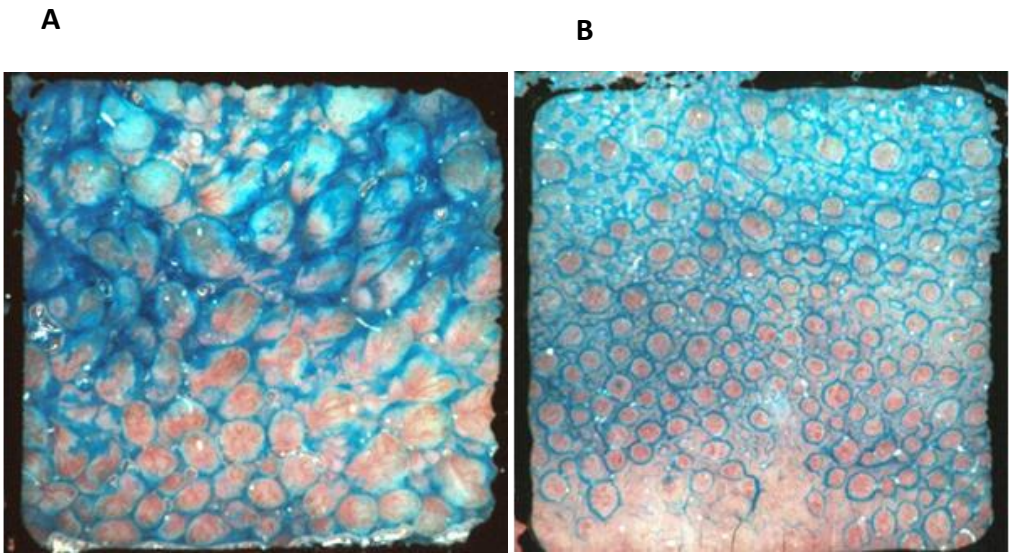


Figure 2.8 FP densities for tolerant-tasters (**A**) and hyper-tasters (**B**). FP are the pale pink-stained circular structures surrounded by blue-stained, non-gustatory filiform papillae. The average density for tolerant-tasters was 72 papillae/cm^2 and 179 papillae/cm^2 (Essick, Chopra, Guest & McGlone, 2003).

Yet in recent years this synonymous relationship has come into question. As much as there are clearly volumes of research supporting the relationship between hyper-taster status and FP densities there is also some contradicting evidence (Delwiche, Buletic & Breslin, 2001; Fischer, Cruickshanks, Schubert, Pinto, Klein & Pankratz *et al.,* 2013). Most recently, the lack of correlation between taster status and FP densities was highlighted in Garneau, Nuessle, Sloan, Santorico, Coughlin and Hayes (2014). In a 300+ participant study done with visitors to the Denver Museum of Nature and Science's permanent Expedition Health exhibit they found no support to substantiate the prior reports FP density varied across taster status or could be used to predict taster status (Garneau, Nuessle, Sloan, Santorico, Coughlin & Hayes 2014). The key differences between the studies that found relationships between taster status and FP density and those that didn't are participant numbers. Both Garneau, Nuessle, Sloan, Santorico, Coughlin and Hayes (2014) and Fischer, Cruickshanks, Schubert, Pinto, Klein & Pankratz *et al.,* (2013) had more than 300 participants in their studies. Other than participant numbers the studies all followed similar procedures and analyses of the data so the differing finings remain largely unexplained.

FP densities and taster status have also been used to explain differences in intensity perception of other tastes and oral sensations. Research supports that the greater the density of FP the greater the perceived intensity is of various other substances (Miller & Reedy, 1990; Delwiche, Buletic & Breslin, 2001) including sweet, salt (Bartoshuk, Duffy, Lucchina, Prutkin & Fast, 1998), chemo-sensation (Prescott & Swain-Campbell 2000; Pickering & Gordon 2006), and somatosensation (Essick, Chopra, Guest & McGlone 2003; Prutkin, Duffy, Etter, Fast, Gardner & Lucchina *et al.,* 2000; Hayes & Duffy 2007). Associations between bitter perception, chemo-sensation and somatosensation are expected due to FP being innervated by both taste and trigeminal fibres (Hayes, Bartoshuk, Kidd & Duffy, 2008) so with a great density of fibres a greater signal would be sent.

Approximately 75% of FP innervation has been found to arise from the lingual nerve of the trigeminal (Beidler, 1969). Data from hamsters (Whitehead, Beeman & Kinsella, 1985) indicate a proportional relationship between the number of FP and trigeminal fibres. Combined with the suggestion that FP density is linked

to PROP sensitivity indicates that hyper-tasters possess more trigeminal nerve endings than tolerant-tasters (Manrique & Zald, 2006). This means that the different taster's groups have differing levels innervation with the mouth which may translate into more than greater taste and chemosensory sensitivity in hyper-tasters but also the other sensations that the trigeminal nerve perceive including pain.

Spatial summation is an important aspect of the perception and processing of several cutaneous senses (Stevens & Marks, 1979). Spatial summation refers to the increase in sensation, especially pain, when stimulating a larger area. Hyper-tasters are thought to possess an increased number of FP and as such would have greater density of trigeminal fibres. This reflects an increased region of stimulation in hyper-tasters over tolerant-tasters which would imply that hyper-tasters are anatomically more susceptible to the effects of spatial summation and could explain the perceptual differences between the taster groups.

2.5.1 Taster Status and Genetics

Bitter taste perception evolved as a mechanism to detect and thus avoid a range of toxins that often possess a bitter taste (Sternini, 2007). This implies that a high sensitivity to bitter taste perceptions may have survival implication (Behrens & Meyerhof, 2013).

The ability to taste varies considerably across individuals and in some cases has been seen to be inherited (Bartoshuk, Duffy & Miller, 1994). Taste blindness is specific to bitter tasting substances, which is why they are used to assess taster status. In humans, bitter detection is mediated by a family of 25 bitter receptors (TAS2Rs) (Adler, Hoon, Mueller, Chandrashekar, Ryba & Zuker, 2000). Taster status is a genetic polymorphism meaning it is a DNA sequence variation that is common within the population. In the case of taste blindness there is no single allele regarded as making up the standard genetic sequence but there are two or more acceptable alternatives to the sequence (Twyman, 2003). To be classed as a polymorphism and not a mutation the least common allele must appear in 1% or

more of the population, if the frequency is lower than this it is classed as a mutation in the genetic sequence (Twyman, 2003).

There have been more than 25 different genes identified as being involved in taste perception but polymorphisms on chromosome 7q have been found to explain some of the variability in P.T.C (Kim, Jorgenson, Coon, Leppert, Risch & Drayna, 2003) and PROP taste perception (Duffy, Davidson, Kidd, Kidd, Speed & Pakstis *et al.,* 2004). It is thought that the TAS2R38 gene was found to explain variability in bitter taste perceptions (Kim, Jorgenson, Coon, Leppert, Risch & Drayna, 2003; Timpson, Heron, Day, Ring, Bartoshuk & Horwood *et al.,* 2007; Bufe, Breslin, Kuhn, Reed, Tharp & Slack *et al.,* 2005). Other genes have however been identified to play a role in taster phenotyping (Hayes, Bartoshuk, Kidd & Duffy, 2008; Reed, Nanthakumar, North, Bell, Bartoshuk & Price, 1999).

Research into the heritability of PROP sensitivity suggests that it follows an incomplete dominant pattern. There are 2 common forms of the TAS2R38 gene based the single-nucleotide polymorphisms that result in 3 amino acid substitutions; the proline-alanine-valine (PAV) haplotype and the alanine-valine-isoleucine (AVI) haplotype. It is a combination of these haplotypes that results in the three taster types, super-tasters are PAV homozygotes and non-tasters are AVI homozygotes, tasters are heterozygotes (Kim, Jorgenson, Coon, Leppert, Risch & Drayna 2003). Duffy, Davidson, Kidd, Kidd, Speed & Pakstis *et al.,* (2004) found that although PAV homozygotes perceive a greater bitter taste than heterozygotes those who are genotypic of taster status had smaller intergroup difference than phenotypic divisions of taster status. This suggests that TAS2R38 gene only accounts for approximately 85% of the phenotype variability in P.T.C bitter taste perception (Wooding, Kim, Bamshad, Larsen, Jorde & Drayna, 2004). There is some argument about how influential the genotype is to FP densities with Duffy, Davidson, Kidd, Kidd, Speed & Pakstis *et al.,* (2004) demonstrating that genotype and FP number make independent contributions to PROP bitterness perception yet conversely Hayes, Bartoshuk, Kidd and Duffy, (2008) found a relationship between not only FP density and PROP intensity only in the homozygote groups and not the heterozygote groups. There could be potential alternative factors that may be

involved in the perception of PROP (Bufe, Breslin, Kuhn, Reed, Tharp, & Slack *et al.,* 2005).

2.6 Summary

Understanding of the oral anatomy helps to explain why there are different taster groups within the population. It highlights that it is more than simply a difference of taste perception but the driving force behind the differences is in our human design. Different regions of the mouth are innervated with different branches and sub-branches of the cranial nerves. Given differences between the taster groups reflecting in different quantities of FP and as such different levels of innervations which in turn could implicate different levels of neurotransmitters with the hyper-taster population. The oral anatomy is the foundation on which all the taste and oral sensation research is built.

The tongue has a nerve supply from both the trigeminal and facial nerves and as such is innervated with different quantities of sensory fibres. It is acknowledged that hyper-tasters possess a greater density of FP and this makes them better with discriminative touch on the tongue but the role of touch as a whole on the perception of stimuli in the mouth remains largely unexplored. This is explored in chapter four of the thesis where different regions of the tongue and mouth are examined for their responses to different sensations and the role that touch plays on their perception.

From the anatomical understanding of the lip anatomy, questions were raised about the which type of skin they are comprised of. The different skin types have different innervation and the difference in innervation leads to a difference in sensory and affective experiences. This is examined in more detail in chapter five.

Finally, the possibility that taster status is further influenced by or has influence on the neurotransmitters that are involved in taste perception are explored in chapter six. Serotonin is thought to be involved in the transduction of taste in the taste bud so this is explored.

Chapter 3 : Methodology

Abstract

This chapter details the methodology used in this thesis. It begins with a discussion of psychophysical techniques, highlighting those techniques applied in this thesis. The second part of this chapter covers quantitative sensory testing with particular focus on the specific procedure used in chapter five. The development of sensory measuring scales is discussed. Finally, the chapter concludes by explaining the evolution of the taster status test and the various procedures used to classify taster status with specific information regarding the approach use in this thesis.

3.1 Quantitative Sensory Testing

Where questionnaires were developed to be the quantitative method of assessing usually qualitative information, quantitative sensory testing (QST) is a set of methods used in the neurological examination of somatosensory function (Greenspan, 2001). It mostly refers to a set of techniques that allows a researcher to determine a person's perceptual thresholds and utilises a variety of psychophysics approaches including the method of limits, the method of levels and the staircase procedure (Greenspan, 2001).

The QST protocol consists of 13 tests that measure different aspects of somatosenation and was compiled by Rolke , Mageri, Campbell, Schalber, Caspari & Birklein *et al.,* (2006). A nationwide multicentre research network (German Research Network on neuropathic pain – DFNS; http://www.neuro.med.tu-muenchen.de/dfns/e_index.html) compiled a protocol that would provide somatosensory profiles for two body area within a one-hour protocol. Only one test of the QST standardised battery is used in this thesis and that is thermal detection and pain thresholds. The other tests are out of the scope of this thesis so for further information regards the entire QST battery see Baad-Hansen, Pigg, Yang, List and Svensson *et al.,* (2015), Rolke , Mageri, Campbell, Schalber, Caspari & Birklein *et al.,* (2006) and Rolke, Baron, Maier, Tölle, Treede & Beyer et al., (2006).

3.1.1 Thermal detection, thermal pain thresholds and paradoxical heat sensations

QST tests for thermal sensation are performed using a Medoc thermal sensory testing device (Fruhstorfer, Lindblom & Schmidt, 1976; Yarnitsky, Sprecher, Zaslansky & Hemli 1995). The protocol requires measuring cold detection (CDT) and warm detection (WDT) first. Paradoxical heat sensations (PHS) are determined during the thermal sensory limen procedure (TSL, the difference limen for alternating cold and warm stimuli). This is then followed by cold pain threshold (CPT) and hot pain threshold (HPT).

All of the thresholds are obtained with ramped stimuli (1°C/s) that terminates when participants press a trigger. Standard safety temperatures are

usually set as 0 and 50°C with measurements starting at a baseline body temperature of 32°C. The standard thermode for this procedure is 7.84cm^2. The mean threshold temperature is calculated from three consecutive measurement for each for the CDT, WDT, CPT and HPT

This QST test was adapted for use in chapter 6 for exploring the thermal thresholds and pain experiences on the cheek, lip vermillion and oral mucosa. A smaller thermode was use measuring 1.5cm^2, the vermillion of the lip is a small region of the body so ensuring only the target region is stimulated required a smaller surface area thermode. The thermode used has previously been used in similar research (see Essick, Guest, Martinez, Chen & McGlone, 2004).

Base temperatures were also changed for the intra-oral region as starting at 37°C for intra-oral thermal research (oral mucosal surface) because the mouth is a warmer environment than skin surface.

3.2 Scales

Scales have been developed to quantify the subjective differences in sensory perception. The labels and anchor points on scales are derived from the way we use language and describe sensory experiences in everyday life (Bartoshuk, Duffy, Green, Hoffman, Ko, Lucchina, Marks, Snyder & Weiffenbach, 2004). People experience the sensory world differently so in order to conduct research across individuals and groups various labelled scales have been developed (Bartoshuk, Duffy, Chapo, Fast, Yiee, & Hoffman *et al.,*, 2004).

3.2.1 Visual Analogue Scale (VAS)

The graphic rating scale is a combination of ratings on a straight line that has labels under it that has descriptive phrases indicating varying degrees of a trait (Freyd, 1923).

The visual analogue scale (VAS) is essentially a graphic rating scale without category labels (Bartoshuk, Duffy, Chapo, Fast, Yiee & Hoffman *et al.,* 2004). It is comprised of a lined graphic scale with relevant anchors at the extreme ends related to the attribute being studied (Bartoshuk, 2004).

When exploring taste hedonics, it is common to use a VAS measuring how pleasant or unpleasant a taste is (Miura, Morita, Koizumi & Shingai, 2009; Yeomans, Tepper, Rietzschel & Prescott, 2007). These scales usually have three anchor points; two extreme anchors being very pleasant and very unpleasant and a neutral middle, occasionally marked with 0 or neutral. To assess taste pleasantness in Chapter 6 the VAS shown in image 5 was used. Participants were instructed to indicate on the scale how pleasant or unpleasant they found the taste with -50 very unpleasant being the most unpleasant extreme and +50 very pleasant being the most pleasant extreme. The centre of the scale represents neutral meaning the taste is neither pleasant nor unpleasant (see Figure 3.1). This scale is used for collecting hedonic ratings of tastes in chapter 6.

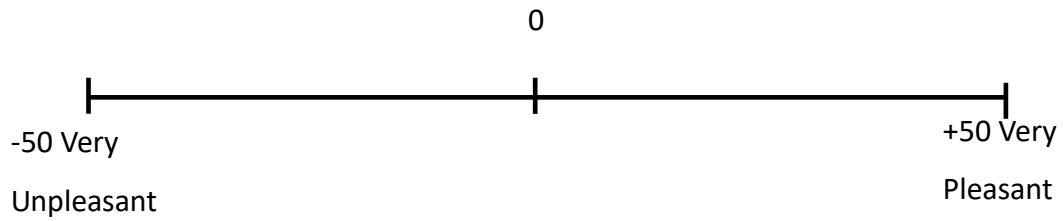


Figure 3.1 VAS scale used in thesis chapter 6 to explore the hedonics of taste perception. Participants were instructed to rate on the scale how pleasant or unpleasant the taste they experienced was with -50 very unpleasant being the negative extreme and +50 very pleasant being the positive extreme. The zero in the centre represents neutral meaning the taste was neither pleasant nor unpleasant.

3.2.2 Ratio Scales

A ratio scale is one where the scale anchor points are proportional to the perceived intensities (see Bartoshuk, Duffy, Fast, Green, Prutkin & Snyder, 2003 for a review).

3.2.2.1 Labelled Magnitude Scale (LMS)

Green, Shaffer and Gilmore (1993) constructed a semantically labelled scale of sensation magnitude that would generate data on perceived intensity that was equivalent to the data produced via magnitude estimation (ME). The scale that was

developed was called the LMS. It is characterized by a non-linear spacing among the verbal descriptors of barely detectable, weak, moderate, strong, and very strong. Reliability assessment of the LMS was undertaken by comparing it with the method of ME. The scale anchor points are placed according to their associated geometric means and participants were asked to rate the intensity of three kinds of oral stimuli, gustatory, thermal and nociceptive (Green, Shaffer & Gilmore, 1993). These were chosen to assess if the scale could be used to assess three sensory modalities simultaneously. After normalizing the data to eliminate the effects of personal number usage in ME no significant difference between the methods of ME and the LMS were identified indicating that the LMS provided ratio-level data that was comparable to that of magnitude estimation (Green, Shaffer & Gilmore, 1993).

The LMS was used in chapters 4, 5, 6, and 7 to assess taster status (see Figure 3.2). The specific scale used was The Oral LMS replicating that developed by Green, Shaffer and Gilmore (1993) which was developed specifically for use in examining oral stomato-sensation and gustation. It was further used in chapter 6 to measure taste intensity, the LMS has previously been validated for research into intensity responses and is commonly used in taste research (Dinehart, Hayes, Bartoshuk, Lanier & Duffy, 2006; Duffy & Bartoshuk, 2000; Green, Dalton, Cowart, Shaffer, Rankin & Higgins, 1996; Hayes, Allen & Bennett, 2013).

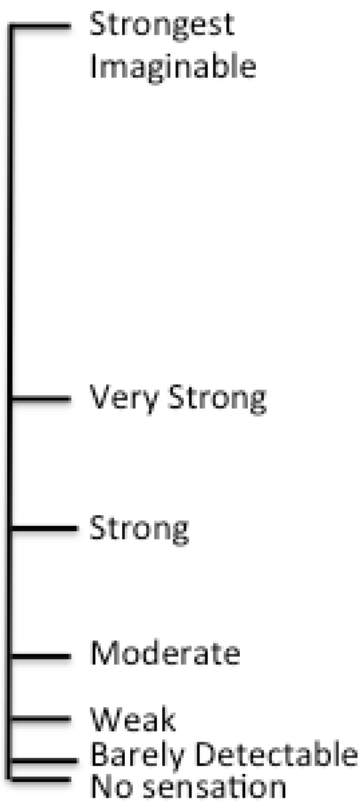


Figure 3.2 The LMS used in thesis chapters 4, 5, 6 and 7 to assess taster status and again in chapter 6 to measure the intensity of the tastes participants perceived. The scale was explained to participants in that they were to make on the scale how intense the sensation they perceived was. The top of the scale is labelled strongest imaginable, specifically strongest imaginable oral sensation but that could come from oral health care products, foods consumed, simply whatever they considered their strongest imaginable oral sensation. The bottom of the scale was no sensation, meaning they perceived no sensation at all. They were told they could mark anywhere on the main line going up the scale, it did not have to be on an anchor point.

In explaining the scale to participants, they were told to rate the intensity of the sensation they perceived. At the top of the scale was strongest imaginable sensation, meaning specifically strongest imaginable oral sensation but that could come from oral health care products, food consumed, dental procedures, simply whatever they imagined to be the strongest oral sensation. The bottom of the scale was no sensation, meaning they perceived nothing at all from the stimuli. Participants were also advised that they could mark anywhere on the line going up the scale, it did not have to be on an anchor point.

3.3 Taster Status Classification

Taster status was assessed in chapters 4, 5, 6, and 7. Tepper, Christensen and Cao (2001) compared a three-solution (0.0032, 0.32 and 3.2 mmol/l) PROP and three-solutions (0.01, 0.1, 1.0 mol/l) of NaCl test with a one-solution version consisting of the middle concentration of the three solutions. Participants used an LMS to rate the intensity of the solutions. The LMS cut off points were established for the one-solution test by calculating the ± 95% confidence interval around the PROP group means leaving hyper-tasters giving a rating of 51 (''very strong'' on the LMS) or higher and hypo-tasters giving 15.5 (approximately ''moderate'' on the LMS) or lower and the classification for the three-solution test was made independently. There was a significant agreement between the methods for classification indicating that one solution test was equally as reliable as the three-solution test (Tepper, Christensen & Cao, 2001).

Further simplification of the procedure using filter paper was also developed. The paper disc procedure utilised a similar approach the classification procedure of the one-solution test. It was first successfully trialled by Zhao, Kirkmeyer and Tepper (2003). They used Tepper, Christensen and Cao (2001) three-solution test as a standard for comparing filter papers with. PROP is known to be poorly soluble in water but possesses a saturation point in boiling water of 59mmol/l. The first filter paper concentration came from the highest concentration of the three-solution test (3.2mmol/l); further filter papers were made by increasing the concentration by a factor of 10 (Zhao, Kirkmeyer and Tepper, 2003).

The primary finding was that paper disks impregnated with a 32mmol/l or 42mmol/l concentration of PROP were unable to distinguish the taster groups. It was only at a concentration of 50mmol/l that separate taster groups could be identified. Comparison of the taster groups assessed by both the three-solution test and the 50mmol/l paper disk test were found to be highly reliable (Zhao, Kirkmeyer and Tepper, 2003).

The method of taster status assessment in this thesis was by the filter paper method using the concentration concluded as the most appropriate for the delivery method by Zhao, Kirkmeyer and Tepper (2003). A 50mmol/L 6-*n*-Propylthiouracil

(PROP) solution was prepared by dissolving 0.75g of PROP powder in 100ml of rapidly boiling water on a stirring hotplate until the solution was clear. Filter paper disks (15mm in diameter, Whatman, Qualitative filter paper Grade 1, Sigma) were threaded onto cotton threads with a sterilised sewing needle. Plastic straw segments (~0.5cm) were used as spacers to separate papers for impregnation. The disks and separators were soaked in the solution for 30 seconds and then removed, excess solution was lightly shaken off and the impregnated disks were left to dry on a plastic catering tray for at least 2 hours or until completely dry. Additionally, pre-made filter papers soaked in a 1 Mol (58.44g/L) concentration of sodium chloride (NaCl; salt) were prepared using the same method for making the PROP papers. Filter papers were removed from the cotton and stored in sealed Glassine Envelopes (Lindner, 45x60mm, Germany).

To assess taster status the filter papers were given to the participant and they were asked to place them as close to the tip of the tongue as they could but ensuring the whole filter paper was on the tongue. They were instructed to soak the paper in saliva and leave it on the tongue for a timed period of 10 seconds. After the 10 seconds they removed the paper and swallowed any saliva while waiting a further 10 seconds before rating the intensity of the perceived taste on the LMS. The LMS used was a replicant of that developed by Green, Shaffer and Gilmore (1993) for use specifically in examining oral somatosensation and gustation, was developed in photo shop and used to rate the sensation intensity for the taster status test (see section LMS 3.2.2.1 pg 89).

Participants first rated the NaCl paper followed by the PROP paper. Bitter scores from the PROP paper are compared against previous researchers pre-determined LMS taster status cut offs (Prescott & Swain-Campbell, 2000). NaCl scores are only used for participants whose PROP intensity rating falls on the cut-off between the taster groups to establish which side of the taster division they belong (Daştan, Durna & Daştan 2015). PROP is a useful means to stratify taster status as it is widely considered safe to for human consumption as it is commonly used in the treatment of hyperthyroidism in doses up to 1000mg (Medscape, 2016) and has been used in taste research for over thirty years

3.4 Acute Tryptophan Depletion (ATD)

Acute tryptophan depletion (ATD) is an experimental procedure that has been used substantially in research (see Reilly, McTavish & Young, 1997 for review). The goal of ATD is to allow the behavioural and cognitive consequences of acute reductions in plasma tryptophan (TRP). The premise behind the procedure is that depletion of plasma TRP, which is the precursor of serotonin (5-HT), leads to the depletion of brain 5-HT (Hood, Bell & Nutt, 2005).

3.4.1 Tryptophan

Tryptophan is an essential amino acid and is only obtained through diet (Khaliq, Haider, Ahmed, Perveen & Haleem, 2006; Figure 3.3). It is found in many common foods including meats, seeds, nuts, eggs, dairy products and other high protein foods. The essential amino acids help the body to produce the non-essential kind and together they are important for building and repairing muscle tissue, helping neurotransmitter function, supply energy to the brain and balance blood sugars. Once consumed tryptophan is distributed throughout the human body in the circulatory system as it has relatively low tissue storage (Reilly, McTavish & Young, 1997) and is one of the amino acids with the lowest concentration within the human body (Young & Stoll, 2003 as cited in Richard, Dawes, Mathias, Acheson, Hill-Kapturczak & Dougherty, 2009). Tryptophan is a precursor to two very important metabolic pathways, kynurenine synthesis and 5-HT synthesis (Richard, Dawes, Mathias, Acheson, Hill-Kapturczak & Dougherty, 2009). It is estimated that only 3% of dietary tryptophan is absorbed into the body and approximately 1% of that is used in 5-HT synthesis (Bender, 1983).

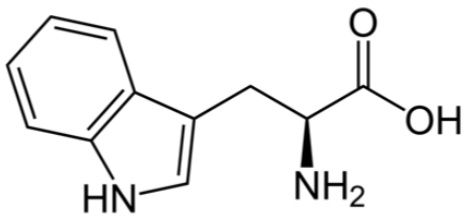


Figure 3.3 Tryptophan chemical structure

3.4.2 Serotonin (5-HT)

5-HT is biochemically derived from tryptophan and is most commonly associated with mood, feelings of well-being and happiness (Young, Smith, Pihl &

Ervin, 1985; Owens & Nemeroff, 1994). It is a broad impact monoamine neurotransmitter and neuromodulator which has been implicated in numerous psychiatric conditions and psychological processes but is found in relatively low concentration in the brain compared to the rest of the body (Richard, Dawes, Mathias, Acheson, Hill-Kapturczak & Dougherty 2009). When it comes to the human body 5-HT is found in the blood platelets and central nervous system of animals (González-Flores, Velardo, Garrido, González-Gómez, Lazno & Ayuso *et al.,* 2011) but approximately 90% of all mammalian 5-HT is located in the gastrointestinal tract and enterochromaffin cells of the gut where it is used to regulate intestinal movements (Gershon & Tack, 2007).

Monoamine neurotransmitters are synthesized from essential large neutral amino acids (LNAAa). 5-HT cannot cross the blood brain barrier (BBB) therefore all neuronal 5-HT in the central nervous system (CNS) must be synthesized in the neurone. This synthesis is a two-step process, the first is that its precursor, TRP is hydroxylated to 5-HT by the enzyme TRP hydroxylase. This is followed by decarboxylation involving a universal enzyme, L-aromatic acid decarboxylase (Green & Grahame-Smith, 1975).

The primary focus when looking to manipulate brain 5-HT levels is to limit the step of TRP hydroxylation by both enzyme inhibition and alerting the substrate available (Reilly, McTavish & Young, 1997). An increase in brain TRP will increase 5-HT synthesis (Wurtman, Wurtman, Growdon, Henry, Lipscomb & Zeisel 1981). In order to enter the brain however, TRP must compete with other LNAAs for transport across the BBB. Availability of transport across the BBB is dependent on the relative peripheral availability of TRP in comparison to its other LNAA competitors.

To alter 5-HT levels, the central substrate availability and consequently central 5-HT synthesis can be manipulated. This is done by altering the availability of TRP or other LNAAs via changing the levels of dietary TRP, changing the dietary levels of other LNAAs, changing the overall rate of protein synthesis or a combination of all these methods (Reilly, McTavish & Young, 1997).

3.4.3 Depletion

The ATD technique was used in chapter 6 of this thesis. ATD manipulation used was a combination of a low TRP diet and a TRP-deficient protein load containing large amounts of other LNAAs. This combination of approaches produces the maximal brain TRP depletion (Reilly, McTavish & Young, 1997).

The diet control portion of the manipulation began 24 hours before the test day and participants were instructed to consume a low protein diet following Delgado, Charney, Price, Aghajanian, Landis & Heninger, (1990) guidance. Participants were not allowed to consume anything but water after midnight the night before the experiment and the fast continued throughout the testing day. A small lunch was provided for the participants containing only 2.8g of protein to keep the TRP levels low.

15 amino acids make up the amino acid drink. This reflects the same proportions as contained in human milk. The key difference is that TRP is missing from the depletion drink but contained in the control drink. Aspartic acid and glutamic acid were omitted from both drinks due to toxicity concerns (Hood, Bell & Nutt, 2005). After ingestion of the amino acid drink peak effects aren't usually seen until 5 to 7 hours later. This approach was chosen as TRP levels are reduced by 70 to 90% when a LNAA load mixture is used. For detailed experimental procedure see chapter 6 pg 168.

3.5 CT Touch

C-tactile (CT) afferents which were first discovered in human facial skin (Johansson, Trulsson, Olsson, & Westberg, 1988) and respond optimally to a slow-gentle stroking touch (between 1cm/s and 10cm/s) with decreased activity at the lower and higher speeds (Löken, Wessberg, Morrison, McGlone, & Olausson, 2009) responding most vigorously to slow and soft stroking between 3-5cm/sec (Nordin, 1990; Essick, McGlone, Dancer, Fabricant, Ragin & Phillips *et al.,* 2010).

They are considered as coding for the rewarding aspects of interpersonal touch (Löken, Wessberg, Morrison, McGlone, & Olausson 2009) and conduct at a velocity that is approximately 50 times slower than the myelinated fibres (McGlone,

Wessberg & Olausson, 2014). CTs respond to the affective aspects of touch encountered in grooming behaviours (Olausson, Lamarre, Backlund, Morin, Wallin & Starck *et al.,* 2002).

CTs have been shown to be highly sensitive to harmless tactile stimulation (Nordin, 1990; Vallbo, Olausson & Wessberg, 1999; Wessberg, Olausson, Fernström, & Vallbo, 2003). These unmyelinated afferents are found in the hairy skin, like that of the arm and torso, but are absent in the glabrous skin of palm (McGlone, Vallbo, Olausson, Löken, & Wessberg, 2007; Olausson, Wessberg, Morrison, McGlone & Vallbo, 2010). They are slow-conducting afferents are easily fatigued with repeated stimulation and may continue firing for several seconds after the stimuli has been removed (Vallbo, Olausson & Wessberg 1999). Microneurographic studies, measuring the receptors electrical signals during stimulation, identified CTs respond optimally to a slow-gentle stroking touch (between 1cm/s and 10cm/s) with decreased activity at the lower and higher speeds (Löken, Wessberg, Morrison, McGlone, & Olausson, 2009) responding most vigorously to slow and soft stroking between 3-5cm/sec (Nordin, 1990; Essick, McGlone, Dancer, Fabricant, Ragin & Phillips *et al.,* 2010). These electric signals have been shown to generate an inverted U in response to the tactile stimulation across velocities (see Figure 3.4).

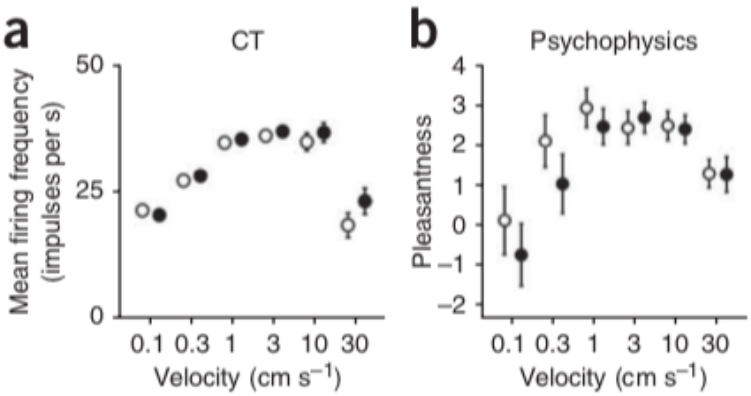


Figure 3.4 Firing rate of the CT afferents (**A**) at slow CT non-optimal, CT optimal and fast CT non-optimal velocities are matched by the hedonic rating obtained from strokes administered at the different velocities (**B**) generating what is acknowledged as the classic inverted U. The white dots represent a stroke force of 0.2N and the black dots are force of 0.4N (Löken, Wessberg, Morrison, McGlone & Olausson, 2009).

Essick, James and McGlone (1999) were the first to identify the velocity/pleasantness interaction of gentle touch finding a high correlation between CT firing and hedonic ratings of touch. It was Löken, Wessberg, Morrison, McGlone and Olausson (2009) who first showed the CT afferent firing is essential for this psychophysical relationship.

Using a rotary tactile stimulator (RTS; Dancer Design, St Helens, UK) has become the standard approach to assessing affective touch. The RTS can administer various controlled velocities at various forces across skin surfaces. Löken, Wessberg, Morrison, McGlone and Olausson (2009) used the now standardised approach of stroking the forearm with a brush at 6 velocities (0.1, 0.3, 1, 3, 10, 30 cm/s) starting at CT non-optimal and progressing through CT optimal to fast CT non-optimal. Hedonic ratings of the touch were collected on a visual analogue scale (VAS). Highest hedonic ratings were identified at velocities in ranges 1 to 10cm/s peaking at 3cm/s and are reflected in the CT firing responses (see Figure 3.4A and 3.4B). This inverted U has repeatedly been identified in research (Vallbo, Olausson & Wessberg 1999; Essick, McGlone, Dancer, Fabricant, Ragin & Phillips *et al.,* 2010; Löken, Evert & Wessberg, 2011; Liljencrantz & Olausson, 2014; Morrison, Löken, Minde, Wessberg, Perini, & Nennesmo, *et al.,* 2011).

CT touch is examined in Chapter Five (pg 140) of this thesis. An adaptation of the standardised CT approach was utilised. Strokes were administered by hand using a 10ml glass rollette bottle topped with a plastic rollerball (see Figure 3.5). When using an RTS the computer controls the velocities but hand delivery requires the use of a metronome. The metronome was programmed for copying a velocity across a specific distance, in this case 3cm. Strokes were given at approximately three different speeds: 0.5cm/s (CT non-optimal), 3cm/s (CT optimal) and 20cm/s (CT non-

Figure 3.5 Roller ball used for stroke administration

optimal). Participants experienced each stroke for a period of 6 seconds before providing a hedonic rating on a VAS.

Triscoli, Olausson, Sailer, Ignell & Croy (2013) compared the pleasantness ratings of CT-optimised touch obtained by hand stroke administration and robotic stroking. They identified that pleasantness ratings were similar in both conditions and across velocities meaning that the effects are comparable.

Experimental Chapters

The following four chapters cover the experimental part of the thesis with each chapter building on or expanding on the knowledge gained from the previous. Together they examine the role of somatosensation and hedonic experiences in oral perception.

Chapter 4 : Examining the impact of taster status on oral sensory processing

Abstract

There are three types of tasters within the population; hyper-taster, tasters and tolerant-tasters. Each taster status experiences the orosensory world differently with hyper-tasters being most sensitive to tastes and chemo-stimulants in the mouth. These perceptual differences between the taster groups are reflected in anatomical differences including innervation and sensory receptor quantities within the mouth.

Using psychophysics this study first explored (1) the role of taster status on chemosensory perception and (2) detection of sensations on the median sulcus, side and tip of the tongue, frenulum and vermillion of the lower lip. The final aim of this study was to (3) see if a dynamic touch increased the intensity of the perceived sensations.

(1) The taster groups experienced all chemosensory stimulant significantly differently with hyper-tasters experiencing a greater intensity than tasters and tolerant-tasters. (2) The different regions of the mouth experienced the sensations differently with the vermillion of the lip being the least sensitive and the tongue tip generally being the most sensitive. (3) No main effect for touch type was identified but it was found to interact with taster status and location with 10ppm capsaicin and Sichuan pepper. A significant interaction between location and touch type with the mint oil was also found with a dynamic touch on the vermillion of the lip being significantly more intense than a static touch. This is possibly explained by activation of C tactile afferents.

4.1 Introduction

Research has established the presence of three taster groups within the population (Bartoshuk, 1993), hyper-tasters, tasters and tolerant-tasters are distinguished by their sensitivity to the bitter taste elicited from PROP. Hyper-tasters find the bitter taste significantly more intense than their taster and tolerant-taster counterparts (Bartoshuk, 1993). These perceptual differences are reflected in anatomical differences with hyper-tasters possessing a greater density of FP and as such taste pores, on the tongue (Bartoshuk, Duffy & Miller, 1994). Comparison between the taster groups has identified that tasters possess double that of tolerant-tasters and supertasters have double that of tasters (Bartoshuk, Duffy, & Miller, 1994).

Expanding on this, data collected in animal research suggests a relationship between the number of FP and the density of V^{th} nerves (Farbman & Hellekant, 1978; Whitehead, Beeman, & Kinsella, 1985; Whitehead, Ganchrow, Ganchrow, & Yao, 1999). The V^{th} nerve supplies a large proportion of innervation to the tongue. The lingual nerve branch supplies the anterior two-thirds of the tongue with tactile sensation. Microneurography studies have highlighted that some lingual nerve fibres have low mechanical thresholds and small receptive fields considered to terminate near the tongue surface (Trulsson & Essick, 1997). The neuroepithelial connections are located at the taste bud. Taste buds are the chemosensory organs of the tongue with receptor synapses on specific gustatory nerves and when stimulated by a variety of chemical substances generate electrical signals (see Roper, 1992).

Combined this all implied that hyper-tasters may express greater oral sensitivities than a tolerant-taster due to greater densities of trigeminal innervation of the oral cavity (Manrique & Zald, 2006; see chapter 2 section 2.3 pg. 66 regarding oral innervation).

PROP sensitivity allows the examination of differences in oral sensory perception because it is possible to experience the oral sensory world without gustatory input (Breslin, Gilmore, Beuchamp, & Green, 1993). It has widely been reported that taster status influences more than bitter taste perception but also

differing levels of sensitivity to the main taste groups and chemosensory stimulants (Alimohammadi & Silver, 2002; Bartoshuk, Duffy, Lucchina, Prutkin, & Fast, 1998; Looy & Weingarten, 1992). Of these chemosensory stimulants, four are primarily used when exploring the oral chemosensory and trigeminal systems (Green & Schullery, 2003). These stimulants are capsaicin that elicits a warm, burning sensation, menthol that elicits a cooling, irritating sensation, sanshool that elicits a tingling sensation and aluminium potassium sulphate that elicits a dry, puckering sensation.

4.1.1 Sensation stimulants: Heat

Capsaicin is naturally found in chili peppers and has long been known to excite nociceptive neurons producing a burning sensation when it comes into contact with the TRPV1 receptors (Yang & Zheng, 2017). When this receptor is activated by capsaicin, Ca^{++} and Na^{+} ions flow through the cell, depolarising the nociceptive neurons leading to an action potential firing and leading to the sensation of spiciness associated with capsaicin (Caterina, Schumacher, Tominaga, Rosen, Levine & Julius, 1997). Capsaicin binds to receptors that are located with trigeminal nerve neurons but limits its effect to a specific type of trigeminal neuron that specifically transmits signals triggered by heat and acidity (Caterina, Schumacher, Tominaga, & Rosen, 1997; Szallasi, Conte, Goso, Blumberg, & Manzini, 1993; Tominaga, Caterina, Malmberg, Rosen, Gilbert & Skinner *et al.*, 1998). Given that the anterior two-thirds of the tongue is innervated by the trigeminal nerve responses to capsaicin are usually limited to this region (Smutzer & Devassy, 2016).

Capsaicin is the most commonly used chemostimulant for probing oral chemosensory perception due to its ability to stimulate somatosensory neurons without affecting the gustatory ones (Hettinger & Frank, 1992) and its effects on the trigeminal system. Repeated TRPV1 exposures of capsaicin leads to a desensitisation of the receptor and induces it to either minimally activate or fail to activate altogether (Dessirier, Simons, O'Mahony, & Carstens, 2001; Liu, Wang, & Simon, 1996; Ho, Ward & Calkins, 2012). However, successive brief exposures of capsaicin can also enhance the response to some stimulants (Green, 1993b).

Capsaicin's limited affect and its influence on other stimulants is why it is the most often used to support PROP sensitivity as an argument for the presence of taster status perceptual differences within the population.

Capsaicin perception and its relationship to PROP sensitivity are somewhat inconclusive. Karrer and Bartoshuk (1991) found that PROP non-tasters rated the burn from capsaicin as lower than PROP tasters but this difference was not seen in every condition they tested. That finding was supported by Prescott and Swain-Campbell (2000) who found tolerant-tasters rated the burn lower than hyper-tasters but that there was no difference between tasters and hyper-tasters. This is a common and highly supported finding within the literature (Bartoshuk, Conner, Grubin, Karrer, Kochenbach, & Palcso, et al., 1993) but there are however, contradictory findings like that of McBurney, Balaban, Popp, and Rosenkranz (2001) who found no difference in burn intensity perception from capsaicin between tolerant-tasters and hyper-tasters, a finding supported by Törnwall, Silventoinen, Kaprio, and Tuorila (2012). Research is still inconclusive of the role that taster status plays on the sensory perception of capsaicin but the key difference between the old and recent research mentioned here is the method used to classify taster status. The literature that finds a difference between the taster groups and capsaicin perception use a rating of bitter solutions approach for taster status classification where the recent research uses saturation-soaked filter papers on the tongue.

4.1.2 Sensation stimulants: Cool

Menthol is commonly found at low concentrations in sweets, cigarettes and oral health care products for its cooling and refreshing sensory properties when inhaled, consumed or applied to the skin. It could be considered the chemosensory opposite to capsaicin due to its elicitation of a cooling sensation rather than a heat sensation (Cliff & Green, 1996; Dessirier, O'Mahony, & Carstens, 2001; Eccles, 1994; Green & McAuliffe, 2000).

Similar to capsaicin, menthol has been found to activate its own specific TRP channel. The TRPM8 and TRPA1 are both members of the same subfamily and are

activated at cool temperatures. The TRPM8 is sensitive to harmless temperature decreases (McKemy, Neuhausser & Julius, 2002; Peier, Moqrich, Hergarden, Reeve, Andersson & Story, *et al.,* 2002a) of less than 25°C and the TRPA1 less than 17°C (Peier, Moqrich, Hergarden, Reeve, Andersson & Story, *et al.,* 2002a; Bautista, Jordt, Nikai, Tsuruda, Read, & Poblete *et al.,* 2005). The TRPM8 are expressed as innocuous cold fibres but are co-expressed with TRPV1 in nociceptors (McKemy, Neuhausser & Julius, 2002) possibly explaining why oral menthol can create both a cooling and irritation sensation (Cliff & Green 1994, 1996; Dessirier, O'Mahony & Carstens, 2001).

Despite its wide spread use, the perceptual effects of menthol remain practically unexplored. To the best of my knowledge the earliest published study conducted by Watson, Hems, Rowsell, and Spring (1978) describes the cooling characteristics of menthol and various other artificial coolants but focused more on the molecular properties rather than the perceptual ones. Although it has never been studied psychophysically as a gustatory stimulus, the electrophysiological experiments indicate that menthol excites the chorda tympani nerve in rodents, but eventually begins supressing the sensation (Lundy & Contreras, 1993).

The majority of physiological studies regarding menthol explore the sensory effects of menthol and the psychophysical studies that are available explore the effect of menthol on temperature perception highlighting several complicated interactions. Green (1985) found that while menthol enhances cooling sensations when at room temperatures at temperatures above 37°C warm sensations are enhanced. Pre-exposure to liquid menthol has also been seed to enhance cooling and suppress warmth on lip (Green, 1986) and forearm (Green, 1992b).

Like capsaicin, applications of menthol in quick succession have been seen to increase irritation but to also possess similar desensitizing properties to capsaicin, particularly when applied to the mucosal surfaces of the oral cavity (Cliff & Green, 1996) . A further similarity that capsaicin and menthol possess is that it has been found to cross-desensitize other chemical irritants particularly capsaicin itself (Cliff & Green, 1996).

In their study, Cliff and Green (1996) obtained intensity of capsaicin or liquid menthol administered to the tip of the tongue of participants. They proceeded to

treat the participants with mouthwash samples of the same substance before waiting 15 minutes and assessing the intensity perceived on the tip of the tongue again. Amongst their findings they confirmed self-desensitisation for both substances, cross desensitisation between the substances. This finding was replicated even with inter-stimulus intervals of 5 minutes, high menthol concentrations were seen to decrease mean sensory irritation ratings with repeated application, indicating desensitization rather than adaptation (Prescott & Swain-Campbell 2000; Green & McAuliffe, 2000). Together this implies that the neurochemical processes that underpin both menthol and capsaicin's sensory irritation share some underlying characteristics (Cliff & Green, 1996).

There is very little literature available that explores the oral perception of menthol; this is probably due to the research that is available indicating that menthols coolness remained considerably consistent under experimental conditions (Cliff & Green, 1996). Further to this there is limited research on the impact that taster status has on the perception of oral menthol. Yet there is research that indicates both capsaicin and menthol can generate a bitter taste. Green and Schullery (2003) found that fifteen of twenty-five participants reported, on average, that capsaicin and menthol produced a moderate bitter taste when applied to the circumvallate region and a weaker bitterness on tip and side of the tongue. This suggests that both menthol and capsaicin are able to stimulate bitter taste neurons.

Touch has also been identified as having a role in menthol perception. A study conducted by Green and Schoen (2007) found that when menthol was applied to the skin the irritation that was experienced by participants was reduced when a dynamic thermal cool touch was applied over the treated area.

4.1.3 Sensation stimulants: Tingle

The third common chemo-stimulant that elicits a sensation when applied to mucosal surfaces comes from Alkylamides (Bryant & Mezine, 1999). There are naturally occurring alkylamides like that of hydroxy-α-sanshool (HαSS) found in Sichuan pepper (*Xanthoxylum piperitum*) which are often used in food to provide a

unique tingling sensation during consumption (Ramsewak, Erickson, & Nair, 1999; Yang, 2008). This tingling and often buzzing and numbing sensations appear to be qualitatively different from the burning sensation elicited from capsaicin (Bryant & Mezine, 1999; Sugai, Morimitsu, Iwasaki, Morita, Watanabe & Kubota, 2005; Sugai, Morimitsu, & Kubota, 2005). This suggests that sanshool activates a different set of sensory receptors than capsaicin (Sawyer, Carstens, Simons, Slack, McClusky, Furrer & Carstens, 2009) . Electrophysiological studies in rats indicate that the activated afferents are low and high threshold cold sensitive fibres as well as low threshold mechanoreceptive fibres (Bryant & Mezine, 1999).

HαSS has been shown to activate two sensory cell types, the first being nociceptive neurons which express TRPV1 and some debate remains as to if it also activates TRPA1 (Koo, Jang, Cho, Lee & Jang *et al.,* 2007; Riera, Menozzi-Smarrito, Affolter, Michlig & Munari *et al.,* 2009). The second being large diameter TrkC-expressing mechanosensitive neurons. Activation of both the mechanosensitive and nociceptive cells occurs due to alkylamides ability to inhibit potassium conductance through potassium channels (Bautista, Sigal, Milstein, Garrison, Zorn *&* Tsuruda *et al.,* 2008).

The impact of taster status on the perception of HαSS has not previously been explored but given the suggested relationship between taster status, FP density, number of V^{th} fibres and the specific sensory cells it has been identified as activating it would be expected that taster status would have some impact on sensory perception.

4.1.4 Sensation stimulants: Astringency

A final common sensation experienced by people who often consume certain types of fruits and beverages like tea and red wine is that of astringency. It is often described as a dry, puckering sensation (Prinz & Lucas, 2000) and though it is generally considered an unpleasant sensation in certain circumstances, like in the case of red wine, it is considered desirable as it extends the taste of the wine (Jiang, Gong, & Matsunami, 2014). Neurologically the sensation activates the chorda tympani taste nerve as well as the glossopharyngeal (Schiffman, Suggs, Sostman, &

Simon, 1992) implying that astringency is a taste sensation. Psychophysical studies with astringents suggest that it could also be a somatosensory sensation as it could be perceived on non-taste oral tissues (Breslin, Gilmore, Beauchamp & Green, 1993; Green, 1993a; Lim & Lawless, 2005).

To test this theory Breslin, Gilmore, Beauchamp and Green (1993) applied aluminium potassium sulphate (Alum) to a non-gustatory surface between the gum and upper lip. They found that the astringent sensation could be perceived on this location thus indicting it is not a gustatory sensation alone, in fact the sensation was better identified when applied to a surface that is moved against another rather than to an isolated surface. A finding that is supported by Lim and Lawless (2005) who generated a perceivable astringent sensation at the same location using a different substance. In addition to this the perception of the astringent sensation increases with repetitive administration (Green, 1993a; Ishikawa & Noble, 1995; Lyman and Green 1990; des Gachons, Mura, Speziale, Favreau, Dubreuil & Breslin, 2012) which is a typical feature of the trigeminal system.

Currently it is thought that the perception of the astringent sensation comes from reduction of oral lubrication by the interaction of polyphenols with basic salivary proline-rich proteins (Jöbstl O'Connell, Fairclough & Williamson, 2004). In turn, this activates mechanosensors of somatosensory nerves which leads to the dryness sensation (Lyman & Green, 1990).

Research concerning taster status and astringency perception is inconclusive. Two studies established taster status using PROP threshold sensitivity and found that ratings were not related to the intensity of the astringent sensation elicited by red wine (Ishikawa & Noble, 1995) or grape seeds (Smith, June & Noble, 1996). Contrastingly one study that assessed taster status and astringent sensations using a labelled magnitude scale for intensity ratings of three wines found that tolerant-tasters perceived a significantly lower astringency sensation than hyper-tasters (Pickering, Simunkowa & DiBattista, 2004). This difference in findings could be due to the different approaches used to assess taster status. To further link the astringent sensation to taster status several astringent phenols have been seen to activate bitter taste receptors (Soares, Kohl, Thalmann, Mateus, Meyerhof & De Freitas, 2013).

4.1.5 Aims and Hypothesis

The sensations elicited by chemo-stimulants are dependent on the neuroanatomy and associated receptors, with specific receptors being activated by specific chemo-stimulants. With this in mind all chemo-stimulants should be perceived differently based on taster status as the anatomy associated with taster status shows that hyper-tasters have greater innervation of the tongue which would reflect increased quantities of receptors. Furthermore, due to the level of movement within the oral cavity a dynamic touch would be expected to change the chemosensory experience.

The main aims and hypotheses were:

1) Confirm that hyper-tasters experience a greater sensation intensity than tasters and tolerant-tasters, particularly from capsaicin and aluminium potassium sulphate (Alum).

2) As taster status has not previously been explored in menthol and HαSS it is hypothesised that hyper-tasters will experience significantly more intense sensations than tasters and tolerant-tasters.

3) Published research indicates differences in innervation and thus receptors across the tongue therefore the current study targets specific small locations on the tongue, gum and lip to identify regions that experience chemosensations differently and explore if taster status influences the intensity. The primary hypothesis tested being that regions with a greater density of innervation (i.e tongue tip) perceive a greater sensation intensity and hyper-tasters experience greater intensity across locations than tasters and tolerant-tasters.

4) Finally, there is suggestion that astringent sensations are tactile sensations rather than taste sensations so this study examined if creating friction by rubbing the oral surface against another oral surface (dynamic touch) or by doing nothing once the stimuli is administered (static touch) changed the perceived intensity.

4.2 Methods

4.2.1 Participants

Data from 44 participants was collected, although four of the participant's data was excluded from the analysis due to either failure to complete sessions or missing data. All analyses were conducted on the remaining 40 participants who completed the entire study. Of the participants there were 11 males (27.5%) and 29 females (72.5%) with a mean age of 20.55 years (SD = 3.87). This consisted of 10 hyper-tasters, 18 tasters and 12 tolerant-tasters. This reflected a 25%:45%:30% population split which closely reflects the expect population division of taster groups. Participants were recruited through Liverpool John Moores University (LJMU) research participants scheme for first year psychology undergraduates, university department emails and via the snowball sampling.

The inclusion criterion was that all participants were non-smokers aged between 18 and 35 years. All participants were screened for allergies to pepper and mint, must have never been diagnosed with a neurological disorder that affects sense of taste or touch or being treated for an under/over active thyroid or dry mouth syndrome. Participants who were taking antihistamines or medication that has the side effect of creating dry mouth or if the participant was/may be pregnant were excluded from participation.

Participants who were recruited from the LJMU research participants scheme for first year psychology undergraduates were given 6 credits for each session they attended and participants recruited through email were given £30 Amazon vouchers for completing both sessions. Vouchers were not given out until the end of the second session

This study was granted full ethical approval by the LJMU Ethics Committee on 8th April 2015 (Ref: 14/NSP/017).

4.2.2 Materials

4.2.2.1 Stimuli:

Six stimuli were used in this study. With the exception of the mint oil all the other stimuli were delivered on premade swabs.

4.2.2.2 Swabs:

Primary delivery method used for the stimuli was through swabs. Cosmetic buds (Boots 100% pure cotton tips) were used as they have a flat face on one end allowing for precision delivery. Once the substances were completely dissolved in a solution, clean swabs were impregnated with the solution by soaking them for 30 seconds, excess solution was lightly shaken off and the impregnated swabs were laid on an alcohol sterilised catering tray to dry. Swabs were stored in sealed Glassine envelopes (Lindner, 45x60mm, Germany) for a period no longer than 3 months.

Alum: A 21.1mM solution of aluminium potassium sulphate ([AIK(SO4)2.12H20] – Alum, Fischer brand) was used in this study. To obtain the desired concentration 0.99g of alum was dissolved in 100ml of water on a magnetic mixing plate.

***Menthol*:** A 100mM solution of menthol (C10H20O, Sigma) was used in this study. Menthol is not water-soluble so the menthol crystals were dissolved first in 5ml of 100% ethanol and 95ml of water. The solution was mixed and heated on a mixing plate until all the crystals were dissolved.

Capsaicin: Two concentrations of capsaicin ((CH3)2CHCH=CH(CH2)4CONHCH2C6H3-4-(OH)-3-(OCH3), Sigma) were used in this study. Capsaicin is not a water-soluble substance therefore for each concentration 5ml of 100% ethanol was used and 95ml of water were added to create a solution of 100ml. The lower concentration of 10 ppm (0.0327mMol/L) was made by dissolving 0.00099g of capsaicin in the ethanol and water solution and the higher concentration of 100ppm (0.327mMol/L) was made by dissolving 0.0099g of capsaicin in a separate solution. All solutions were mixed on a magnetic mixing plate. Both concentrations were delivered during testing by cotton swab.

Sichuan Pepper: The active component of sanshool is found in Sichuan Pepper and generates a tingling sensation. To access this component a method similar to that used by Hagura, Barber, and Haggard (2013) was employed. Five grams of Ground Sichuan Pepper (Just Ingredients) was mixed with 40 ml of 100%

ethanol and 60 ml of water. A magnetic mixing plate was used to combine the suspension before the swabs were inseminated.

***Mint Oil*:** The mint oil swabs were not pre-prepared like the other swabs. Dr. Oetker Natural Extract, American Peppermint flavour was used in the study, with swabs being dipped in the pure oil when needed during the experimental session. Swabs were soaked for 10 seconds before being immediately applied to the desired oral location during the experimental session.

***Sucrose*:** A sucrose solution was used to reset the mouth between swabs. Sucrose (C12H22O11, Fisher Brand) is an aqueous solution so a 1 litre solution was made by dissolving 1.7g of sucrose in 1000ml of water.

4.2.3 Measures

4.2.3.1 Taster Status:

Taster status was assessed using the standard PROP soaked filter paper method outlined in the methodology chapter 3 section 3.3 pg 92.

4.2.3.2 Intensity Rating:

A labelled magnitude scale (LMS), replicating that developed by Green, Shaffer and Gilmore (1993) for use specifically in examining oral somatosensation and gustation, was developed in photo shop and used to rate the sensation intensity. This scale was used for assessment of taster status and was presented for every swab for participants to rate the intensity of the sensation they experienced from the delivered stimuli. For further information on the scales used see methodology chapter section 3.2 pg 88.

4.2.3.3 Qualitative Descriptions

Participants were provided with sheets of plain paper and asked to write down any words that they could think of that described the sensations perceived. Blank sheets of paper were provided for each swab.

4.2.4 Procedure:

Testing sessions commenced at 9.30 and 13:00. Due to the number of swabs involved in this study, delivery was divided into two sessions held between 5 and 10 days apart. Both sessions were booked at the same time. The same procedure was used in both sessions and each consisted of a PROP filter paper test and either swabs of:

Session A: 10ppm Capsaicin, Alum, Mint Oil.

Session B: 100ppm Capsaicin, Sichuan Pepper, Menthol.

Session order was randomised and counterbalanced and the delivery order of the swabs within each session was randomised, with the exception of 100ppm capsaicin in session B which was left to the end of that testing session as it has desensitising properties at high concentration.

Upon entering the laboratory participants were given a brief description of the experimental process and their rights as a participant, including their right to withdraw. Consent was obtained and a health screening measure was complete to ensure suitability to participate. The LMS and its extreme anchor points were explained to participants (see methodology section 3.2.2.1). They were also advised that they could mark anywhere on the line going up the scale, it did not have to be on one of the anchor points. Participants were asked to try and discount any tastes that they perceive when making their ratings but to focus on the sensations/feelings at the location the stimuli was applied to.

Participants were presented with Figure 4.1 showing them the locations within the mouth that the stimuli would be delivered to. Before the presentation of each swab participants were informed of the target location.

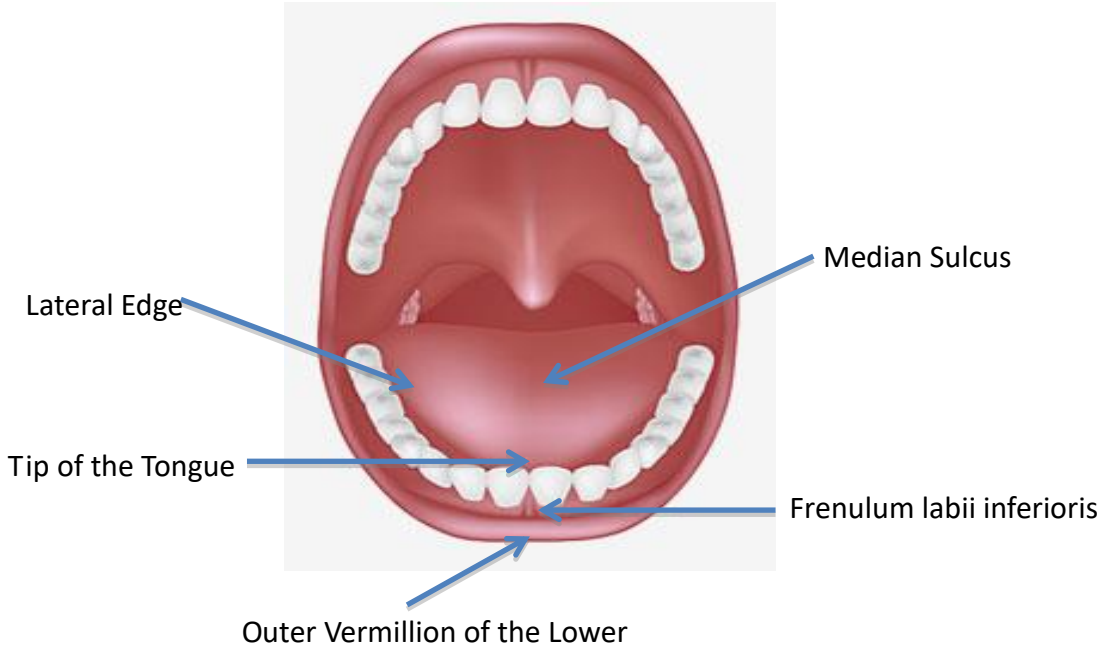


Figure 4.1 The image that was shown to participants during the experimental session to inform them of the 5 different oral locations that would be targeted during the session. These locations consisted of the median sulcus, the lateral edge of the tongue, the tip of the tongue, the outer vermillion of the lower lip and the frenulum labii inferioris.

Participants first completed the taste test with the PROP soaked filter paper and NaCl filter paper. The premade swabs were dipped in water to moisten them and then one side of the flat faced swab was placed on the oral location and turned over, applying the other flat surface to the same location. On half of the occasions participants were asked the rub the surface the swab had been applied to against another surface of the mouth, either the backs of the teeth, cheek or hard pallet of the mouth depending on the oral location the swab had been applied. Participants then rated on the LMS how intense the sensation they experienced was. Swabs were randomly applied to the five different oral locations; median sulcus, side of the tongue, tongue tip, frenulum labii inferioris and outer vermillion of the lower lip (see Figure 4.1). Each stimulus was experienced in each location twice, once where the participant was requested to do nothing and once where the participant was asked to rub the surface it had been applied to against another surface of the mouth.

An enforced two-minute wait was programmed into the computer program collecting the ratings, ensuring the next LMS was not presented until the two minutes were over. Participants were told they had a mandatory 2-minute wait but if their mouth had not returned to normal they should let the researcher know and continue to wait until the sensation had completely dissipated. Participants were encouraged to rinse their mouth between swabs, this could be done with either a swill and spit with a sucrose solution, spitting into the provided wine spittoon, or drinking some fresh water that was also provided. Session A took approximately 1.5 hours to complete and session B took up to 2 hours to complete, this was due to session 2 having the 100ppm capsaicin swabs which elicited a stronger sensation and participants requesting a longer wait between each swab delivery.

At the end of each session participants were debriefed, getting a full debrief at the end of the second session. Participants who were recruited from the LJMU SONA system were given 6 credits for each session they attended and participants recruited through email were given £30 Amazon vouchers for completing both sessions. Vouchers were not given out until the end of the second session.

4.2.5 Statistical Analysis:

The data was assessed for normality, which indicated that the data was non-normally distributed. Attempts to correct this with transformation were unsuccessful therefore analysis was run on the original data. Levene's test was run on all the data and Mauchly's tests of sphericity were examined and where appropriate Greenhouse Geisser corrections to degrees of freedom are reported. Mixed measures ANOVA's were run on the data with taster status being a between participants variable of three levels (hyper-taster, taster and tolerant-taster). There were two within participants factors, the first touch type which had two levels (static touch and dynamic touch) and the second was oral location that consisted of five levels (median sulcus, side of the tongue, tongue tip, frenulum labii inferioris and outer vermillion of the lower lip). Each of the stimuli were analysed separately and where appropriate with identified main effects and interactions, further

investigation was done with Mixed and Repeated Measures ANOVA's, t-tests and pairwise comparisons.

4.3 Results

Socio-demographic information about the participants and the taster groups they belong to are shown in Table 4.1.

Table 4.1 Demographic information for the 40 participants divided by taster status.

	Hypo-taster	**Taster**	**Hyper-taster**
Males: n (%)	2 (16.7)	7 (38.9)	2 (20)
Age (SD)	22.50 (6.01)	19.44 (1.46)	20.13 (2.75)

The primary analysis was exploring the experimental data as a whole, looking to identify main effects and interactions between the substance, location, touch type and taster status. Mixed Measures ANOVAs revealed a significant main effect for substance and the intensity of the sensation experienced (F(2.97, 109.80) = 70.33, p<.001, ηp^2= .66, Power = 1.00). There was also a significant effect for oral location and intensity ratings (F(4, 148) = 82.84, p<.001, ηp^2 = .69, Power = 1.00). There was no significant main effect for touch type indicating that ratings for the static and dynamic touch type were in general the same (F(1, 37) = .72, p>.05, Power = .13) though with such a small observed power this is likely not the case. A significant main effect of taster status was also identified (F(2,37) = 11.89, p<.001, ηp^2 = .39, Power = .99). Taster comparisons indicate that the intensity scores were significantly higher (ps<.01) for hyper-tasters (M=30.59, SD = 2.70) than both tasters (M = 17.59, SD = 2.02) and tolerant-tasters (M= 13.33, SD = 2.47).

No significant main interactions were found between taster status and substance, taster status and location or taster status and touch type (ps>.05). There were, however significant interactions identified between substance and location (F(9.69, 358.41) = 19.85, p<.001, ηp^2 = .35, op = 1.00) and location and touch type (F(4, 148) = 3.86, p<.01, ηp^2 = .09, op = .89). These lead to the identification of significant 3-way interactions between substance, location and touch type (F(10.48, 387.59) = 2.48, p<.01, ηp2 = .6, op = .96). Also, a significant

four-way interaction between substance, location, touch type and taster status (F(20.95, 387.59) = 1.65, p<.05, ηp2 = .08, op = .96) was also identified.

The following analysis was a breakdown of the four-way interaction with the aim of identifying exactly where in the data the interactions took place. This was done by examining each substance separately and testing for location, touch type and taster status main effects within the substance and any interactions. It was hypothesised that each location would experience the substances differently and those perceptions would be further altered by the different types of touch. Furthermore, it was expected that the different taster groups would experience the induced sensations differently.

4.3.1 Aluminium Potassium Sulphate

There were no significant interactions between taster status, location or touch type at this location so only main effects are explored.

4.3.1.1 Location

A significant main effect for location was identified for the Alum (F(3.25, 120.09) = 6.53, p<.001, ηp^2 = .15, Power = .98) with the intensity perception on the vermillion of the lip being considered significantly less intense than all other oral locations (ps<.05; Figure 4.2).

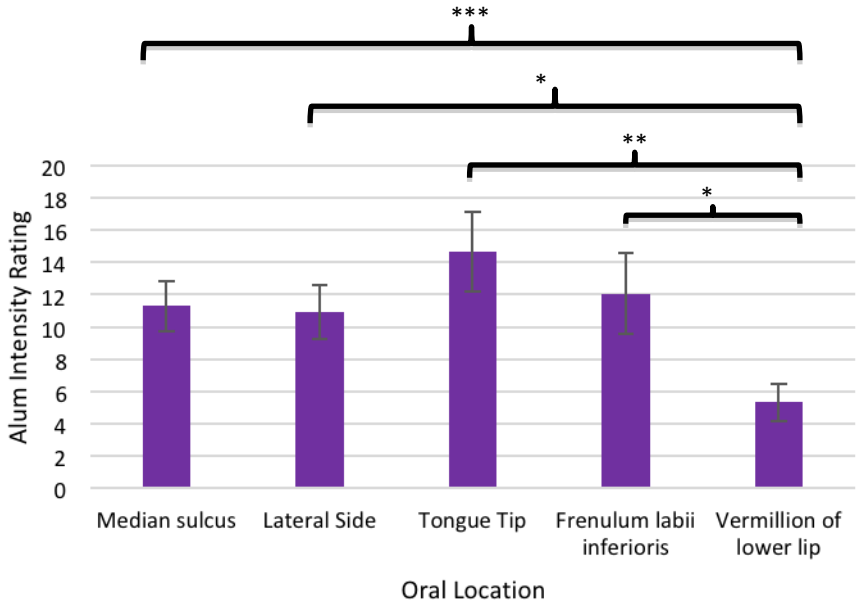


Figure 4.2 The mean intensity ratings reported by participants from the Alum across locations. Sensations elicited from Alum were significantly lower on the vermillion of the lower lip compared to other locations (*p<.05, **p<.01, ***p<.001).

4.3.1.2 Taster Status

A significant main effect of taster status was identified ($F(2, 37) = 5.17$, $p<.01$, $\eta p^2 = .22$, Power = .80) with hyper-tasters considering the sensation significantly more intense (M= 17.63, SE = 2.77) than both tasters (M = 8.97, SE = 2.06) and tolerant-tasters (M = 6.00, SE = 2.53; ps<.05).

4.3.1.3 Touch Type

No significant differences between the touch types were identified for the Alum.

4.3.1.4 Supposition

The data presented here supports the hypotheses that the regions of the mouth experience the dry puckering oral sensation elicited by alum differently with the vermillion of the lower lip perceiving the least intense sensation. Hyper-tasters experienced a significantly more intense sensation than both the tasters and tolerant-tasters but there is no evidence to indicate that static or dynamic touch influences the intensity of the sensation.

4.3.2 Mint Oil

4.3.2.1 Location

A significant effect of location ($F(4, 148) = 8.41$, $p<.001$, $\eta p^2 = .19$, Power = 1) was found with all locations being considered to generate a significantly more intense sensation than the outer vermillion of the lip (ps<.05; see Figure 4.3).

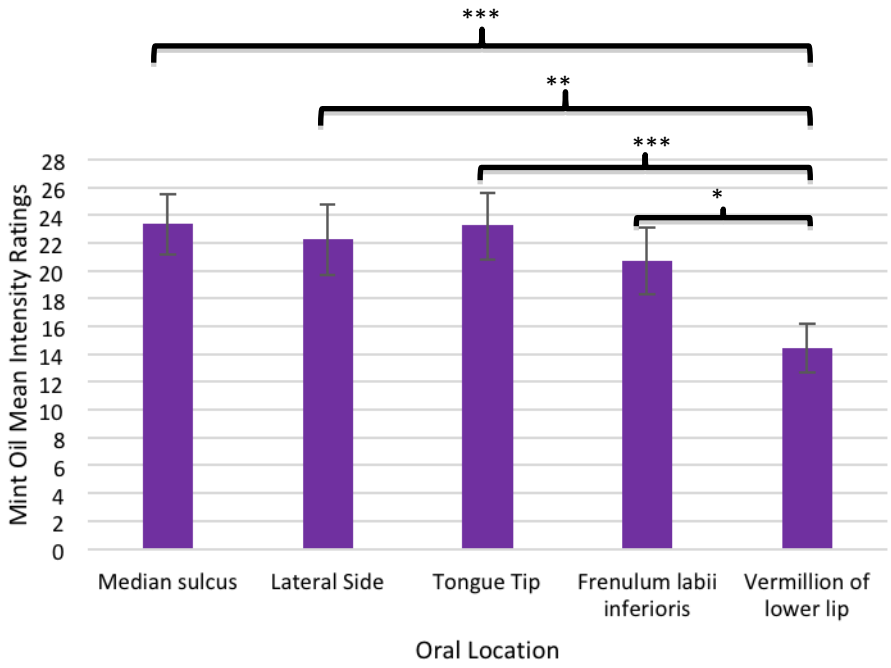


Figure 4.3 The mean intensity ratings reported by participants from the Mint Oil sensation across locations. Sensations elicited from Mint Oil were significantly lower on the vermillion of the lower lip compared to other locations (*p<.05, **p<.01, ***p<.001).

4.3.2.2 Taster Status

There was a significant effect for taster status ($F(2, 37) = 9.44$, $p<.001$, $\eta p^2 = .34$, Power = .97) with hyper-tasters considering sensory perception of mint oil as significantly more intense (M=33.66, SE=3.82) than tasters (M=15.81, SE=2.85) and non-tasters (M=13.03, SE=3.49; ps<.01).

4.3.2.3 Touch Type

No significant main effect touch types were identified at this location

4.3.2.4 Interactions

A significant interaction between location and touch type ($F(4, 148) = 4.93$, $p<.001$, $\eta p^2 = .12$, Power = .96) was also identified.

This interaction was broken down to explore the effect of the touch type on each location the mint oil was applied as the touch type may have only been influential on the experience at some locations. Paired t-tests identified that significant differences between the static and dynamic touch for the median sulcus ($t(39) = -2.17$, $p<.05$), tip of the tongue ($t(39) = 2.35$, $p<.05$) and the vermillion of the lower lip ($t(39) = -2.74$, $p<.01$). Figure 4.4 shows that for the tip of the tongue the static touch was rated as significantly more intense than the dynamic but the opposite effect is seen on the median sulcus and the vermillion of the lip.

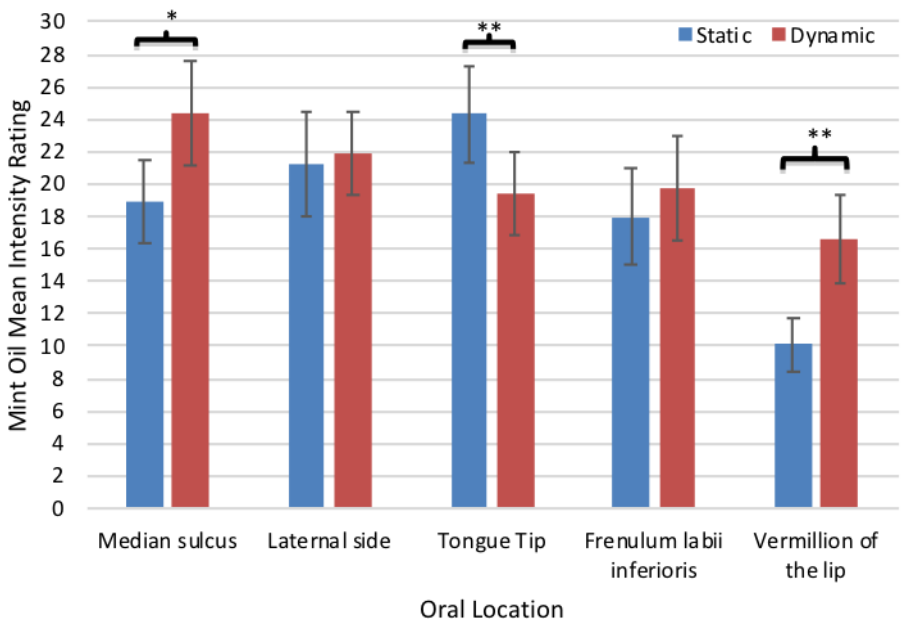


Figure 4.4 The mean mint oil intensity ratings at each location depending on the type of touch associated with administration. Significant differences in sensation intensity ratings were seen on the median sulcus, tip of the tongue and vermillion of the lip with the static touch being more intense than dynamic touch on the tip of the tongue and the dynamic touch was more intense than the static touch on both the median sulcus and vermillion of the lip (*p<.05, **p<.01).

4.3.2.5 Supposition

The results from the mint oil only partially supported the hypothesis that the regions of the oral cavity, tested in this study, experience the sensation differently as only one location was significantly different to all the others and that was the vermillion of the lip as less intense than the other locations.

Hyper-tasters also reported to perceive a significantly more intense sensation than both the tasters and tolerant-tasters supporting the hypothesis that taster status would influence perception. The hypothesis that touch type would impact on intensity was supported with a greater intensity of static touch on the tip of the tongue, this is likely due to the greater density of FP on the tongue tip and as such greater innervation. Interesting, the dynamic touch being more intense on the vermillion of the lip indicating that non-discriminative touch plays a pivotal role in perception on the lip.

4.3.3 10ppm Capsaicin

4.3.3.1 Location

A significant main effect for location was identified (F(2.90, 107.45)=69.16, $p<.001$, $\eta p^2=.65$, Power = 1.00) with all locations being significantly different to each other (ps<.001). As can be seen in Figure 4.5 the frenulum and vermillion of the lip were rated significantly less intense than the other three locations but the frenulum was rated significantly more intense than the vermillion of the lip (see Figure 4.5).

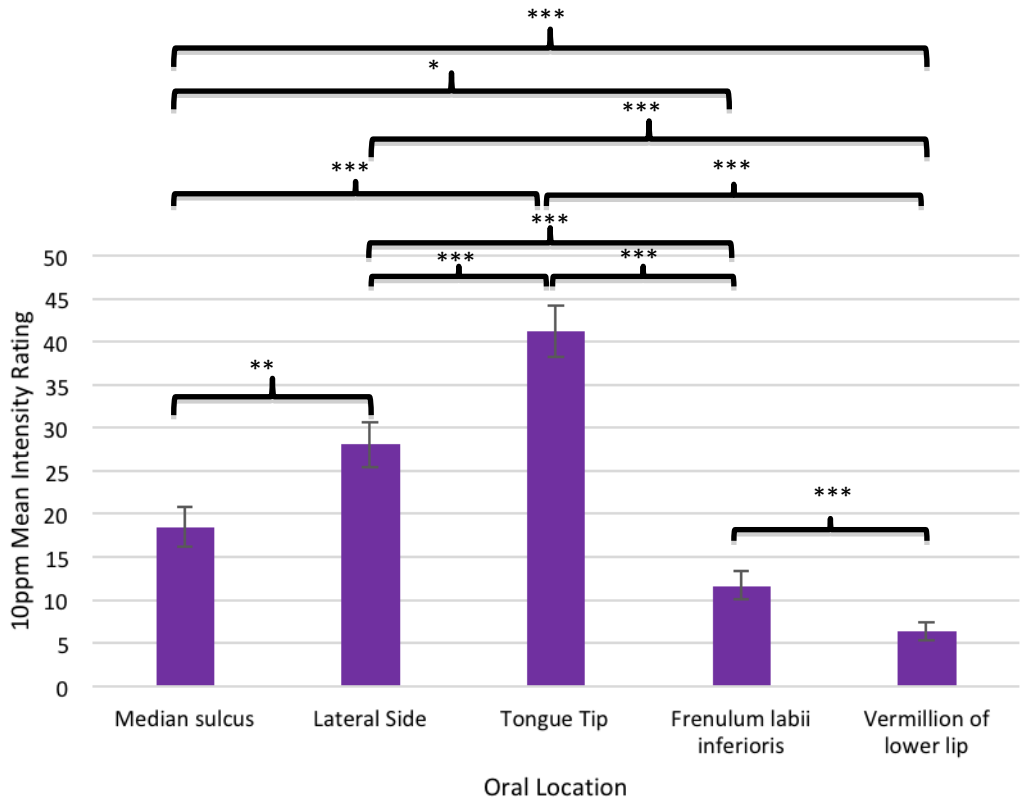


Figure 4.5 The mean intensity ratings reported by participants from the 10ppm capsaicin sensation across locations. Sensations elicited from the 10ppm capsaicin concentration were significantly lower on the vermillion of the lower lip compared to other locations and the frenulum was significantly more intense than the vermillion (*p<.05, **p<.01, ***p<.001).

4.3.3.2 Taster Status

A significant main effect for taster status ($F(2,37) = 7.06$, $p<.01$, $\eta p^2 = .28$, Power = .91) was identified with hyper-tasters rating the perceived intensity (M=30.51, SE=3.22) as significantly more intense than the tasters (M=17.24, SE=2.40) and tolerant-tasters (M=15.68, SE=2.94; ps<.01).

4.3.3.3 Touch Type

No significant main effect touch type was identified for 10ppm capsaicin.

4.3.3.4 Interactions

A significant three-way interaction between location, touch type and taster status were found ($F(8,148)=1.99$, $p<.05$, $\eta p^2=.10$, Power = .80). This interaction was broken down to examine each location separately to identify, which touch type created the greater intensity of sensation and between which of the taster groups the difference was found. Hyper-tasters rated the intensity of the 10ppm capsaicin as significantly more intense than both tasters and tolerant-tasters with a static touch on the median sulcus. Hyper-tasters experienced a greater intensity burn on the side of the tongue than tolerant-tasters when the touch was static and finally hyper-tasters experienced a more intense sensation than tasters and tolerant-tasters on the tip of the tongue and vermillion of the lower lip in the dynamic touch (see Figure 4.6).

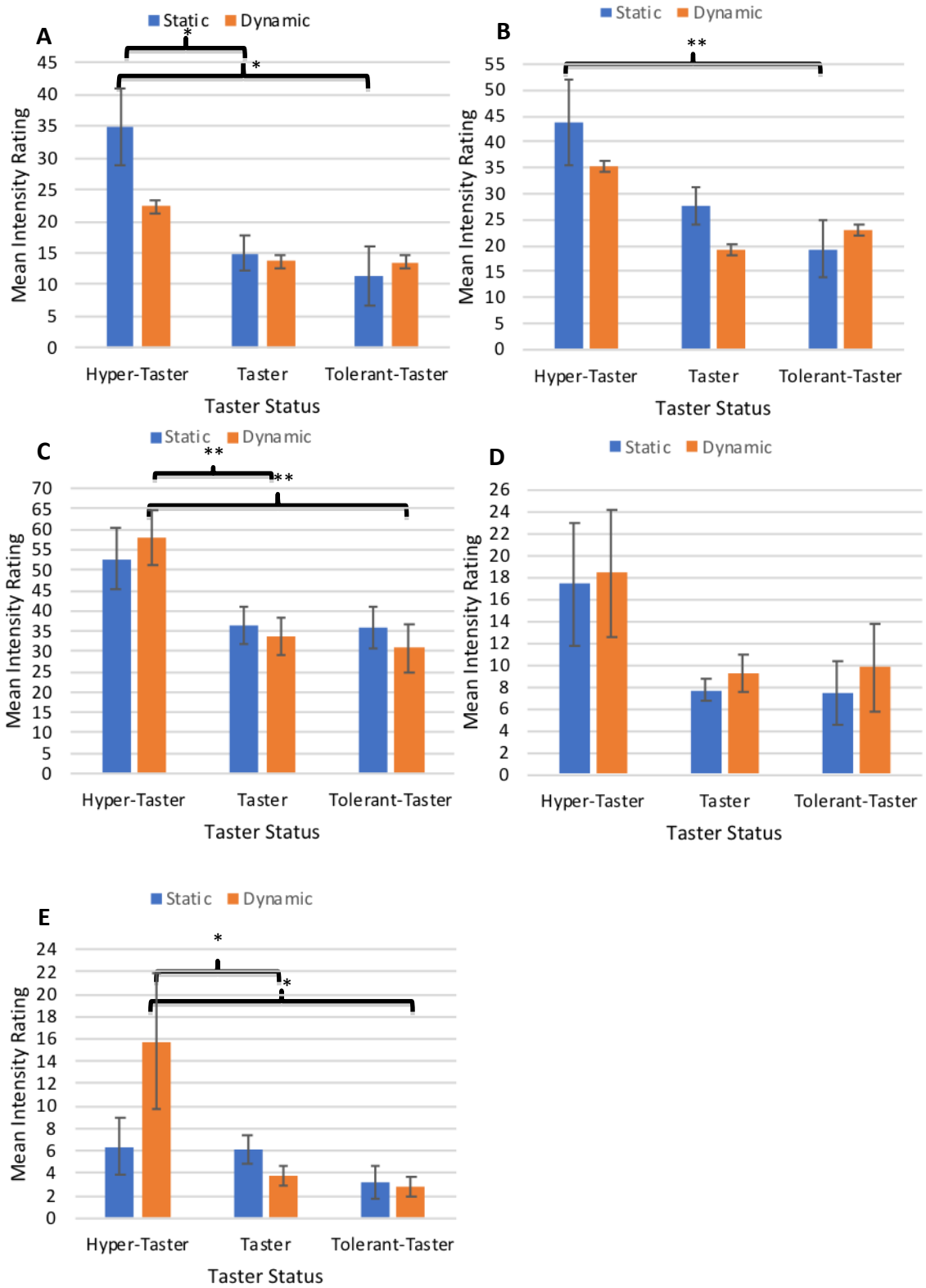


Figure 4.6 The mean intensity ratings reported by participants from the 10ppm capsaicin sensation across locations. Sensations elicited from the 10ppm capsaicin concentration were more intense for hyper-tasters than tasters and tolerant-tasters with a static touch on the median sulcus (**A**), and dynamic touch on the tip of the tongue (**C**) and vermillion of the lip (**E**). Hyper-tasters also rated the static touch to create a significantly more intense than tolerant-tasters on the side of the tongue (**B**). There was no significant effect of taster status on the frenulum (**D**) (*p<.05, **p<.01, ***p<.001).

4.3.3.5 Supposition

The hypothesis that there would be differences in perceptions across oral locations is supported with the 10ppm capsaicin being considered significantly different across all locations and the outer vermillion of the lower lip being the least sensitive to sensation. Significant taster status differences support the hypothesis that hyper-tasters experience a greater intensity than tasters and tolerant-tasters but there was no effect of touch type. The interactions highlight that the hyper-tasters perceived a greater intensity sensation than tasters and tolerant-tasters at various locations across the mouth but dependent on if the touch was a static or dynamic. From the mean intensity ratings, a dynamic touch on the outer vermillion of the lower lip and top of the tongue decreases the intensity of the touch for the tasters and tolerant-tasters but increased the intensity for the hyper-tasters.

4.3.4 Sichuan Pepper

4.3.4.1 Location

There was a significant effect for location identified ($F(4, 148) = 13.00$, $p<.001$, $\eta p^2 = .26$, Power = 1). The outer vermilion of the lower lip was rated significantly less intensely than the median sulcus ($p<.001$) and side of the tongue ($p<.001$). Significant differences in ratings were also found between the frenulum and the side of the tongue ($p<.01$) and tip of the tongue ($p<.01$) again with the mean scores indicating that the perception of the frenulum was less intense than the side and tip of the tongue (Figure 4.7).

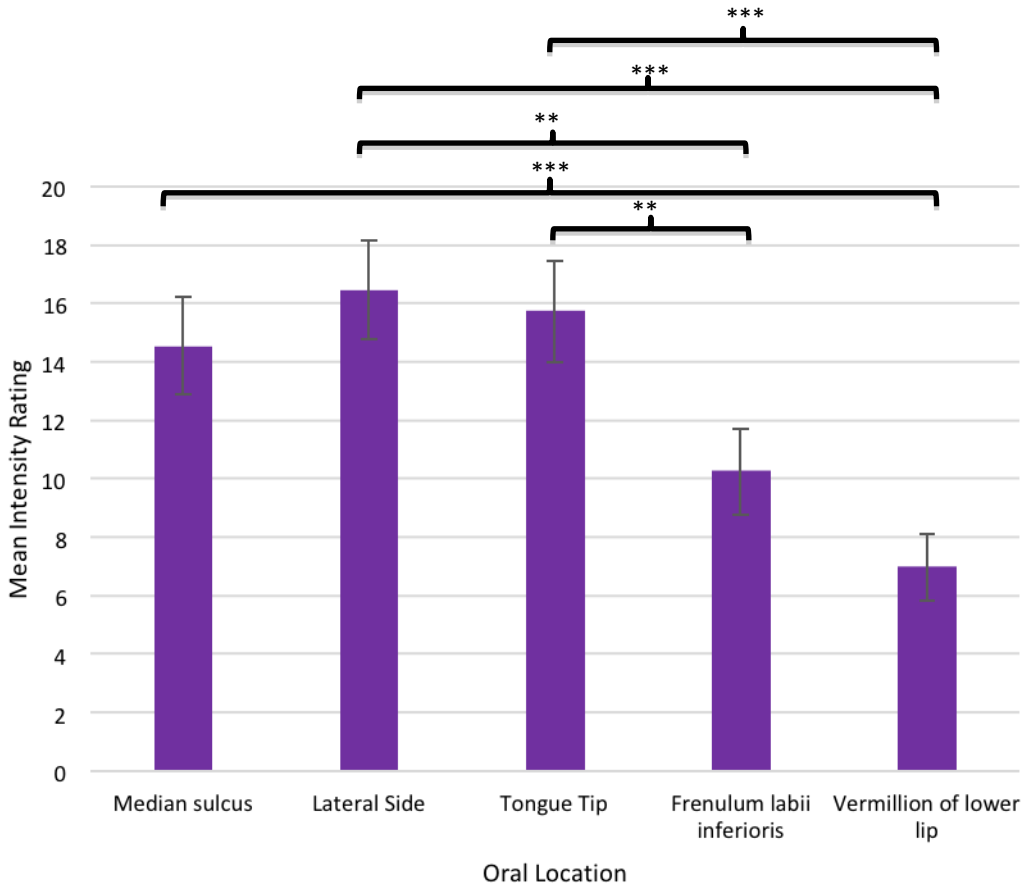


Figure 4.7 The mean intensity ratings reported by participants from the Sichuan pepper sensation across locations. Sensations elicited from the Sichuan pepper concentration were significantly lower on the vermillion of the lower lip compared to the median sulcus ($p<.001$) and side of the tongue ($p<.001$). Significant differences in ratings were also found between the frenulum and the side of the tongue ($p<.01$) and tip of the tongue ($p<.01$). (*$p<.05$, **$p<.01$, ***$p<.001$)

4.3.4.2 Taster Status

There was a significant effect for taster status ($F(2, 37) = 8.06$, $p<.001$, $\eta p^2 = .30$, Power = .94) with hyper-tasters (M=19.51, SE = 2.30) considering the sensations as significantly more intense than both the tasters (M = 11.83, SE = 1.72; $p<.05$) and hypo-tasters (M = 7.04, SE = 2.10; $p<.001$).

4.3.4.3 Touch Type

No significant differences between the touch types were identified with sanshool pepper intensity perception.

4.3.4.4 Interactions

A significant interaction of location and taster status ($F(8, 148) = 2.11$, $p<.05$, $\eta p^2 = .10$, Power = .83), location and touch type ($F(2.63, 97.21) = 7.72$, $p<.001$, $\eta p^2 = .17$, Power = .98) and a three-way interaction between location, touch type and taster status ($F(5.25, 97.21) = 4.76$, $p<.01$, $\eta p^2 = .20$, Power = .98) was also identified (see Figure 4.8). As a three-way interaction was identified as per the hypothesis that the different oral regions would experience the sensation intensity differently, the touch type would change the perceived intensity and which taster groups experience different levels of intensity. Each location was examined separately with mixed measures ANOVA.

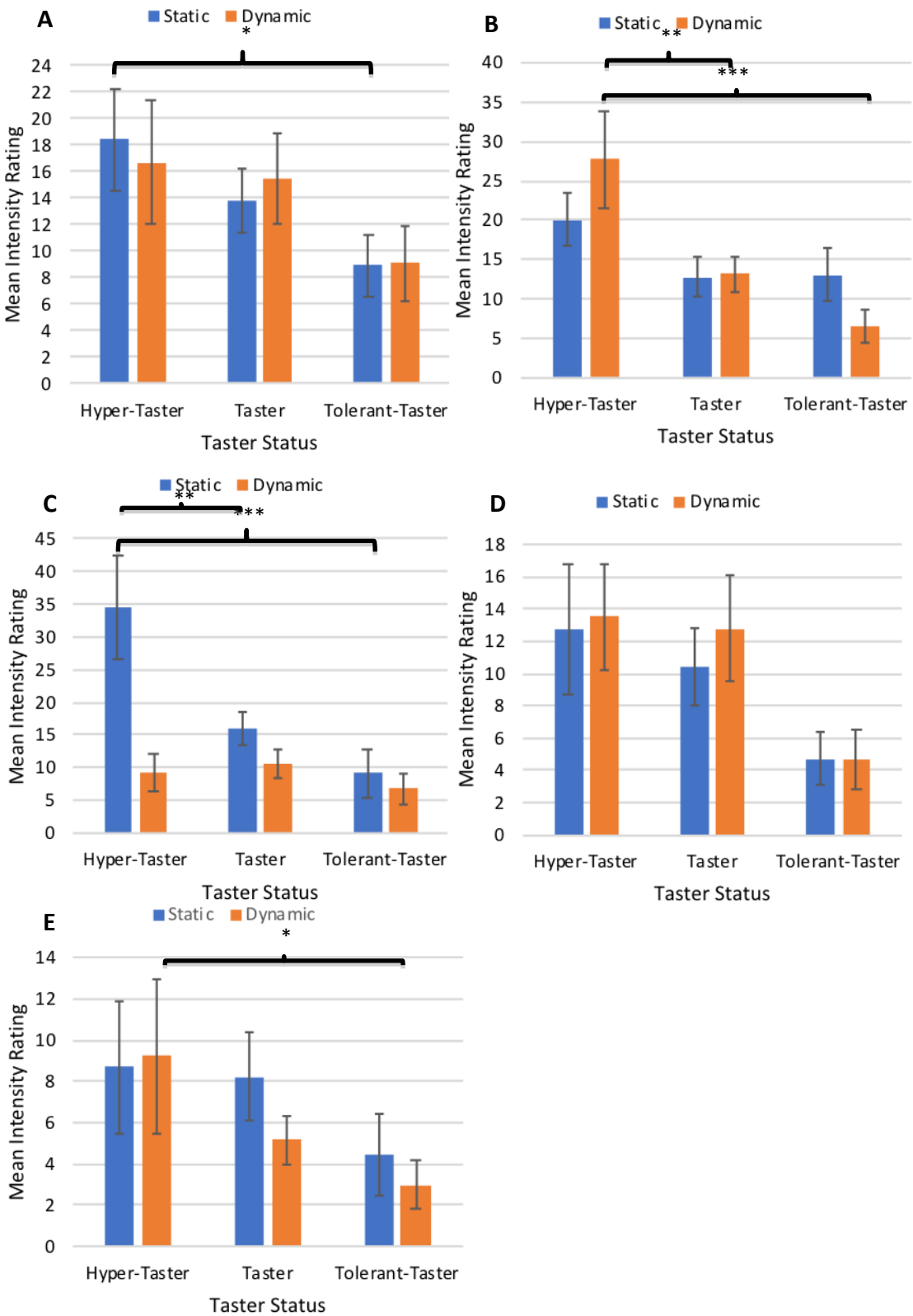


Figure 4.8 The mean intensity ratings reported by participants from the Sichuan pepper sensation across locations. Sensations elicited from the Sichuan pepper concentration were more intense for hyper-tasters than tolerant-tasters with a static touch on the median sulcus (**A**) and tongue tip (**C**) and dynamic touch on the vermillion of the lip (**E**). Hyper-tasters also rated sensations as significantly more intense than tasters and tolerant-tasters with a dynamic touch on the side of the tongue (**B**) and static touch on the tip of the tongue (**C**) There was no significant effect of taster status on the frenulum (**D**) (*p<.05, **p<.01, ***p<.001).

4.3.4.5 Supposition

The tingling sensation elicited by Sichuan pepper is seen to be similar on the side and tip of the tongue but the hypothesis that the oral regions experience the oral sensation differently is supported.

4.3.5 Menthol

4.3.5.1 Location

A significant main effect for location was identified ($F(4, 148) = 18.18$, $p<.001$, $\eta p2 = .33$, Power = 1) . The outer vermillion of lower lip was rated as significantly different from all other locations (ps<.01), examining the mean scores shows that the outer vermillion of the lip was again rated as being the less intense than all the other oral regions (ps<.01). The side of tongue when menthol was applied also rated significantly different from the frenulum (p<.01) with mean scores indicating the intensity perceived on the side of the tongue was more intense than on the frenulum (Figure 4.9).

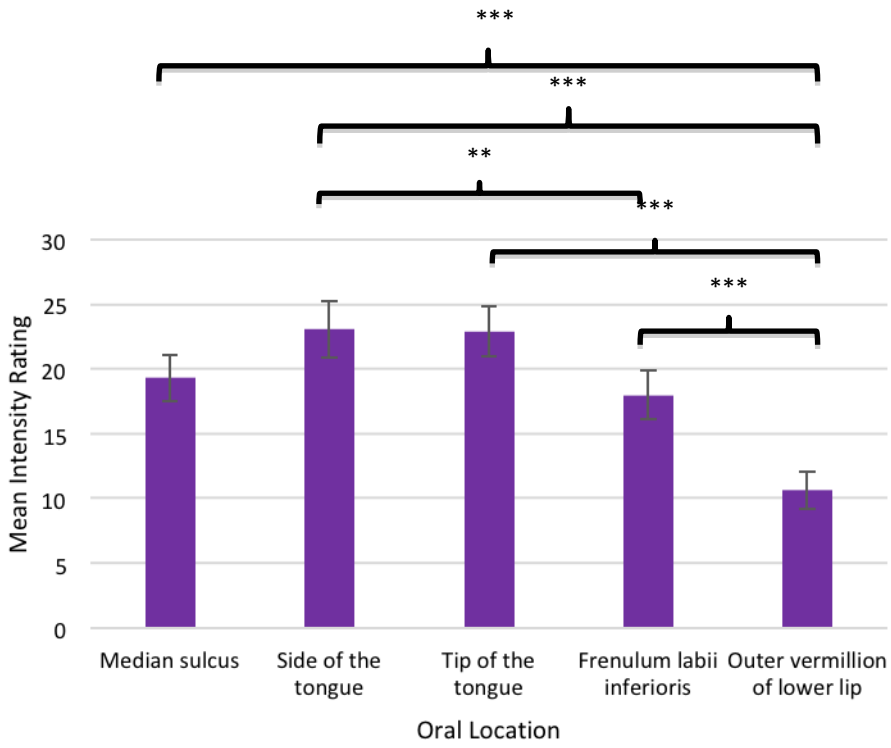


Figure 4.9 The mean intensity ratings reported by participants from the Menthol induced sensation across locations. Sensations elicited from the Menthol were significantly lower on the vermillion of the lower lip compared to all other locations (ps<.01) and side of the tongue was rated significantly more intense than the frenulum (p<.01) (*p<.05, **p<.01, ***p<.001).

4.3.5.2 Taster Status

There was a significant effect for taster status ($F(2, 37) = 13.62$, p<.001, ηp2 = .42, Power = 1.00) with hyper-taster (M = 30.58, SE = 3.00) considering the sensations as significantly more intense than both the tasters (M= 13.77, SE = 2.23; p<.001) and tolerant-tasters (M = 9.95, SE = 2.74; p<.001).

4.3.5.3 Touch Type

No significant differences between the touch types were identified for menthol.

4.3.5.4 Supposition

Menthol perception was evidently different across oral locations supporting the first hypothesis and hyper-tasters reported to find the intensity induced by menthol as significantly more intense than both tasters and tolerant-tasters. There

were no interactions with touch type meaning that static and dynamic touch had little effect on perceived menthol intensity.

4.3.6 100ppm Capsaicin

4.3.6.1 Location

A significant main effect for location was identified ($F(4, 148) = 56.91$, $p<.001$, $\eta p2 = .61$, Power = 1) . With the exception of the frenulum which was not considered to being significantly different from the median sulcus, all other locations were significantly different from each other in intensity perceived (ps<.01). The mean scores demonstrate that the outer vermillion of the lower lip was significantly less intense than all locations and the tip of the tongue had the greatest intensity ratings of all the locations (see Figure 4.10).

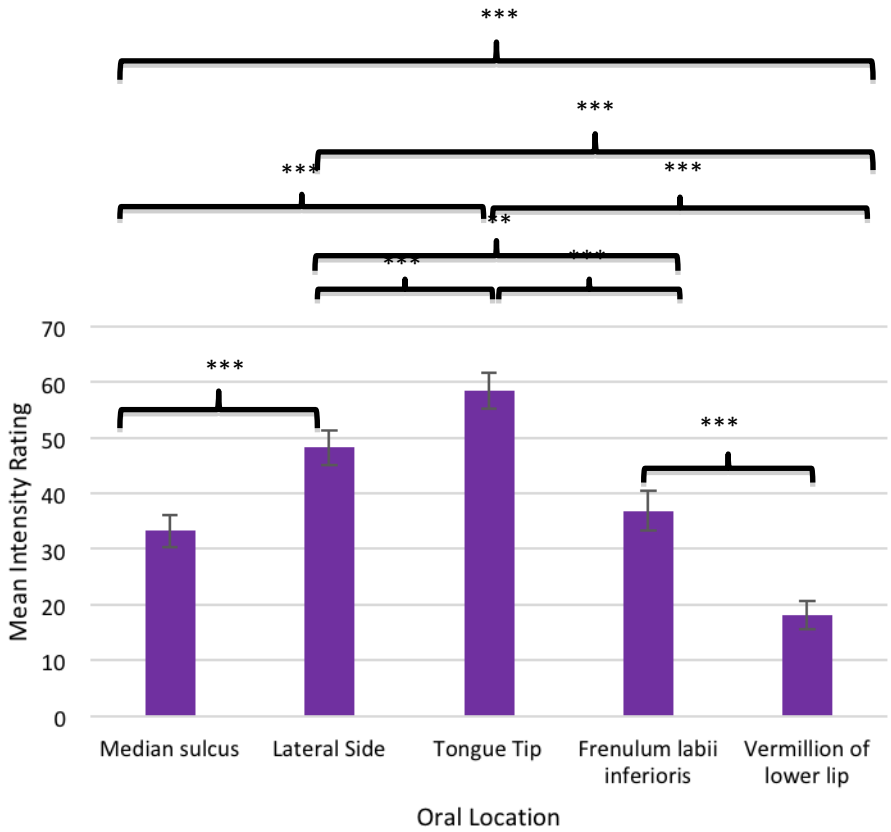


Figure 4.10 The mean intensity ratings reported by participants from the 100ppm capsaicin sensation across locations. Sensations elicited from the 100ppm capsaicin across all locations were significantly different from each other (ps<.01) with the exception of the frenulum which was consider to not be significantly different to the median sulcus (*p<.05, **p<.01, ***p<.001).

4.3.6.2 Taster Status

There was a significant effect for taster status ($F(2, 37) = 6.37$, $p<.01$, $\eta p2 = .26$, Power = .88) with hyper-taster (M = 28.24, SE = 4.44) considering the sensations as significantly more intense than tolerant-tasters (M = 51.63, SE = 4.86; $p<.01$).

4.3.5.3 Touch Type

No significant differences between the touch types were identified with 100ppm capsaicin intensity perception.

4.3.5.4 Supposition

The 100ppm capsaicin was experienced differently across the regions of the mouth and the different taster groups experienced the sensations differently but touch type played no role in perceived intensity.

4.4 Discussion

The overall findings of this study support that the different oral regions experienced sensations differently with the majority of stimuli indicating that the vermillion of the lip was the least sensitive to the induced sensations and the tongue tip the most sensitive. Taster status was also identified as having an influence on the perception of the induced sensations with the hyper-tasters experiencing a greater intensity of all sensations than tasters and tolerant-tasters. Anatomically, the tip of the tongue has a greatest density of FP than the rest of the tongue and as such greater innervation than other regions therefore more receptors will have been activated by the stimuli. Hyper-tasters have been found to have a greater density of FP (Bartoshuk, Duffy & Miller, 1994) and as such greater V^{th} innervation therefore although the same area was stimulated for each participant a spatial summation mechanism may explain some of the taster status differences due to the increased number of stimulated receptors for hyper-tasters in comparison to tasters and tolerant-tasters. No main effect of touch type was identified but touch type was seen to have interactions with oral location and taster status for the 10ppm concentration of capsaicin and Sichuan pepper. Hyper-tasters had a mixed interaction with location and touch type within these substances. Touch type also interacted with location in the mint oil. Together the data suggests that while touch plays a role in oral perceptions it is dependent on the substance involved and the location that is being stimulated.

4.4.1 Alum

Breslin, Gilmore, Beauchamp and Green (1993) and Lim and Lawless (2005) found that astringent sensations are not only a taste sensation but also a somatosensory one which is supported by the mean scores obtained in this study. The frenulum of the lower lip, a non-gustatory surface, reported a strong sensation induced by the Alum though these scores are not statistically significantly different from other location scores it does score second highest in mean scores for intensity. It was also expected that the type of touch would increase the intensity perception, especially with Alum due to previous research finding that applying an

astringent stimulant to a surface which rubs against another increases the intensity of the sensation (Breslin, Gilmore, Beauchamp & Green, 1993). The Alum data here, however does not support the research as no effect of touch type was established during data analysis, but taster status was seen to influence intensity perception. Tasters status does however appear to influence the sensation as hyper-tasters reported perceiving a significantly greater intensity of sensation than tolerant-tasters supporting the previous research (Pickering, Simunkowa & DiBattista, 2004).

This data may not support the previous literature but it does show that taster status influences the Alum perception. If hyper-tasters experience a greater intensity of sensation from alum due to increased chorda tympani innervation, it may add to the argument of taster status being a protective factor against alcoholism. Red wine is a common astringent; therefore, if hyper-tasters experience a greater intensity of sensation from alum they may consume less red wine than tolerant-tasters.

4.4.2 Menthol

No significant interactions or effect of touch type were found in the menthol data but the data reported here demonstrated that the intensity of menthol was significantly lower for the vermillion of the lip and the frenulum compared to the locations on the tongue. Both of the locations that were significantly different to the other regions were non-gustatory surfaces. Also, hyper-tasters perceived the menthol sensation as significantly more intense than both the tasters and tolerant-tasters. There was no effect of touch type identified with menthol and touch type was not found to interact with location or taster status. This finding supports Cliff and Green's (1996) assertion that the limited volume of research for menthol's effect in the oral region is due the coolness induced remaining consistent under experimental conditions as the tongue locations did not experience the sensations differently and taster status did not impact on the sensations either.

A further possible explanation about the lack of touch type influence on the cool menthol sensation is that the previous research linking the two comes from

menthol applied to the volar surface of the forearm. Green and Schoen (2007) found that when menthol is applied to the skin the irritation is reduced with dynamic cool thermal touch on the skin. The current study found touch alone had no influence on the sensory perception so the additional thermal sensory stimulant may be required for touch to influence menthol perception.

The findings of intensity across oral location with all locations being rated more intense than the vermillion of the lower lip may be due to role of retronasal olfaction. When menthol is applied to the lip, the sensation is cool and rubbing the surface does not significantly change the intensity. What is different regarding the location is that the lip has an olfactory response but the other regions located within the mouth experience a retronasal response. This different olfactory receptive response may explain some of the differences. Furthermore, the surfaces within the mouth are mucosal and as such are likely more permeable to substances like menthol than the vermillion of the lip would be, thus generating a stronger intensity of sensation.

4.4.3 Sichuan Pepper

The tingle sensation induced by Sichuan pepper was felt significantly different across the mouth with the vermillion of the lip again being the least sensitive to the tingle sensation and the frenulum being less intense than the side of the tongue.

The tingle from Sichuan pepper is relatively new to perception research so as with menthol the research available for comparison is limited and with taster status not previously done before. This study has highlighted that hyper-tasters experience a greater tingle sensation particularly on the side of the tongue with a rubbing touch and tip of the tongue static touch, both regions of the tongue where the FP are densest. In fact, the hyper-tasters rated the intensity of all of the locations more intense than the tolerant-tasters with the exception of the frenulum where no taster status effect was seen. Intensity on the vermillion of the lip was significantly different between the hyper-tasters and tolerant-tasters with a dynamic touch. For the perception of the tingle to be changed on the basis of

taster status would imply that the innervation level of the tongue is important, the greater innervation suggested to be present in hyper-tasters than tolerant tasters would include a greater density of mechanoreceptors to be activated by the Sichuan pepper.

4.4.4 Capsaicin

10ppm capsaicin was the lowest concentration of capsaicin used as it was at a low enough level for guaranteed perception of heat but not high enough to cause pain (Karrer & Bartoshuk, 1991). All of the targeted oral locations experienced the burn from the 10ppm capsaicin significantly differently from each other, with the intensity of sensation on the vermillion of the lip being the lowest and the tip of the tongue the highest. When the capsaicin concentration was increased ten-fold to 100ppm capsaicin, this location effect remained, however the frenulum was no longer significantly different from the median sulcus of the tongue.

There was no main effect of touch type found in either of the capsaicin concentrations however touch type interacted with location and taster status in the 10ppm concentration. A dynamic touch on the vermillion of the lip and tip of the tongue were rated as significantly more intense for hyper-tasters than both tasters and tolerant-tasters.

The 100ppm capsaicin was only rated as significantly more intense by hyper-tasters than tolerant-tasters but at 10ppm hyper-tasters rated the burning sensation as significantly greater than their taster and tolerant-taster counterparts supporting research by Karrer and Bartoshuk (1991) amongst others (Prescott & Swain-Campbell, 2000). This finding supports the early research of a taster status influence on capsaicin burn perception but there is still ample published research that contradicts this finding (McBurney, Balaban, Popp, & Rosenkranz, 2001; Törnwall, Silventoinen, Kaprio, & Tuorila, 2012) so it may be sometime until a consensus is established regarding taster statuses influence on capsaicin burn perception.

While a main effect of taster status in the 100ppm concentration was identified the taster status effect was reflected across different oral regions like was

see in the 10ppm concentration. This means that whilst, innervation differences between the taster groups does play a role and influence the perception of heat and burning from capsaicin, at a high concentration the effect is negligible. Interestingly with 10ppm capsaicin a three-way interaction between taster status, oral location and touch type indicated that regions where movement would commonly occurred, hyper-tasters found the sensation significantly more intense than tolerant-tasters. Examination of mean scores indicate very little difference between the static and dynamic ratings of tolerant-tasters at both these locations. This could be due to the intra-oral location experiencing a simple dilution in the capsaicin concentration when combined with the saliva of the mouth.

A potential alternative explanation for the 10ppm capsaicin findings may relate to the role touch plays in soothing pain; hyper-tasters experience the static sensation on the side and median sulcus of the tongue more intensely because the dynamic touch helps to sooth the pain and irritation caused by the capsaicin. Conversely, the dynamic touch combined with taster status and vermillion of the lip leads to a significantly more intense sensation for hyper-tasters than tolerant tasters, showing an opposite effect to the intra-oral location. This could be due to lack of saliva dilution which would be experience within the mouth but also an effect of spatial summation, by rubbing the lips together the capsaicin likely spreads and thus a greater density of receptors are activated, potentially supporting the argument that taster status innervation increases extend beyond the tongue and intra-oral cavity.

4.4.5 Mint oil

The sensations induced from the mint oil were significantly different dependent on taster status with hyper-tasters rating the intensity as significantly more intense than both tasters and tolerant-tasters. The vermillion of the lip was rated as significantly less intense than all other locations and significant interactions were found between location and touch type with static touch on the tip of the tongue being rated more intensely but dynamic touch on the median sulcus and vermillion of the lip being rated more intense.

What makes the mint oil most interesting is that it should have had similar effects of menthol but actually more activity and interactions were found with the mint oil. This would indicate that more receptors or different receptor types are activated with the mint oil than with the menthol.

4.4.6 Limitations

The biggest limitation of the reported study is that though participants were asked to disregard taste as far as possible they may have failed to do so. Flavour perception is 80% olfaction (Stuckey, 2012) which could account for some of the mint oil, Sichuan pepper and menthol findings as being an interaction between the sensation and retronasal olfaction. Flavour consists of interactions between the perceptions of smells, texture and tastes. Mint oil, Sichuan pepper and menthol activate receptors of taste but also somatosensation and olfaction.

Inhalation of menthol produces a cooling sensation that is mediated by the trigeminal nerve branches associated with the olfactory epithelium. The aroma of menthol is distinct and again has been directly linked to stimulation of the olfactory nerves (Eccles, 1994). Very few chemostimulants produce exclusively trigeminal or olfactory sensations but possess characteristics of both odour and irritation (Hummel & Livermore, 2002). Davidson, Linfort, Hollowood and Taylor (1999) asked participants to rate the intensity of flavour they perceived while chewing mint-flavoured gum. The mint taste they perceived came from the sugar contained within the gum while the menthol gave rise to olfactory and trigeminal components. The intensity of the menthol odour rapidly increased when chewing was initiated and intensity was reported to readily decrease over a 4-5-minute period of chewing, even though the actual intensity remained fairly consistent. The rapid decrease in menthol perception tracked the decline in sugar taste in the mouth and was found to return with the addition of more sugar (the mint-flavoured tastant which had no smell). This highlights that the intensity perception of menthol flavour is driven by the release of sugar in their mouth and the detection on the tongue.

Together this could suggest that either the intensity reported in the current study from the menthol is over exaggerated based on the sudden olfactory irritation experienced from menthol exposure. To reduce this effect the nose would need to be blocked however in the current study the nose was not blocked due to the length of the testing sessions. This was due to concern that the nose plug tightness would distract from the task and cause distracting discomfort for participants. Future research may wish to use a shorter protocol and block the nose to guarantee the olfactory influence is limited.

The findings within the Alum data may be explained by the stimuli delivery method used in this study. All, but the mint oil, stimuli were given using premade swabs that were rewetted before applications. Using freshly saturated swabs like that utilised by Breslin, Gilmore, Beauchamp and Green (1993) may have increased the astringent sensations. Additionally, a specific limitation to the Sichuan pepper administration could be that unlike the other chemo-stimulants where the active chemical components that generate the sensation was used, ground Sichuan pepper was used rather than the tingle inducing chemical HαSS. Use of HαSS would have removed the possible effect of taste interfering with the perception of the sensation and may have led to more intense sensation.

4.4.7 Future directions

This study confirmed the previous research that taster status affects the perception of chemo-stimulants (Prescott & Swain-Campbell, 2000; Pickering, Simunkowa & DiBattista, 2004) with hyper-tasters finding the induced sensations significantly more intense than tasters and tolerant-tasters. Yet the taster statuses interaction with location was only identified with the 10ppm capsaicin and Sichuan pepper. The frenulum of the lower lip (gum) one of the non-gustatory surfaces, was not significantly influenced by taster status but the lower lip was which is unexpected as it is a non-gustatory surface and lacks the FP innervation. The locations with the greater density of V^{th} and VII^{th} innervation, like the tongue tip, experienced a greater intensity of sensation as expected and hyper-tasters

experienced the greatest intensity on the regions that possess greater V^{th} and VII^{th} innervation.

Differences in the location perceptions may be due to different innervation densities from the V^{th} and VII^{th}, using a nerve knock out approach by anesthetising the lingual branch of the V^{th} or chorda tympani branch of the VII^{th} on one side of the tongue would allow the examination of the precise roles they play in the intensity experience. Only anesthetising the one side of the mouth would also allow for within participant comparisons and allow the opposite side of the tongue to act as a control location.

The identified link between sensation intensity and touch type, particularly on the vermillion of the lip warrants further investigation. The vermillion of the lip is a non-gustatory surface therefore taster status would not generally be considered to have influence over its sensory detection ability. Yet, 10ppm capsaicin and Sichuan pepper all established a touch type interaction with location and taster status. Taster status involves a greater level of V^{th} innervation within the mouth as identified by the increased FP densities on the tongue (Bartoshuk, Duffy & Miller, 1994; Essick, Chopra, Guest & McGlone, 2003) but these findings suggest that it may extend further than the mouth to the lips.

Interestingly, the mint oil identified that a dynamic touch on the vermillion of the lip was significantly more intense than the static touch. One possible explanation for this finding is that there could be C tactile afferents (CTs) present in the vermillion skin. CTs are low-threshold mechanoreceptive afferents that have previously been found in the hair skin of the body and respond most optimally to a slow-gentle stroking touch like that experienced during a human caress (Löken, Wessberg, Morrison, McGlone & Olausson, 2009). This could help explain the pleasure experienced during lip to lip contact and is explored in chapter 5.

Chapter 5 : Lips, A Social Organ?

Abstract

C-tactile (CT) afferents have been found to be present in the hairy skin and to code for pleasant touch. The firing activity of CTs have been seen in microneurography responding most optimally to a slow gentle stroke of 1 - 10cm/s. Participant ratings of a touch to the hairy skin at this CT optimal velocity report respond with the highest pleasantness ratings. These ratings and firing activity have come to be identified as an inverted U, with CT optimal velocity rated the highest and velocities faster or slower a significantly less pleasant.

Applying the standardised psychophysical approach to CT pleasantness rating to three facial locations, the cheek, vermillion of the lower lip and the mucosa of the lower lip the aim of this study was to identify if CT afferent like behaviours were present in the vermillion of the lower lip. It is hypothesised that CTs are present in the vermillion of the lip explaining why people engage in lip-to-lip contact and the findings from Chapter Four where intensity ratings of mint oil of the vermillion of the lip increased with a gentle dynamic touch.

This study identified a classic inverted-U to the strokes administered on the vermillion of the lip and cheek. While CTs are known to be present in the cheek the finding an inverted-U on the lip indicates CT like behavioural responses to stroking indicating the potential presence of CTs in the lip but further investigation is required.

5.1 Introduction

The cutaneous senses are crucial mediators of social interaction contributing to both sensation and emotion. Touch perception has various functions, it provides information about the structure, temperature and shape of the world around us. This is discriminative touch and is supported by specific neural circuitry (McGlone, Wessberg, & Olausson, 2014). Its other function is social with Morrison, Löken, and Olausson (2010) identifying three types of social functions within touch: 1) emotional communication, 2) forming/maintaining bonds and 3) affiliate behaviour (seeking close contact with others).

Social touch is supported by specialised neural pathway (McGlone, Wessberg & Olausson, 2014) identified from research both on the peripheral and central levels. Peripherally, a type of unmyelinated C fibre, the C tactile (CT) afferent, were first discovered in human facial skin (Johansson, Trulsson, Olsson, & Westberg, 1988). They have been shown to be exclusively located in the hairy skin, like that of the face and arm (Nordin, 1990; Vallbo & Wessberg, 1993; Vallbo, Olausson & Wessberg, 1999; Johansson, Trulsson, Olsson, & Westberg, 1988) and not the glabrous skin of the palm, soles and lips (Morrison, 2012; Olausson, Wessberg, Morrison, McGlone, & Vallbo, 2010). Skin biopsies of the human facial skin have found that the hairy skin shares some characteristics of glabrous skin with a rich innervation of mechanoreceptors and myelinated afferents (Nolano, Provitera, Capor, Stancanelli, Leandri & Biasiotta *et al.,* 2013).

CTs are slow-conducting afferents that are easily fatigued with repeated stimulation and may continue firing for several seconds after the stimuli has been removed (Vallbo, Olausson & Wessberg, 1999). Microneurography studies, measuring the electrical signal elicited from nerves during stimulation identified CTs preferentially responding to stroking over the skin within a velocity range of 1-10cm/s and this range is rated most pleasant compared to slower and faster velocities (Vallbo, Olausson & Wessberg, 1999; Löken, Wessberg, Morrison, McGlone & Olausson, 2009; Liljencrantz & Olausson, 2014; Wessberg, Olausson, Fernström & Vallbo, 2003). Johansson, Trulsson, Olsson and Westberg (1988)

identified slow adapting afferents in the skin of the transitional zone of the upper lip.

On a central level the CT pathway projects to the insular and orbitofrontal cortex for processing (Olausson, Lamarre, Backlund, Wallin & Strack *et al.,* 2002; Olausson, Cole, Vallbo, McGlone, Elam & Krämer *et al.,* 2008; Morrison, Löken & Olausson, 2010). Stimulation of CTs in the arm and thigh elicit somatotopically organised activation of the postier insular cortex (Björnsdotter, Löken, Olausson, Vallbo & Wessberg, 2009) a region known to play an important role in representing information relevant to well-being (Craig, 2003). The slow gentle stimulation that activates the CTs of the hairy skin is likely to occur during social interactions such as affiliative interactions between a parent and child, siblings, trusted friends and significant partners (Morrison, Löken & Olausson, 2010).

Social touch has a characteristic subjective quality with the hedonic values of it being innately related to the physical characteristics of the touch such as its softness (Rolls, O'Doherty, Kringelbach, Francis, & Bowtell *et al.,* 2003), temperature (Ackerley, Backlund Wasling, Liljencrantz, Olausson & Johnson *et al.*, 2014) and force and velocity (Löken, Wessberg, Morrison, McGlone & Olausson, 2009).

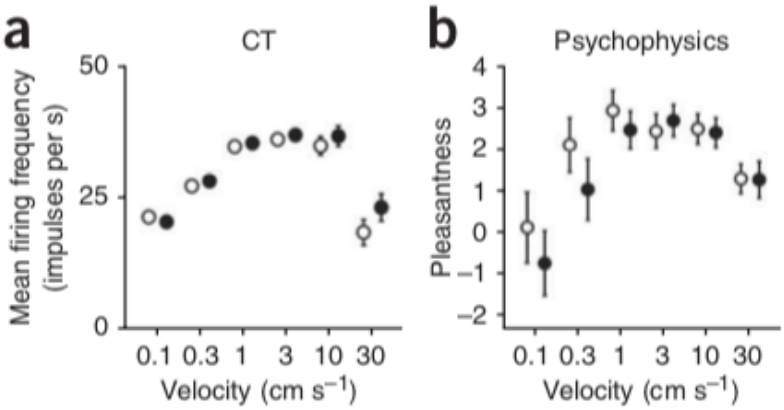


Figure 5.1 Löken, Wessberg, Morrison, McGlone & Olausson (2009) identified that the firing rate of the CT afferents (**A**) are matched by the pleasantness rating obtained from strokes administered at the different velocities (**B**) generating what is acknowledged as the classic inverted U. The white dots represent a stroke force of 0.2N and the black dots are force of 0.4N.

CT firing has been found to highly correlate to the subjective pleasantness rating of touch. Essick, James and McGlone (1999) were the first to identify the velocity/pleasantness interaction of gentle touch, however it was Löken, Wessberg, Morrison, McGlone and Olausson (2009) who first showed the CT afferent firing is essential for this psychophysical relationship. By using a rotary tactile stimulator (RTS; Dancer Design, St Helens, UK) they administered brush strokes across the dorsal forearm at 6 velocities (0.1, 0.3, 1, 3, 10, 30 cm/s). Hedonic ratings were collected on a visual analogue scale (VAS). The greatest CT firing were identified within the velocity ranges of 1 to 10cm/s peaking at 3cm/s and reflected the highest pleasantness ratings within these velocities. Velocities that were slower or faster were rated significantly less pleasant and mirrored the invert U-shaped CT firing rates. This finding has since been replicated multiple times (Vallbo, Olausson & Wessberg, 1999; Essick, McGlone, Dancer, Fabricant, Ragin & Phillips *et al.,* 2010; Löken, Evert & Wessberg, 2011; Liljencrantz & Olausson, 2014; Morrison, Löken, Minde, Wessberg, Perini & Nennesmo *et al.,* 2011; see Figure 5.1)

This has led to the "social touch hypothesis" that states CT fibres are specifically tuned to respond to comforting interpersonal touch (Olausson, Wessberg, Morrison, McGlone, & Vallbo, 2010). It suggests that despite the CTs reduced discriminative capabilities it is able to extract specific stoking velocities which are socially relevant (Morrison, Löken, & Olausson, 2010). This hypothesis is an intuitive explanation for the way slow gentle stroking tend to enact close affiliative interactions such as between parent and children (Morrison, Löken & Olausson, 2010).

Other than between parent and child, the majority of interpersonal communication via touch has been documented in romantic relationships (Gulledge, Gullege & Stahmann, 2003). Gulledge, Gullege and Stahmann (2003) used questionnaires asking about preferences and attitudes to different expressions of romantic physical affection and found that tactile physical affection was highly correlated with overall relationship and partner satisfaction. More recently using a standard CT activity stroking paradigm Jönsson, Backlund Wasling, Wagnbeck, Dimitriadis, & Georgiadis *et al.,* (2015) found that ratings of both touch

pleasantness and touch eroticism were significantly higher at CT optimum velocities.

Romantic kissing is defined as the "lip to lip contact" between individuals in a sexual or intimate setting (Jankowiak, Volsche, & Garcia, 2015). Although kissing is considered both common and significant it has seldom been the primary focus of research. The research that has been done exploring the lips has primarily focused on the discriminative abilities of the lips.

Anatomically, the lip skin's discriminative function is served by fast conducting low threshold mechanoreceptors (Nordin & Hagbrath, 1989) but contain a wide range of specialized sensory neurons that are sensitive to temperature, pressure, irritation, itch, pain and touch (McGlone & Reilly, 2010; see Oral Anatomy Chapter Two section 2.2 pg 56). Psychophysical studies examining the discriminative abilities of the lips found that the tactile acuity on the lip and tongue was higher than the fingertip in numerous studies (Johnson & Phillips, 1981; Van Boven & Johnson, 1994; Sathian & Zangaladze, 1996). This focus on discriminative perception is most likely due to the lips being considered glabrous skin and as such, affective qualities are often disregarded, but this disregard does not explain lip to lip contact.

Despite kissing's frequent depiction in art and literature, there is no consensus as to whether or not romantic kissing is a human universal. Evolutionary anthropologists and psychologists (Hughes, Harrison & Gallup, 2007; Wlodarski & Dunbar 2013, 2014) argue that lip kissing may be an adaptive tactic to assess a potential mates health and genetic compatibility as well as a partner's romantic interest. Most studies, when exploring kissing, assess the occurrence of a person's first romantic kiss identifying that adolescent couples who engaged in more kissing report a significantly higher relationship satisfaction (Welsh, Haugen, Widman, Darling & Grello, 2005) or examining the importance level ascribed to romantic kissing (Wlodarski & Dunbar, 2013).

Kissing has also been found to be used at different time for different purposes. Hughes and Kruger (2011) assessed sex differences in pre and post coital activities in pair bonding with long term partners. They found that males were more likely to initiate kissing before sex, where it may be used for arousal purposes

and females were more likely to initiate kissing after sex, where it might possess a relationship maintenance function (Hughes & Kruger, 2011). Together this highlights lip-to-lip contact possessing a strong underlying affective quality. The primary factor that the majority of this research ignores is the underlying CT afferents involved in affective perception on the lip. Other C fibres, however, have been reasonably widely researched.

Within the oral cavity, it is thought that the different taster groups possess differing levels of trigeminal innervation, explaining the anatomy behind the different intensity perceptions of sensations and tastes between the taster status groups. If this were the case, it is possible that the trigeminal innervation differences expand beyond mucosal surfaces of the mouth and into the outer vermillion surface of the lip, the opening to the oral cavity. It could then be expected that different sensations may be perceived differently on the lip dependent on an individuals taster status.

5.1.1 Quantitative Sensory Processing (QST)

Thermal quantitative sensory testing (QST; see Methodology chapter three section 3.1 pg 87 for an overview) allows separate testing of warm perception thresholds (reflecting the function of unmyelinated C-fibres) and cold perception thresholds (reflecting the function of A fibres and subgroups of C-fibres) (Yarnitsky & Pud, 1997).

The earliest studies assessing the perioral thermal sensitivity asked participants to scale the sensation of intensity of non-painful hot and cold contact stimuli. When comparing the upper and lower vermillion of the lip it was found that warmth ratings were higher on the upper than lower lip (Green, 1984). However, Essick, Guest, Martinez, Chen and McGlone (2004) suggest that the difference between these ratings might not be present when using a threshold approach to the data collection. Stevens and Choo (1998) found that the lips were more sensitive than the cheeks to both warming and cooling.

The application of thermal QST to the lower lip border identified that it was significantly more sensitive to hot and cold stimuli than the surrounding skin

(Renton, Thexton, Hankins & McGurk, 2003). The healthy control participants in Renton, Thexton, Hankins & McGurk, (2003) showed that the lip, chin and tongue were all more sensitive to cooling than to warming, a finding supported by numerous other studies (Green, 1984; Green & Gelhard, 1987; Van Sickels, Zysset, Nishioka, & Thrash, 1989).

Essick, Guest, Martinez, Chen & McGlone (2004) examined the thermal threshold across 10 different facial locations. The thermal thresholds varied across sites but the upper and lower lip vermillion was found to be the most sensitive sites to both warming and cooling, including at noxious temperatures. The preauricular skin the least sensitive. Overall Essick, Guest, Martinez, Chen & McGlone, (2004) found that the further from the mouth that was tested, the more thermal sensitivity decreased. Skin morphology could explain this as previous research observes higher pain thresholds on the glabrous skin opposed to hairy skin, suggesting the thicker epithelium of glabrous skin absorbs more thermal energy than hairy thus raising thermal pain thresholds (Taylor, Mcgillis & Greenspan 1993).

Later assessments of thermal perception on the tongue and lip were made by Manrique and Zald (2006). They identified a weak association for both the tongue and lip between the warm and hot stimuli. Warm detection threshold explained 11% of the variance in the hot supra-threshold intensity measures but was an effect that vanished when controlling for outliers. Cold detection and supra-threshold intensity measurements were not associated. The supra-threshold ratings of cooling on the tongue were found to be modestly associated with the intensity ratings of PROP but a similar was association was also identified for the lower lip.

Temperature changes cause neuronal depolarization though activation of receptor channels. Transient receptor potential (TRP) ion channels are expressed in the nerve endings and respond to distinct thermal thresholds (Kim, Jung, Park & Lee, 2017). Temperatures above 33°C activate the TRPV3 ion channel (Schepers & Ringkamp, 2009). TRPV3 responds to both innocuous and noxious heat (Green 2004).

5.1.2 Aims/Hypothesis

Reported here is a study that applies the standard psychophysical approach in affective touch research to the lower lip in order to investigate if CT responses are present. Previous findings in Chapter Four identified that significant interactions between the intensity ratings of mint oil on the vermillion of the lip and touch type. A dynamic touch on the lip increased the intensity perceived and as such it is hypothesised that CT afferents may have been involved in this and could potentially explain some these findings.

The main hypotheses of this study were that:

1) The lips are regularly used in affiliative/romantic interactions and as such the pleasant rewards experienced from lip-to-lip contact could be due to CT innervation of the lips glabrous skin, even though they are not found in the glabrous skin of the palm. Therefore, CT optimal touch (3cm/s) should be more pleasant than CT non-optimal touch (0.5 and 20cm/s).

2) This study also sought to confirm what is already known about the thermal sensitivity of the lip being the most sensitive to thermal change.

3) However, given that taster status influences the chemosensory perception of warming and cooling agents this study also intends to expand knowledge by examining if taster status influences thermal perception on the lip and mucosa. This is expected due to an increased level of trigeminal innervation in hyper-tasters than tolerant-tasters.

5.2 Methods

5.2.1 Participants:

Data from 46 participants was collected. Of the participants there were 14 males (30.4%) and 32 females (69.6%) with a mean age of 23.07 years (SD = 3.43), although four of the participant's chose not to disclose their age but did confirm they were within the age boundaries of the study. This consists of 13 hyper-tasters, 17 Tasters and 16 tolerant-tasters, making a 28.3%:37%:34.8% population split which closely reflects the expected wider population taster status divisions. Participants were recruited through Liverpool John Moores University (LJMU) research participants scheme for first year psychology undergraduates, university department emails and via the snowball sampling.

The inclusion criterion was that all participants were aged between 18 and 35 years and non-smokers. All participants must never have been diagnosed with a neurological disorder that affects sense of taste or touch or be being treated for an under/over active thyroid or dry mouth syndrome. Anyone with a lip piercing and allergy/intolerance to food colouring and participants who were taking antihistamines or medication that has the side effect of creating dry mouth or if the participant was/may be pregnant were excluded from participation.

This study was granted full ethical approval by the Liverpool John Moores University Research Ethics Committee on 8th April 2015 (Ref: 16/NSP/034).

5.2.2 Materials:

5.2.2.1 Roller ball:

Stroke was administered with a glass rollette bottle with plastic screw on roller ball. The bottle was 85mm tall, 18mm wide and could hold 10ml of liquid. For the purpose of this study the bottles were kept empty and each participant was given a fresh bottle and rollerball (see Figure 5.2).

Figure 5.2 roller ball used for stroke administration

5.2.2.2 Papillae Density:

This was assessed using Dr Oetker Blue food colouring as done by Miller and Reedy (1990). The blue food colouring coats the filiform papillae allowing the FP to be counted. Photographs of the stained tongue will be taken with a Canon 750D with a 105mm F2.8 EX DG Macro OS lens attached. No identifying information was in the photograph; only an image of the tongue was taken.

5.2.2.3 Metronome

A laptop was used running a metronome designed in Psychopy. The metronome was designed to show the stroke speed for each of the strokes to be administered. The metronome randomized strokes at speeds of 0.5cm/s, 3cm/s and 20cm/s across three locations, the outer vermillion of the lip, the mucosal surface of the lower lip and the cheek. It was programmed to give the speed correctly timed to cover a 3cm distance.

5.2.3 Stimuli:

5.2.3.1 Stroke Velocity:

Strokes were given at three different approximate speeds (0.5cm/s, 3cm/s and 20cm/s) by a glass rollette bottle topped with a plastic rollerball. The strokes were administered to three locations of the mouth and face (the outer vermillion of the lower lip, the mucosa of the lower lip and the cheek). Each stroke lasted for a period of approximately 6 seconds and covered an area of approximately 3cm.

5.2.3.2 Thermal Perception

Participants were asked to gently press a small, specifically designed for oral use, thermode (1.5 x 1.5 cm^2 stimulus area; see Essick, Guest, Martinez, Chen & McGlone, 2004) which was wrapped in cling film for hygiene, on the outer vermillion of the lower lip, mucosal surface of the lower lip or the cheek. Starting temperatures for external thermal research (outer vermillion of the lower and cheek) was 32°C (body temperature) and 37°C for intra-oral thermal research (oral mucosal surface). The thermode warmed up or cooled down at a rate of 1°C/s.

Figure 5.3 Medoc thermode for intraoral QST with a 1.5cm^2 plate.

The safety protocols built into the Medoc Pathway are that it has an automatic cut off point of 55°C for hot temperatures and -10°C for cold temperatures, this ensures that it will not burn participants. In obtaining ethical approval for this study, LJMU REC insisted that the participant have complete control over the thermode. This meant that contrary to the published research and standard procedure, participants held the thermode against the skin. This was to allow them ease of withdrawal from the thermal stimuli if required.

This procedure, including the cling film cover, was previously used by Manrique and Zald (2006) who also noted that the cling film cover produced a mild slowing of the thermal conduction (approximately 0.2-0.3°C). This will be accounted for in the data.

5.2.4 Measures

5.2.4.1 Taster Status:

Taster status was assessed using the standard PROP soaked filter paper method outlined in the methodology chapter (section 3.3 pg 92).

5.2.4.2 Hedonics

The pleasantness ratings for the stroking stimuli were collected using a Visual Analogue Scale (VAS) (see methodology chapter section 3.2.1 pg 88). The middle of the scale was labelled 0, the two extreme anchor points on the scale were 'very unpleasant' on the left and 'very pleasant' on the right. Participants indicated with a click anywhere on the scale indicating how pleasant or unpleasant they found the stroke.

5.2.4.3 Questionnaire

A questionnaire was also included in this study to measure aspects that may influence the pleasantness of touch (The Touch Experience and Attitudes Questionnaire: TEAQ; Trotter, McGlone, Reniers & Deakin, 2018).

The Touch Experience and Attitudes Questionnaire (TEAQ; Trotter, McGlone, Reniers & Deakin, 2018): A 57 item questionnaire that asks individuals to strongly agree to strongly disagree with statements related to touch experiences and attitudes. Questions relate to current social touch ("I find it natural to greet my friends and family with a kiss on the cheek"), current intimate touch ("I am often given a shoulder massage"), childhood touch ("As a child my parents would often hold my hand when I was walking along with them"), attitude to personal grooming ("I like exfoliating my skin"), attitude to intimate touch ("I like to fall asleep in the arms of someone I am close to") and attitude to unfamiliar touch ("I dislike people being very physically affectionate towards me"). An individual's past experiences of touch and their attitude to touch is likely to influence their response to touch.

5.2.5 Procedure:

Upon entering the laboratory participants were given a brief description of the experimental process and their rights as a participant including their right to withdraw. Consent was obtained and a health screening measure was complete to ensure suitability to participate. Participants were given a paper version of the

TEAQ and no time limit to complete it was given but the majority of participants completed it within 5 minutes.

Taster Status Test: Participants sat comfortably in a dental chair for the remainder of the experimental session. The next part of the study undertaken was the taster status test by using the procedure outline in the methodology section 3.3 pg 92.

Touch Task: Participants had an empty and never before used 10ml rollerball aromatherapy bottle rubbed along 3 locations (the lower lip, the mucosal lip surface on the inside of the lower lip and the cheek) at approximately 3 different speeds (0.5cm/s, 3cm/s and 20cm/s). Between each stroke participants were asked to rate how pleasant or unpleasant they found the stroke on a visual analogue scale (VAS). Participants experienced each stroke for a period of 6 seconds before providing a rating and experience each stroke velocity at each location three times to allow for a mean pleasantness rating to be calculated. The anchor points on the scale were explained to the participants in that the more central they clicked the more neutral they found the touch, so it was neither pleasant nor unpleasant, further to the right of the centre point they marked it the more pleasant they found the stroke with very pleasant being the extreme. Rollerball bottles and heads were disposed of between participants.

Thermal Detection/Thresholds: Participants were asked to gently press a small, specifically designed for oral use thermode (1.5cm x 1.5cm^2 stimulus area; see Essick, Guest, Martinez, Chen & McGlone, 2004) which was wrapped in cling film for hygiene on the outer vermillion of the lower lip, mucosal surface of the lower lip or the cheek. Starting temperatures for external thermal research (outer vermillion of the lower lip and cheek) was 32^{0}C and 37^{o}C for intra-oral location (oral mucosal surface).

Warm and cold thresholds and cold pain and hot pain were all measured three times. It was explained to the participant that the thermode would warm up or cool down at a rate of 1^{o}C/s. Participants were asked to indicate when they perceived a temperature change by clicking a mouse button which will stop the temperature change and bring it back to a baseline normal/body temperature. Cold and hot pain thresholds were established next. The process is the same as

with detection thresholds except participants were asked to click the mouse when the temperature became painful. Participants had full control over the temperature change, by pressing a mouse button as soon as the sensation becomes detectable or painful (depending on which is being measured) and returning the temperature back to baseline. Before testing commenced the safety protocols for were explained to the participant and they were advised that as they were holding the thermode they were free to remove it from the location if they felt the need.

Papillae Density: The final part of the study was to assess the density of the FP of the participants. This was done using a cotton swab coated in blue food colouring and applying the dye to the surface of the tongue. Vaseline was coated onto the lower lip to minimise staining on other tissues. The food colouring stains only the filiform papillae but leave the FP unstained. Participants were asked to stick their tongue out as far as they could while keeping their mouth open. Several images were taken of the tongue to ensure correct location, clarity of the photograph and size and density of the FP were calculated (Essick, Chopra, Guest & McGlone, 2003).

At the end of each session participants were fully debriefed. Participants who were recruited from the LJMU research participants system were given 3 credits and participants recruited through email were given £10 Amazon vouchers as compensation for their time.

5.2.6 Statistical Analysis

Levenes test for homegeity of variance was run on all the data indicating that the variance between the groups was equal (ps>.05) and Mauchly's tests of sphericity were examined and where appropriate Greenhouse Geisser corrections to degrees of freedom are reported.

5.2.6.1 Touch data

The data was assessed for normality that indicated the data was non-normally distributed. Attempts to correct this with transformation were unsuccessful therefore analysis was run on the original data. Repeated measures

ANOVA were run on the data with two within participants factors, the first being location which had three levels (cheek, outer vermillion of lower lip, mucosal surface of lower lip) and the second was stroke velocity that consisted of three levels (0.5cm/s, 3cm/s and 20cm/s). Interactions between the variables were explored and where appropriate with identified main effects and interactions, further investigations included Mixed and Repeated Measures ANOVA's, t-tests and pairwise comparisons were run. To assess the pleasantness perception over the stroking velocities a curve estimation analysis was run on the data as per the standard analysis procedure in CT data (Essick, James & McGlone, 1999; Löken, Wessberg, Morrison, McGlone & Olausson, 2009; Löken, Evert, & Wessberg 2011).

5.2.6.2 Thermal data

Mean thresholds were calculated from the consecutive measurements. Warm detection (WDT) and cold detection threshold (CDT) the absolute value was subtracted from the base temperature of 32°C for the lip and cheek and 37°C for the mucosa to calculate the degrees of change. Scores were then $\log_{10}$ transformed and z-scored following the standard procedure and compared with normative values as set out by Rolke, Magerl, Campbell, Schalber, Caspari & Birklein *et al.,* (2006). Scores for the hot pain (HPT) and cold pain threshold (CPT) were z scored before the analysis was run.

Separate repeated measures ANOVA's were run on thermal data examining the differences in thermal perception between oral locations. Where appropriate with identified main effects and interactions, further investigations including ANOVA's, t-tests and pairwise comparisons were run.

5.2.6.3 Questionnaires

Questionnaire data was assessed for normality and ANOVA analysis was run on the data examining differences between taster status and TEAQ scores.

5.2.6.4 Fungiform Papillae Count

Photographs were uploaded onto a computer. A square was marked out measuring 4 x 4cm^2 with the midline of the tongue being central in the square. The square was placed so that it was completely on the tongue but as close to the tip as possible. The image was cleaned in attempts to make the image sharper and reduce flashback from the tongue. Fungiform papillae were counted within the square. The image colouring was then inverted and the papillae were counted for comparison. Mean scores were taken if the counts did not match.

5.3 Results

Demographic information about the participants and the taster groups they belong to are shown in Table 5.1.

Table 5.1 Demographic information representing the percentage of tested population and segmented by taster status.

		Tolerant taster	Taster	Hyper-taster
Gender	Male: n (%)	6 (42.9)	5 (35.7)	3 (21.4)
	Female: n (%)	10 (31.3)	12 (37.5)	6 (31.3)

5.3.1 Touch Task Full Model: Mixed Measures ANOVA

Mixed measures ANOVAs were run on the data to test if the CT afferent behaviour occurs when stroking is administered to the vermillion of the lower lip at a CT optimal velocity in comparison to CT non-optimal velocities. It further tested if there was any significant difference between the three locations where the strokes were administered. The Adult/Adolescent Sensory Profile as a covariant to account for different liking levels of touch. Significant differences between Locations (F(1.51, 58.82) = 11.69, p<.001, ηp^2= .23, Power = .98), Velocity (F(1.56, 60.90) = 16.00, p<.001, ηp^2= .29, Power = 1.00) and their interaction (F(4, 156) = 7.65, p<.001, ηp^2= .16, Power = 1.00) was identified. Scores on the Adult/Adolescent Sensory Profile were used as a covariant and no significant effect for Location and Adult/Adolescent Sensory Profile, Velocity and Adult/Adolescent Sensory Profile (ps>.05) or their interactions with Taster Status (ps>.05) were identified and as such was removed from the remaining analysis. No main effect of taster status was found (p>.05).

Mixed measures ANOVAs tested the hypothesis that CT afferent behaviour occurs when stroking is administered to the vermillion of the lower lip at a CT optimal velocity in comparison to two CT non-optimal velocities. It further tested if there was any significant difference between stroke pleasantness ratings across the vermillion of the lip, cheek and musoca. A significant main effect for Velocity on

the lip ($F(2, 90) = 25.29$, $p<.001$, $\eta p^2 = .36$, Power = 1.00) and cheek ($F(2, 90) = 23.62$, $p<.001$, $\eta p^2 = .34$, Power = 1.00) but not the oral mucosa ($p>.05$). For both the lip and cheek, pairwise comparisons indicate that the CT-optimal 3cm/s stroke was significantly more pleasant than the non-optimal 0.5cm/s and 20cm/s stroke (p's<.001). No significant difference was seen between the pleasantness ratings for 0.5cm/s and 20cm/s stroke ($p>.05$) (see Figure 1). This highlights that the manipulation works in that both the non-CT-optimal velocity of 0.5cm/s and 20cm/s were significantly less pleasant than the 3cm/s CT-optimal velocity.

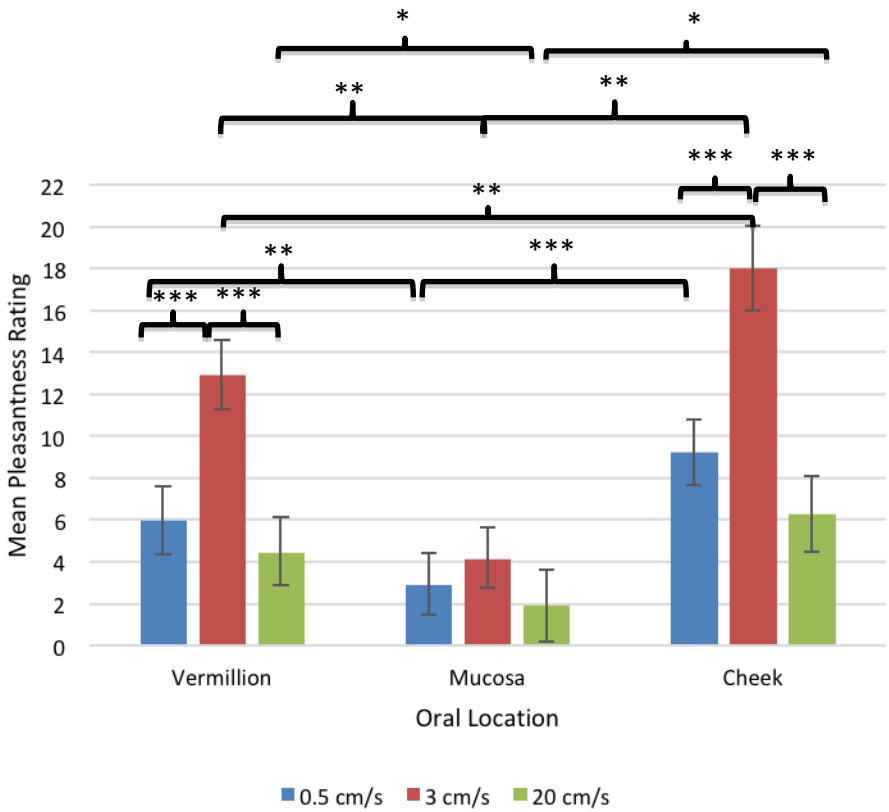


Figure 5.4 Mean and Standard Error ratings for touch pleasantness for each location. A significant difference in pleasantness rating was found between all velocities when applied to vermillion of the lip and the cheek (ps<.001). All velocities on the mucosa were not significantly different (ps>.05). The velocities were significantly different across locations with the 3cm/s stroke significantly different across all locations (ps<.01). The slow 0.5cm/s stroke was significantly less pleasant on the mucosa than the lip (p<.01) and cheek (p<.001) but there was no difference between the lip and cheek ratings at 0.5cm/s (p>.05). 20cm/s velocity was again found to be significantly less pleasant on the mucosa than the lip and cheek (ps<.05) (* p<.05 level, ** p<.01 level, *** p<.001).

locations (p's<.01) with mean scores showing that the oral mucosa was rated less

pleasant than the lip and cheek but also that the lip was less pleasant than the cheek. The non-optimal slow velocity of 0.5cm/s was a significantly less pleasant on the oral mucosa than the lip ($p<.01$) and cheek ($p<.001$) but no difference was found between the cheek and lip at 0.5cm/s ($p>.05$). The other non-optimal velocity of 20cm/s again found that the mucosa was significantly less pleasant than the lip and cheek ($ps<.05$) (Figure 5.4).

As can be seen in Figure 5.4 the significant location x velocity interaction reflects differences in between pleasantness ratings at the different locations at different velocities. This implies that each location is differently innervated by CTs with it appearing highly unlikely that they are present in the oral mucosa but that they may be present in the lip due to CT afferent like behavioural responses obtained from the strokes.

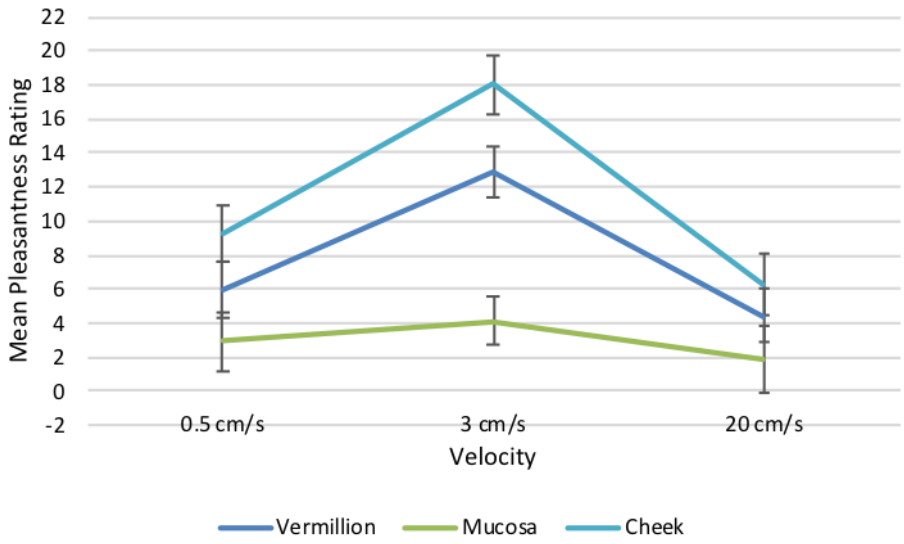


Figure 5.4 Mean and Standard Error scores across velocity highlighting a quadratic fit for both the lip and cheek ($ps<.001$). The ratings show that pleasantness on the cheek was significantly more pleasant This reflects the inverted U commonly found in CT research.

The pleasantness perception over the stroking velocities at each location were examined. A curve estimation analysis was conducted to establish the relationship between stroking velocities and pleasantness ratings at each location (i.e. a velocity-pleasantness profile). Both linear and quadratic models were tested

to define the velocity-pleasantness profile. The profiles for the vermillion of the lower lip and cheek were best fit by quadratic models, rather than linear models, giving the characteristic "inverted- U" shaped curves, as found in previous studies investigating pleasantness of different velocity stroking stimuli (Essick, James & McGlone, 1999; Löken, Wessberg, Morrison, McGlone & Olausson, 2009; Löken, Evert, & Wessberg 2011; Ackerley, Carlsson, Wester, Olausson & Wasling, 2014; Walker, Trotter, Woods & McGlone, 2017). Neither the linear or quadratic model fit the mucosal data ($p>.05$). The identified quadratic regressions for the vermillion and cheek were significant (vermillion: $R^2 = 0.101$, $p = .001$, cheek: $R^2 = .145$, $p = 0.000$; Figure 5.5).

Finally, to ensure that the gender of the researcher did not influence the touch ratings as a social cue given the location being tested gender differences were explored and no significant differences between the genders ratings were found ($p>.05$).

5.3.2 Thermal detection and thermal pain thresholds

Mixed ANOVAs were used to examine the difference in thermal detection and pain across locations and to explore if taster status impacts on thermal detection. A significant difference between the thermal measures was identified ($F(2.08, 87.48) = 21.09$, $p<.001$, $\eta p^2 = .33$, Power = 1.00), between the locations ($F(2, 84) = 174.74$, $p<.001$, $\eta p^2 = .81$, Power = 1.00) and their interaction with each other ($F(4.22, 177.20) = 48.33$, $p<.001$, $\eta p^2 = .54$, Power = 1.00). No significant main effect of taster status was identified ($p>.05$) and taster status was found to not be related to the thermal tests or the location or a three-way interaction ($ps>.05$). Figure 5.6 shows the participant profiles for responses to the thermal tests. The black lines indicate the upper and lower 95% confidence intervals for normative values and the blue dots are where the participants rated within these norms as set out by Rolke, Magerl, Campbell, Schalber, Caspari and Birklein *et al.,* (2006) for the facial skin. The scores that do not fall within the 95% confidence interval are likely due to effects of spatial summation. The reference values used for calculating the scores are based on a larger thermode than the one utilised in the reported study. This

change in thermode was required to make assessment of thermal sensation on the vermillion and mucosa practical.

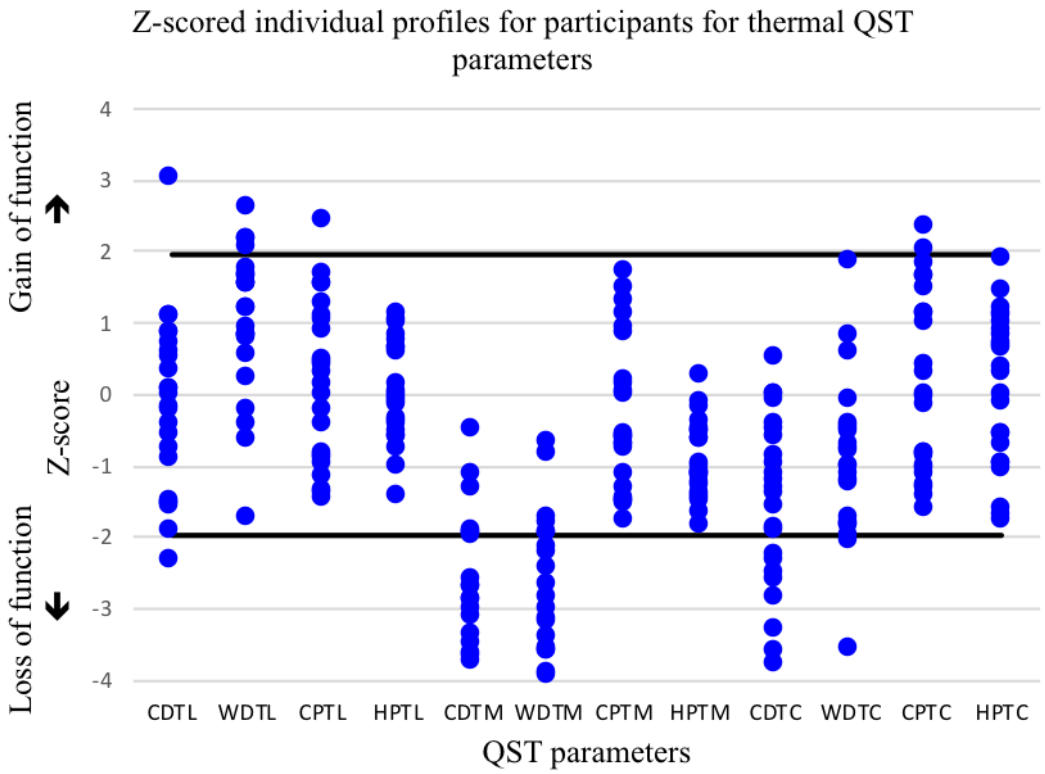


Figure 5.5 The participant profiles obtained for the Thermal QST. Black lines indicate the upper and lower 95% confidence interval for normative values and the blue dots are where the participants rated within these norms as set out by Rolke, Magerl, Campbell, Schalber, Caspari and Birklein *et al.,* (2006) for the facial skin. Scores should be between the two black lines. The scores on the mucosal surfaces particularly the cold detection (CDTM) and warm detection (WDTM), fall outside the norms likely due to the norms being associated with facial skin as intra-oral norms are not yet established. The outliers at other locations such as the cold detection on the cheek (CDTC) and warm detection on the cheek (WDTC) are likely due to the effect of spatial summation, the normative values were calculated using a larger thermode than the one utilised in this study. The hot pain on the lip (HPTL), mucosa (HPTM) and cheek (HPTC) falls between the standard deviation norms as do the majority of the cold pain on the lip (CPTL), mucosa (CPTM) and cheek (CPTC).

Table 5.2 Mean and standard error thermal responses to the thermal QST procedures. CDT and WDT are reported as degrees of change from baseline temperatures (from 32 °C for the vermillion and cheek and 37 °C for the mucosa. The CPT and HPT are the recorded mean absolute temperatures. Significant differences in CDT and WDT for all locations were identified (ps<.001) with temperature change baseline indicating the vermillion of the lip was best at detecting a cold and warm temperature changes and the mucosa the least sensitive to temperature change. The mucosa was also significantly different the vermillion (ps<.001) and cheek (ps<.05) in both CPT and HPT. Temperatures indicate the mucosa felt the cold pain before the vermillion and cheek but the hot pain later.

	CDT °C change from baseline (SE)	**WDT °C change from baseline (SE)**	**CPT °C (SE)**	**HPT °C (SE)**
Vermillion	-1.24 (0.11)	1.01 (0.11)	14.81 (1.36)	43.23 (0.41)
Mucosa	-4.00 (0.35)	5.46 (0.38)	18.05 (1.36)	46.62 (0.33)
Cheek	-2.09 (0.20)	3.03 (0.36)	16.30 (1.47)	42.99 (0.64)

Each QST thermal test was further analysed to identify if there were specific differences in thermal detection and threshold levels between the locations. Significant differences between the locations were identified with the CDT ($F(2,90) = 84.74$, $p<.001$, $\eta p^2 = .65$, Power = 1.00), WDT ($F(2,90) = 132.99$, $p<.001$, $\eta p^2 = .75$, Power = 1.00), CPT ($F(2,88) = 9.24$, $p<.001$, $\eta p^2 = .17$, Power = .97) and HPT ($F(1.39, 62.44) = 45.41$, $p<.001$, $\eta p^2 = .50$, Power = 1.00). Pairwise comparisons indicate that for each of the thermal QST tests, significant differences were identified with all locations being significantly different for CDT and WDT (ps<.001) with scores indicating that vermillion of the lip was best at detecting a cold and warm change in temperature and the mucosa the least sensitive. The mucosal CPT and HPT were both significantly different to the vermillion (ps<.001) and cheek (ps<.05). Mean temperatures indicate that the mucosa felt cold pain before the vermillion and cheek but hot pain threshold was greater on the mucosa than vermillion and cheek. The vermillion and cheek were not significantly different from each other at CPT and HPT (ps>.05) (see Table 5.2).

5.3.3 Fungiform Papillae Count and Taster Status

ANOVA analysis tested the hypothesis that fungiform papillae density varied across the taster groups and found that there were no significant differences between the taster groups density of fungiform papillae on the tongue ($F(2, 41) = .70$, $p>.05$).

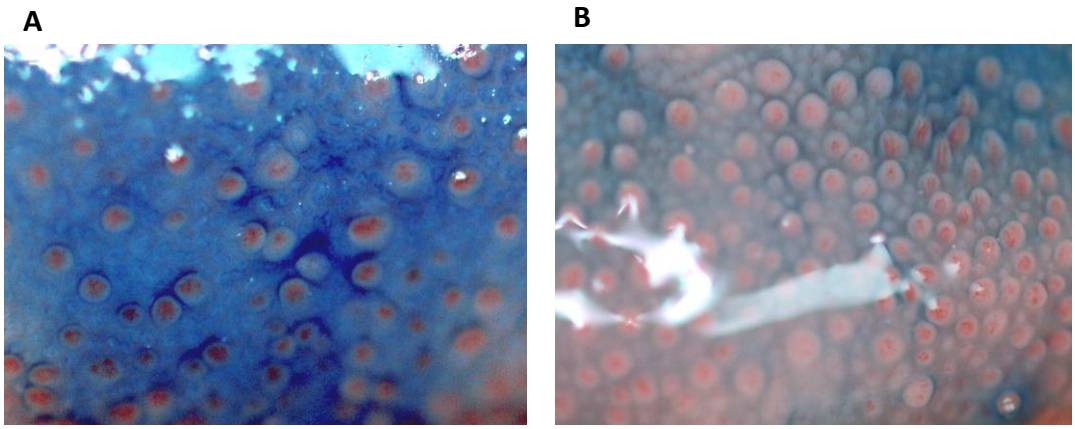


Figure 5.7 Example photographs of the FP tongue density from participants in the current study. FP are the pink lumps on the tongue that have not held to blue food colouring. Image **A** represents a tolerant-taster tongue with approximately 46 FP/cm^2 and **B** represents a hyper-tasters tongue with approximately 71 FP/cm^2.

5.3.4 Touch Experience and Attitudes Questionnaire (TEAQ)

Analysis of the TEAQ and taster status was conducted to examine if the attitudes and experiences of touch differed between the taster groups. Different taster groups were identified as experiencing different Current Intimate Touch ($F(2,43) = 3.39$, $p<.05$, $\eta p^2 = .14$, Power = .61) and Childhood Touch ($F(2, 43) = 6.08$, $p<.01$, $\eta p^2 = .22$, Power = .86). Pairwise comparisons found no significant differences between the taster groups and Current Intimate Touch. This could be a false positive indicated by a moderate observed power for this factor and low effect size. Within the aspect of childhood touch it was found that hyper-tasters (M = 4.37, SD =.21) experienced significantly more touch in childhood than tasters ($p<.05$; M = 3.80, SD = .18) and tolerant-tasters ($p<.01$; M = 3.78, SD = .19).

Table 5.3 Mean and standard error for the factors of the TEAQ segregated by taster status. The taster groups were observed as scoring significantly different for current intimate touch ($p<.05$) and childhood touch ($p<.01$) with hyper-tasters reporting to have experienced more childhood touch than tasters and tolerant-tasters. Mean scores indicate that hyper-tasters report have more current intimate touch than tasters and tolerant-tasters but further analysis didn't indicate this difference was significant.

	TASTER STATUS	MEAN	SE
CURRENT SOCIAL TOUCH	Hyper-taster	3.65	.25
	Taster	3.40	.22
	Tolerant-Taster	3.35	.22
CURRENT INTIMATE TOUCH	Hyper-taster	3.96	.26
	Taster	3.20	.23
	Tolerant-Taster	3.13	.23
CHILDHOOD TOUCH	Hyper-taster	4.58	.21
	Taster	3.80	.18
	Tolerant-Taster	3.67	.19
ATTITUDE TO INTIMATE TOUCH	Hyper-taster	4.37	.23
	Taster	4.00	.20
	Tolerant-Taster	3.98	.20
ATTITUDE TO UNFAMILIAR TOUCH	Hyper-taster	3.37	.26
	Taster	2.79	.23
	Tolerant-Taster	2.89	.23
ATTITUDE TO SKIN CARE	Hyper-taster	3.66	.34
	Taster	3.44	30
	Tolerant-Taster	3.00	.30

5.4 Discussion

The main goal of this study was to apply the standard psychophysical approach in affective touch research to the lower lip in order to investigate if behaviour consistent with the potential presence of CTs occurred when strokes were administered to the lip. If the responses were present then it would indicate the potential presence of CT afferents and indicate if further investigation was worthwhile. It was hypothesised that CT afferent behaviours may explain the previous findings in Chapter four which identified a significant interaction between the intensity ratings of mint oil on the vermillion of the lip and touch type. This hypothesis was supported in the data of this study as pleasantness ratings for the administered touch was rated as significantly more pleasant on the cheek and lip than on the mucosa. The lack of the inverted-U on the mucosal surface of the mouth may not represent a potential absence of CTs in this surface but could simply reflect that the experimental procedure of stroking that specific surface was a highly alien experience. The pleasantness of the strokes did not differ based on taster status.

CT responses to touch have previously been found to highly correlate with the subjective rating of touch pleasantness. The greatest CT firing were identified at a stroking velocity of 3cm/s, a velocity that was used in the current study, and reflected the highest pleasantness ratings within these velocities. Velocities that were slower or faster were rated significantly less pleasant and mirrored the invert U-shaped CT firing rates (Löken, Wessberg, Morrison, McGlone, & Olausson, 2009). This finding has since been replicated multiple times (Vallbo, Olausson, & Wessberg 1999; Essick, McGlone, Dancer, Fabricant, Ragin & Phillios *et al.,* 2010; Löken, Evert & Wessberg, 2011; Liljencrantz & Olausson, 2014; Morrison, Löken, Minde, Wessberg, Perini, & Nennesmo *et al.,* 2011; see Figure 5.1). The data collected in the current study identified the classic pleasantness inverted-U associated with CT responses to affective touch on the cheek, where CTs are known to already be present (Ackerley, Saar, McGlone & Wasling, 2014). The behaviour response obtained indicates the potential presence of CTs in the lip, which could explain why

people engage in lip-to-lip contact/romantic kissing regularly and imply the lip skin is not glabrous, contrary to popular opinion.

The most obvious concern within this part of the study is experimenter consistency of stroke delivery which is often addressed by using a rotary tactile stimulator to apply the touch, however Triscoli, Olausson, Sailer, Ignell and Croy (2013) compared the pleasantness of CT-optimised touch between hand and robotic stroking and found that pleasantness ratings were similar in both conditions and across velocities. This means that impact of manual delivery should be minimal given that it was controlled for by the use of a metronome.

The second aspect of the study reported here was exploring the thermal perceptual abilities of the cheek, lip vermillion and mucosa. The lip was identified in the present study as being the most sensitive to warm and cold detection and also having the highest cold pain tolerance, but the mucosa possessed the highest hot pain tolerance. Hot pain thresholds were higher on the lip than cheek in the current study but they were not significantly different. These findings support that of Renton, Thexton, Hankins and McGurk (2003) of the lip being the most sensitive to thermal warming and cooling. The lip being more sensitive than the cheek supports the findings of Essick, Guest, Martinez, Chen and McGlone (2004). It is suggested that thermal perceptual differences could be linked to differences in skin type. The glabrous skin is generally less sensitive to pain and as such possesses higher pain thresholds due to the thicker epithelium absorbing more thermal energy (Taylor, Mcgillis & Greenspan, 1993). However, the epithelium on the lip is thin compared to other skin (Hand & Frank, 2014) so that cannot explain why the lip has a higher cold pain threshold in this study.

Within the thermal testing in the current study it was expected to see an effect of taster status on the thermal perceptual abilities, with hyper-taster being better able to detect temperature change and having lower pain thresholds due to increased trigeminally innervation within the mouth. This, however, was not supported in the current data suggesting that the increased trigeminal innervation due to taster status is limited to inside the oral cavity, specifically the tongue as the mucosal surface was also not identified as being influenced by taster status.

It was clear that the data did not entirely fit within the QST norms outlined by Rolke, Magerl, Campbell, Schalber and Caspari et al., (2006). There are possible explanations for this however, the norms were developed from using a larger thermode. This would change the results on the grounds of spatial summation, that is spatial variations in the density of receptors may underlie differences in thermal sensitivity. Research indicated that the larger the area being stimulated by noxious thermal stimuli, the sooner it becomes painful (Nielsen & Arendt-Nielsen, 1997). The spatial effects on the basis of the thermoreceptor density has repeatedly been proposed for cool (Stevens & Marks, 1979), warm (Stevens & Marks, 1971; Marks & Stevens, 1973), cold pain (Westcott, Huesz, Boswell & Herold, 1977;) and hot pain (Douglass, Carstens & Watkins, 1992). There is also some evidence that skin type influences the effects of spatial summation. Defrin, Petrini and Arendt-Nielsen (2009) found that warm detection thresholds were reached at a higher temperature with a small thermode compared to a large, however the opposite effect was found with cold detection thresholds. They compared responses on the hairy and glabrous skin and found that the effect of spatial summation in the glabrous skin was larger than in the hairy skin, specifically for cold thresholds.

Together the thermal and stroking data indicate that the effects of taster status, though vast in the literature regarding intra-oral perceptions, does not extend beyond the oral cavity.

FP densities in the current study were found to not be related to taster status supporting the research conducted most recently (Fischer, Cruickshanks, Schubert, Pinto, Klein, & Pankratz *et al.,* 2013; Garneau, Nuessle, Sloan, Santorico, Coughlin & Hayes, 2014) but disagreeing with early studies (Duffy, Hayes, Davidson, Kidd & Bartoshuk, 2010; Essick, Chopra, Guest & McGlone, 2003; Hayes & Keast, 2011). Attempts were made to clear the images with photo software but this did not allow for the FP counting to be precise. The failure to find the link between FP densities and taster status is due to methodological problems. The photographs obtained in this study were done using a macro lens and were occasionally blurry and the shine on the tongue often caused distortion. Previous studies used a medical camera lens (Essick, Chopra, Guest & McGlone, 2003) for taking images of

the tongue, this magnifies the FP and would have improved the photo quality above that which was available for this study. If the FP density photographs were to be repeated with a high-quality medical lens it may generate clearer image quality and different results which is essential for future FP assessments and to aid in clarifying if the relationship between FP density and taster status does exist.

Interestingly, a relationship between taster status and the TEAQ subscale of childhood touch was found with hyper-tasters reporting to receive more childhood touch than tolerant-tasters. A possible explanation for this finding could be due to the affective quality of touch. Hyper-tasters are repeatedly linked to increased emotionality in both human and rat research (Dess & Chapman, 1990; Dess & Edelheit, 1998; Macht & Mueller, 2007) therefore, if hyper-tasters are more emotionally reactive it is possible they did not technically receive more childhood touch, but the touch they did receive could have been interpreted with a more emotional internal processing making it more prominent for them. To the best of our knowledge touch experience has not been linked to taster status before and could possibly indicate a wider effect of increased innervation

5.4.1 Conclusions and Future directions

The epithelium of the lip is considered glabrous skin and as such should not possess CT afferents, this study however, indicates that CT like behavioural responses are present in the lower lip. This could explain the makeup industry's lipstick success, the extensive use of lip balms and be associated with the pleasure of kissing. Further detailed investigation is required to replicate the findings before certainty of their presence can be confirmed. This could be done utilising an RTS to allow for greater control over velocity and stroke pressure, it would also remove researcher influence on the data over repetitive stimulations.

Ideally the search for CTs in the lip would be done through the method of microneurography that allows for direct single unit nerve recordings in responses to stimulation (Vallbo, Hagbarth & Wallin, 2004) and has previously been used to find CTs in other areas of the body (Liljencrantz & Olausson, 2014; Löken,, Wessberg, Morrison, McGlone & Olausson, 2009).

CT activity has been seen to change on the basis of temperature of stimulation with a greater neural response and hedonic rating obtained from neutral body temperature of 32^{o}C compared to warmer and cooler temperatures even at non-CT optimal velocities (Ackerly, Backlund Wasling, Liljencrantz, Olausson, Johnson & Wessberg, 2014). Although temperature is unlikely to have influenced the hedonic ratings obtained during the current study, the lip skin structure being considerably thinner than the skin of the arm, the location used by Ackerly, Backlund Wasling, Liljencrantz, Olausson, Johnson and Wessberg (2014), the CTs of the lips may respond differently to a warmer or cooler touch and warrants exploration.

Chapter 6 : The Role of Serotonin in Taste Perception

Abstract

Taste thresholds are frequently altered by illnesses such as depression and anxiety with research indicating that depressed individuals report a decrease in sensitivity to all tastes (Amsterdam, Settle, Doty, Abelman & Winokur, 1987) but this is rectified with medication.

SSRIs have been identified as reducing the threshold levels in human participants for sweet and bitter tastes but not sour or salt (Heath et al., 2006). Some studies have also suggested a relationship between depression and PROP taste sensitivity with hyper-taster status providing a protective factor against depression (Joiner and Perez, 2004).

A tryptophan depleting (TRP-) amino acid drink was administered to 25 healthy females. After 4 hours, participants underwent a series of tests examining the effect of TRP- on touch, pain and taste perception. Only the taste perception data is reported here. A series of concentrations of sweet, sour, salt and bitter tastes were presented to participants and they were asked if they could detect the taste, how intense the taste was and how pleasant they found the taste.

Mixed measures ANOVAs were run on the data revealing no significant effect of TRP- on detection levels though on average percentage, TRP- reduced detection thresholds. For suprathreshold stimuli, intensity ratings were significantly higher and pleasantness ratings significantly lower for the bitter taste in the TRP- session than the TRP+ and taster status indicated the tolerant-tasters experienced a greater intensity of bitter in TRP- than TRP+. Tasters rated the pleasantness of the sweet taste as significantly more pleasant than tolerant-tasters.

The enhancement of the bitter intensity and increase in unpleasantness may be explained as an affective attentional bias. During depression, research has indicated that attention is increased towards negative stimuli.

6.1 Introduction

Taste perception plays an important protective role in the evolutionary survival of species. It evolved to drive the intake of nutrients but also to aid in the avoidance of poison. Taste thresholds are genetically determined and do not greatly vary day to day (Heath, Melichar, Nutt & Donaldson, 2006). It is this lack of variation that exposed the genetic polymorphism of taster status and the identification of tolerant-tasters and hyper-tasters (Bartoshuk, 2000). Taster status is assessed by the sensitivity to the bitterness elicited by PROP/P.T.C with tolerant-tasters finding the intensity of the bitter taste minimal while hyper-tasters experience a significantly greater intensity of taste from the same stimuli (Bartoshuk, 1993 also see literature review chapter 1 section 1.3 pg 29 for further information). This genetic taste difference is reflected anatomically with varying densities of fungiform papillae (FP) on the tongue (Miller & Reedy, 1990). Individuals who have the highest density of FP are classed as hyper-tasters and those with the lowest density of FP are classed as tolerant-tasters (Bartoshuk, Duffy, & Miller, 1994; Oral Anatomy chapter 2 section 2.5 pg 79 for further information).

In the last two decades however, observations of plasticity and environmental modulations within the taste system has been identified (Kobayashi & Kennedy, 2002; Kobayashi, Kennedy & Halpern, 2006). Taste thresholds are frequently reported to be altered by illnesses like depression and anxiety (Miller & Naylor, 1989). Existing research indicates that depressed individuals report a decrease in sensitivity to all tastes, particularly sweet tastes in which they display blunted intensity ratings of supra-threshold stimuli which normalize on recovery (Amsterdam, Settle, Doty, Abelman & Winokur, 1987). Sweet tastes are the oldest natural reward and their hedonic evaluation is often regarded as the indicator of reward system function in both animals and humans (Berridge, 2000)

Anhedonia is a decreased experience of pleasure and is thought to be a central symptom of major depressive disorder (Snaith, Hamilton, Morley, Humayan, Hargreaves & Trigwell, 1995; American Psychiatric Association, 2000). Self-report data indicate depressed individuals may be less sensitive to aversive life events

(Wertheim & Schwarz, 1983). Behaviours reminiscent of anhedonia in humans have been found in rats who experience unpredictable chronic mild stress (CMS) as they demonstrate a decreased interest in dilute sucrose solutions (Willner, 1990), an effect that Willner has found to be maintained over a period of weeks and months if application of the stress is continued (Willner, Muscat, & Papp 1992; Willner 1997).

Some studies have also suggested a relationship between depression and PROP taste sensitivity with hyper-taster status providing a protective factor against depression (Joiner & Perez, 2004). Joiner and Perez (2004) suggest that a hyper-taster's greater aversion to the bitterness of alcohol (DiCarlo & Powers, 1998) may be a protective factor against heavy alcohol consumption thus, indirectly, reducing risk of depression associated with heavy alcohol use (see Graham, Massak, Demers & Rehm, 2007 for review). Segmenting participants based on taster status and asking about familial depression history, Joiner and Perez (2004) found that hyper-tasters reported significantly lower rates of depressive illness in their 1st degree relatives, than both tasters and tolerant-tasters. It is suggested that the taste sensitivity of hyper-tasters may lead them to experience more intense pleasure from tastes than their taster and tolerant-taster counterparts (Joiner & Perez, 2004) and as such, protect them from anhedonia, which has been identified as a central symptom of major depressive disorder (Snaith, Hamilton, Morley, Humayan, Hargreaves & Trigwell, 1995; American Psychiatric Association, 2000) and a vulnerability marker for the disorder's onset (Loas, 1996; Schrader, 1997; Shankman, Nelson, Harrow & Faull, 2010).

More recently, hedonic capacity has been found to positively correlate with P.T.C sensitivity, specifically hyper-taster status was associated with heightened hedonic capacity and tolerant-tasters had significantly lower hedonic capacity than hyper-tasters. This implies that P.T.C taste sensitivity may represent a peripheral risk factor for anhedonia (Thomas, Al-Mesaabi, Bahusain & Mutawa, 2014).

The 'monoamine theory of depression' argues that depression is a consequence of reduced circulation and concentrations of monoamines and thus neurotransmitters like NA, dopamine and 5-HT (Hirschfeld, 2000). Over recent years, these monoamines have been linked to taste on a peripheral level with taste

buds being found to release 5-HT upon taste stimulation (Huang, Maruyama, Lu, Pereira, Plonsky, Baur, Wu, & Roper, 2005). When mammalian taste buds are stimulated, the taste cells release neurotransmitters that excite the primary afferent fibres and transmit gustatory signals to the CNS. These transmitters mediate cell-to-cell interactions on the periphery and play important roles shaping the output and generating taste code for gustatory stimuli. Studies have shown that taste cells synthesize or take up a number of candidate neurotransmitters but to-date only serotonin (5-HT; Clapp, Yang, Stoick, Kinnamon & Kinnamon, 2004; Huang, Maruyama, Lu, Pereira, Plonsky, Baur, Wu, & Roper 2005), adenosine 5'-triphosphate (ATP; Finger, Danilova, Barrows, Bartel, Vigers & Stone *et al.,* 2005) and norepinephrine (NE; Huang, Maruyama & Roper, 2008) have been specifically identified as involved in the taste transduction process and are released in response to taste stimulation (Huang, Maruyama, Lu, Pereira, Plonsky, Baur, Wu, & Roper 2005).

The secretion of these transmitters have been linked to separate classes of taste cells (see anatomy chapter 2 section 2.4.4 pg 74) and specific tastes have been found to activate specific cells, thus activating transmitter secretion. Sweet, bitter and umami tastes trigger receptor (type 2) cells to secrete ATP (Huang, Maruyama, Dvoryanchikov, Pereira, Chaudhari & Roper, 2007; Tomchik, Berg, Kim, Chaudhari, & Roper, 2007) however 5-HT is released indirectly from type 2 cells with sweet and bitter stimuli (Meredith, Corcoran & Roper, 2015). ATP further modulates the function of adjacent taste cells, by exciting sensory afferents (Finger, Danilova, Barrows, Bartel & Vigers *et al.,* 2005) as well as stimulating the presynaptic (type 3) cells to release 5-HT (Huang, Maruyama, Dvoryanchikov, Pereira, Chaudhari & Roper, 2007).

Using calcium imaging with biosensor cells on single, isolated, taste cells Huang, Maruyama, Stimac, & Roper, (2008) found that the presynaptic (Type 3) cells specifically respond to sour taste stimulation by releasing 5-HT. This 5-HT release was later identified as coming directly from the cell and furthermore the sour stimulation triggered the release of NE (Huang, Maruyama, Lu, Pereira & Plonsky et al., 2005; Huang, Maruyama & Roper, 2008; Huang, Maruyama, Stimac, & Roper, 2008).

In mammals, many of the taste cells that synapse with the nerve fibres are serotonergic. These cells take up the 5-HT precursor 5-hydroxytryptophan (5-HTP; Kim & Roper, 1995). 5-HT was found to be present in the rat taste cells (Kim & Roper, 1995) a finding that was further supported by Clapp, Yang, Stoick, Kinnamon & Kinnamon, (2004) when they examined rat circumvallate papillae and found that when stimulated they showed 5-HT immunoreceptive activity. Furthermore, in Chinese hamster cells Huang, Maruyama, Lu, Pereira, Plonsky, Baur, Wu, & Roper, (2005) found that taste buds released 5-HT when depolarized by potassium chloride (KCl) or stimulated with bitter, sweet and sour tastants.

Human research exploring the role of neurotransmitters involved in taste perception is limited, however in illnesses like anxiety and depression, that alter 5-HT and NA, disturbances in taste perception is often reported. Treatment of these illnesses often involves the administration of repeated doses of SSRIs, such as citalopram, that with prolonged use increases tonic levels of 5-HT. Research conducted using SSRIs are often done by administering an acute dose of an SSRI, which reduces tonic 5-HT levels (Chamberlain, Müller, Blackwell, Clark, Robbins, & Sahakian 2006) rather than increasing it as repeated doses would (see Cools, Roberts & Robbins, 2007).

Heath, Melichar, Nutt and Donaldson (2006) measured the taste function of 20 healthy individuals before and after treatment with the SSRI paroxetine, the NA reuptake inhibitor (NARI) reboxetine or a placebo. Participants were presented with various concentrations of sweet, sour, salt and bitter tastes and they were asked to report if they could detect the taste. The acute dose of SSRI significantly reduced the threshold levels for sweet and bitter tastes by 27% and 53% respectively, while there was no significant effect of the SSRI on sour and salt taste thresholds. In contrast, the NARI significantly reduced detection thresholds for bitter and sour tastes by 39% & 22% respectively. Overall, while detection thresholds for salt were not affected by either the SSRI or the NARI, both increased bitter thresholds. This implies that levels of these neurotransmitters determine normal taste threshold (Heath, Melichar, Nutt & Donaldson, 2006).

Together this demonstrates that 5-HT has an important role in the peripheral and central perception of taste. Previous research focuses on the

general presence of 5-HT in the taste cells and when it is released with the human experimental studies looking at the peripheral effects of 5-HT on taste detection. This study looks to expand on this by changing tonic 5-HT levels using acute tryptophan depletion (ATD). This technique is based on the premise that by depleting plasma tryptophan (TRP) levels, the precursor of 5-HT, depletion in brain 5-HT is observed (Hood, Bell & Nutt, 2005).

The aims of the current study were to examine not only peripheral taste perception under the influence of reduced 5-HT but also the central perceptions of intensity and pleasantness. It was hypothesised that the ATD manipulation would:

1) Reduce detection thresholds of tastes, specifically sweet and bitter as identified by the previous research.
2) Increase the perceived intensity of the tastes.
3) Decrease the perceived pleasantness of the tastes.
4) Finally, taster status plays an important role in taste perception and given that previous research speculates that taster status works as a protective feature from depression it is hypothesised that taster status will influence taste perception with hyper-tasters reporting lower detection thresholds and higher intensity of tastes. However, if taster status has a protective function then hyper-tasters should experience less of an effect of ATD manipulation on detection, intensity and pleasantness ratings than their taster and tolerant taster counterpart.

6.2 Methods

6.2.1 Participants:

Twenty-five healthy female participants with a mean age of 20.92 years (SD = 0.44) were recruited for this study. Only female participants were included in this study to avoid the confound of gender on the data. Females are also twice as likely to be affected by depression as males (Hamet & Tremblay, 2005) and are seen to be more susceptible to the effects of the ATD (Nishizawa, Benkelfat, Young, Leyton, Mzengeza, & de Montigny, *et al*. 1997; Bell, Hood & Nutt 2005). Participants were recruited through Liverpool John Moores University (LJMU) and University of Liverpool recruitment emails, poster adverts and the snowball effect

The inclusion criterion were that all participants had to be non-smoking females aged between 18 and 45 years. Participants could not have any history of psychiatric illness and needed to score less than nine on the Beck Depression Inventory (Beck, Ward, Mendelson, Mock & Erbaugh, 1961) at screening, have no history of any neurological disorder, no medical conditions including heart abnormalities or heart conditions. Participants could not currently be using any medication except non-steroidal asthma inhalers or hormonal contraceptive and have normal or corrected to normal vision. In the 4-week period leading up to testing, participants must not have taken any street drugs, must drink less caffeine than the equivalent of 6 strong cups of tea/coffee per day, consume less than 30 units of alcohol per week, not suffer from chronic sinusitis, diabetes, have any disorder affecting taste or dry mouth or affecting pain perception. Finally, participants were screened for known relevant food allergies and must not be or suspect they may be pregnant. The participant was reimbursed for their time in Love2Shop vouchers at the end of the testing sessions.

This study was granted ethical approval by the Liverpool John Moores University Research Ethics Committee on 8th April 2015 (REF: 15/NSP/034).

6.2.2 Materials:

6.2.2.1 Amino Acid Drink

The amino acid drink was based on that of Young, Smith, Pihl & Ervin (1985). Due to the lower average body weight of females than males the quantities of amino acids used were 80% of the original quantities (Hood, Bell & Nutt, 2005). The additional benefit of reducing the quantities is that it reduces the nausea and vomiting side effects of the drink (see Table 6.1).

Table 6.1 Amino Acid drink quantities.

Amino Acid	Quantity (g)	Amino Acid	Quantity (g)
l-Alanine	4.58	**l-Arginine**	4.08
l-Cystine	2.25	**l-Glycine**	2.67
l-Histidine	2.67	**l-Isoleucine**	6.67
l-Leucine	11.25	**l-Lysine monohydrochloride**	9.17
l-Methionine	2.50	**l-Phenylalanine**	4.75
l-Proline	10.17	**l-Serine**	5.75
l-Threonine	5.42	**l-Tyrosine**	5.75
l-Valine	7.42	**l-Tryptophan (T+ group only)**	1.92

The control (placebo; TRP+) drink contained all of the amino acids in the quantities listed in Table 1 and the tryptophan depleting (TRP-) drink did not contain the 1.92g of tryptophan. Every participant completed two experimental sessions, during one session they received the tryptophan depleting drink (TRP-) and during the other session they received the control drink (TRP+). Drink order delivery was randomized and double blinded.

The amino acids for each drink totalling, 100g of amino acids, were weighed out in advance of the experimental session. The drink was made just before consumption on the morning of the testing session. Using a blender, the amino acids were mixed with 150 ml of water and some flavouring (chocolate or strawberry ice cream syrup) to make the amino acid drink more palatable.

6.2.2.2 Tastants

All of the stimuli were made by dissolving the required tastant in 100ml of filtered water. The samples were stored in a refrigerator for a maximum period of two weeks before being disposed of and replaced. Eight concentrations of both sweet (sucrose; Sigma) and sour (citric acid; Sigma) ranging from 1M to 0.316mM were made and divided into half log steps (see Table 6.2).

Table 6.2 Sweet (sucrose) and sour (citric acid) taste millimolar concentrations, quantity of each dissolved in water to create the concentration and the respective log step. Eight concentrations of each taste were used in this study.

Log step	mM	Sucrose g/100ml	Citric Acid g/100ml
0.0	1000	34.229	19.212
-0.5	316	10.816	6.071
-1.0	100	3.423	1.921
-1.5	31.6	1.082	0.607
-2.0	10	0.342	0.192
-2.5	3.16	0.108	0.061
-3.0	1	0.034	0.019
-3.5	0.316	0.011	0.0061

Seven concentrations ranging from 3.16M (saturation point) to 3.16mM of salt (NaCl; Sigma; see Table 6.**3A**) and seven concentrations of bitter (quinine; Sigma; see Table 6.**3B**) ranging from 3mM to 0.00316mM were made and divided into half log steps.

Table 6.3 **A**) Salt (sodium chloride) **B**) bitter (quinine) taste millimolar concentrations, quantity of NaCl dissolved in water to create the concentration and the respective log steps.

A

Log Steps	mM	g/100ml
0.25	3160	18.46704
0.0	1000	5.844
-0.5	316	1.846704
-1.0	100	0.5844
-1.5	31.6	0.1846704
-2.0	20	0.05844
-2.5	3.16	0.01846704

B

Log Steps	mM	g/100ml
-2.5	3	0.125
-3.0	1	0.040
-3.5	0.316	0.0125
-4.0	0.1	0.004
-4.5	0.0316	0.0013
-5.0	0.01	0.00040
-5.5	0.00316	0.000125

6.2.3 Measures:

6.2.3.1 Taster Status:

Taster status was assessed using the standard PROP soaked filter paper method outlined in the methodology chapter (section 3.3 pg 92). The 25 participants consisted of 16 Tasters and 9 tolerant-tasters.

For the taste task participants responded to three questions presented on a computer running E-Prime. The first asked them to indicate if they detected a taste and responded with a Y for yes and N for no. The second was to rate the intensity of the taste and used the same LMS as the taster status and the final question asked them to indicate how pleasant or unpleasant they found the taste using a Visual Analogue Scale (VAS). The middle of the scale was labelled 0, the two extreme anchor points on the scale were 'very unpleasant' on the left and 'very pleasant' on the right. For further information on the scales used, see methodology chapter (methodology chapter 3-section 3.2.1 pg 88).

6.2.3.2 Questionnaires

Mood was assessed using two self-report questionnaires, the Profile of Mood States (POMS) (McNair & Lorr 1971) and the Fawcett-Clark Pleasure Scale (FCPS) (Fawcett, Clark, Scheftner & Gibbons 1983). These questionnaires were completed a total of three times, first before the administration of the amino acid

drink to obtain a baseline mood measurement, second before the main experimental session commences 4.5 hours after drink administration and finally before the leaving the laboratory at the end of the experiment.

6.2.3.3 Taste Experimental task

The taste experimental protocol utilised in this study was adapted from that of Heath, Melichar, Nutt and Donaldson (2006). Four of the five basic tastes were used in the study (sweet, sour, salty and bitter) at increasing levels of concentration from very low/almost undetectable to high and in some cases saturation point. The tastes were generated from sucrose, citric acid, sodium chloride and quinine respectively and representing half log steps. A small adaptation from Heath, Melichar, Nutt and Donaldson (2006) who used citric acid for eliciting a sour taste and divided the tastes into quarter log steps.

Tastant delivery order and concentration within each taste were randomized. Low concentrations of one tastant may be misidentified as another (Pilkova, Novakova & Pokorný 1991) so participants were informed about which taste modality they were receiving but not told whether it was expected that they would be able to identify the taste from the concentration. The first concentration of each taste that was experienced was at supra-threshold level so the participant could be sure of the taste they were looking for.

The tastants were applied in solution form to the tip of the tongue using a cotton bud that was saturated in the solution. The cotton buds were placed on the tongue for approximately 5 seconds (Prutkin, Fast, Lucchina & Bartoshuk, 1999) and participants were asked to respond to three questions on the computer. The first question was to identify if they could perceive a taste with a simple yes/no response. The second questions asked the participant to rate the intensity of the taste they perceived and the final question asked them to rate how pleasant or unpleasant they found the taste. Each concentration of taste was presented to the participant three times. This approach differed from Heath, Melichar, Nutt and Donaldson (2006) in that they only asked participants to indicate if they could detect a taste and they repeated the ratings for each concentration 5 times.

6.2.4 Procedure:

The following reported study was conducted as part of a larger ATD project. Only the parts of the procedure relevant to this study are reported here. For further general information regarding ATD, see Methodology chapter 3 section 3.4 pg 94.

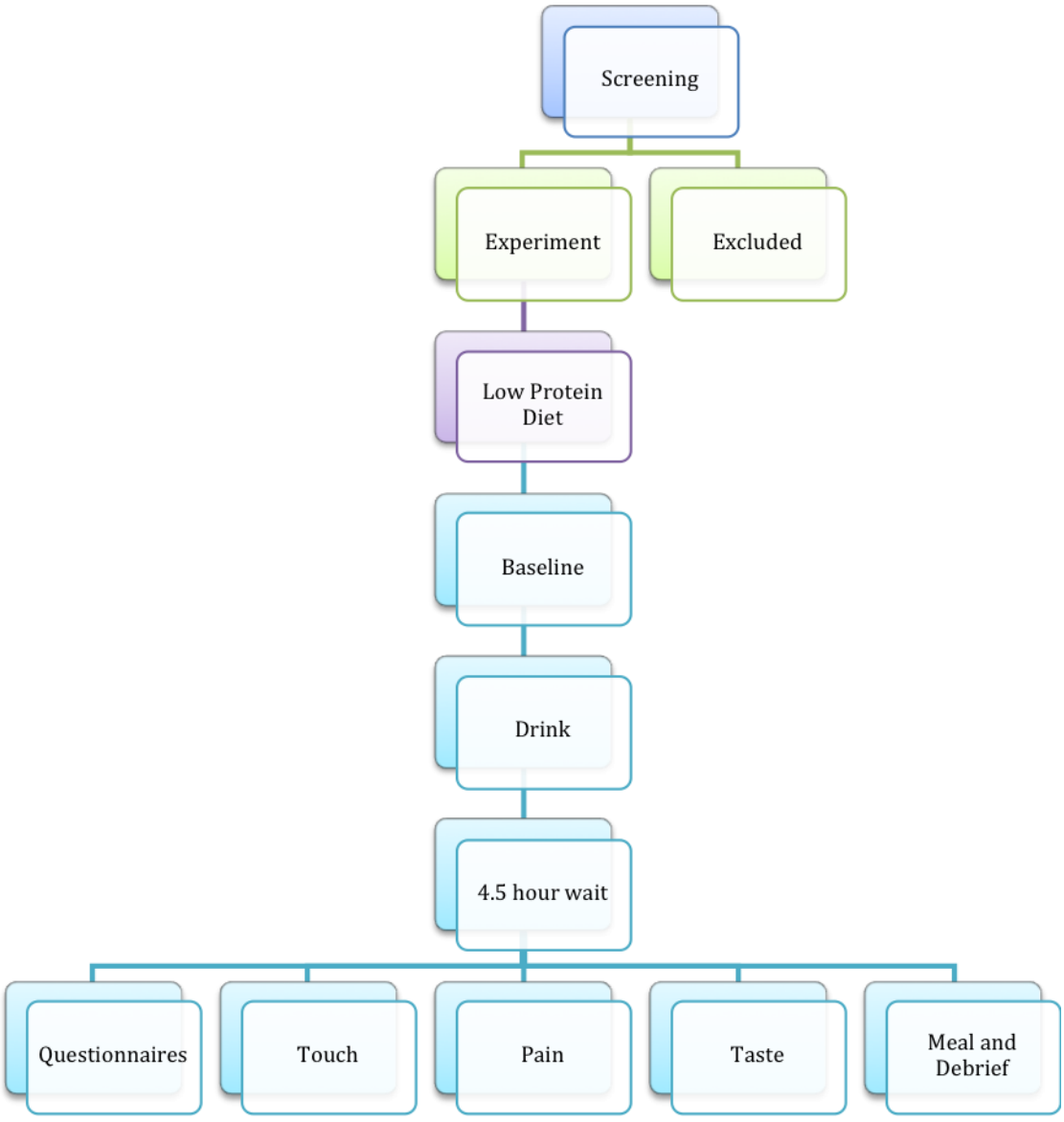


Figure 6.1 Flow chart for the entire experimental procedure that participants underwent from screening to debrief.

6.2.4.1 Screening Session

A screening session was completed at least 2 days before the first experimental session. During the session, the participant was informed about what was involved in the study and informed consent was obtained. The screening session was to ensure that participants met the inclusion criteria. The Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, text revision (DSM-IV-TR) (American Psychiatric Association, 2000) Axis I Disorders – Non-patient edition (SCID-I/NP) (First, Spitzer, Gibbon & Williams, 2002) was used to determine whether or not participants have a psychiatric history. This tool is used for research purposes only and is not a diagnostic tool. The Beck Depression Inventory (BDI) (Beck, Ward, Medelson, Mock & Erbaugh, 1961), a self-report questionnaire, was used to verify participants were not currently depressed, participants with a score greater than 9 were excluded. Taster status was assessed using the standard protocol outlined in methodology chapter 3 section 3.3 pg 92.

Participants were asked to follow a low-protein diet the day before each testing session and to not eat from midnight onwards on the day of the testing session, not to drink alcohol for 24 hours before the session and not to drink any caffeinated drinks on the morning of the session. They were also asked to not take any pain-relieving medication on the morning of the testing session. Participants were encouraged to use public transport rather than drive themselves to the experimental sessions in case they experience side effects from the amino acid drink such as nausea and/or fatigue.

6.2.4.2 Experimental Sessions

On the test days, participants were asked to arrive at the laboratory between 8.30 and 9am. Before the amino acid drink was administered several baseline measurements were taken. These included blood glucose and pressure levels taken at baseline, after lunch before the main testing session began and finally after the tryptophan repleating meal at the end of the session before participants left. To measure blood glucose the participant's fingertip was cleaned

with an alcohol wipe, then a sterile, single use lancet was used to prick the participant's finger. A drop of blood was taken from the finger and placed in the machine used to measure blood glucose. The machine provided a readout of the blood glucose level immediately. An electronic blood pressure monitor was used to determine blood pressure.

The POMS (McNair & Lorr, 1971) and FCPS (Fawcett, Clark, Scheftner & Gibbons 1983) were completed as baseline measurements. Two blood samples were obtained by venepuncture. Samples were collected in the morning before testing began and 4.5 hours after drink administrations before the experimental session began. Blood samples were stored according to the Human Tissue Act (HTA) regulations on the day of testing. The samples were centrifuged at 10 000 rpm for 5 minutes to allow separation of blood cells from plasma. Plasma was removed from the cells and stored separately to the blood cells with the blood cells stored at -20^{o}C and the plasma stored at -70^{o}C until it could be analysed. The blood cell analysis is not part of this study but the plasma was analysed using an Enzyme Linked Immunosorbent Assay (ELISA) to determine total plasma tryptophan concentration.

The participant was given the amino acid drink in a randomized order so that half of the participants receive the tryptophan depleting drink during the first testing session and the other half received the balanced drink during first testing session. The drinks were administered double blind, so both participant and experimenter did not know which drink has been administered. Participants were asked to drink all of the mixture within 15 minutes.

Participants were then required to wait for 4.5 hours for the drink to take effect. During this time, participants were provided with a bed to lie on and rest if they liked and with neutral films to pass the time. Participants were allowed to bring in their own reading materials, work etc. to do during this time as long as it was emotionally neutral and did not significantly reduce or elevate their mood. Participants were given lunch which contained less than 2 g of protein and consisted of 5 crackers, a teaspoon of jam and jelly pot.

During the following three hours the participants were involved in experimental tasks. One of these tasks was a taste perception task that is outlined in measures.

6.2.4.3 Tryptophan Repletion

Once the experimental session was complete, final blood glucose and pressure readings were taken and participants were given a meal that contained tryptophan. While eating the meal, participants were given advice about what to do should they feel unwell after they return home including the researcher's contact telephone number. The researcher conducted a leaving interview to check the participant felt well enough both physically and mentally to leave the lab and return home. If the participant did not feel well enough to leave the researcher remained with the participant until they felt well enough.

6.2.5 Statistical Analysis:

The data was assessed for outliers and Q-Q plots highlighted that the data was non-normally distributed. Attempts to correct this with transformation were unsuccessful, therefore further analysis was run on the original data. Assessment of the data's skewness and kurtosis by z scoring and dividing by the SE indicated two thirds of the data were within allowable limits below 1.96 (Field, 2009). Levenes test for homogeneity of variance also indicated that the majority of group variances were equal and Mauchly's tests of sphericity were examined and where appropriate Greenhouse Geisser corrections to degrees of freedom are reported.

Analysis was conducted to assess threshold detection and explore the differences in ratings between the tryptophan (TRP) manipulations and the relationship between the TRP intensity and pleasantness perceived by the participant. Where appropriate with identified main effects, further investigations were completed using Mixed and Repeated measures ANOVA's and paired sample t-tests. Further exploratory Repeated measures ANOVA analysis was conducted to examine the effect of taster status on the detection and perceive intensity and pleasantness.

6.3 Results

Analysis of the blood samples shows that the total plasma tryptophan increased significantly after the control TRP+ drink ($p<.001$) and decreased significantly after the TRP- drink ($p<.001$). When compared, the tryptophan levels were found to be significantly higher in the TRP+ session than in the TRP- session 4 hours after drink consumption (see Table 6.4).

Table 6.4 Total plasma tryptophan before and after amino acid drink consumption for both the control (TRP+) and tryptophan depletion (TRP-) sessions.

	TRP+		**TRP-**	
	0 hours	+4 hours	0 hours	+4 hours
Plasma TRP (µmol/l)	74.6 (0.66)	185.6 (2.99)	77.9 (0.85)	22.7 (0.32)

6.3.1 Detection

The threshold detection level was established as the point at which each taste was detected 50% of the time.

The data was analysed to test the hypothesis that the taste detection ability will be reduced in the TRP- session over the TRP+. Data were analysed using a separate Repeated measures ANOVAs for each tastant. For all four tastants there was a significant main effect for concentration, with detectability increasing significantly with concentration (see Figure 6.2): sweet, (Figure 6.2**A**: $F(2.16, 51.79) = 60.10$, $p<.001$, $\eta p^2 = .72$, Power = 1.00), sour (Figure 6.2**B**: $F(2.21, 53.14) = 46.33$, $p<.001$, $\eta p^2 = .66$, Power = 1.00), salt (Figure 6.2**C**: $F(1.78, 42.81) = 75.27$, $p<.001$, $\eta p^2 = .76$, Power = 1.00) and bitter (Figure 6.2**D**: $F(2.67, 64.06) = 38.56$, $p<.001$, $\eta p^2 = .62$, Power = 1.00).

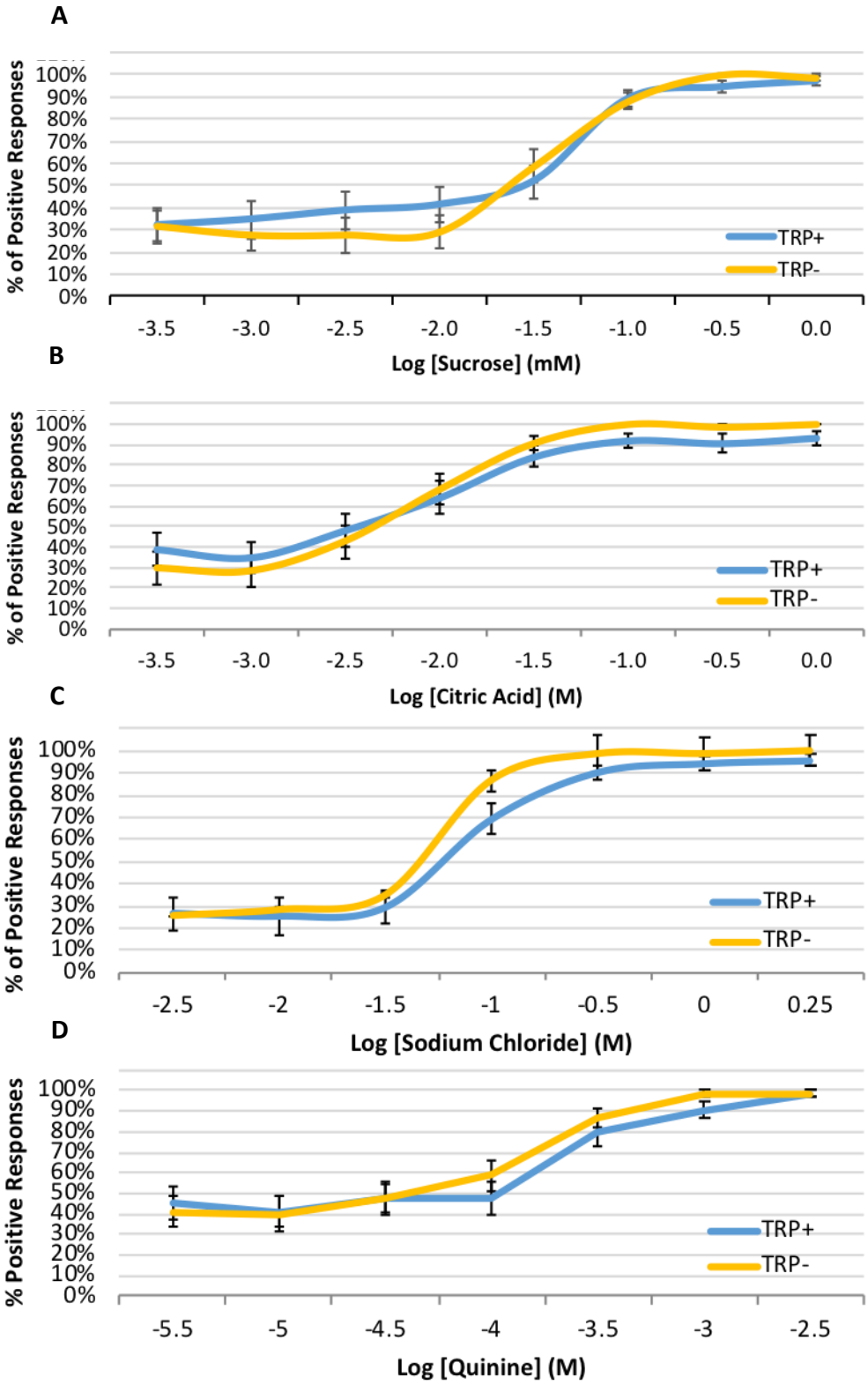


Figure 6.2 The effects of tryptophan depletion on detection thresholds for each of the 4 tastes (A Sweet; B Sour; C Salt; D Bitter) collapsed across taster status. The blue line represents the control TRP+ session and the orange line represents the TRP- session with the x-axis representing the concentration steps and the y-axis the percentage of responses confirming detection of the concentration. Significant effects for concentration were identified in all tastes (ps<.001).

Table 6.5 The percentage of supra threshold detections (i.e. concentrations detected 50% of the time more) for the TRP manipulation. Detection ability decreased in the TRP- session for the sweet (3.47%), sour (7.29%), salt (8.68%) and bitter (7.39%) tastes. The concentrations at which the taste was detected at least 50% of time were taken to be the detection threshold for each taste and that log level and over were used in subsequent analysis of intensity and pleasantness ratings.

Taste	TRP+	TRP-	% detection change	50% detection threshold
Sweet	83.33%	86.33%	-3.47%	-1.5
Sour	84.80%	91.47%	-7.29%	-2.0
Salt	87.67%	96.00%	-8.68%	-1.0
Bitter	79.33%	85.67%	-7.39%	-4.0

However, the only taste the tryptophan manipulation had a significant effect of detection threshold on was salt, (F(1, 24) = 6.83, $p<.05$, $\eta p^2 = .22$, Power = .71), with salt detection thresholds being significantly lower in the TRP- session (M = 67.43%, SE = .14) than the control session (M = 61.71%, SE = .13).

6.3.1.1 Detection with Taster Status

A subsequent analysis was conducted to test the hypothesis that a participants' taster status alters their detection thresholds. Thus 4 further Repeated measures ANOVAs were conducted, one for responses to each taste, this time with the additional between group factor of taster status, consisting of 16 tasters and 9 tolerant-tasters. However, no main effects of taster status or its interaction with either concentration or tryptophan manipulation were identified (ps>.05).

Table 6.6 The percentage of supra threshold detections (i.e. concentrations detected 50% of the time more) for the TRP manipulation depending on the respondent's taster status. Sweet taste detection decreased for both taster groups in the TRP- session. Detectability increased in the TRP- session for both taster groups and within all tastes. The concentrations at which the taste was detected at least 50% of the time was chose to be the detection threshold for each taste and that log level and over were where analysis of intensity and pleasantness data were run from.

Taste	Taster Status	TRP+	TRP-	% detection change	50% detection threshold
Sweet	Tolerant Taster	89.87%	90.74%	0.97%	-1.5
	Taster	78.25%	84.00%	7.35%	
Sour	Tolerant Taster	92.00%	94.07%	2.20%	-2.0
	Taster	81.00%	90.20%	10.20%	
Salt	Tolerant Taster	90.75%	98.25%	7.63%	-1.0
	Taster	86.25%	94.25%	8.97%	
Bitter	Tolerant Taster	75.93%	89.81%	2.40%	-4.0
	Taster	81.25%	83.25%	15.46%	

Concentrations were assessed for the percentage of detections. Though there was no significant effect of taster status the average percentage of positive taste identifications showed a pattern of TRP- reducing detection thresholds for both taster groups.

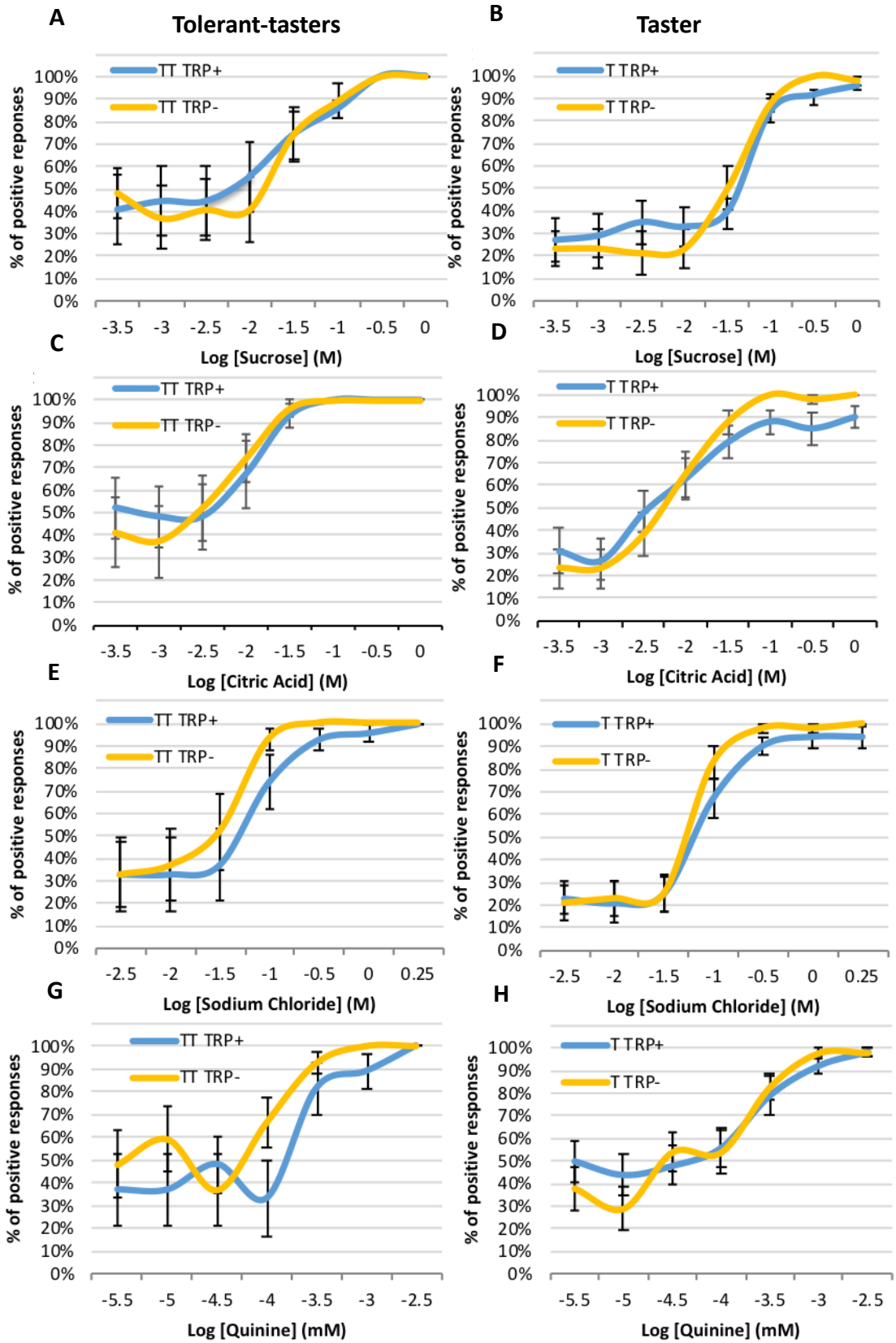


Figure 6.3 Effects of TRP- on detection thresholds for each of the 4 tastes split between the tolerant-tasters (A: sweet; C: Sour; E: Salt; G: Bitter) and tasters (B: Sweet; D: Sour; F: Salt; H: Bitter). The blue line represents the control TRP+ session and the green line represents the TRP- manipulation.

6.3.2 Intensity

Analysis of taste intensity was conducted on those concentrations that were detected 50% of the time or more (see Figure 6.4 A, B, C & D). The data was analysed to test the hypothesis that the taste intensity will increase in the TRP- session over the TRP+. As expected, for all tastes, there was a significant main effect of concentration on perceived intensity of all 4 tastes: sweet ($F(1.72, 41.33) = 74.28$, $p<.001$, $\eta p^2 = .76$, Power = 1.00), sour ($F(2.21, 50.80) = 103.36$, $p<.001$, $\eta p^2 = .82$, Power = 1.00), salt ($F(1.82, 43.58) = 119.66$, $p<.001$, $\eta p^2 = .83$, Power = 1.00) and bitter ($F(1.82, 41.81) = 72.35$, $p<.001$, $\eta p^2 = .76$, Power = 1.00).

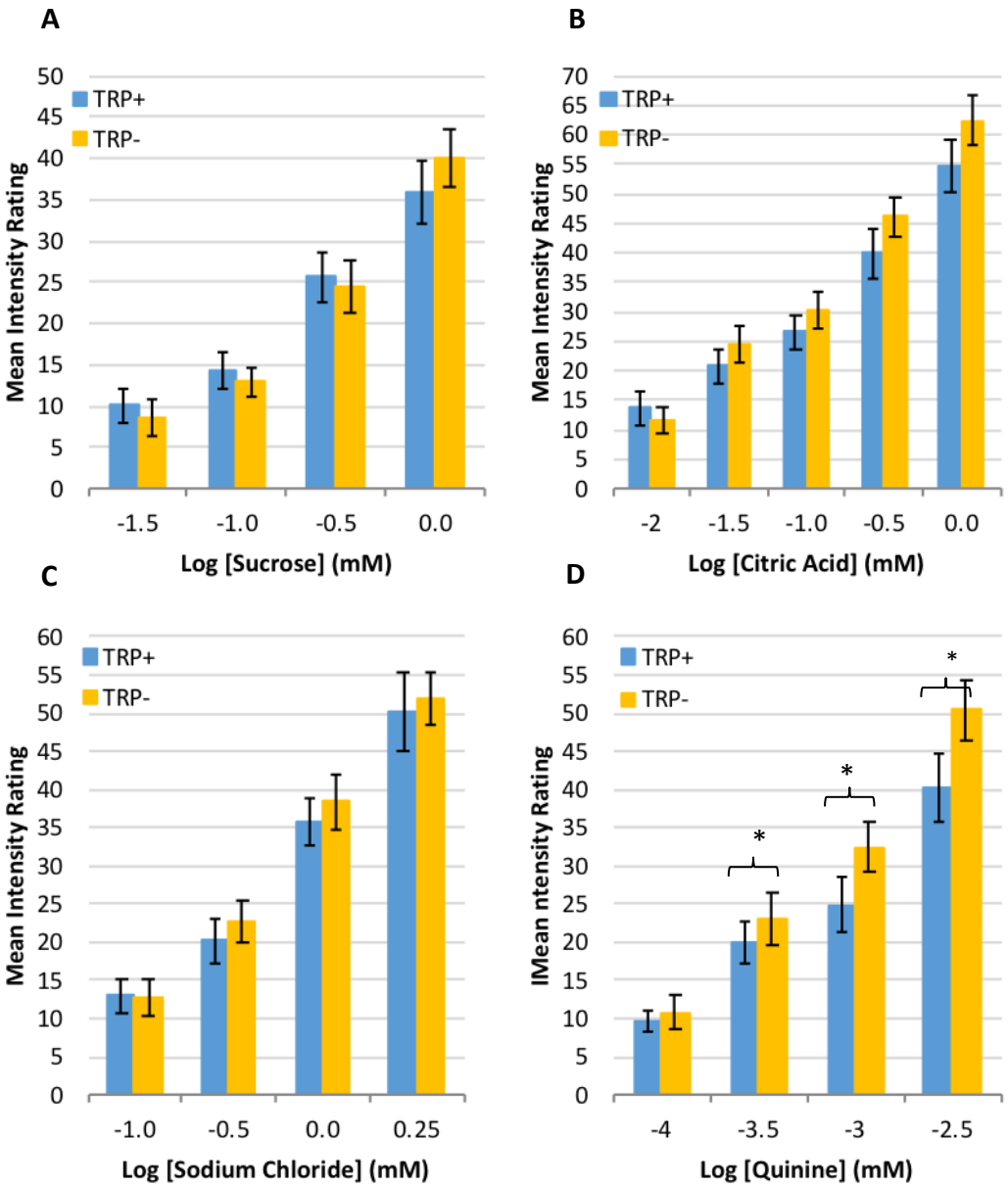


Figure 6.4 Mean intensity ratings of the above threshold concentrations for each of the 4 tastes (A: Sweet; B: Sour; C: Salt; D: Bitter). Significant main effects for concentration were identified with each taste (ps<.001).

In addition, there was a significant main effect for tryptophan manipulation on intensity ratings of the bitter quinine ($F(1, 23) = 9.86$, $p<.01$, $\eta p^2 = .30$, Power = .85). As can be seen in Figure 6.4 **D** this reflects the fact, intensity ratings were higher following tryptophan depletion (Bitter: TRP- M = 29.10, SE = 2.86, TRP+ M = 21.97, SE = 2.58). Paired samples t-test showed that TRP manipulation has a significant effect on perceived taste intensity of the 3 highest concentrations of quinine (ps<.05; see Figure 6.4**D**).

6.3.2.1 Intensity with Taster Status

A further analysis was run to investigate the effects of taster status on perceived intensity and its interaction with the tryptophan manipulation using all above threshold concentrations. However, no main effect of taster status was identified on intensity ratings of any taste (ps>.05).

A further exploratory analysis was conducted examining the effect of taster status on the perceived intensity of the highest concentration of the four tastes as shown in Figure 6.5. Significant effect of manipulation was identified in the highest concentration of bitter taste ($F(1,23) = 13.14$, $p<.001$, $\eta p^2 = .36$, Power = .93) and as shown in Figure 6.5 D with tolerant-tasters rating the bitter taste significantly more intense in the TRP depletion session.

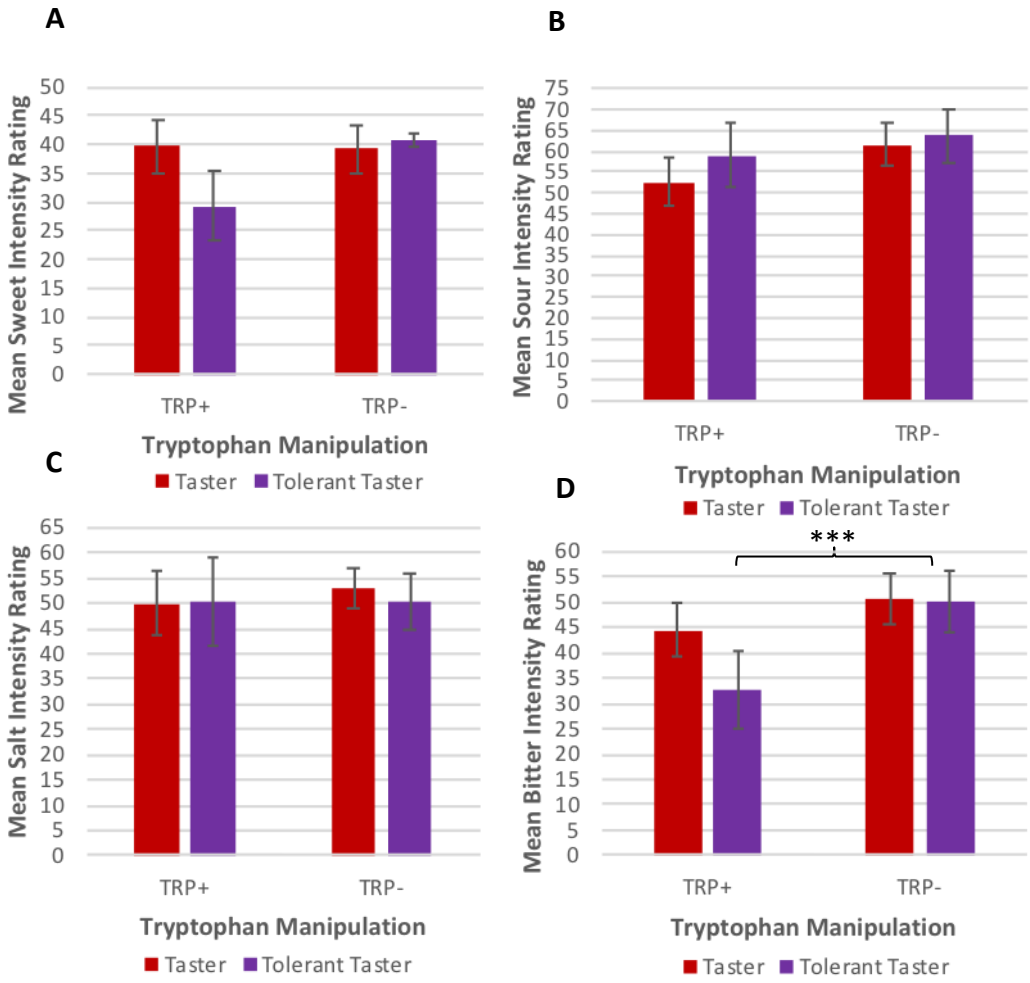
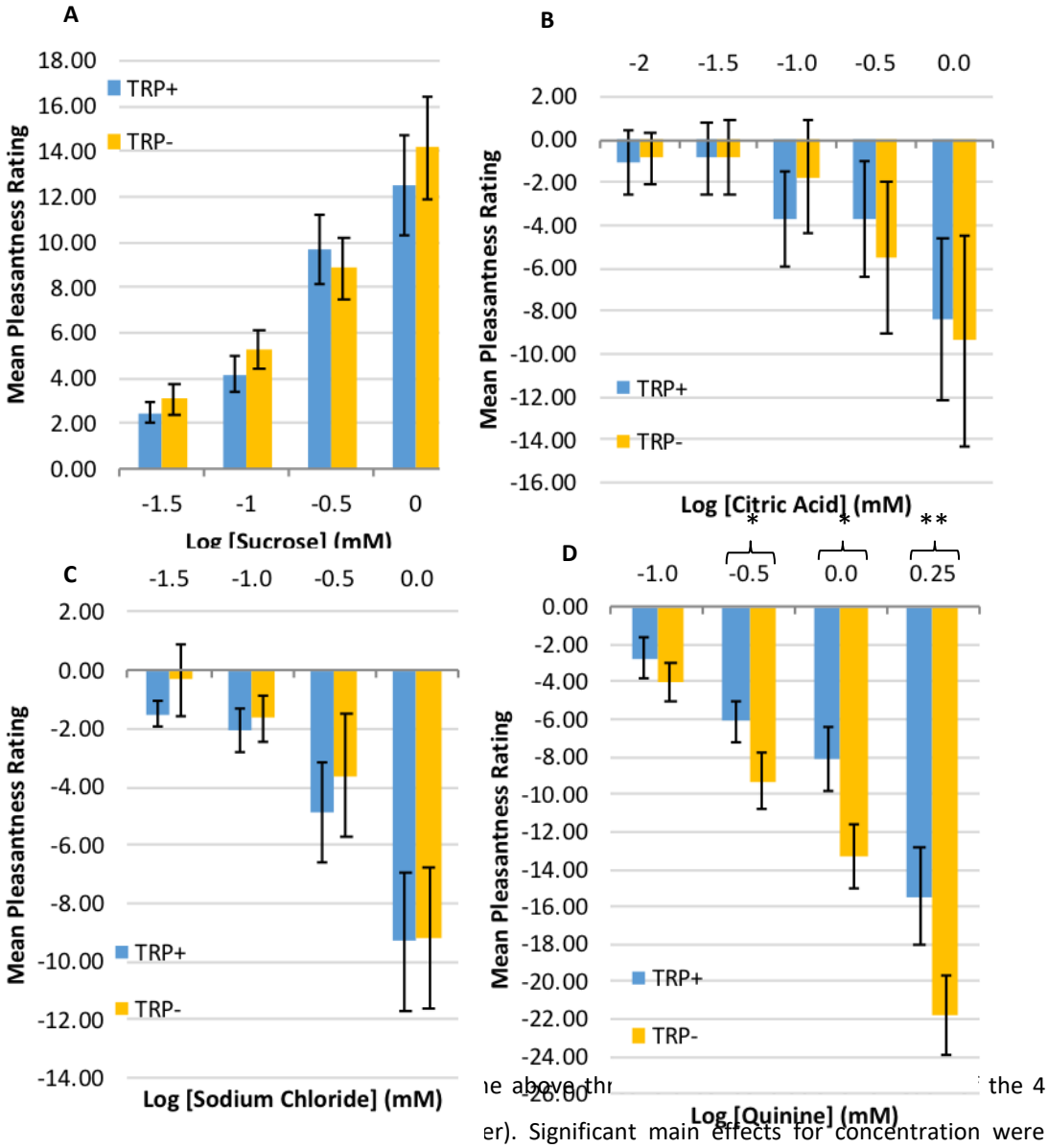


Figure 6.5 The effects of the TRP manipulation on the highest concentration of each taste split over taster status. No significant differences were found between the taster groups for any of the four tastes (A: Sweet; B: Sour; C: Salt; D: Bitter) however a significant difference for manipulation was found in the bitter taste ($p<.001$).

6.3.3 Pleasantness

Initial analysis of taste pleasantness was run on all concentrations where detection rate was 50% or more. The data was analysed to test the hypothesis that the tastes pleasantness will be reduced by the TRP-. As can be seen from Figure 6.6, there was a significant main effect of concentration on mean pleasantness ratings for each of the 4 tastes. ($F(1.35, 41.11) = 31.55$, $p<.001$, $\eta p^2 = .58$, Power = 1.00), sour ($F(1.42, 31.34) = 6.42$, $p<.01$, $\eta p^2 = .23$, Power = .79), salt ($F(1.48, 35.42) = 3.49$, $p<.05$, $\eta p^2 = .13$, Power = .53) and bitter ($F(1.49, 34.16) = 54.68$, $p<.001$, $\eta p^2 = .70$, Power = 1.00). While ratings for the sweet tastant increased with increasing

concentration, perceived pleasantness decreased with increased concentration of the other 3 identified in the pleasantness ratings of the bitter quinine ($F(1, 23) = 10.67$, $p<.01$, $\eta p^2 = .32$, Power = .88). As can be seen in the pattern of Figure 6.6 D, this reflects the fact, quinine was rated as significantly less pleasant following tryptophan depletion (TRP- : M = -12.02, SE = 1.34; TRP+ : M = -8.52, SE = 1.06). Paired samples t-test showed that TRP manipulation has a significant effect on perceived pleasantness of the 3 highest concentrations of quinine (ps<.05; see Figure 6.4D).



ıe above thr the 4 er). Significant main effects for concentration were identified with each taste (ps<.05).

6.3.3.1 Pleasantness with Taster Status

To investigate the effect of taster status on pleasantness ratings, an additional ANOVA was run with the added between subject factor of taster status.

A significant main effect of taster status was identified for the pleasantness rating of sweet tastes ($F(1, 23) = 5.22$, $p<.05$, $\eta p^2 = .19$, Power = .59). In the sweet taste, tasters (M = 8.83, SE = 1.05) rated sweet as more pleasant than tolerant-tasters (M = 4.92, SE = 1.48). A trend towards an effect of taster status with in the salt rating pleasantness was identified ($F(1, 23) = 3.95$, $p = .06$, $\eta p^2 = .15$, Power = .48) with tolerant-tasters finding the salt taste more unpleasant (M = -9.66, SE = 3.41) than tasters (M = -1.18, SE = 2.56).

A further exploratory analysis was conducted examining the effect of taster status on the perceived pleasantness of the highest concentrations of the four tastes as shown in Figure 6.7 Significant effect of manipulation was identified in the highest concentration of bitter taste ($F(1, 23) = 7.57$, $p<.01$, $\eta p^2 = .25$, Power = .75) with TRP- significantly more unpleasant than TRP+ but no significant effect of taster status was found in any of the tastes. Paired t-tests shown in Figure 6.7 D that tasters rated the bitter of quinine to be significantly less pleasant in the TRP- than the TRP+.

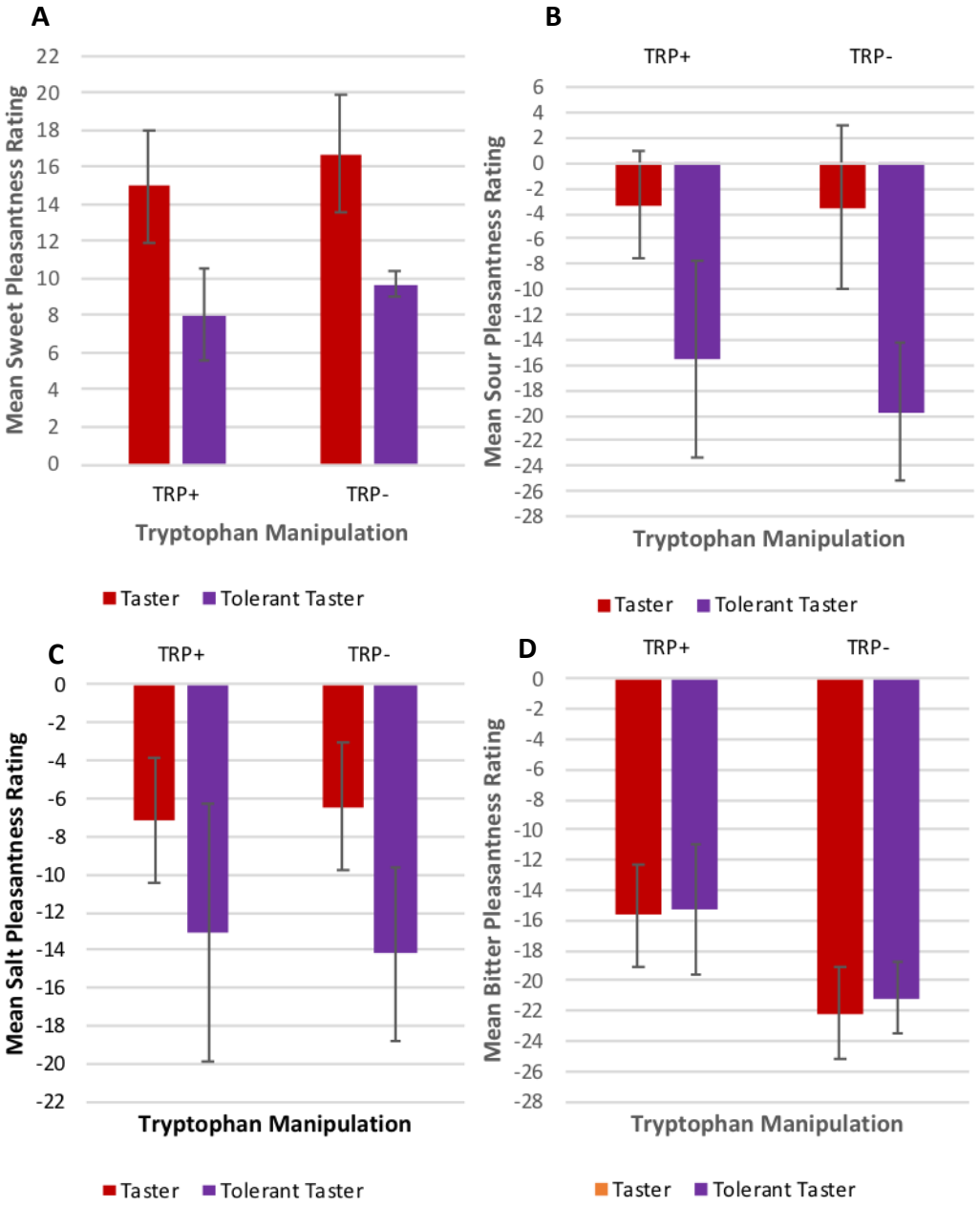


Figure 6.7 The effects of the TRP manipulation highest concentration pleasantness ratings split over taster status. A significant difference in rating was found with bitter (**D**) being rated as significantly less pleasant overall in the TRP- session than the control TRP+ session ($p<.05$). Taster status did not significantly affect the pleasantness ratings between the sessions for either the sweet (**A**), sour (**B**) , salt (**C**) or bitter (**D**) tastes ($ps>.05$).

6.3.4 Hyper-tasters

The data was further explored over three taster groups but with only 3 hyper-tasters, the power of the analysis was significantly decreased. No main effect of taster status was identified in either, detection, intensity and pleasantness. The only changes to the data come in the pleasantness ratings of bitter. When the data is segmented over three taster groups the effect of tasters rating bitter as significantly less pleasantness in the TRP- than the TRP+ is lost with all taster groups not possessing a significant difference in pleasantness ratings between sessions. This also occurs when the hyper-taster data is removed from the analysis.

6.4 Discussion

The primary aim of this study was to explore the effects of an ATD manipulation on the peripheral and central perceptions of tastes and to examine the role taster status plays in taste perception under these conditions. It was hypothesised that the TRP- would reduce detection thresholds specifically for the sweet and bitter tastes. In the current study, however, this was not identified. No significant difference in detection threshold was seen between the TRP+ and TRP- sessions. This contrasts with the findings of Heath, Melichar, Nutt and Donaldson (2006) who reported a significant reduction in threshold levels for both sweet and bitter tastes. Examination of the raw percentage detection scores in the current study however, do indicate a trend towards reduction in taste threshold in all tastes in the TRP- session compared to TRP+ with the only manipulation effect on detection being seen is salt detection with a reduced detection threshold in the TRP- session. Taster status had no effect detection threshold with the ATD manipulation. Heath, Melichar, Nutt and Donaldson (2006) did not consider taster status in their study; however, the large effects they found may have varied between taster statuses. The different approaches to 5-HT manipulation between the current study and Heath, Melichar, Nutt and Donaldson (2006) may explain the different findings. The ATD approach reduces tryptophan levels and consequently tonic 5-HT levels, whereas Heath, Melichar, Nutt and Donaldson (2006) administered an acute dose of an SSRI that directly reduces 5-HT.

The second hypothesis was that the TRP- would increase the perceived intensity of the tastes was supported. Bitter taste concentrations were rated as significantly more intense during the TRP- than the TRP+ session. Examination of the highest concentrations of each taste identified that tolerant-tasters perceived a greater intensity from the bitter quinine during TRP- than TRP+. No effect was found in the other tastes or between tasters and tolerant-tasters.

Finally, the effect of TRP- on perceived pleasantness of tastes was assessed hypothesising that the TRP- would decrease the pleasantness/increase the unpleasantness of the tastes. Furthermore, previous research suggests that taster status may have a protective function against anhedonia and depression (Joiner

and Perez, 2004; Thomas, Al-Mesaabi, Bahusain & Mutawa, 2014) therefore it was hypothesised that hyper-tasters would less likely aware of the effects of the TRP-. Overall, this study only found an effect of perceived pleasantness in the bitter taste with the reported taste being significantly less pleasant in the TRP- than the TRP+ session. Within the highest concentration and effect of taster status was found in the bitter quinine with tasters perceiving a significantly less pleasant taste in the TRP- than the TRP+. Taster status also had an effect on the sweet taste with tasters perceiving sweet as significantly more pleasant than tolerant-tasters when manipulation is disregarded. This is an unusual finding as tasters and hyper-tasters tend to show less liking for foods that have a high sweet content (Duffy & Bartoshuk, 2000; Looy & Weingarten, 1992) and tolerant-tasters report consuming more sweet food than tasters (Duffy, Peterson, Dinehart & Bartoshuk, 2003). It does not offer support for the hypothesis that taster status offers protection from the effects of depression anhedonia, however, as no interaction between taster status and manipulation was identified.

Combined the overall effect of ATD on taste perception and their interactions with taster status are mixed. Though the ATD manipulation had no effect on participants ability to detect any of the tastes it did alter perceived taste intensity and pleasantness.

It is possible that the findings of Heath, Melichar, Nutt and Donaldson (2006) aren't significantly different because the effect isn't large enough, only a small amount of 5-HT may be involved in taste detection but there was a percentage of change between treatment and control. This could be why the ATD manipulation didn't effect detection. Altering 5-HT levels is a by-product of the ATD so the level of change may not have been great enough to alter the involvement of the 5-HT in taste detection. However, the level was great enough to alter perceptions of intensity and pleasantness which are subjective aspects of oral perception. The majority of the effects of ATD were found in the bitter tastant with an increase in intensity and decreased pleasantness. A reduction in tryptophan could trigger a primitive defence mechanism indicated by the changes in bitter perception. Where food is concerned bitter tastes commonly indicate poison from

plant life and should be avoided, the increase in sensitivity could indicate that the body is already in a weakened state and as such potential toxins should be avoided. Interestingly this effect was also seen to be different on the basis of taster status. As a tolerant-taster the general oral feedback from foods is dulled compared to tasters however, the tolerant-tasters in the current study reported a greater intensity at supra-threshold potentially a further defence mechanism specific to the tolerant-taster status amplifying the intensity of bitter tastes during the tryptophan depletion session to encourage food avoidance as tolerant-tasters generally don't experience bitter tasters.

Future research could expand on the current study's findings by examining if 5-HT has any influence on the perception of the heat perception from capsaicin, the astringent sensation from alum or the tingle from sanshool. There may not be a peripheral effect as it is unknown if 5-HT plays a role in the transduction of these sensations but it is likely to have a central effect of the perceived intensity and pleasantness of the.

Furthermore, the data in the present study was only analysed regarding tasters and tolerant-tasters due to there only being three hyper-tasters identified in the participants. Though the data was examined over three taster groups, it did not change the outcome in a significant way and the primary outcomes remained the same even when analysed without the data from the hyper-tasters. Increasing the number of participants would increase the number of hyper-tasters in the experimental manipulation. It would be advantageous to be able to examine the effects of reduced 5-HT over the three groups even though an effect is still established with two groups.

This study highlighted that affective responses are altered during depression. The enhancement of the bitter intensity and decrease in perceived pleasantness may be explained as an affective attentional bias. During depression, research has indicated that attention is increased towards negative stimuli (Gotlib, Kasch, Traill, Joormann, Arnow, & Johnson, 2004; Gotlib, Krasnoperova, Yue & Joormann, 2004). Furthermore, the induced conditions from the ATD may signal a weakness or illness and as such these adaptations reduce the risk of further

damage from consumption of poisonous plant compounds that commonly taste bitter (Reed & Knaapila, 2010).

Chapter 7 : The Oral Lexicon

Abstract

The McGill pain questionnaire was a ground-breaking sensory lexicon when it was developed. It acknowledged that pain was more than just an evaluative experience, it was also a sensory and affective one. This made it an ideal framework for development of other lexicons and it was successfully applied to create a touch perception task lexicon.

Over the years numerous oral sensory lexicons have been developed for assessing the qualities of specific products like red and white wines. To be correctly applied these lexicons usually require a specially trained panel. The aim of this study was to apply the procedures that were used to develop the McGill Pain Questionnaire and the touch perception task in order to generate a candidate oral sensory lexicon.

The candidate oral lexicon that was developed in this study highlights that the procedure set out for developing the McGill pain questionnaire is an applicable method to create a tool that can generate a standardised language applicable to oral sensations. This is probably as close as possible to assessing in detail the oral experience as there is no mechanical or physical device that can do what the mouth, nose and brain can do at detecting and evaluating tastes.

7.1 Introduction

Sensory flavour testing uses panels or groups of people to measure flavour. This type of research necessitates the use of human tasters as there are no mechanical or physical devices that can do the combined work of the mouth, nose and brain in evaluating flavour. As such tools have been developed for use by human participants to try and study the sensation of flavour and these are termed lexicons.

A sensory lexicon is a standardised vocabulary that can be used to facilitate communication (Lawless & Civille, 2013). The initial requirement for lexicons arose from the need of industry and manufactures wanting to reliably evaluate products across locations (Lawless & Civille, 2013). Lexicons allow product researchers and developers to understand product attributes (Koppel & Chambers 2010) and to quantify variability within a product type (Civille, Lapsley, Huang, Yada & Seltsam, 2010).

Arthur D Little is accredited with developing the first standardised terminological approach to quantifying oral sensation in the 1940's with the Flavour Profile Method (FPM). This was not published until the 1950's when Jean Caul described the method (Caul, 1957). In the FPM, trained panellists evaluate the intensities of the flavour, aroma and aftertaste of products by rating on a 7-point scale (Caul, 1957). The principles of the FPM were later used to develop the Texture Profile Method (TPM; Brandt, Skinner & Coleman, 1963) that measured the mechanical, moisture and fat characteristics of foods.

Lexicons have also been developed to evaluate specific products, as sensation variation within certain products are clearly present. Wine is particularly known for its variety and complexity of sensations between different brands and types. This complexity is often termed mouth feel and refers to the sensations that are characterised by tactile response in the mouth (Pickering & Demiglio, 2008). In acknowledging these differences Gawel, Oberholster and Francis (2000) developed the red wine 'mouth feel wheel' which was designed to assist in the identification and classification of the complex oral sensations elicited from red wine. This was designed specifically for the red wine industry in order to have a standardised

terminology that can be applied by red wine panels to different brands and types for comparison of qualitative differences. Pickering and Demiglio (2008) later expanded on this with the development of a white 'wine wheel' aiming to ease classification and description of the qualities of white wine in a similar manner to the red wine mouth feel wheel.

Over the years multiple other lexicons related to specific products have been developed such as the McCormick Spice Wheel (Lawless, Hottenstein & Ellingsworth, 2012), a lexicon to describe the flavour of pomegranate juice (Koppel & Chambers, 2010), beer flavour terminology (Clapperton, Dalgliesh & Meilgaard, 1976; Meilgaard, Reid & Wyborski, 1982) and coffee (Hayakawa, Kazami, Wakayama, Obishi, Tanaka & Maeda *et al.,* 2010). There is one problem with all of these lexicons and that is they were all developed and used by specially trained panels of experts in their field who considered and rated the sensations elicited by the products being tested. Using these lexicons appropriately after they have been developed often requires specialised training.

This leaves the majority of oral related research that isn't conducted using the panel-developed lexicon to rely on Labelled Magnitude Scales (LMS) or Visual Analogue Scales (VAS) to collect the desired information. The VAS evolved from category and graphic scales and consists of a line with a minimum and maximum rating at either end (Bartoshuk, 2004). The removal of the category ratings on the line gave the VAS the appearance of a ratio scale (Price, McGrath, Rafii & Buckingham, 1983) and today they are extremely popular and used in a variety of fields of research. VAS's are a valuable tool when exploring the perceived pleasantness of foods (Schutz & Cardello, 2001). The LMS was originally developed by Green, Shaffer and Gilmore (1993) for rating the intensity of general oral stimuli and is a valuable tool used regularly in the assessing bitter intensity to classify taster status of individuals (Bartoshuk, 2000) (see methodology chapter 3 section 3.2 pg 88 for further information on scales). Though LMS's have substantial popularity in the field of sensory evaluation and multiple variations have been developed (e.g. Cardello, Schutz, Lesher & Merrill, 2005; Guest, Essick, Patel, Prajapati & McGlone, 2007; Lim, Wood & Green, 2009) they only specify the

evaluative or overall subjective intensity perceived and lack the ability to assess the qualitative differences between sensations in a fast and systematic way (Melzack, 1975).

The most well-known sensory lexicon that was developed to address multidimensional sensory experience rather than just intensity is the McGill Pain Questionnaire (MPQ; Melzack, 1975). What makes the MPQ so different from other lexicons and scales is that it accounts for multiple aspects of the sensory experience and has been the framework for the development of other lexicons. The MPQ was developed by utilising a systematic and scientific approach which began with Melzack and Torgerson (1971 as cited in Melzack, 1975).

In the first phase of the study, 102 words were compiled from descriptors previously recorded from patients experiencing pain from various conditions including phantom limb, reflex sympathetic dystrophy and back pain (Melzack, 2005) and were classified into smaller groups that described different aspects of the pain experience. This led to the identification of 3 major classes and 16 subclasses of pain descriptors each class consisting of words that were considered qualitatively similar. The largest class consisted of words that describe the *sensory qualities* of the pain experience which included the subclasses pressure, thermal, brightness and spatial among others. The second major class describes the *affective qualities* such as the subclasses of tension, fear and punishment and the third final major class consisted of *evaluative* words which describe the overall intensity of the pain experience. The second phase of the MPQ development was to determine the pain intensity implied by the words which was done by participants assigning values of intensity on a numeric scale to each word. Though some of the words were synonyms the word intensity rating demonstrated that the words reflected different levels of intensity of the same sensation for example a shooting pain represented a more intense sensation than a flashing pain which was, in turn, rated to reflect a more intense pain than a jumping one (Melzack and Torgerson 1971 as cited Melzack, 1975). Melzack (1975) assessed the usefulness of the MPQ as a tool for examining dimensions of pain and found that it provided quantitative data that was sensitive enough to detect differences among different

methods of pain relief and the effects of pain relief on the sensory, affective and evaluative dimensions of pain.

With its simple format and ease of use the MPQ can be utilised by experts and amateurs alike, providing both support in a clinical setting and information for researchers, while providing quantitative data that explores the qualitative experience highlighting that pain is both a sensory discriminatory and affective experience. Whilst this tool can assess the dimensions of pain, when it comes to peripheral nerve injury it fails to capture the subjective changes from simple tactile stimulation which can be considered painful. The hedonic attributes of touch are important to the quality of a person's life. Both the pleasant and unpleasant aspects of touch form the cornerstone of social and affiliative behaviours in both humans and other primates (Björnsdotter, Larsson & Ljungberg, 2000). Early experimental studies indicate that soft and smooth materials were considered pleasant where as those that are stiff, rough and coarse are unpleasant (Essick, James & McGlone, 1999). A specific set of C fibres termed C-tactile afferents (CT's) were identified for coding for pleasant touch and responded to a touch that reflected gentle caressing touch at a velocity between 1 and 10cm/s (Löken, Wessberg, Morrison, McGlone & Olausson, 2009).

Little was known beyond the observed pleasantness of the softness and the velocity of CT touch which is associated with the emotional experience of touch (Essick, McGlone, Dancer, Fabricant, Ragin & Phillips *et al.,* 2010) when the discovery of nerve fibres specifically designed to respond to affective touch were identified. To gain further insight into the subjective experience of touch, pleasant or unpleasant, a measure similar to that of the MPQ was developed. This measure was called the Touch Perception Task (TPT; Guest, Dessirier, Mehrabyan, McGlone, Essick, & Gescheider *et al.,* 2011) and was designed to assist with understanding the complexity of tactile experience.

Guest, Dessirier, Mehrabyan, McGlone, Essick, & Gescheider *et al.,* (2011) conducted three experiments replicating Melzack's (1975) approach to develop a touch lexicon that describes the complexity of tactile experience. Collating a word list was done by reviewing the scientific literature on tactile perception and

standard linguistic reference tools such as the dictionary, a thesaurus and encyclopaedia. This generated 262 words for participants to rate on a 4-point scale the extent to which each word described the sensory, emotional and evaluative aspects of touch (Guest, Dessirier, Mehrabyan, McGlone, Essick, & Gescheider *et al.,* 2011) following the definitions outlined by Melzack (1975). Words that obtained a mean score of 3 or more on either describing the emotional, sensory or evaluative aspect of touch were retained. They next identified dimensions of semantic-perceptual space underlying the sensory and emotional words by applying a multi-dimensional scaling (MDS) method of analysis. To do this participant rated the words on a 15-point scale of dissimilarity and the MDS analysis organises ratings into a perceptual space so that differences in meaning between words are reflected in their perceptual distinctiveness. Validation of the TPT was done by stroking participants with 5 sensory distinctive fabrics (polyester with a silky finish, with a textured finish, unpowdered latex, cotton t-shirt material and hessian) at four different body sites (upper limb, index finger pad, volar forearm, fossa of the axilla and vault of the axilla). These body sites were chosen because different these different locations vary in their tactile perception with site wise differences in both affective and sensory responses. For example, when it comes to texture perception, the finger is most adept at discriminating fine differences (Sathian & Zangaladze, 1996) whereas the forearm has a larger affective response to stimulation (Löken, Wessberg, Morrison, McGlone, & Olausson, 2009). These perceptual differences relate to differences in innervation with the hairy skin of the forearm possessing both low-threshold CTs and mechanoreceptors important for the conveyance of affective touch (Löken, Wessberg, Morrison, McGlone, & Olausson, 2009; McGlone, Vallbo, Olausson, Löken, & Wessberg, 2007; Vallbo, Olausson, & Wessberg, 1999) whereas the glabrous skin of the fingertip lacks CT afferents (Liu, Vrontou, Rice, Zylka, Dong & Anderson, 2007).

Factor analysis extracted four distinct factors from the data, roughness, slip, firmness and pile for sensory aspects and comfort and arousal for emotional aspects of touch. Responses to these dimensions varied across body site and across

the location they were being applied to (Guest, Dessirier, Mehrabyan, McGlone, Essick, & Gescheider *et al.,* 2011).

Lexicons developed using the principles and approach of Melzack (1975) in the development of the MPQ are clearly reliable and replicable as demonstrated by Guest, Dessirier, Mehrabyan, McGlone, Essick, & Gescheider *et al.,* (2011). The previously developed oral lexicons used within consumer research only consider the *evaluative* aspects of oral sensations and not the *sensory* and *affective* qualities generated. To be an effective oral lexicon the words contained within it must be able to describe the sensations that are being perceived, the emotions that it elicits, and the evaluative aspects that describe the extent to which the sensation is being perceived.

The primary aim of this study was to adopt the approach of Melzack (1975) with the MPQ and Guest, Dessirier, Mehrabyan, McGlone, Essick, & Gescheider *et al.,* (2011) with the TPT to develop and validate an oral sensory lexicon based on a limited set of oral sensory stimuli. A functioning oral lexicon would assist with teasing out the different facets of the oral experience, including more than the sensory perception and overall experience but also considering the emotional responses to the how the mouth feels.

7.2 Experiment 1: Identifying adjectives that describe oral sensations

The objective for experiment 1 was to produce a list of English language adjectives related to both oral and tactile sensation and that can be applied to describing oral sensation. This list would then be examined for how representative the descriptive words were of the different aspects of sensory perception (i.e. sensory, emotional and evaluative; see Melzack, 1975) are represented by the descriptive words.

7.2.1 Methods

7.2.1.1 Participants

Table 7.1 Demographic information of participants in Experiment 1 divided by word set. The age ranges for each set of participants were similar and consisted of almost equal number of females within each set.

	AGE RANGE (YEARS)	MEAN (YEARS)	SD	FEMALE (%)
WORD SET 1	18 - 58	25.13	1.13	46 (67)
WORD SET 2	18 - 60	25.83	1.27	34 (65)

Participants were recruited through the university in exchange for course credit and via Prolific Academic (https://prolificacademic.co.uk/) in exchange for £5. Prolific Academic is a crowdsourcing platform based in the UK that is designed to assist researchers for online study recruitment. Of the participants in the study, word set one was completed by sixty-nine university students and a further 25 from prolific academic. Word set two was completed by fifty-two university students and a further 23 from prolific academic (see Table 7.1 for demographics).

7.2.1.2 Design and Procedure:

The list of adjectives was generated by collating the words used in Study 1 Exploring the Impact of Taster Status on Oral Sensation (see Chapter 4 pg 101), other published sensory lexicons including the TPT (Guest, Dessirier, Mehrabyan, McGlone, Essick, & Gescheider *et al.,* 2011), the mouthfeel/taste section of the McCormick Spice Wheel (Lawless, Hottenstein & Ellingsworth, 2012) and the Surface Texture section of the White Wine Mouthfeel Wheel (Pickering & Demiglio, 2008). Replicating the approach of Guest, Dessirier, Mehrabyan, McGlone, Essick, & Gescheider *et al.,* (2011) standard linguistic reference tools were also read, this included the Oxford English Dictionary (2011) and examining the words with a thesaurus for alternative words with the same meaning.

This approach generated a list of 302 adjectives that were used in experiment one (see Table 7.2). The words were randomly allocated to two separate lists consisting of 151 words each. To assess the reliability of ratings one word in each word set was included twice, in word set one the adjective 'vibrating' appeared twice and 'irritating' appeared twice in word set two set.

Table 7.2 302 candidate words for an oral sensation lexicon that was generated from the TPT, McCormick Spice Wheel, White Wine Mouthfeel and reading the Concise Oxford English Dictionary (2011).

Ablaze	Coolth	Fine	Ice-Cold	Overheated	Scraping	Tender
Abrasive	Cottony	Firm	Icky	Painful	Scratchy	Tense
Achy	Crawling	Flabby	Icy	Parched	Searing	Tension
Acute	Creamy	Fleecy	Impacting	Pat	Sensual	Tepid
Airy	Creepy	Fleeting	Important	Pebbly	Sensuous	Textured
Aggravating	Crisp	Fleshy	Indented	Persistent	Sexy	Thick
Annoying	Crispy	Flexible	Infernal	Pert	Shaggy	Thorny
Arid	Crumbly	Florid	Inflexible	Placid	Shallow	Thrilling
Arousing	Crusty	Fluffy	Intense	Plastic	Sharp	Throbbing
Astringent	Cushy	Fluttering	Irie	Pleasurable	Significant	Tickling
Attending	Damp	Focused	Irksome	Pliable	Silky	Ticklish
Aversive	Deadened	Fragile	Irregular	Plush	Sinuous	Tickly
Balmy	Decisive	Freezing	Irritate	Pointed	Sizzling	Tight
Biting	Dehydrated	Fresh	Irritable	Pointy	Slack	Tingly
Blissful	Delicate	Friction	Irritating	Poked	Slick	Tortuous
Blunt	Demanding	Frigid	Itchy	Polished	Slimy	Tough
Bothersome	Dense	Frisky	Jagged	Porous	Slippery	Tranquil
Braw	Desirable	Frosty	Leathery	Pounding	Slippy	Transient
Breezy	Determined	Furry	Light	Powdery	Sloshy	Translucent
Bristly	Diffuse	Fuzzy	Liquidly	Pressed	Sludgy	Trim
Brittle	Dirty	Gauzy	Lively	Pressure	Slushy	Unpleasant
Bumpy	Discomfort	Gelatinous	Localized	Prickly	Smear	Uneven
Burn	Distinctive	Gentle	Lumpy	Provocative	Smooth	Unyielding
Burning	Distressing	Glassy	Luscious	Puckery	Soapy	Vague
Bushy	Doughy	Glossy	Lush	Pulpy	Soft	Velvety
Buzzing	Downy	Gooey	Malleable	Purposeful	Solid	Veneered
Callous	Drenched	Goopy	Matted	Raw	Soothing	Vibrating
Calming	Dry	Grainy	Mealy	Refreshing	Spiky	Viny
Chafed	Dull	Granular	Meaningful	Relaxing	Spiny	Viscous
Chalky	Effervescent	Grating	Meaty	Resolute	Spongy	Vivid
Chapped	Elastic	Greasy	Mild	Ribbed	Springy	Warm
Chilling	Enjoyable	Grimy	Moderate	Rigid	Squeezed	Watery
Chilly	Emollient	Gritty	Moist	Ripley	Squishy	Waxy
Clammy	Erotic	Grooved	Mushy	Robust	Steely	Weird
Clean	Evanescent	Gunky	Nappy	Rotten	Sticky	Wet
Clear	Evocative	Gummy	Nasty	Rough	Stinging	Wiggly
Cloggy	Exciting	Hairy	Nice	Rubbery	Stringy	Woodsy
Coarse	Excruciating	Hard	Nippy	Rugged	Supple	Woody
Cold	Execrable	Harsh	Notable	Sandy	Sweaty	Woolly
Comfortable	Faint	Heavenly	Noticeable	Satiny	Sweeping	Worn
Compliant	Feathery	Horny	Numb	Scabby	Tacky	Wrinkly
Compressed	Feel-Good	Hot	Odious	Scalding	Tactual	Yielding

Consequential Contact Cool	Fiery Filmy	Hurting Hydrous	Oily Oozy	Scaly Scorching	Tap Taut	Yucky Yummy

For ease of word organisation, collection and delivery to participants it was decided to deliver the rating task electronically. Qualtrics (Qualtrics, Provo, UT) is a computerised research software that was developed in 2001. It is a flexible survey tool which has a variety of question formats, embedded data and display logics. It can also be used offline, on mobile devices and provides multiple advanced features including randomisation and advanced branching of questions based on response.

The words lists were presented to participants using Qualtrics (Qualtrics, Provo, UT) and consisted of a participant information sheet, consent form, health screening, demographic information collection, rating task instructions, the words for rating and a debrief. The health screening asked participants to declare smoking status and if they had been diagnosed with a neurological disorder that affects sense of taste or touch, participants who responded yes to these items were excluded from the study.

Participants accessed one of two links associated with a single survey containing 152 words each. Participants were asked to rate the words on the extent to which they referred to sensory, emotional and evaluative aspects of oral sensory perception (after Melzack, 1975; Guest, Dessirier, Mehrabyan, McGlone, Essick & Gescheider et al., 2011) and how applicable and important the descriptor is to oral sensation. Definitions for the five aspects were given as follows:

Sensory: refers to the pure sensation resulting from oral sensory experience.

Emotional: refers to the feelings that occur from oral sensory experience.

Evaluative: refers to the overall significance and importance of the sensory experience.

Applicable: refers to how well the word applies to oral sensation.

Importance: refers to how important to the overall oral sensory experience it is.

The participant rated each word on a 4-point Likert type scale with scale values of: 1 “Has nothing to do with this aspect of oral sensory experience”; 2, “Refers slightly to this aspect of oral experiences”; 3, “Refers moderately to this aspect of oral sensation”; 4, “Refers strongly to this aspect of oral sensation”. Participants were also given the option to respond saying they do not know the meaning of the word; this was given a value of 0.

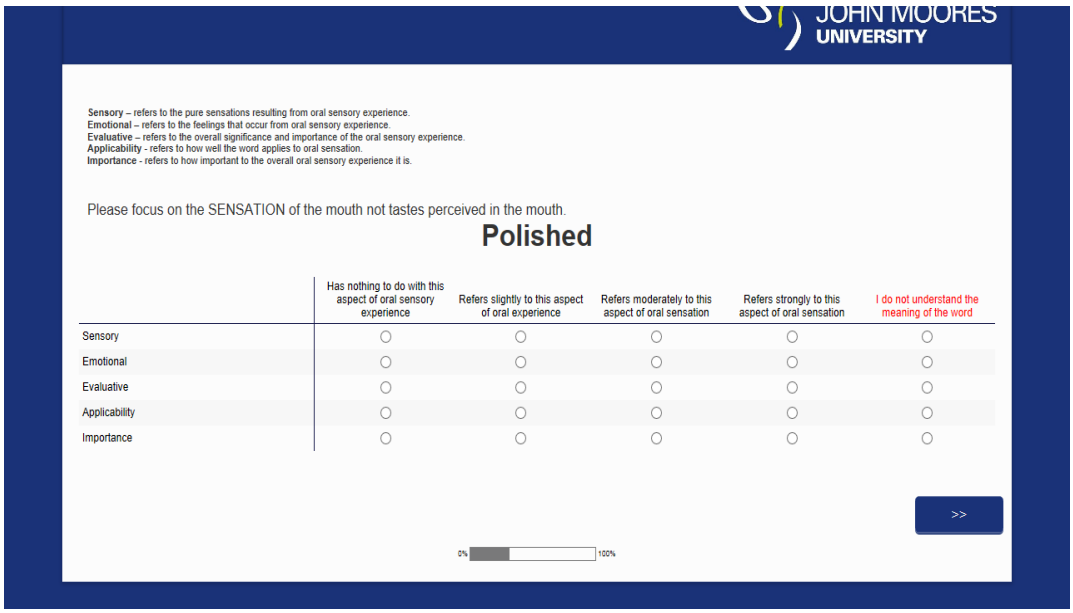


Figure 7.1 Example of question layout definitions as they display to participants in Qualtrics

7.2.2 Results and Discussion

The original full word set consisted of 302 words and was divided into two sets of 151 each. The aim of the first phase of data analysis was to assess the modal rating of words in order to reduce the number of words. Histograms were generated for each word that separately showed the rating frequencies for each aspect of the oral sensory experience. Initial culling of the words was done via excluding words from further analysis if its modal rating was less than 3 (i.e. “Refers moderately to this aspect of oral sensation”) for at least one aspect of the sensory experience (i.e. sensory, emotional or evaluative) and possessed a modal rating of less than 3 on either importance or applicable aspects. To check consistency in

participants ratings paired t-test were run on the vibrating and irritating, which both appeared twice in rating task and were found to be consistently rated.

To further reduce the descriptors the remaining words were ranked for each participant via two different schemes. For each scheme the ranking reflected that a lower rank represented more applicable aspects of oral sensation. The first scheme was within aspect, this gave each aspect (i.e. sensory, emotional and evaluative) a word list containing all the words that passed the initial reduction resulting in three orderings for each participant: there was one-word ordering for the sensory aspect ratings, one for the emotional aspect rating and one for evaluative aspects rating.

The second scheme was within word and had each aspect ordered for each individual word. This means that the sensory, emotional and evaluative rating for each word was inspected and the aspect that received the largest rating was ranked in first position and the aspect that received that smallest rating was ranked in third position.

A breakdown of both word sets, for the three aspects of oral sensation and the frequency of words that met the criteria for modal ratings, are shown in Tables 7.3 and 7.4. A substantial proportion of the words were found to have mean ranks that overlapped over more than one aspect. Both word sets each had 3 words that were found to load onto only one aspect.

Table 7.3 Distribution of 42 words of word set one selected as moderately or strongly descriptive of the three aspects of oral sensation (sensory, affective and evaluative). The table is symmetrical across the diagonal intersection of the table. On the basis of the ratings some words loaded onto two or more aspects.

		Aspect of Oral Sensation			
		Sensory	Emotional	Evaluative	All Three
Aspect of Oral Sensation	Sensory	1	0	1	
	Emotional	0	0	25	
	Evaluative	1	25	2	
	All Three				13

Table 7.4 Distribution of 53 words of word set Two selected as moderately or strongly descriptive of the three aspects of oral sensation (sensory, affective and evaluative). The table is symmetrical across the diagonal intersection of the table. On the basis of the ratings some words loaded onto two or more aspects.

		Aspect of Oral Sensation			
		Sensory	Emotional	Evaluative	All Three
Aspect of Oral Sensation	Sensory	0	3	2	
	Emotional	3	0	31	
	Evaluative	2	31	3	
	All Three				14

For further analysis the separate word lists need to be combined. As the words were collected from two different participants sets in order to combine them the data was z-scored and mean ranks were then calculated. The words were then allocated to the aspect that they ranked in first position.

To combine the words, ratings were z-scored and the combined data was ranked by mean identifying 6 words that loaded solely onto one aspect, this was in line with the analysis done on the previous separate data and consisted of the same words. Those single loading words in the separate analysis consisted of one sensory word (exciting) and five evaluative words (liquidly, scaly, fluffy, sharp and grainy) which was replicated in the combined data analysis.

Table 7.5 Distribution of 95 z-scored candidate words selected as moderately or strongly descriptive of the three aspects of oral sensation (sensory, affective and evaluative). The table is symmetrical across the diagonal intersection of the table. On the basis of the ratings some words loaded onto two or more aspects 5 words loaded solely onto the evaluative aspect and 1 word loaded solely onto the sensory aspect.

		Aspect of Oral Sensation			
		Sensory	Emotional	Evaluative	All Three
Aspect of Oral Sensation	Sensory	1	3	3	
	Emotional	3	0	56	
	Evaluative	3	56	5	
	All Three				27

The 95 remaining lexicon candidate words that remained in the oral lexicon is fewer than were retained at this stage of the TPT lexicon (Guest, Dessirier, Mehrabyan, McGlone, Essick & Gescheider et al., 2011). This means that participants at this stage of the development procedure found fewer words to describe the oral sensations they perceived than were considered descriptive of touch sensations.

The current number of words remaining does not make a viable or practical tool for use so further reduction of the word list was required. The words that loaded onto one factor alone were carried through to the validation stage (Experiment 4) of the oral lexicon development. The remaining 89 words, which loaded onto two or more aspects, needed to be assigned to only one aspect so further data analysis was required.

7.3 Experiment 2: Distributions

The objective for Experiment 2 was to separate out the words that in Experiment 1 were found to load onto more than one of the sensory, emotional or evaluative aspects of oral sensation. With the words separated out it would mean, in the completed lexicon, specific words are seen to relate to a specific aspect of the oral sensory experience. The six words that already loaded onto one factor alone were not considered in this section of data collection and analysis and can be seen highlighted in grey in Table 7.6.

7.3.1 Methods

7.3.1.1 Participants

A total of 102 participants with an age range of 18 to 60 years (M = 31.78, SD = 10.78) participated in this phase of data collection. Of the participants 52 reported to be male (51%), 48 reported as female (47%) and 2 (2%) said they would prefer not to say. All the data was collected through Prolific Academic with participants receiving £5 as compensation for their time.

7.3.1.2 Design and Procedure

A total of 95 words that resulted from Experiment 1 only 6 of them loaded onto one aspect of oral sensation with 89 words loading onto two or more. The remaining words can be seen in Table 7.6 with the words that loaded onto only one aspect highlighted in grey. Those words that were single loading were not used in this stage of data culling.

Table 7.6 The 95 candidate words. Words that single loaded and were not used in Experiment 2 are highlighted in grey.

Bristly	Delicate	Fresh	Liquidly	Pleasurable	Sharp	Tender
Burn	Desirable	Furry	Lumpy	Powdery	Sizzling	Textured
Burning	Discomfort	Gelatinous	Luscious	Prickly	Slimy	Tickly
Chilling	Doughy	Gooey	Meaty	Pulpy	Slippery	Tingly
Clear	Dry	Grainy	Mild	Raw	Sludgy	Tough
Coarse	Enjoyable	Granular	Moist	Refreshing	Slushy	Unpleasant
Cold	Exciting	Greasy	Mushy	Rough	Smooth	Velvety
Cool	Excruciating	Gritty	Nasty	Rubbery	Soft	Viscous
Creamy	Feel-good	Hard	Nice	Scalding	Soothing	Warm
Crisp	Fiery	Heavenly	Numb	Scaly	Spongy	Watery
Crispy	Firm	Hot	Oily	Scorching	Squishy	Wet
Crumbly	Fleshy	Hurting	Oozy	Searing	Sticky	Yucky
Crusty	Fluffy	Icy	Painful	Sensuous	Stinging	Yummy
Dehydrated	Freezing	Intense	Parched			

The remaining 89 words were once again set up in Qualtrics (Qualtrics, Provo, UT) with a participant information sheet, consent form, demographics, health screening and debrief. This phase of data collection consisted of presenting the participant with a word and giving them a forced choice question to choose which aspect of the oral sensory experience the word was most descriptive of. Each word was only presented once and was only presented with the answer options associated with the aspects upon which it had previously been rated as loading onto. If a word duel loaded onto emotional and evaluative they were the only options in response to the question. Descriptions for each aspect were as follows:

Sensory: – The pure sensations that are experienced during oral experiences.

Emotional: – The feelings that you experience during oral experiences.

Evaluative: – This applies when the word can be applied to describe the overall significance and importance of the oral sensory experience.

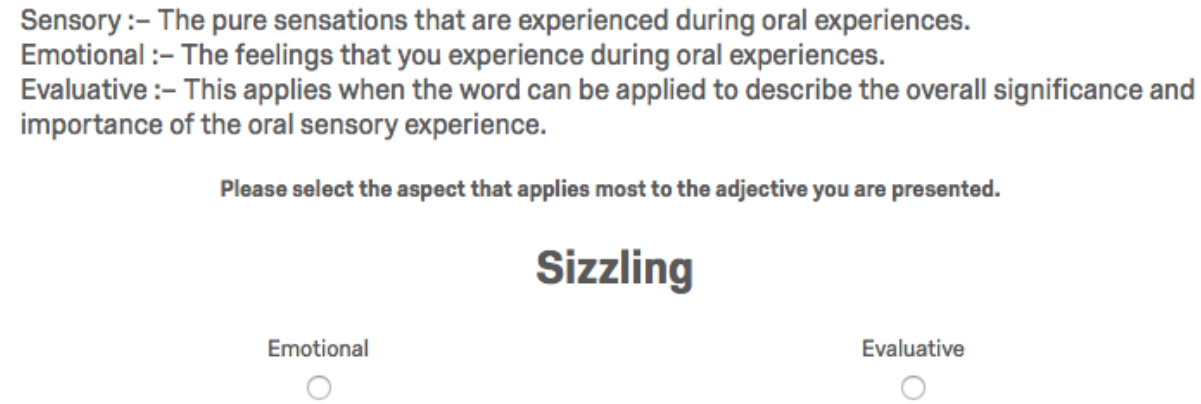


Figure 7.2 Example of question layout and definitions as they display to participants in Qualtrics.

7.3.2 Results and discussion

The aim of this analysis was to assess which aspect of oral sensation the words loaded onto and as such were considered descriptive of that aspect of oral sensation. The data was analysed via frequency for each aspect selection. The percentage of respondents that allocated each word to each optional aspect was calculated. The words that reached the level of 65% of respondents allocating it to a specific assessed aspect of oral sensation were retained and those that failed to reach the minimum level of 65% on any aspect were removed from the study (Table 7.7)

Table 7.7 Words were assessed for a 65% agreement of which aspect it reflects. 27 words failed to reach the level of 65% and were dropped from the lexicon. Each of the other words were allocated to one aspect of the oral sensation.

		Aspect of Oral Sensation			
		Sensory	Emotional	Evaluative	Excluded
Aspect of Oral Sensation	Sensory	9	0	0	
	Emotional	0	5	0	
	Evaluative	0	0	48	
	Excluded				27

After analysis, 27 words were excluded for not meeting the criteria of 65% of respondents allocating it an aspect. This means that 62 words were retained at the end of this phase of data analysis. The words had a loading divided over the three aspects of oral sensation but the majority of words loaded in the evaluative aspect. The total number of words is too many to make a quick and effective lexicon so the words that were considered to be sensory or emotional were carried forward to Experiment 4 for validation. Words loading onto evaluative needed to be further reduced and this was done with another experiment assessing word similarity.

7.4 Experiment 3: Word Similarity

The objective of Experiment 3 was to assess the similarity in word meaning to further reduce the words in the evaluative aspect of oral sensation.

7.4.1 Methods

7.4.1.1 Participants

A total of 6 participants with an age range of 22 to 42 year (M = 28.50, SD = 3.05) completed this part of the study. Of the participants 2 reported to be male (33%), 4 reported as female (67%).

7.4.1.2 Design and Procedure

This experiment was designed to assess the similarity in word meaning between all the words that up to this stage have loaded onto the evaluative aspect of oral sensory perception. The words were presented in Qualtrics with participants presented with one word and asked to indicate which, if any, of the other remaining words were similar in meaning by ticking a box. Every word was compared against every other word.

7.4.2 Results and Discussion

The aim of this analysis was to reduce the words that were considered to be evaluative of oral sensation by assessing their meaning and removing words that were similar in meaning. Each participant's responses were assessed for which words they considered to have similarity in meaning. Words the participants considered similar in meaning were explored for dictionary definitions and checked for frequency used in the English language on the Corpus of Contemporary American English (COCA; Davies, 2008). Of the words considered similar in meaning the word that was most frequently used according to COCA was retained. If participants generally agreed with each other words were removed from the final lexicon.

Table 7.8 Shows the distribution of the words retained from all experiments. When combined with the previous experiments retained words, a total of 10 sensory, 5 emotional and 27 evaluative words make up the candidate oral lexicon.

		Aspect of Oral Sensation		
		Sensory	Emotional	Evaluative
Aspect of Oral Sensation	Sensory	10		
	Emotional		5	
	Evaluative			27

This resulted in a total of 23 evaluative words being retained during this phrase of data culling. Table 7.8 shows the distribution of all retained words from all experiments up to this point across the three aspects of oral sensation. When combined the candidate lexicon consists of 10 sensory, 5 emotional and 27 evaluative words. Table 7.9 lists the final candidate words for the lexicon that will be tested in Experiment 4s validation study.

Table 7.9 The 42 words that are contained in the candidate oral sensory lexicon.

Burning	Crusty	Fluffy	Heavenly	Painful	Scaly	Squishy
Coarse	Desirable	Freezing	Intense	Pleasurable	Scorching	Sticky
Cold	Dry	Furry	Lumpy	Powdery	Sharp	Stinging
Cool	Enjoyable	Grainy	Moist	Prickly	Slimy	Warm
Creamy	Exciting	Gritty	Numb	Raw	Slippery	Watery
Crispy	Fleshy	Hard	Oily	Scalding	Spongy	Wet

7.5 Experiment 4: Validation

7.5.1 Introduction

Experiments 1 through 3 identified a set of words that are considered to be attributes of the sensory, emotional and evaluative aspects of oral sensation. The words that generated this lexicon however, were not generated with actual reference to experienced oral sensation but rather to words only. Only certain subsets of the words may apply to specific circumstances and sensations so it is important to test empirically the use of the words in describing oral sensation. Therefore, the aim of experiment 4 is to use the lexicon and obtain ratings on different oral sensations.

The mouth is a highly complex region of the human body and one of the most densely innervated (Haggard & de Boer, 2014). Unlike the development of other similar other tools, which are specifically, designed for use with specific foods like the lexicon for red wine (Gawel, 1998; Pickering & Robert, 2006) and white wine (Pickering & Demiglio, 2008) this lexicon was designed using the procedure outlined by Melzack (1975) for the development of the McGill Pain Questionnaire, and by Guest, Dessirier, Mehrabyan, McGlone, Essick & Gescheider et al., (2011) to develop the TPT for general use. This general use makes the validation process slightly more complex. The MPQ was validated by having the questionnaire completed by individuals diagnosed with various pain conditions including arthritis, cancer, menstrual, phantom limb and neurological pain (Melzack, 1975). Each of these conditions have pain associated with it and each of the pain experiences is qualitatively different. Validation of the TPT involved administering a stroking touch with five different materials (polyester with a silky finish, polyester with a textured finish, unpowdered latex, cotton t-shirt and hessian) to four different body sites (upper limb, index finger pad, volar forearm, fossa of the axilla (the annulus surrounding the hairy central part of the underarm) and the vault of the axilla (the central, hairy portion of the underarm). Immediately after each stroke participant competed the TPT (Guest, Dessirier, Mehrabyan, McGlone, Essick & Gescheider et al., 2011). Both the validation process of the MPQ and TPT was fit for purpose for each individual lexicon and as such the validation procedure for the candidate oral

lexicon must follow suit, however stroking the inside of the mouth with various fabrics is not practical.

In order to validate the candidate oral lexicon certain factors must be considered, primarily taster status. Taster status is a genetic polymorphism which impacts of the taste and sensation perception within the mouth. Research has firmly established the presence of three taster groups within the population (Bartoshuk, 1993). Hyper-tasters have been found to be more sensitive to the burn from capsaicin (Prescott & Swain-Campbell, 2000) coolness and stinging from menthol (Manrique & Zald, 2006) and astringent sensations (Pickering, Simunkowa & DiBattista, 2004) amongst others (see Chapter 1 literature review and Chapter 4 Examining the impact of taster status for further information). Due to these perceptual differences taster status and its influence on the oral experience must be considered.

The proposed candidate oral lexicon was not designed with the intention of being applied to a specific sensation. Manipulation of mouthfeel by applying chemo-stimulants or tastes however is sensitive to the influence of taster status, which means that hyper-tasters may utilize the scale in a different manner to tolerant-tasters. The easiest and most effective way to manipulate mouthfeel is simply via cleaning. Comparing how individual's mouths feel first thing in the morning before they clean their teeth and after they undertake their usual oral cleansing routine should be uninfluenced by taster status and as such be more comparable and controllable.

7.5.2 Method

7.5.2.2 Participants

A total of 87 participants were involved in this study. Of these 32 came from the Glaxo-Smith Kline research and development team and were collected at their research site in Weybridge. A further 55 participants data was collected at Liverpool John Moores University and of these 24 participants were collected while they were in the university laboratory participating in another study. Due to the

Glaxo Smith Kline laboratory rules, age and gender were not collected from participants.

7.5.2.3 Materials

Taster Status: This was established using the filter paper method with the concentration concluded as the most appropriate for the delivery method by Zhao, Kirkmeyer and Tepper (2003) and outlined in the methodology chapter 3 section 3.3 pg 92).

Teeth Cleaning Products: Aqua Fresh Intense Clean toothpaste, dental floss and Aqua Fresh mouth wash.

Candidate Oral Sensory Lexicon*:* The core candidate oral lexicon consists of 42 words. To check the data filtering process an additional 41 words previously excluded were also included. Three of the core words (lumpy, sticky and gritty) were included twice for rating validity checks.

7.5.2.4 Design and Procedure

Participants were invited to attend two laboratory sessions. Sessions were randomised in delivery with half the participants experiencing the good day first and half the participants experiencing the bad day first.

A bad oral health day consisted of participants brushing their teeth before they went to bed the night before the testing session but not cleaning them the following morning. The bad day session was usually run 8.30am to 10am. Participants came in to the laboratory having not brushed that morning and were asked to complete the oral lexicon. They were then provided with a toothbrush and toothpaste to clean their teeth before continuing with their day.

The good day sessions ran 1pm to 5pm. Participants would come to the lab and be given the taster status test. The test would be explained to them and they would be provided with a toothbrush and toothpaste, dental floss and mouthwash. They were asked to clean their teeth to a high quality and if they would normally

use dental floss and mouth wash to do so. It was not enforced that they had to use the floss and mouth wash due to the potential damage to the gums and oral feeling if they had not used them before. Once clean participants were given an oral lexicon to complete.

Taster status was assessed on the good day when participants were given a PROP soaked filter and asked to asked to place them as close to the tip of the tongue as they could but ensuring the whole filter paper was on the tongue. They were instructed to soak the paper in saliva and leave it on the tongue for a timed period of 10 seconds. After the 10 seconds they removed the paper and swallowed any saliva in their mouth, while waiting a further 10 seconds before rating the bitter sensation intensity which it invoked. The bitter taste was rated on a labelled magnitude scale (LMS) asking how intense the sensation was (Guest, Essick, Patel, Prajapati & McGlone, 2007).

The Participants who only participated in the lexicon validation study completed both sessions in one sitting with the bad day session first. Participants attended the laboratory having not cleaned their teeth and were given a lexicon to complete. They were then provided with the same products and instructions as the GSK participants and cleaned their teeth before completing the lexicon for the final time and undertaking the taster status test.

Participants whose data was collected as part of another study were emailed an electronic lexicon and asked to complete the bad day session from home a day before the other studies testing session. The good day was completed at the start of the testing session for the other study.

7.5.2.5 Statistical Analysis

Several words were repeatedly included in the Oral lexicon to allow for reliability checks on the word ratings with t-test analysis. Due to the limited number of words associated with the sensory and emotional aspects of oral sensation the lexicon was analysed as a whole rather than as separate aspects

The goal of the initial analysis was to identify descriptive components that can summarise the data. This can be done with either Factor Analysis (FA) or

Principle Component Analysis (PCA). According to Tabachnick and Fidell (2007) there is no readily available criteria to assess which approach will provide the best solution, however they do outline differences between FA and PCA. The primary difference is that PCA analyses all the variance in the observed variance of the data so is best if what is wanted is an empirical summary of the data, whereas in FA only shared variance is analysed so is best when a theoretical solution uncontaminated by unique and error variability is desired (Tabachnick & Fidell, 2007). For this analysis a PCA approach was used as it has previously been used to facilitate grouping of relative attributes (Koppel, Timberg, Salumets & Paalme, 2011; Koppel & Chambers, 2010).

The next decision involves solution rotation. As the extracted components should not be related an orthogonal rotation was used via either a varimax, quartimax or equamax rotation. A varimax rotation was chosen as it attempts to maximise the dispersion of item loadings so that a smaller number of items load highly onto each component (Field, 2009).

To establish if an omnibus PCA was valid a separate analysis was also conducted on the good day and bad day data. As similar results were obtained in the separate analysis as the omnibus only the omnibus analysis is reported. Once appropriate components were identified the data was assessed for taster status to see if the different taster groups responded differently and to see in what ways the lexicon was different depending on the day the data was collected.

7.5.3 Results

The analysis reported below examined the essence of the words contained within the lexicon. Principle component analysis was used to identify the descriptive word grouping for sensations that individuals used to describe mouthfeel. The core lexicon words as identified by the three previous experimental phases and the non-core words were analyses separately to see if similar factors are identified within the word groupings.

7.5.3.1 Omnibus Analysis Core Lexicon

To examine consistence in ratings of words paired sample t-tests were run on the words *lumpy, sticky* and *gritty* that appeared at 2 different times in the lexicon. There was no significant difference found the ratings obtained at time 1 and time 2 of lexicon appearance ($p>.05$).

The Kolmogorov-Smirnov test of normality indicated that all 42 words within the lexicon significantly deviated from normal ($p<.001$) therefore a non-parametric correlation of Spearman's rho was used instead of the standard Pearson's correlation that is run as part of the PCA. Assessment of the correlation table indicates if there is a problem with multicollinearity so words that correlate at a level of .9 were removed from further analysis and any word that does not correlate with at least 3 other words or at a level of at least .3 is also removed. No issue of multicollinearity was identified but the word *numb* was removed as no other words correlated with it.

7.5.3.2 Principle Components Analysis Core Lexicon

A principle components analysis was conducted on the 42 items of the oral lexicon with an orthogonal rotation (varimax). The Kaiser-Meyer-Olkin measure verified the sampling adequacy for the analysis (KMO = .81) rated as good according to Field (2009). Bartlett's test of sphericity ($X^2(820) = 3870.38$, $p<.001$) indicated that correlations between items were sufficiently large for PCA.

The anti-image matrix showed that all the items correlated above the .5 level. Nine components were identified as having eigenvalues over Kaiser's criterion of 1 and in combination explained 70.11% of the variance. The scree plot

indicated inflexions that would justify retaining components 6 and 8. The rotated component matrix indicated that only one item loaded onto factor 9 so supressing the rotation onto 8 components is justifiable. When the rotation is suppressed onto 8 components 67.63% of the variance is explained, however the item *intense* not only duel loads it does so with only 0.02 differences in scores. *Slippery* was then noted to not load on any components so was also removed from the model. Assessment of the item loadings

The Kaiser-Meyer-Olkin measure verified the sampling adequacy for the analysis (KMO = .83) rated as good according to Field (2009). Bartlett's test of sphericity ($X^2(741) = 3993.32$, $p<.001$) indicated that correlations between items were sufficiently large for PCA. The anti-image matrix showed that all the items correlated above the .5 level. Supressing the loadings onto 8 components explained 67.25% of the variance.

The Cronbach's alpha for the component of Moisture is low due to it containing *dry*, the opposite of the other words. Removal of the word would increase the alpha to .85 but as it is related to the component as a whole does not seem justified. The same applies for Thermal, as the item warm is included with items at the other end of the spectrum. Removal of warm would increase the alpha to .87 but as it also fits with the structure of the component removing it does not seem justified. Table 7.10 shows the final component loading after rotation.

Table 7.10 The component loading for the candidate Oral Lexicon with related eigenvalues and Cronbach's alphas scores highlight the presence of 8 factors comprising the oral lexicon, each describing a different sensation experienced in the mouth.

	Component							
	Emotional	Pain	Texture: Granularity	Texture: Consistency	Texture: Consistency 2	Moisture	Texture: Firmness	Thermal
Heavenly	.89							
Pleasurable	.87							
Enjoyable	.81							
Desirable	.81							
Exciting	.72							
Scorching		.83						
Stinging		.83						
Burning		.79						
Scalding		.75						
Prickly		.64						
Sharp		.51					.46	
Scaly			.75					
Grainy			.73					
Coarse			.70					
Powdery			.64					
Gritty			.61					
Furry			.48	.45				
Raw			.44					
Slimy				.76				
Fluffy				.72				
Lumpy			.41	.63				
Oily				.63				
Sticky				.54	.41			
Spongy					.71			
Fleshy					.70			
Squishy					.68			
Creamy				.42	.50			
Wet						.87		
Moist						.82		
Watery						.77		
Dry			.43			-.49		
Hard							.74	
Painful							.63	
Crispy							.57	
Crusty				.45			.55	
Cold	.43							.72
Cool	.55							.64
Freezing	.49							.61

Warm					.44			-.53
Eigenvalues	4.78	3.94	3.85	3.49	2.68	2.65	2.65	2.19
% of variance	12.26	10.11	9.86	8.95	6.87	6.80	6.79	5.62
α	.92	.86	.81	.83	.72	.48	.73	.34

Mixed measures ANOVAs were used to explore the subscales. Mauchly's test indicated that the assumptions of sphericity were violated for subscale ($X^2(27) = 77.26$, $p<.001$, $\varepsilon = .75$) and subscales interaction with day ($X^2(27) = 191.31$, $p<.001$, $\varepsilon = .49$) therefore Greenhouse Geisser corrections to the degrees of freedom. A significant main effect of Day ($F(1.00, 59.00) = 28.46$, $p<.001$, $\eta p2 = .33$, op = 1.00) and subscale ($F(5.22, 307.92) = 56.94$, $p<.001$, $\eta p2 = .49$, op = 1.00) and their interaction ($F(3.45, 203.65) = 66.07$, $p<.001$, $\eta p2 = .53$, op = 1.00) but there was no main effect of taster status or its interaction with day and subscale (ps>.05).

Pairwise comparisons between the subscales indicated that the subscale of emotional is significantly different to all the other subscales (ps<.01) with the exception of the Thermal subscale (p>.05) and the Thermal subscale was significantly different to all subscales (ps<.001) except the Emotional subscale (p>.05). The pain subscale was found to only be significantly different from Moisture, Texture: Firmness and Thermal (ps<.001) a pattern reflected in Texture: Granularity, Consistency and Consistency2 (ps<.001). The Moisture subscale was significantly different to Texture: Firmness (p<.001).

Exploring the lexicon subscales indicate that significant differences were seen between the good day and bad day ratings on the Emotional subscale ($F(1, 69) = 153.89$, $p<.001$, $\eta p2 = .69$, Power = 1.00) with mean scores indicating that the scores obtained on a good day were more emotional than on a bad day. Ratings associated with the Pain subscale were also significantly different depending on if it was a good day or bad day rating ($F(1, 80) = 23.90$, $p<.001$, $\eta p2 = .23$, Power = 1.00) with mean scores showing that the pain subscale was higher on a good day than a bad day. A significant interaction between day and the location the data was collected from was also identified ($F(1, 75) = 17.60$, $p<.001$, $\eta p2 = .19$, Power = .99). Further analysis showed this interaction was located in the good day ratings of the

pain subscale (F(1, 82) = 17.75, p<.001) with the mean scores showing that data collected from the GSK participants (M = 1.92, SE = 0.14) was significantly higher than the LJMU participants (M = 1.34, SE = 0.07).

The various Texture subscales also differed in rating depending on day with Granularity rated significantly higher on a bad day (F(1, 81) = 56.46, p<.001, ηp2 = 4.1, Power = 1.00). Texture subscale Consistency was significantly different depending on day (F(1, 81) = 50.48, p<.001, ηp2 = .38, Power = 1.00) with bad day ratings being higher than on the good day. A significant interaction between days interaction with taster status was also identified (F(2, 81) = 3.66, p<.05, ηp2 = .08, Power = .66). One-way ANOVAs show this difference to be located in the Bad day ratings (F(2, 82) = 3.99, p<.05) with a significant difference in rating between tolerant-tasters and hyper-tasters (p<.05) with tolerant-tasters scoring lower on the Consistency subscale (M = 1.51, SE = .09) than hyper-tasters (M = 1.97, SE = .14). The Consistency2 texture rating was also significantly different for the day (F(1, 83) = 30.04, p<.001, ηp2 = .27, Power = 1.00) with mean scores highlighting increased scores and the interaction between day and taster status (F(2, 83) = 5.43, p<.01, ηp2 = .12, Power = .83). Further one-way ANOVA analysis found that in the taster status differences in the good day ratings were not close to significant but the bad day ratings were (F(2, 84) = 2.95, p=.058), however the pairwise analysis in the bad day data highlighted no significant differences between the taster status so this interaction appears to be a false positive. There was no significant difference between the ratings for the moisture subscale (p>.05) but a significant difference between the good and bad day was seen with the texture: Firmness (F(1, 82) = 5.26, p<.05, ηp2 = .06, Power = .62) and Thermal subscale (F(1, 76) = 102.62, p<.001, ηp2 = .58, Power = 1.00) with mean scores indicating that scores obtain on both subscales were higher on the good day than the bad (see Table 7.10).

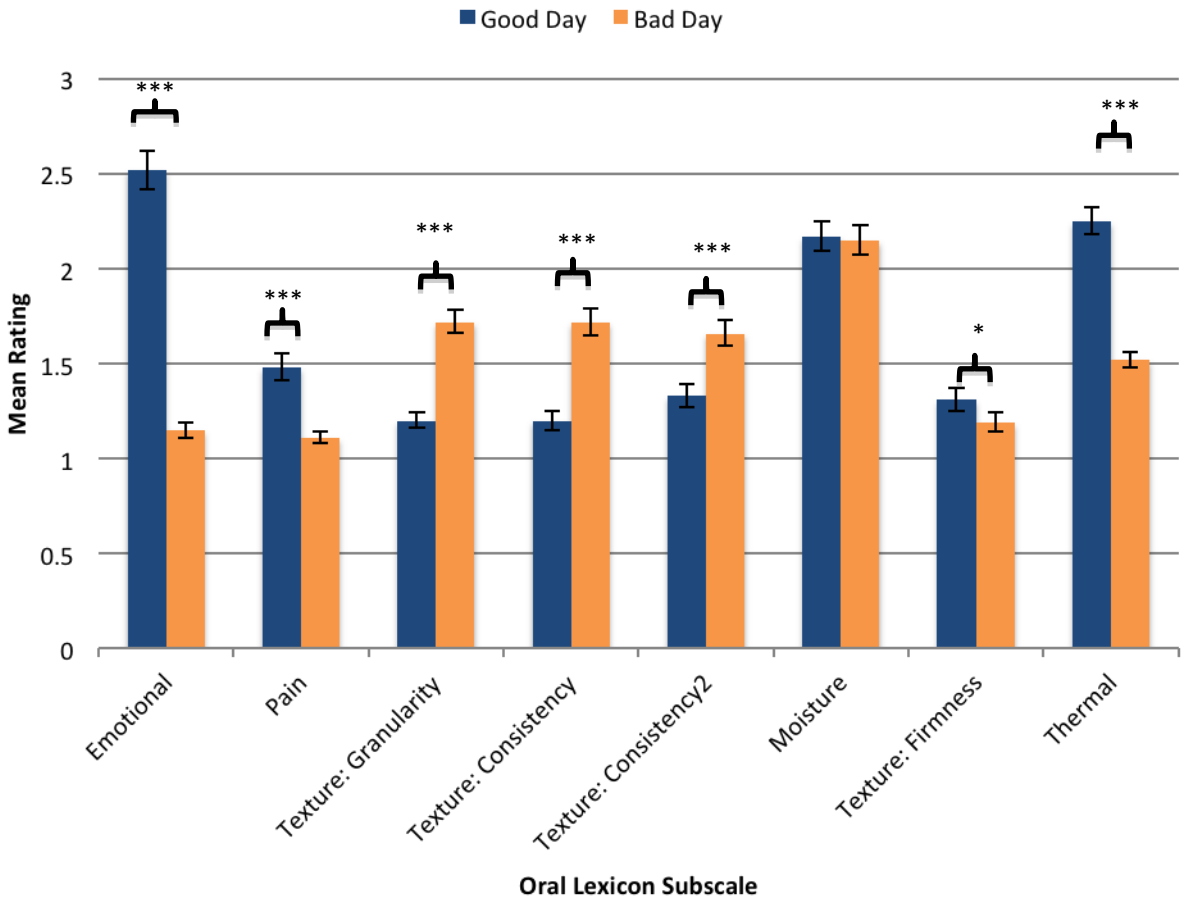


Figure 7.3 The mean ratings obtained on the good oral health and bad oral health day. These scores indicate that different factors of the candidate oral lexicon are used depending on the oral health day. Factors associated with more negative aspects were rated higher on a bad day than good day. Emotional words were rated higher on the good day than the bad (*p<.05, **p<.01, ***p<.001).

Together these findings show a preliminary functioning oral lexicon with the scores obtained on the good day and bad being significantly different. There is also support that the taster statuses use the lexicon differently but only on the one identified factor of Ick. The influence of the location the data was collected from was explored and was found to have an effect on the Pain subscale but this is unlikely to have much of an influence on the core lexicon results.

7.5.3.3 Principle Comparisons Analysis Non-core words – omnibus

The Kolmogorov-Smirnov test of normality indicated that the additional 41 words within the lexicon significantly deviated from normal ($p<.001$) therefore a non-parametric correlation of Spearman's rho was used instead of the standard Pearson's correlation that is run as part of the PCA. Assessment of the correlation table indicates no issue of multicollinearity and no words were removed.

A principle components analysis was conducted on the 41 items of the oral lexicon with an orthogonal rotation (varimax). The Kaiser-Meyer-Olkin measure verified the sampling adequacy for the analysis (KMO = .86) rated as good according to Field (2009). Bartlett's test of sphericity ($X^2(820) = 4979.08$, $p<.001$) indicated that correlations between items were sufficiently large for PCA.

The anti-image matrix showed that all the items correlated above the .5 level. Nine components were identified as having eigenvalues over Kaiser's criterion of 1 and in combination explained 71.46% of the variance. The scree plot indicated inflexions that would justify retaining components 6 and 7. The rotated component matrix indicated that only two items loaded onto factors 8 and 9 so supressing the rotation onto 7 components is justifiable. When the rotation is suppressed onto 7 components 66.40% of the variance is explained, however the items *crisp* and *viscous* duel load and the duel loading does not make sense with the component structures so there were removed from the analysis.

With those words removed the Kaiser-Meyer-Olkin measure verified the sampling adequacy for the analysis (KMO = .87) rated as good according to Field (2009). Bartlett's test of sphericity ($X^2(741) = 4687.98$, $p<.001$) indicated that correlations between items were sufficiently large for PCA.

The anti-image matrix showed that all the items correlated above the .5 level. Seven components were identified as explaining 67.38% of the variance. The scree plot indicated inflexions that would justify retaining components 6. The rotated component matrix indicated that only one item loaded onto factor 7 so supressing the rotation onto 6 components is justifiable. When the rotation is

suppressed onto 6 components 64.71% of the variance is explained, however the item *tickly* does not load onto any component so needs removing from analysis.

With *tickly* removed words the Kaiser-Meyer-Olkin measure verified the sampling adequacy for the analysis (KMO = .86) rated as good according to Field (2009). Bartlett's test of sphericity ($X^2(703) = 4865.14$, $p<.001$) indicated that correlations between items were sufficiently large for PCA. The anti-image matrix showed that all the items correlated above the .5 level. Six components were identified as explaining 65.34% of the variance.

The Cronbach's alpha for the component 1 is high but would be higher if the words *yucky* and *unpleasant* were removed. However, this does not make sense, as those words are simply the opposite end of the spectrum for that component.

Table 7.11 The component loading for the non-core words with related eigenvalues and Cronbach's alphas scores highlight the presence of 6 factors. There is some overlap in factors, for example there are two consistency factors,

	Component					
	Affective	Texture: Consistency	Pain	Texture: Consistency2	Texture: Granularity	Texture: Smoothness
Feel-Good	.89					
Refreshing	.88					
Fresh	.87					
Nice	.85					
Icy	.83					
Soothing	.70					
Chilling	.69					
Clear	.68					
Smooth	.59					.49
Oozy		.80				
Mushy		.80				
Gooey		.77				
Rubbery		.68				
Slushy		.68				
Sludgy		.68		.46		
Gelatinous		.67				
Doughy		.59				
Pulpy		.58				
Meaty		.55				
Greasy		.46		.42		
Burn			.84			
Sizzling			.80			
Fiery			.78			
Searing			.77			
Hurting			.69			
Nasty				.77		
Yucky	-.42			.77		
Unpleasant	-.45			.75		
Textured				.52		
Rough				.50	.43	
Bristly					.80	
Crumbly					.69	
Granular					.67	
Firm					.48	.43
Soft						.72
Velvety						.63
Tender			.49			.51
Mild						.45

Eigenvalues	6.79	5.65	3.94	3.16	2.74	2.55
% of variance	17.88	14.86	10.36	8.31	7.22	6.71
α	.80	.89	.87	.88	.72	.75

Mixed measures ANOVAs were used to explore the non-core words subscales. Mauchly's test indicated that the assumptions of sphericity were violated for subscale ($X^2(14) = 50.515$, $p<.001$, $\varepsilon = .79$) and subscales interaction with day ($X^2(14) = 130.80$, $p<.001$, $\varepsilon = .49$) therefore Greenhouse Geisser corrections to the degrees of freedom. A significant main effect of subscale ($F(5.22, 286.81) = 74.43$, $p<.001$, $\eta p2 = .51$, Power = 1.00) and their interaction with taster status ($F(7.86, 286.81) = 2.05$, $p<.05$, $\eta p2 = .05$, Power = .82). An interaction between type of day and subscale was also identified ($F(2.46, 4.93) = 75.43$, $p<.001$, $\eta p2 = .51$, Power = 1.00).

Pairwise comparisons between the subscales indicated that the Affective factor is significantly different to all the other factors (ps<.001). The non-core words Texture: Consistency and Pain were significantly different to Texture: Consistency 2 and Smoothness (ps<.001) and subscale Texture: Consistency 2 was significantly different to all subscales (ps<.001) except Smoothness.

Exploring the non-core word lexicon subscales indicates significant differences were seen between the good day and bad day ratings on Affective subscale ($F(1, 80) = 195.47$, $p<.001$, $\eta p2 = .71$, Power = 1.00) with mean scores indicating that the scores obtained on a good day were higher than on a bad day. A significant main effect of taster status was identified in Affective subscale ($F(2, 80) = 3.62$, $p<.05$, $\eta p2 = .08$, Power = .65). Further ANOVA analysis shows that this significant difference is located within the bad day ratings ($F(2, 83) = 4.89$, $p<.01$) with tolerant-tasters rating higher on Affective subscale (M = 1.38, SE = .04) than the tasters (M = 1.63, SE = .08).

Subscale of Texture: Consistency of the non-core words identified a significant difference between the days ($F(1, 77) = 22.04$, $p<.001$, $\eta p2 = .22$, Power = 1.00) and for the interaction between the day type and the location the data was collected from ($F(1, 77) = 5.64$, $p<.05$, $\eta p2 = .07$, Power = .65). Mean scores

highlight that scores were higher on the bad day than on the good day but further analysis could not identify where the interaction between day type and location was located (ps>.05).

Ratings associated with Pain subscale were also significantly different depending on if it was a good day or bad day rating (F(1, 79) = 20.71, p<.001, ηp2 = .21, Power = .99) and for days interaction with location the data was collected (F(1, 79) = 4.69, p<.05, ηp2 = .06, Power = .57). Mean scores highlight that the significant difference between the days is that higher scores were obtained on the good day than the bad day. ANOVA analysis showed that the significant difference in ratings was obtain on the good day (F(1, 84) = 5.25, p<.05) with mean scores showing that significantly higher scores were obtained from the participants from GSK (M = 1.55, SE = 0.13) than the LJMU participants (M = 1.24, SE = 0.07). The ratings from Texture: Consistency 2 show a significant difference in ratings for day (F(1, 80) = 91.74, p<.001, ηp2 = .53, Power = 1.00) with the bad day scores being significantly higher than the good day. Granularity subscale also showed the same effect of type of day (F(1, 81) = 5.95, p<.05, ηp2 = .07, Power = .67) with mean scores obtained higher on a bad day than the good day.

Finally, Smoothness subscale of the non-core words found a significant difference between the days (F(1, 80) = 19.94, p<.001, ηp2 = .20, Power = .99) with the mean scores showing that scores were higher on the good day than on the bad day.

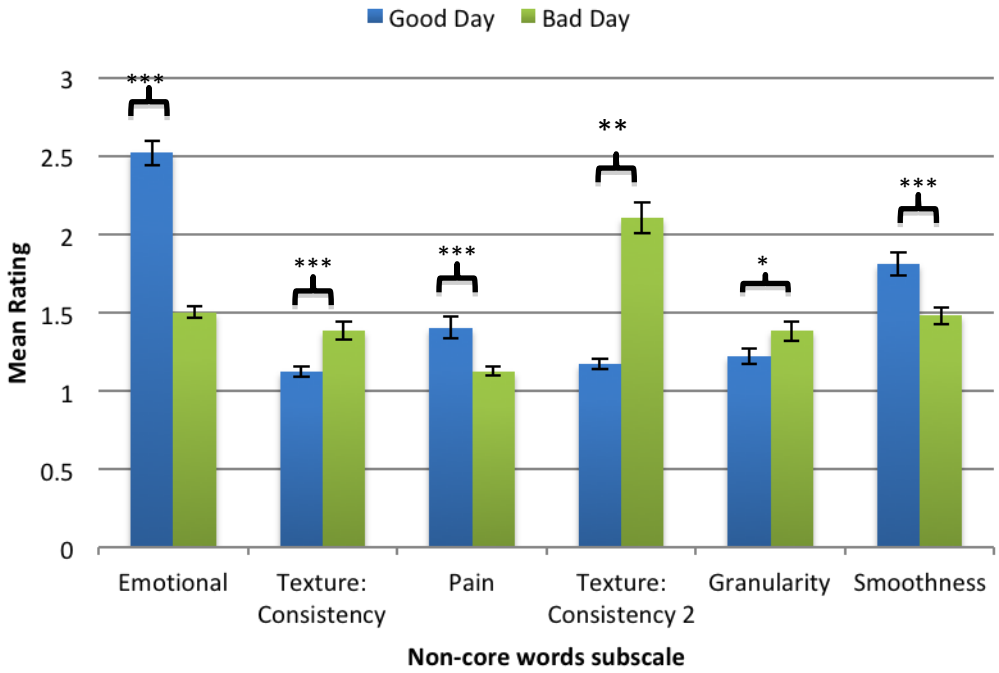


Figure 7.4 The mean ratings obtained on the good oral health and bad oral health day from the non-core candidate lexicon words. These scores indicate that different factors of the candidate oral lexicon are used depending on the oral health day. Factors associated with more negative aspects were rated higher on a bad day than good day. Emotional words were rated higher on the good day than the bad (*p<.05, **p<.01, ***p<.001).

As with the core words analysis an effect of taster status was identified as having an effect within one factor with tolerant-tasters using more emotive words than tasters. Data analysis was unable to establish if the location the data was collected from influenced the findings.

7.5.4 General Discussion

The approach outlined by Melzack (1975) and replicated by Guest, Dessirier, Mehrabyan, McGlone, Essick & Gescheider *et al.,* (2011) can appropriately be applied to generate a preliminary oral lexicon. The approach previously used established both affective and discriminative aspects of pain in the MPQ Melzack (1975) and touch with the TPT (Guest, Dessirier, Mehrabyan, McGlone, Essick & Gescheider *et al.,* 2011) sensations respectively. The oral lexicon that was developed in this study established that oral sensations also consist of affective and discriminative aspects. The words that comprise the oral lexicon describe the texture, the sensory and the emotive sensations experienced in the mouth. This tool is important as it can standardise the language used across oral sensations because as Caul (1957) said there is no mechanical or physical device that combined can do the work of the mouth, nose and brain in detecting and evaluating flavour so human tasters remain necessary.

The oral health manipulation worked with the responses on the candidate oral lexicon being significantly different depending on if it was completed before or after cleaning, a finding replicated with the non-core words of the lexicon. The decision to retain the extra non-core words for the validation process which were excluded during earlier experiments was due to concerns regarding the word filtering processes.

Factors identified in the non-core words of the lexicon divided into similar factors as the core words do with some factors reflecting almost opposite ends of the spectrum of the same category, for example within the core words, a factor or firmness was identified and within the non-core words smoothness. Both word sets also had factors of granularity, consistency, pain and emotion. Together this could indicate that core preliminary lexicon in its current form does not entirely serve its purpose and may be missing certain details and that some words should possibly not have been excluded during the word filtering and rating processes.

The lexicon that was developed possessed core factors that highlight both emotional and sensory words are used to describe the oral sensations experienced between a bad oral health care day and a good one. This was further reflected in

the division of the words over factors in the non-core word section of the validation lexicon.

Looking back at Figures 7.3 and 7.4, the subscales of emotion possess a higher mean score on a good oral health day than any other subscale on either the core or non-core candidate lexicon words. This highlights that when describing how the mouth feels the emotional experience is clearly emphasised and important to respondents even if they are not aware of it on an everyday basis. On an evolutionary stand point the emotional responses, be they positive or negative, to oral experience is essential for survival. From birth, babies use their mouths to explore world and must engage in suckling behaviour to obtain all their nutrients. Negative oral experiences, such as long-time frames on assisted breathing apparatus or tube feedings, often translate into oral aversive behaviours (Dellert, Hyams, Treem & Geertsma, 1993).

Higher scores were obtained on good days from the pain subscales with both the core and non-core lexicon words. This finding may be explained by the use of mouthwash on the good days. Some people find that the alcohol content in mouthwash makes their mouth sting and if it is either a sensation they are not used to or more intense than they are used to it may have created a burning sensation that some people found painful. With chemosensory burning sensations elicited from capsaicin being rated as significantly more intense for hyper-tasters than tolerant-tasters (Karrer & Bartoshuk, 1991) it is unexpected that the pain subscale rating on both the core and non-core lexicon did not differ with taster status.

Though the good oral health, bad oral health day manipulation was successfully quantitatively different on the candidate oral lexicon it was not influenced by taster status, which was one of the goals of the chosen method. In fact, the effect of taster status was limited to the bad oral health day responses with core word candidate lexicon Texture: Consistency sensation being stronger for hyper-tasters than tolerant-tasters and in the non-core words, tolerant-tasters used more emotive words to describe mouth feel than tasters did. This finding raises questions regarding taster statuses relationship with emotions. Where these findings find links with oral emotional responses and tolerant taster status previous

research in rats find that emotional reactivity in PROP-tasters appear to elicit more negative emotions, which trigger actions such as fight, flight, defence and avoidance (Dess & Minor, 1996). Further exploration of possible relationships between taster status and emotional responses would help clarify the disagreements between the research.

The primary concern within the data rises from Experiment 4: Validation. The data was collected at multiple locations one of which was the GSK research and development laboratory. People who work for the GSK Oral Health Care team and participated in the study tend to be more orally focused and aware than the general population, this is due to assessing mouthfeel being a large part of their job. The concern was that the data collected from the GSK participants would unduly influence the ratings of how descriptive the words were of the sensations elicited during the good and bad oral health day. Hayakawa, Kazami, Wakayama, Oboshi, Tanaka & Maeda *et al*., (2010) suggest that words which are appropriate for trained tasters, which the GSK participants are, may not be appropriate to the consumer. During data analysis it was found that the location the data was collected from did not influenced the core candidate lexicon words which suggests that the candidate oral lexicon works as designed and can successfully be applied to bother trained tasters and consumers in the same manner. There was, however, an influence of location on the non-core lexicon responses but it was not possible to establish more than an interaction between location and oral health day.

Reflecting on the entire data collection processes some other concerns should be considered as they deviate from the published procedures and as such may have influenced the reliability of the scale. Within Experiment 1 there was a large overlap in word ratings which may be explained by participants not understanding what was meant by the provided explanations of sensory, emotional, evaluative, applicable and importance. It is also possible that this overlap is because when it comes to explaining the oral sensory experience it is not as simple as being only sensory, only emotional or only evaluative. When it comes to the mouth and how things feel relating to it, when put on the spot and asked to describe mouthfeel many people struggle to describe sensations (as demonstrated

in chapter 4 Examining the role of taster status on oral sensory processing pg 101). In turn this overlap led to complications further down the candidate oral lexicon development that had to be overcome and led to an additional data collection phase being Experiment 2: Distributions (section 7.3 pg 215).

Further concerns with the data collection process is that due to the volume of words that were collated for Experiment 1 the initial ratings of them was done by two different groups of people on two different word lists. This is unlike what was successfully applied in the development of the TPT where all the words were rated by all study participants on the three dimensions sensory, emotional and evaluative (Guest, Dessirier, Mahrabyan, McGlone, Essick & Gescheider *et al.,* 2011). Ideally to create a more reliable tool the ratings of the all the original words would be done as one complete set with all words being rated by all participants, replicating the TPT developmental approach. This together with the subjective nature of the word filtering procedure used in Experiment 3 led to the inclusion of some of the previously removed words, termed the non-core words, in the validation study to assess if there was a possibility they should have been retained. Given that the factors of the validation study loaded similarly for both the core and non-core candidate lexicon words it is not unreasonable to presume that the word filtering process for the core words was not precise.

This study has verified that the approach used to create the MPQ (Melzack, 1975) and the TPT (Guest, Dessirier, Mahrabyan, McGlone, Essick & Gescheider *et al.,* 2011) could be successfully applied to the generation of a full oral lexicon. The candidate oral lexicon identified that oral sensory perception is more than an evaluative experience but also a sensory and emotive one and that the procedure used can identify these different dimensions of the oral experience. If the concerns with the data filtering process are addressed by providing examples with the descriptions of what is meant by the terms sensory, emotional, evaluative, importance and applicable in Experiment 1 then it is possible the overlap of rating will be reduced. Having only one set of participants spending a longer time rating all the words may also assist with dealing this overlap. The hope would be that once a reliable Oral Lexicon was developed it could be applied to multiple sensations and

may be able to further identify perceptual differences between the taster groups.

Chapter 8 : General Discussion

Abstract

This thesis aimed to explore oral sensory perception. Oral sensation extends far beyond taste perception. Specifically, the aim was to explore the role of somatosensation by utilising classic psychophysical techniques. Furthermore, it looked to see if taster status had any impact on oral somatosensory perception as there is on oral chemosensation.

This final chapter summarises the results of the experimental work and discusses them in the context of the previous research and its implications for the understanding of the oral somatosensation. Overall, the results of the thesis indicate the somatosensation alone had no influence over the intensity of chemostimulants but it did interact with the different oral regions, reflecting the differences in oral anatomy. Taster status was also seen to potentially have a wider influence than on the oral cavity.

Extending the somatosensation field, C tactile afferents were identified in the lip possibility explaining increased lip sensation intensity identified in chapter 4 and the pleasure experienced in lip-to-lip contact. It also furthered the understanding of neurotransmitter, 5-HTs, role on peripheral and central perception of four basic tastes in chapter 6. Finding that bitter tastes intensity and pleasantness were highly influenced by TRP-.

Lastly, the development of a candidate oral lexicon reported in the final experimental chapter fills a gap in the research. The candidate oral lexicon reported in this thesis replicates the approach previously used successfully and demonstrates that it is an approach that can also be applied to oral sensations.

8.1 Summary of experimental work and implications

The aim of this thesis was to explore the role of somatosensation on oral perception by utilising classic psychophysical techniques. A series of studies investigated the role of somatosensation in oral perception and examined if taster status influenced somatosensations as it does chemosensation. The influence of innervation, receptor density, taster status and somatosensory impacts on chemostimulant intensity was explored first. The second study looked to identify if CT afferents were present in the lip. The third study used an ATD approach to examine the role of 5-HT has in taste transduction. Finally, it was identified that individuals have a failing in ability to describe how their mouth feels or how things feel in their mouth that led to the development of a candidate oral lexicon.

8.1.1 Study 1: The role of taster status in oral chemosensory perception

Chapter 4 investigated different oral regions for their cheomo-perceptive abilities and with the addition of dynamic touch, investigated if the elicited sensations intensity changed. As chemostimulant perception is highly influenced by taster status it was further explored by establishing the participants taster status to allow assessment of its influence on perception with the inclusion of the somatosensory stimuli. This study found that the regions with greater levels of innervation, and as such greater density of receptors, like the tip of tongue, experienced a greater sensation intensity than locations with reduced receptors chemoreceptors like the lip and frenulum of the lower lip.

An overall main effect of taster status was identified in all five substances tested with hyper-tasters experiencing a greater intensity of sensation than tolerant-tasters across the board. While, it is not substance specific it does support the argument that the taster groups do experience oral sensations differently and that they may possess different levels innervation within the mouth even though it is not reflected at individual location with all substances.

The location the stimuli were applied to and the participants taster status highly interacted with touch type. In the 10ppm capsaicin concentration the

sensations were significantly more intense for the hyper-tasters than tolerant-tasters with a static touch on the side and median sulcus of the tongue. Sichuan pepper applied to the median sulcus and tongue tip with a static touch was also rated a significantly more intense by hyper-tasters than tolerant-tasters and finally mint oil on the tip of the tongue was more intense with a static touch than dynamic, but no interaction with taster status was established in mint oil. The tongue tip has the greatest density of FP and hyper-tasters have significantly more FP on the tongue than tolerant-tasters. This stands to reason that increased FP density equals greater innervation and as such a greater sensation intensity. Regarding the static touch, the intensity is greater in regions within the oral cavity, a region that is more used to experiencing dynamic sensations during eating, therefore the increased static intensity could be due to the lack of movement diluting the sensations.

Astringency has previously been identified as a taste sensation due to activation of the chorda tympani taste nerve and glossopharyngeal nerve (Schiffman, Suggs, Sostman & Simon, 1992) but the sensation could be perceived on non-taste oral tissues indicating the possibility it was a somatosensory sensation (Breslin, Gilmore, Beauchamp & Green, 1993; Green, 1993a; Lim & Lawless, 2005). Within chapter 4 the astringent sensation elicited from Alum was detected on non-gustatory surface of the frenulum of the lower lip indicating that it indeed, may not be a taste sensation alone but may include a somatosensory component to its perception, however it was not altered by touch.

Whilst no interactions with Alum and taster status was found, a main taster status effect was established with hyper-tasters experiencing a greater intensity of sensation compared to tolerant-tasters. Within astringency literature there is evidence suggesting that tasters status influences the intensity of astringency (Pickering, Simunkowa & DiBattista, 2004) and others that don't find differences between the taster groups (Ishikawa & Noble, 1995). These studies often use red wine to elicit the sensation and though a desirable quality in red wine (Jiang, Gong & Matsunami, 2014) it must be considered in a wider context. Repetitive application of astringent eliciting substances increases the intensity, which in the

situation of red wine would probably induce individuals to either take longer consuming the drink or stop. However, when considering taster status if only hyper-tasters are getting the astringent oral feedback at a significant level, as indicated in Chapter 4, then while they may stop drinking or slow down a tolerant-taster may not. Some research indicates that tolerant-tasters are at an increased risk of alcoholism with a disproportionate quantity of them among alcoholic research participants (DiCarlo & Powers, 1998) and that as such hyper-taster status may possess a protective factor against such lifestyle behaviours. This highlights that further understanding of the perceptions of astringency are essential as they may have relationships to some lifestyle behaviours and as such astringent sensations warrant further consideration.

Interestingly, taster status was seen to have an influence outside the mouth with the sensation intensity of the 10ppm capsaicin and Sichuan pepper rated as significantly more intense on the lip by hyper-tasters than tolerant-tasters. This is unexpected and could represent a wider influence of taster status on sensation perception on the basis of increased innervation of the oral region possessed by hyper-tasters. Furthermore, the mint oil dynamic touch on the lip was rated as significantly more intense than a static touch.

In considering the increased intensity found on the lip when a dynamic touch is used it is important to consider the effects of spatial summation. It is possible that the effect is due to spreading the substance across a wider region, thus activating a greater density of receptors and increasing the intensity of the experienced sensation. However, it could also reflect an increase in pleasantness from rubbing the lips together and activating specific nerve fibres associated with pleasant touch. This finding led to the hypothesis that CTs, the known nerve fibre that codes for pleasant touch, may be present in the lip, explain both the findings from Study 1 and how lips are used in social interactions like lip-to-lip contact. It also raises the question if someone expects greater pleasure from lip touching and as such use more lip products like lip balms and lip sticks.

8.1.2 Study 2: Are lips a social organ?

Chapter 5 tested the hypothesis that taster status may have influence that extends beyond the oral cavity. This was done by investigating if there is potential for CT afferents to be present in the lip thus explaining some of the findings from study 1 in chapter 4 and how the lips are used in specific social interactions like lip-to-lip contact. It went on to further investigate if thermal detection and pain levels were different on the basis of location, mucosa, lip or cheek, and looked to confirm the previously established research of taster status oral anatomical differences.

This study did not identify a relationship between taster status and FP density where other researchers did (Bartoshuk, Duffy & Miller 1994; Essick, Chopra, Guest & McGlone, 2003). This lack of finding could be due to poor image quality, the published research has generally used medical grade cameras and lenses but the study in this thesis used a digital camera and macro lens that can be bought in stores.

When comparing these findings to the previous findings it is important to note the differences between the approaches that extend beyond technological availability and participant numbers. The current study, which failed to relate FP density to taster status used an area of $1cm^2$ for FP density counting, replicating the target area previously used to find associations between taster status and FP density by Miller and Reedy (1990) as well as Essick, Chopra, Guest and McGlone, (2003). Others that failed to find relationships between FP density and taster status used 1cm (Garneau, Nuessle, Sloan, Santorico, Coughlin & Hayes, 2014) and 6mm diameter circles (Fischer, Cruickshanks, Schubert, Pinto, & Klein *et al.,* 2013) for FP density counting. Furthermore, Fischer Cruickshanks, Schubert, Pinto and Klein (2013) did not use the pure FP count obtained in the 6mm diameter circle but generated an equation to calculate the FP density of the tongue as a whole. Garneau, Nuessle, Sloan, Santorico, Coughlin and Hayes (2014) used the Denver Papillae Protocol (DPP) to count FP densities, requiring not only multiple counters but also additional training. The different sized areas targeted and the different methods employed for FP counting makes comparisons between the publications complex. A standardised protocol for counting FP like that of the DPP would be

beneficial for conclusively deciding if FP density and taster status are related however, the specialised training and multiple scientist counters limits the use of the DPP in wider research.

The key finding of this study is that the stroking task generated the classic inverted-U of pleasantness commonly associated with CT activity in other body sites. This would suggest that CTs may be present in the lip and should be examined further. This is important as until this study was conducted research assumed that CTs were not present in the lips due to the physiological structure of the lip skin but with no published data available to support either way. The finding of behaviour consistent with the potential presence of CTs in the lips suggests that further investigation is warranted and could have implications for our understanding of the reward mechanisms behind the social behaviour of lip-to-lip contact and it possessing a role within the social touch hypothesis. Furthermore, it may raise questions about the act of cheek kissing in some forms of greeting and lip contact when romantic interaction is not the goal, such as between parent and child. This further exploration would be best achieved by the electrophysiological technique of microneurography that allows for direct nerve recording and stimulation.

The data collected on thermal threshold and suprathreshold across the three locations identified that the lip is highly proficient at thermal detection, mostly likely to the structure of the lip skin and reduced number of layers that it is composed from. Where the mucosal surface is reasonably poor at thermal detection and insensitive to hot thermal pain until it reaches noxious temperatures, the lower lip is highly sensitive to thermal changes and can detect temperature changes within 1 degree of change from body temperature. Furthermore, the hot suprathreshold level, though not significantly different does exceeds that of the cheek. Both of these findings may be related to one of key roles the mouth has and that is food consumption. The mucosal findings could simply be that the location itself, the oral cavity is a naturally warmer environment than the other locations and must hardy enough to withstand the heat experience during food consumption but aware enough to respond when it reaches noxious levels for protection. The

lower lip however, may also be tuned to warm thermal temperature changes for the same reason. Lips act as a protective gate way to the mouth and if the lip detects the temperature to be an extreme the nociceptive activity would induce the individual to not continue to consume the food. It is widely known that the lips discriminative capabilities match or indeed exceed that of the fingertip (Johnson & Phillips, 1981; van Boven & Johnson, 1994; Sathian & Zangaladze, 1996) and the thermal detection capabilities of the lips are likely an extension of this.

No taster status interactions were found in either the stroking or thermal data and although the effects of taster status on the intra-oral perceptions of chemosensation and thermal perception are vast in the literature this study does not support the hypothesis that taster status innervation differences extend beyond the oral cavity.

8.1.3 Study 3: Acute Tryptophan Depletion: Exploring serotonins role in taste perception

5-HT levels have previously been associated with the differentiation between CT touch and discriminatory touch responses (Trotter, McGlone, McKie, McFarquhar, Elliot, Walker & Deakin, 2016). Due to the recent findings that taste cells release 5-HT when stimulated with specific tastes the study reported in chapter 6 used an ATD method to alter TRP levels, and by association tonic 5-HT levels with the aims of examining its effects on the peripheral and central perceptions of four basics tastes.

The overall effect of ATD on taste perception was that it increased the perception of bitter taste intensity and decreased bitter tastes pleasantness. Three hypotheses were tested in this study, the first that TRP- would reduce detection thresholds, specifically for the sweet and bitter tastes as not supported. A trend can be seen in the raw detection scores towards a reduction in taste threshold for all tastes in the TRP- session. It is possible this lack of expected significance and failure to support the findings of Heath, Melichar, Nutt and Donaldson (2006) is due to altering 5-HT levels being a by-product of the ATD manipulation and as such the effect on 5-HT levels may not have been large enough. Although the effect did not

alter detection ability it did alter the perceived intensity and pleasantness of tastes, specifically bitter tastes which are subjective experiences.

The second hypothesis was that TRP- would increase the intensity of tastes but again this was not entirely supported. The opposite effect was actually established with the bitter taste being considered more intense during TRP- than TRP+. Examining taster status showed that the TRP manipulation had a significant effect on the intensity ratings of the highest bitter concentration with tolerant-tasters rating the TRP- significantly more intense than TRP+.

The final hypothesis was that hedonic ratings of the taste was decrease in pleasantness/increase unpleasantness. Again, this effect was only identified in bitter tastes with TRP- being considered significantly more unpleasant than the TRP+ session with the highest concentration reflecting this without an effect of taster status.

This suggest a central mechanism response to the bitter taste with an increase in attention to bitter. Bitter tastes are often associated with foods that should not be consumed due to bitter tastes being associated with poisonous plant life so this increase in intensity and unpleasantness associated with bitter taste during TRP- may be a throwback survival mechanism. Reduced TRP and as such 5-HT levels could indicate that an animal is unwell and by heightening the intensity and unpleasant bitter experience acts a protective mechanism to ensure reduced consumption of something potentially toxic (Reed & Knaapila, 2010). It may also be explained by an affective attentional bias in that during depression attention is increased towards negative stimuli (Gotlib, Kasch, Traill, Joormann, Arnow, & Johnson, 2004; Gotlib, Krasnoperova, Yue & Joormann, 2004).

Interestingly the intensity and pleasantness differ on the basis of taster status with tolerant-tasters experiencing a greater intensity of super-threshold bitter taste from the TRP- session. This could be a specific defence to the tolerant-tasters that as they receive little feedback from their foods so if they consume a poisonous plant they are less likely to notice the taste and as such, when in a weakened state which the ATD manipulation may be a model of, would lead to an increase in bitter intensity perception to encourage the individual to not consume

more of the substance. Furthermore, bitter was experienced as significantly more unpleasant in the TRP- than the TRP+

Taster status was found to impact on the sweet taste pleasantness with tasters rating sweet as more pleasant than tolerant-tasters. This is an unexpected finding as hyper-tasters show less liking for foods possessing high sweet contents (Duffy & Bartoshuk, 2000; Looy & Weingarten, 1992) and tolerant-tasters report to consume more sweet food than tasters (Duffy, Peterson, Dinehart & Bartoshuk, 2003). This could indicate that the TRP- increased the rewards drive. Sweet taste increase in pleasantness could be the body's way of encouraging an individual to try and experience a release of 5-HT to bolster mood. Consumption of sweet serves no benefit for increasing TRP levels so sweet would not help to rebalance the amino acid depletion the body has experienced as a part of the ATD. This is a hypothesis that warrants further investigation.

To the best of knowledge there is no published study that has examined the effect of ATD on human taste detection. Heath, Melichar, Nutt & Donaldson, (2006) conducted a study with humans where participants were given a high dose of SSRI and examined detection ability but the study reported in this thesis goes a step further and examined the central mechanisms of the taste perception by asking about taste intensity and pleasantness. These are things important to consider regarding taste as they are important drives of consumption.

8.1.4 Study 4: The candidate oral lexicon

It became clear early on the development of the thesis that individuals had difficulty describing how their mouth felt. A search of available tools for assessing mouthfeel led to the discovery that those available for describing oral sensations were designed by specialised panels and aimed for use with specific products. To use them effectively specialised training is also often required. Study 4, therefore sort to develop a tool that could be used to aid in the description of oral sensations.

Development of the candidate oral lexicon was complex and involved 3 separate words ratings tasks to generate a lexicon for validation. The entire process from the first experiment to conclusion of the validated candidate oral

lexicon took 3 years. Due to this the lexicon was unavailable for use in the other studies of this thesis.

The candidate lexicon was developed using the approach of Melzack (1975) and Guest, Dessirier, Mehrabyan, McGlone, Essick & Gescheider, *et al.,* (2011) and this approach established that oral sensations also consist of affective and discriminative aspects. Analysis of the words contained in the core lexicon support that the oral lexicon is comprised of words that describe the texture, the sensory and the emotive sensations experienced in the mouth which shows many facets of oral sensation and that oral perception is not just a case of the five basic tastes but includes textures and emotional responses elicited from the experience.

This tool is important as lexicons standardise language use across oral sensations and experiences. This standardisation is important as there is currently no mechanical or physical device that can the work of the mouth, nose and brain in detecting and evaluating flavour (Caul, 1957).

There was some concern after examination of the retained core lexicon words that the words were excluded in experiment 1 that possibly should not have been. This exclusion was thought to have occurred due to confusion with definitions of what evaluative meant as the majority of words were rated 'evaluative' as well as 'sensory' or 'emotional', which led to words duel loading and an additional data collection phase of distribution assessments. Furthermore, the ratings task in experiment 1 split the words over two different word sets which were each rated by different groups of people. This complicated the processes of combining the words ratings together to make one coherent list of rated words. Due to this, experiment 2 needed to be run to make the words load onto a single aspect of the oral experience.

Some words that had been removed in the distribution phase of analysis were included in the validation procedure though not contained in the core lexicon. The words from both the core and non-core lexicons loaded as similar factors however, the words in the non-core lexicon did not always reflect both ends of the sensory spectrum.

The word lists could not be combined for PCA analysis because of lack of power. Furthermore, a lexicon that is comprised of so many words would not be practical therefore results from it would be meaningless. When the core candidate and non-core lexicon were not assessed for their use across the good and bad oral health care days both were found to be used differently between days.

Development of a full Oral Lexicon of Sensation would need to address the concerns of the data collection processes in experiment 1 by taking the additional time to have all participants rate every word. It may also be advised to remove the evaluative aspect of the rating due to the large overlap between evaluative, sensory and emotional.

8.1.5 Future Studies

The findings from this thesis yielded some further questions that if addressed may extend the findings.

Given the supposition from chapter 4 that taster status may have wider influence than the oral cavity further exploration of that would be beneficial. It was identified in chapter 4 that hyper-tasters experience greater oral burn than tolerant-tasters from 10ppm capsaicin on the lip where the intensity was more pronounced with a dynamic touch, however hyper-taster static touch appears similar to taster and tolerant-taster intensity ratings meaning that examination of the role of capsaicin and dynamic touch to the lip should be examined again.

Other aspects of oral somatosensation could also be examined such as oral pain perception. As chemosensory burning perception is affected by taster status, again highlighted in chapter 4 of this thesis. This effect could also be reflected in pain perception with hyper-tasters having perceiving greater oral pain than tasters and tolerant-tasters on the basis of hyper-tasters have oral greater innervation. This could be done by using a QST approach, part of which was used in chapter 2s exploration of lip thermal perception. Using the wider standardised QST protocol as outlined by Rolke , Mageri, Campbell, Schalber, Caspari & Birklein *et al.,* (2006) would allow a detailed assessment of oral pain and touch perception.

The identification of CTs in the lower lip in chapter 5 could be expanded on by the technique of microneurography. Microneurography is a technique used for recording directly from single unit nerve recordings in responses to stimulation (Vallbo, Hagbarth & Wallin, 2004) and been used in other body regions to find CTs (Liljencrantz & Olausson, 2014; Löken, Wessberg, Morrison, McGlone and Olausson 2009). Although no effect of researcher gender was found in the analysis to guarantee no possibility of it using a rotary tactile stimulator (RTS) like that used by Essick, James and McGlone (1999) and Löken, Wessberg, Morrison, McGlone and Olausson (2009). This however raises problems as the lip region is an exceptionally small region to stroke with the robot.

Exploration of the hedonics of lip-to-lip contact should further consider the importance of who is initiating the contact. For example, a lip stroke administered by a significant partner would likely be rated as significantly more pleasant than a lip stroke by a stranger.

8.1.6 Conclusion

This thesis explored some of the somatosensory aspects of oral sensation. Overall, it demonstrated that type of touch, when combined with taster status and oral location can increase the intensity of low concentrations of 10ppm capsaicin and Sichuan pepper chemostimulants. It was also identified that CT afferents were identified as present in the lips. This potentially explains some of the pleasantness associated with lip-to-lip contact and until this study CTs were previously assumed to not be present in the lips due to their type of skin. The ATD study highlighted that by reducing TRP, and as such circulating 5-HT, bitter taste intensity increased and pleasantness decreased but had no effect on detection levels. This highlighted that 5-HT had a role in the central mechanisms of taste perception rather than peripheral mechanisms. The final study recognised a failing in the research and led to the development of a candidate oral lexicon that provides the ability to describe perceptually how the mouth feels and how things feel with the mouth. Somatosensation clearly has a role within the oral cavity and the perception of sensations in the region.

Chapter 9 : References

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