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PSYCHOPHYSICAL AND HEDONIC RESPONSES TO SWEETENERS IN HUMANS

A Thesis in

Food Science

By

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ABSTRACT

Sweetness is an inherently positive sensation that is a highly liked. There are however, individual differences in sweet taste perception that are often overlooked in studies that investigate the intensity perception and hedonics of sweet taste. The objective of this thesis is to investigate human sweet taste perception through psychophysical and physiological responses to sweet stimuli. The present work includes a discussion of the issues concerning the measurement of human physiological and psychophysical responses to sweet taste including methodological inconsistencies, individual differences, and genetic variability. This thesis takes a modern approach to investigating sweet taste perception by using contemporary psychophysical scaling methods. Major experimental findings include: Study 1: Sucrose did not increase cold pain tolerance or threshold times in adult males even when controlling for hand temperature and hunger state. These results agree with reports that sucrose analgesia is an age dependent phenomenon. Study 2: Unlike previous reports, sucrose was found to have a sigmoidal dose-response function. Notably, most non-nutritive sweeteners (NNS) elicited a lower maximal sweetness than sucrose and other nutritive sweeteners. The decrease in NNS sweetness appears to be dependent on mixture suppression due to increasing bitterness. Study 3: Four hedonic groups for sucrose were identified: 'Slope +', 'Slope -', 'Horizontal Line', and 'Inverted-U'. Significant differences in hedonic liking of sweet foods and sweet alcoholic beverages were found between the four sucrose liking groups. Present results suggest that the hedonic liking of sucrose solutions can generalize to sweet food liking. Study 4: Contrary to what was expected, there were no significant differences in the opioid receptor mu-1 (OPRM1) genotype frequency between sucrose hedonic groups. Nor were there significant differences between genotypes in their hedonic ratings of sweet foods.

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Chapter 1

Literature Review

Introduction

In the most primitive way, the ability to taste enables humans and animals to evaluate and make judgments about the nutritional value, quality, and safety of their diet. Touch, vision, and smell are other important sensory modalities that are needed for food and environmental assessment to prevent a harmful item from reaching the mouth. Vision can provide information about the quality of food (e.g. ripeness), smell can provide information about the freshness of food (e.g. rancidity), and tactile information about the textural properties of food that may indicate spoilage. If a noxious item gets beyond keen visual, olfactory, and tactile evaluation of the human, the sense of taste is the next preventative hurdle.

Humans are able to distinguish between the five tastes: sweet, salty, bitter, sour, and umami, all of which serve a nutritional purpose in the omnivorous diet (Glaser, 2002). Although taste and flavor are often used synonymously, in technical usage taste refers only to the five prototypical tastes whereas flavor is a combination of taste, olfaction, and chemesthetic sensations (i.e. cooling, burning, stinging etc.).

Sweet taste is a hedonically positive stimulus that is apparent in early human development prior to and after birth (Ganchrow et al., 1983; Mennella & Beauchamp, 1998). When infants are presented with a sweet tastant their facial expressions and physical movements indicate that the sweet taste is pleasant (Engen et al., 1974). Similar to sweet taste in adults (Drewnowski et al., 2012), infants are able to detect and distinguish between sweet substances (Desor et al., 1973). The ability to detect a wide range of sweet stimuli is attributed to the heteromeric human sweet taste receptor (TAS1R2+TAS1R3). The human sweet taste receptor has

an affinity for multiple sweet compounds (Agarwal & Lucas, 2002; Nelson et al., 2001; Zhao et al., 2003) including non-nutritive sweeteners, natural sugars, and some proteins (e.g. Monellin and Brazzini). It would be unnecessary to have multiple receptors to distinguish between sweet compounds because evolutionarily sweet taste is primarily a marker for energy from mono and disaccharides (Zhao et al., 2003).

In contrast to sweet taste, bitter taste is, generally, a negative stimulus. Unlike sweet taste, it is necessary to have a very wide range of receptors to identify bitter substances to avoid the consumption of toxic stimuli. About 25 (TAS2R) bitter receptors have been identified (Meyerhof et al., 2010) in humans. The bitter taste receptors include generalists and specialists that have an affinity for a wide range of bitter substances with a variety of chemical structures. In human infant studies, bitter substances evoke taste aversion characterized by negative physical features such as mouth gaping, headshaking, and arm flailing (Steiner, 1979; Steiner et al., 2001). In contrast, a study by Desor et al. (1975) found infants to be indifferent to urea suggesting that infants either do not have a hedonic response to bitterness or they are developmentally too young to detect bitter taste.

Umami, “savory” taste, is the perception of mono-sodium glutamate (MSG), (Kurihara & Kashiwayanagi, 2000), which is indicative of amino acids and protein rich foods. Similar to sweet taste, umami is transduced by a heteromeric taste receptor (TAS1R1+TAS1R3) (Nelson et al., 2002). The ability to taste umami is apparent at infancy, however the data on the pleasantness of umami in infants is more variable than that of sweet or bitter taste. MSG (umami) has been reported to be unpleasant to infants when presented alone in aqueous solution (Yamaguchi, 1991). However, in food applications, infants prefer soup containing MSG over soup without MSG (Beauchamp and Pearson, 1991).

Saltiness is the perception of salts, which are necessary for the operation of bodily functions and homeostasis (Daniels & Fluharty, 2004). Salt levels in the body affect the

palatability of salt. Many human and animal studies have illustrated that low sodium diets and salt deficiencies increase the pleasantness and palatability of salt (Daniels & Fluharty, 2004). Unlike sweet or bitter stimuli, neonates do not elicit clear hedonic responses to salt (Desor et al., 1975) and early sensitivity to salt is shown to be age dependent (Beauchamp et al., 1994; Mattes, 1997).

Sourness is induced by acids (e.g. citric acid) and is an aversive stimulus in early stages of life (Desor et al., 1975; Steiner, 1979) although some children can have preferences for sourness (Liem & Mennella, 2003). The purpose of sour taste has been debated in the literature and has been attributed to the need for Vitamin C in the diet (Breslin, 2013) and the need to maintain ion equilibrium for respiration (Huang et al., 2006). The most plausible argument however, is that that sour taste is a protective mechanism as sourness is indicative of food spoilage (Ugawa, 2003).

In summary, the five tastes provide qualitative and hedonic information about food composition and liking. The taste of food evokes an inherent “like” or “dislike” response which indicates if the food is “safe” or “unsafe” for consumption respectively. The innate hedonic response to taste is not limited to humans, but is also observed across species (Ganchrow et al., 1986).

Physiology of taste

Despite common belief, there is no taste map as suggested by previous literature (Hoon et al., 1999). All five prototypical tastes can be perceived on all parts the tongue (Chandrashekar et al., 2006; Collings, 1974) through thousands (2000-5000) of taste buds. Specifically, taste buds are located in papillae and epithelial tissue on the tongue, soft palate and pharynx (Lindemann, 2001).

Papillae are folds or projections located on the front, back, and sides of the tongue (Roper, 2007). There are three main classes of taste-bud containing papillae: fungiform papillae

(front of the tongue), circumvallate papillae (back of the tongue), and foliate papillae (sides near the back of the tongue) (Breslin & Huang, 2006). There is also a fourth class of papillae, filiform papillae, which do not contain taste buds. Despite their lack of taste buds, filiform papillae are the most abundant papillae on the tongue (Nelson, 1998).

Taste cells are held together in a unit called the ‘taste bud’. The number of cells contained in each taste bud is variable ranging from about 30-50 cells (Lawless & Heymann, 2010) to 100 (Lindemann, 2001). A pore at the top of the taste bud allows a tastant, facilitated by saliva, to enter the bud. The taste bud contains four cell types: basal cells, light (type I), intermediate (type II), and dark (type III) taste cells (Breslin & Huang, 2006; Nelson, 1998). Different cell types harbor distinctive proteins and neurotransmitters (Finger et al., 2005) that are responsible for taste signal transduction (Zhang et al., 2003). Type I cells are signal supporting cells that allow for clear taste signal transduction, type II cells are taste receptor cells and express GPCRs for taste perception, and type III cells contain synapses and respond primarily to acids (Roper, 2013). Further information concerning the function of taste cells can be found in a review by Roper (2013).

Taste buds transmit taste signals through nerves. Taste buds are innervated by a variety of cranial nerves including the chorda tympani, glossopharyngeal, and vagus nerves that send information to the brain (Lawless & Heymann, 2010). Taste is a resilient and complex sense and the loss of function in one nerve may not eliminate taste perception entirely (Kveton & Bartoshuk, 1994). Studies that have anesthetized the chorda tympani to imitate taste damage have found that an anesthetized chorda tympani leads to increased taste intensity perception (Lehman et al., 1995), increased chemesthetic intensity perception (Tie et al., 2002), and the presence of ‘phantom’ tastes (Yanagisawa et al., 1998). It should be mentioned that although gustatory nerve damage sometimes results in ageusia (loss of taste function), it is a rare occurrence. Other factors such as age and gender (Mojet et al., 2001), diseases such as Alzheimer’s disease (Schiffman,

1997), and smoking (Vennemann et al., 2008) are more likely than gustatory nerve damage to have a direct negative impact on taste loss as well as flavor and/or taste perception.

The brain is required in order to perceive taste sensations. Rolls and colleagues (2010) present a simple schematic of taste processing in the brain. They defined two primary cortices where taste information is processed, the primary taste cortex and the secondary taste cortex, both of which contain taste neurons. Rolls et al. explain that the primary taste cortex (anterior insula and frontal operculum) has the ability to respond to all five tastes, some texture, and some chemesthetic stimuli, but does not process reward. The secondary taste cortex (caudolateral orbitofrontal cortex) also responds to all five tastes but is involved in reward processing. Taste in the brain is a complex process that involves the perception of five primal tastes and the hedonics that are elicited with each taste. Due to the complex nature of taste processing in the brain, the hedonics and reward associated with taste sensations will be discussed further in the review.

Sweet taste receptors

Two families of human taste G protein-coupled receptors (GPCRs), the TAS1Rs and the TAS2Rs, facilitate sweet, bitter, and umami tastes. The TAS1R family consists of the TAS1R1, TAS1R2, and TAS1R3 GPCR taste receptors. Combinations of two of the previously mentioned GPCRs form heterodimers (a single protein that is made up of two different polypeptide chains). The TAS1R2+3 heterodimer enables the perception of sweet taste and the TAS1R1+3 heterodimer enables umami taste (see figure 1-1). This section will evaluate the literature concerning the TAS1R2+3 heterodimer as it pertains to sweet taste perception.

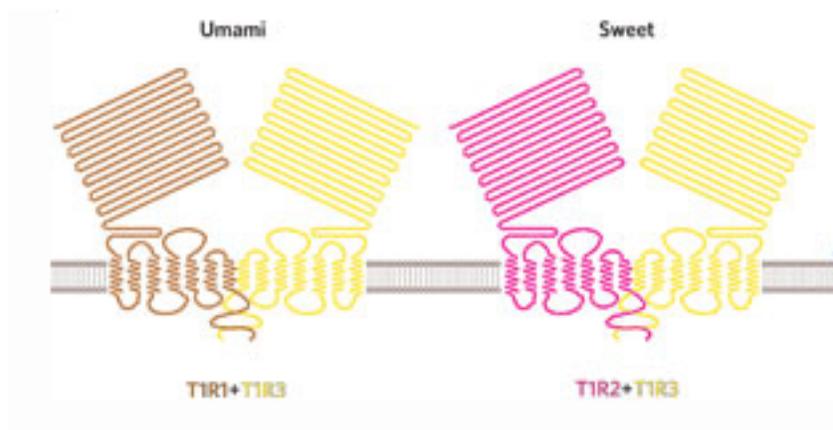


Figure 1-1 Visual representation of the human TAS1R family. TAS1R2+TAS1R3 form the sweet taste receptor and the TAS1R1+TAS1R3 form the umami taste receptor. Figure taken from [(Chandrashekar et al., 2006)]

GPCRs are membrane proteins that “enable cells to respond to their environment” (Roth & Marshall, 2012) by transmitting signals from the outside to the inside of the cell. GPCRs have three main regions: the extracellular, transmembrane, and intracellular region. The extracellular venus flytrap domain (VFD) of the GPCR enables ligand access to the receptor. The transmembrane region of the GPCR is responsible for ligand binding and configuration changes. The intracellular region of the GPCR is responsible for further signal transduction (Venkatakrishnan et al., 2013).

The characterization of the heteromeric sweet taste receptor is a relatively recent discovery. In 1999, Hoon et al. identified two novel GPCRs (TAS1R1 and TAS1R2) expressed in taste cells that were members of the human TAS1R family (Hoon et al., 1999). Two years later in 2001, the human genome database became available and enabled researchers to more easily investigate the genetics of human taste. There was increased interest in finding the gene responsible for the *Sac* locus. The *Sac* locus is a genetic fragment responsible for sweet taste preferences in mice first described by (Fuller, 1974). Many studies were able to conclude that the mouse *Tas1r3* gene was the primary candidate for the mouse *Sac* locus, and the human *TAS1R3*

gene was the candidate gene to code for the human *Sac locus* (Kitagawa et al., 2001; Max et al., 2001; Montmayeur et al., 2001; Sainz et al., 2001).

Animal studies have provided an important gateway into the understanding of the human TAS1R family. In terms of genetic similarity, it should be noted that the genetic similarities between *Tas1r3* gene in mice and *TAS1R3* gene in humans are 72% similar and the *Tas1r1* gene in mice and *TAS1R1* gene in humans are 74% similar (Montmayeur et al., 2001). Although mice and humans share similar sweet taste preferences, they do differ in the variety of sweet ligands that are able bind and evoke sweet taste (Xu et al., 2004). Differences in ligand binding and sweet perception also apply across species (Glaser, 2002; Glaser et al., 1996). Additionally, it must be further noted that polymorphisms in the mouse *Tas1r3* gene *in vivo* only explain some of the variation in phenotypic changes in sweet taste perception documented in the literature (Inoue et al., 2007). Other genes and environmental factors are also responsible for differences in sweet taste perception and phenotypic behavior.

Human TAS1R2 and TAS1R3 receptors are often co-expressed in taste cells, but the TAS1R3 receptor can also be expressed alone (Nelson et al., 2001). The literature that has investigated the individual responsiveness of the human TAS1R2 and TAS1R3 subunits to non-nutritive sweeteners and sweet proteins (Damak et al., 2003; Nie et al., 2005; Zhao et al., 2003) has been variable. It has been suggested that human TAS1R2 and TAS1R3 subunits have unique sweetener affinities (Nie et al., 2005; Zhao et al., 2003). For example, the human TAS1R3 receptor has a higher affinity for sucrose than the TAS1R2 receptor (Nie et al., 2005). Alternatively, the human TAS1R2 receptor has a higher affinity for glucose than the TAS1R3 receptor (Nie et al., 2005).

Many researchers have hypothesized how the human TAS1R2 and TAS1R3 receptors evoke sweet taste (Hoon et al., 1999; Li et al., 2002; Montmayeur et al., 2001; Nelson et al., 2001; Nie et al., 2005; Xu et al., 2004; Zhao et al., 2003). A study by Zhao et al. (2003)

investigated the function of human TAS1R receptors in knockout mice. The study found that the elimination of the human TAS1R2 or the TAS1R3 receptors impaired sweetness perception to sucrose, non-nutritive sweeteners (NNS), and sweet proteins.

A few other critical studies provided information concerning the role of binding sites and sweet ligand binding affinity on the sweet taste TAS1R2+3 heterodimer. Through fluorescence microscopy in human cells expressing TAS1R2+3, Xu et al., (2004) concluded that the TAS1R2+3 heterodimer, rather than the subunits individually, is responsible for the ability to recognize a variety of sweet stimuli. The study also found that there were multiple binding sites on each of the subunits enabling the binding of multiple ligands with different affinities. The ability for sweet ligands to bind to multiple sites on the sweet taste receptor, instead of competing for the same domain, may explain why some sweeteners are able to induce synergistic effects, while others do not (Servant et al., 2011). Additionally, DuBois (2004) showed evidence of multiple receptor binding sites on the sweet taste receptor. The study illustrated that adaptation to aspartame suppresses the sweetness perception of D-tryptophan, but does not affect the sweetness perception of saccharin and cyclamate (DuBois, 2004). This suggests that aspartame and D-tryptophan interact/compete with the same binding site, whereas saccharin and cyclamate bind to alternative areas of the receptors.

Measuring sweet taste perception

How is it possible to measure personal and subjective sensations such as sweet taste intensity and sweet liking? In terms of scaling techniques, researchers have developed many methods and scales over the past 80 years to measure sweet taste intensity perception and liking in adults. Category/hedonic scaling (Peryam & Girardot, 1952), Likert scaling (Likert, 1932), category ratio scaling (Borg, 1982), magnitude estimation (Stevens, 1956), visual analog scales (VAS), and Spectrum descriptive analysis (Meilgaard et al., 2007) have all been used to measure

either intensity or liking constructs. The previously mentioned scales however, do not control for individual differences in experiences, number usage, and semantic understanding.

Taste perception encompasses five attributes: spatial localization, quality, temporal dynamics, intensity, and hedonics (Breslin, 2013). This section will focus on hedonics and intensity. It should be noted that liking and wanting (motivation) are different constructs (Berridge, 1996; Dai et al., 2010). It cannot be assumed that a food is wanted because it is 'liked'. Although liking can impact wanting, motivational wanting will not be discussed further in this review. Also, many studies that are cited in this review erroneously use the terms liking and preference interchangeably. Although a tastant may be highly liked, it does not automatically suggest that there is a preference (Beauchamp & Cowart, 1987).

The relationship between sweetness intensity and sweet taste hedonics has been of interest to many researchers. It has been long known that intensity perception and hedonics are two separate constructs, however, intensity perception can impact liking. Intensity is the perceived magnitude of a taste sensation, whereas hedonic liking is the affective response evoked to that sensation. There are many factors that influence individual differences in sweet taste intensity and hedonics such as age (De Graaf & Zandstra, 1999; Pepino & Mennella, 2005b), weight status (Frijters & Rasmussen-Conrad, 1982), ethnicity (Pepino & Mennella, 2005a; Thai et al., 2011), and genetics (Bachmanov et al., 2011). For additional information on individual differences in sweet taste perception see Hayes (2008).

A brief note on PTC and PROP

As previously mentioned, there are individual differences that influence intensity perception and hedonic liking. The discovery of individual differences in bitter taste perception of the thioureas, PTC (phenylthiocarbamide) and PROP (6-n-propylthiouracil) (Tepper, 1998), opened up a new realm of questions concerning individual differences in taste perception. In

terms of PROP taste perception, PROP bitterness is a continuous trait (Hayes and Pickering 2011; Hayes and Duffy 2007), but historically, individuals were divided into three groups for analysis: supertasters (ST), medium tasters (MT), and non-tasters (NT) (as reviewed by Drewnowski et al., 1997). Some studies have found that ST not only perceive PROP bitterness as more intense than MT and NT groups, but other stimuli as well (e.g. sweetness from sucrose).

The relationship between PROP and sweet liking is unclear (Drewnowski et al., 1997; Duffy et al., 2003; Hayes & Duffy, 2008; Ko et al., 2000; Prescott et al., 2001; Yeomans et al., 2007). Yeomans (2007) conducted a study that found that PROP STs significantly rated sucrose concentrations (0.05, 0.21, 0.42, and 0.83M) as more intense than MT or NT, but did not significantly predict sweet liking status as hypothesized. In this study, there was also a large amount of individual variability in the hedonic ratings of STs as a function of intensity (Yeomans et al., 2007) suggesting that there are other factors, such as additional genetic factors, that influence sweet liking. PROP status will not be further discussed in the review.

Measuring intensity perception

There are differences in the way individuals perceive sensations, use numbers, and understand words. In other words, ‘individuals live in different sensory worlds’ (Bartoshuk et al., 2004; Bartoshuk et al., 2005). In terms of intensity perception, one person’s perception of a taste that is ‘very strong’ might not be equivalent to another person’s perception of a taste that is ‘very strong’. Additionally, one person’s intensity rating of a numerical value of a ‘10’ might not be equivalent to another person’s intensity rating of ‘10’.

Intensity ratings across groups or across individuals can only be compared if they are made “relative to a stimulus that is perceived to be equally intense to all subjects” (Bartoshuk, 1979). Due to the fact that many scaling techniques do not address this issue, Barry Green and colleagues (1993) developed the Labeled Magnitude Scale (LMS). The LMS measures perceived

intensity and produces ratio level data similar to that from magnitude estimation. The scale ranges from 0 (barely detectable) to 100 (strongest imaginable) (see Figure 1-2). The spacing of the semantic labels are empirically determined from the geometric means of numerical magnitude estimates of semantic descriptors. When Green et al. initially developed the scale, the top of the scale (100-strongest imaginable) was to be customized by the researcher to fit the sensory modality being measured (i.e. strongest imaginable ‘taste’ to measure taste; strongest imaginable ‘odor’ to measure odor etc.).

Bartoshuk et al. (2004) resolved the problem with the LMS top anchor by generalizing it across all sensory modalities by replacing the verbal label at 100 “strongest imaginable” (Green et al., 1993) with “strongest imaginable sensation of any kind” and named it the general Labeled Magnitude Scale (gLMS) (see Figure 1-3). The gLMS, similar to the LMS, is a semantically labeled scale with labels located at 0 (no sensation); 1.4 (barely detectable); 6 (weak); 17 (moderate); 34.7 (strong); 52.5 (very strong); and 100 (strongest imaginable sensation of any kind) (Bartoshuk et al., 2004). The development of the gLMS is beneficial tool in identifying individual differences in intensity perception, but it raises questions to whether or not previous studies have made valid comparisons between individuals.

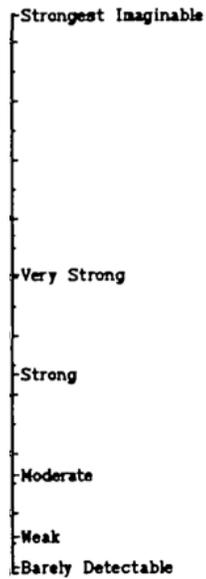


Figure 1-2 Labeled Magnitude Scale (LMS). The LMS is a semantic line scale that scale ranges from 0 to 100 (strongest imaginable). Each descriptor has been assigned a numerical value based upon geometric means of estimated values from participants (n=33). Figure taken from [(Green et al., 2003)] and formatted.

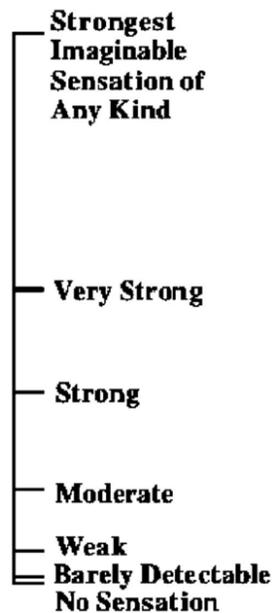


Figure 1-3 Vertical General Labeled Magnitude Scale (gLMS). The gLMS is a semantic line scale that scale ranges from 0 (no sensation) to 100 (strongest imaginable sensation of any kind). The words 'any kind' generalize the scale to all sensory modalities. Figure taken from [(Bartoshuk et al., 2005)] and formatted.

Hedonic Measurement

How is liking measured? Although hedonics is a subjective response, it is not as straightforward as ‘like’ or ‘dislike’. Similar to intensity perception, a variety of scales have been used to measure hedonic response. Hedonic category scales, 9-point hedonic scales (Peryam & Pilgrim, 1957) (see Figure 1-4) and 11-point category scales, with descriptors such as “like extremely” and “dislike extremely”, are popular in industry because they are easy to use. Despite ease of use, 9-point hedonic scales have issues concerning the avoidance of outer anchor categories and preferences in make ratings near the middle of the scale near the ‘like nor dislike’ option (Moskowitz, 1980).

Like extremely
 Like very much
 Like moderately
 Like slightly
 Neither like nor dislike
 Dislike slightly
 Dislike moderately
 Dislike very much
 Dislike extremely

Figure 1-4 Vertical 9-point hedonic scale. Numbers 1-9 are can be assigned to the descriptors starting at either end of the scale (like extremely or dislike extremely). Figure taken from [(Lawless and Heymann, 2010)].

In response to issues with the 9-point Quartermaster scale, the labeled affective magnitude scale (LAM) (Schutz & Cardello, 2001) was developed to measure hedonics. The LAM is a semantic hedonic scale that is similar to the LMS (Green et al., 1993) which is used for

intensity perception. The scale ranges from 100 (greatest imaginable like) to -100 (greatest imaginable dislike) (see Figure 1-5). Many studies have compared the 9pt-hedonic scale and the LAM in their ability to differentiate amongst the liking of different foods. The studies have found variable results concerning discriminability (Dine et al., 2009; Kalva et al., 2014; Lawless et al., 2010; Schutz & Cardello, 2001), however the LMS is more useful in determining differences across people. Additional information on the benefits and limitations of generalized hedonic scaling can be found in a review by Lim (2011).

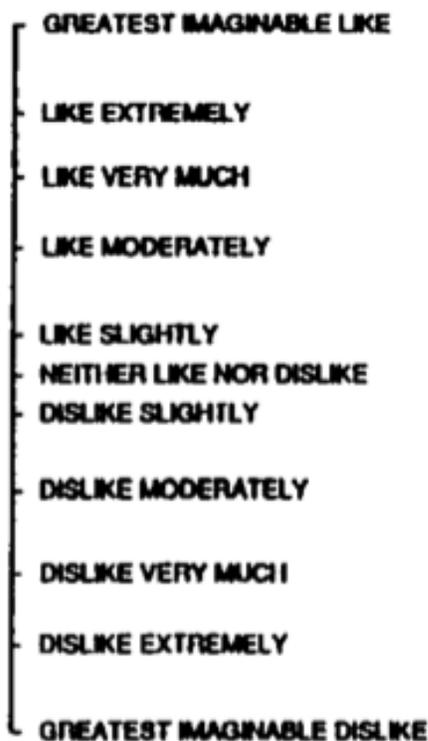


Figure 1-5 Vertical labeled affective magnitude scale (LAM). The scale ranges from -100 (greatest imaginable dislike) to +100 (greatest imaginable like). Figure taken from [(Schutz and Cardello, 2001)].

Sweetness intensity perception and hedonic liking: a function of concentration

The relationship between perceived sweetness and hedonics as a function of sucrose concentration has been investigated in a handful of studies with different methodological approaches. Few studies have been able to replicate results and make similar conclusions because of variability in stimuli, concentrations ranges, and scaling methods.

Intensity perception: dose-response functions

Dose-response functions are often used to illustrate the relationship between sweetness intensity and concentration. Dubois et al. (1991) used Spectrum Descriptive Analysis™ to obtain the dose-response functions of a wide range of nutritive and non-nutritive sweeteners. Nutritive sweeteners are depicted as linear dose-response functions in which sweetness increases as a function of concentration. Critically, Dubois et al. note that sucrose does not follow a linear dose-response function, cautioning “the linearity of the sucrose response with concentration is a result of the panel training” (DuBois et al., 1991) (see Figure 1-6). Artifactual linearization of nutritive sweeteners is not uncommon, as other studies have also linearized nutritive sweetener dose-response curves (Wiet & Beyts, 1992).

Non-nutritive sweeteners in contrast to nutritive sweeteners are hyperbolic dose-response functions, which illustrate that non-nutritive sweeteners fail to increase in sweetness intensity at high concentrations (see figure 1-7) (DuBois et al., 1991). There is room for further investigation concerning dose-response functions of nutritive and non-nutritive sweeteners and individual differences in intensity perception using modern scaling methods.

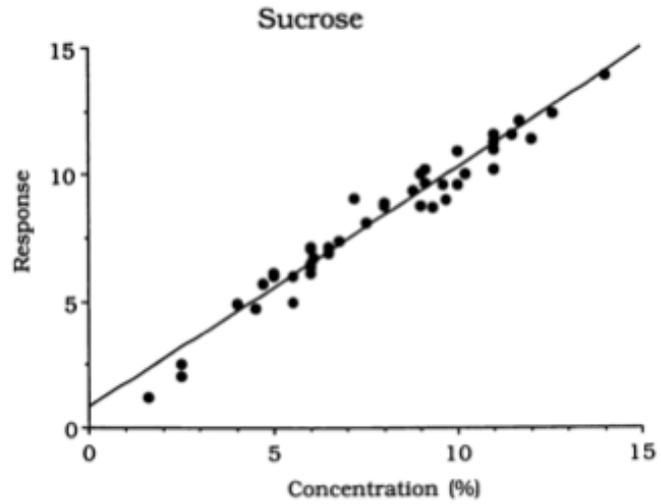


Figure 1-6 Linear sucrose dose-response function. Figure has been taken from [(Dubois et al., 1991)].

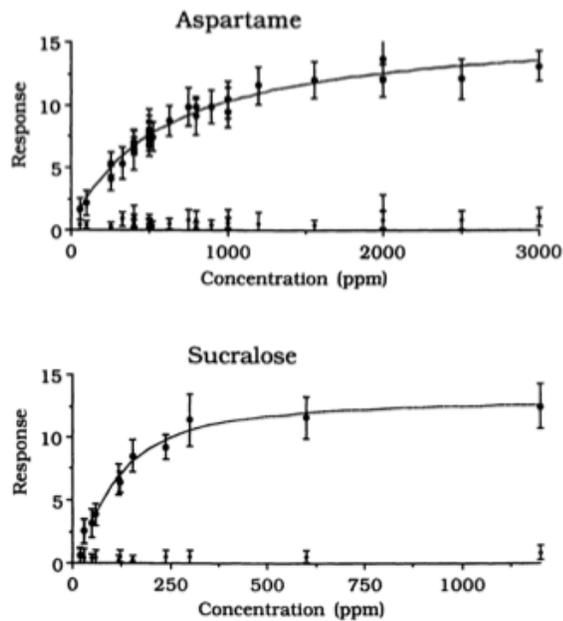


Figure 1- 7 Hyperbolic dose-response functions for non-nutritive sweeteners Aspartame, and Sucralose. Figures have been taken from [(Dubois et al., 1991)].

Hedonic liking curves

Hedonic curves have been used to classify sweet liking status in individuals (e.g. sweet liker vs. sweet disliker). Lundgren et al. (1978) used coffee sweetened with a range of sucrose concentrations (0-10% sucrose) to investigate sucrose concentration and sweet liking. A 17-pt hedonic scale and a 17-pt intensity scale were used to measure liking and intensity respectively (Lundgren et al., 1978). Although the test medium, coffee, also exhibits bitter taste, Lundgren et al. classified their participants into four main sweet liker groups based upon their hedonic ratings of sucrose as a function of concentration: Type I (as concentration increases liking decreases linearly); Type II (liking increases until it reaches an optimum and then decreases); Type III (as concentration increases liking increases linearly); and Type IV (no change in hedonic rating as concentration increases)(see Figure 1-8). Importantly, an average of the four curves (see ‘TOTAL’ figure 1-8) results in a flat curve indicating that averaging hedonic values can mask individual differences. This emphasizes the importance of identifying individual differences in sweet liking analyses.

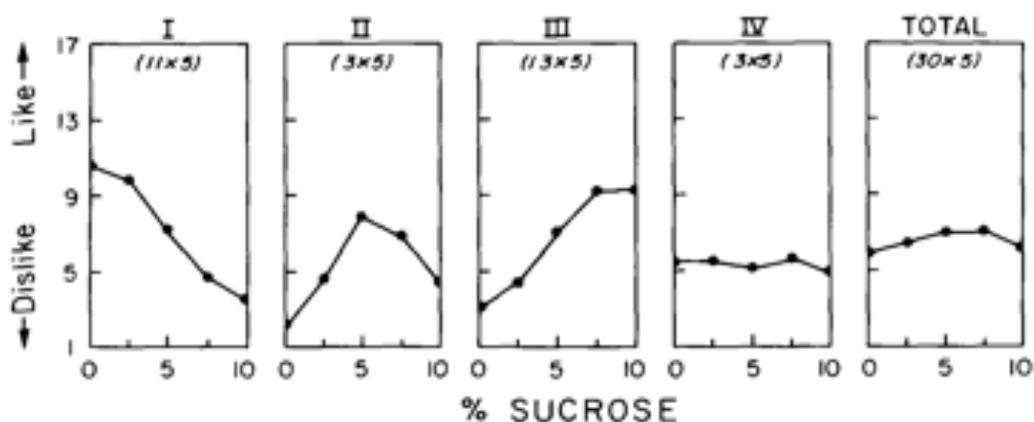


Figure 1-8 Sweet liking curves as a function of sucrose concentration in coffee. There are four curves: I (sweet disliking), II (inverted-U), III (sweet liking), IV (horizontal line). The average of the four groups results in a semi flat curve. Figure taken from [(Lundgren et al., 1978)].

In another study, Stone and Pangborn (1990) were able to identify similar ‘ascending’, ‘inverted-U’, and ‘descending’ hedonic responses to lemonade sweetened with sucrose (4, 6, 8, 10, 14, 20, and 30%(w/v)) on a 20-pt hedonic scale (see Figure 1-9)(Stone & Pangborn, 1990). Ekman and Akesson (1969) also found individual differences and two of the four groups described by Lundgren et al. (1978): inverted-U and sweet dislikers. The inability to find sweet likers and the horizontal line group is probably due to their small sample size (n=8) and range of sucrose solutions tested (0.5-7.4%) (Ekman & Åkesson, 1965).

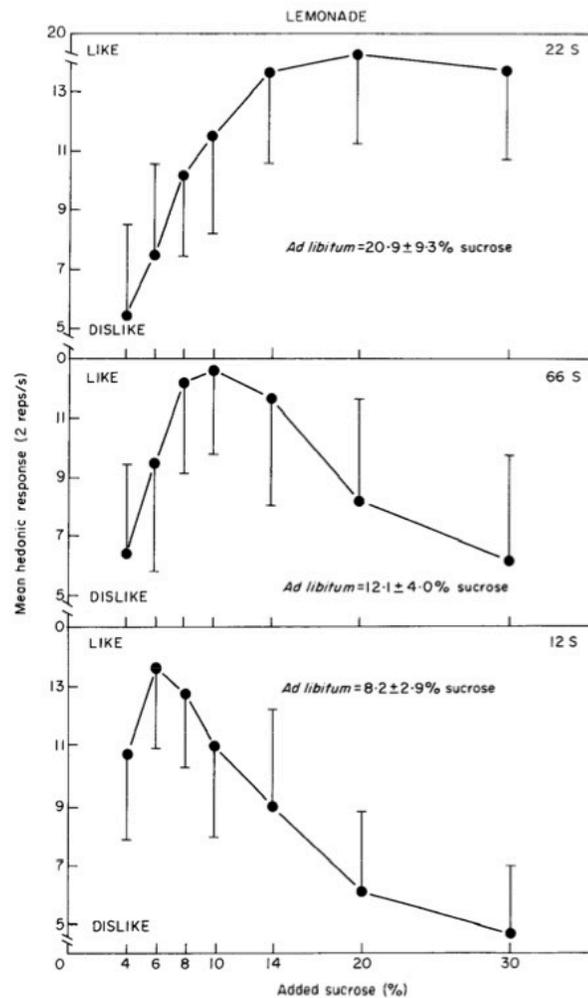


Figure 1-9 Sweet liking curves as a function of sucrose concentration in lemonade. There are three curves: Top (ascending), middle (inverted-U), bottom (descending). Figure taken from [(Stone and Pangborn,1990)].

Some studies have chosen to ignore individual differences in sweet taste hedonics. Yeomans et al. (2007) and Looy et al. (1992) classified participants into two groups, sweet 'likers' or sweet 'dislikers', based upon their hedonic ratings of sucrose solutions. Looy et al. (1992) found that hedonic responses to sucrose solutions (0.05-0.83M) did not explain the liking of sweet foods. This implies that the liking of sugar solutions may not translate to the liking or consumption of real food products. Moskowitz et al. (1974) investigated intensity perception and 'overall pleasantness' of sucrose concentrations in solution and in food applications (yellow cake, vanilla pudding, cherry flavored beverage) and averaged hedonic results instead of looking for individual differences.

Identifying individual differences in hedonic liking with regards to intensity perception can also be used as a segmentation method (Moskowitz et al., 1985). Instead of having 'sweet likers' and 'sweet dislikers' Moskowitz et al. discuss two types of hedonic functions, Type I and II. The Type I group is described as having hedonic ratings that match intensity ratings (i.e. hedonic rating of a 10 is also an intensity rating of a 10). In the Type I group there may be issues in terms of discrimination ability and/or inability to separate hedonic liking from intensity. Type II hedonic functions are described as functions in which hedonic liking does not match the intensity function collinearly. Moskowitz et al. further discuss that there may be a relationship between Type I and Type II hedonic functions in terms of cognitive processing. There are two decisions made with regards to measuring liking: 1. The participant first determines whether or not they like a stimulus or not, and then 2. The participant then determines the magnitude of that liking/disliking. The Type I group may make their decisions more quickly or immediately whereas Type II groups may make hedonic decisions more slowly with more thought. Not only are their differences in terms of intensity or hedonic perception, there may also be differences at a higher decision making level (Kocher & Fisher, 1969).

Despite past work on sweet taste hedonics and intensity perception, much remains inconclusive. There is a large gap in the literature concerning individual hedonics liking curves and sweetness intensity dose-response functions with the use of modern methods and scaling techniques.

Sweet taste and the endogenous opioid system

Taste intensity perception and hedonic liking are processed in the brain. However, pleasure is mediated by the endogenous opioid system and intensity perception is not. The forebrain's nucleus accumbens (Peciña & Berridge, 2005) in the ventral striatum and the ventral pallidum (Peciña et al., 2006) are responsible for the reward processing of sex, food consumption, drug usage, and additional pleasurable stimuli (Kelley et al., 1996). Pleasurable stimuli and behaviors such as sex and eating are innately rewarding because they are needed for survival. The endogenous opioid system and neurobiological pathways in the body mediate sweet pleasantness (Fortuna, 2010; Koob et al., 1992). Although fat has more calories per gram, humans are hardwired to 'like' sweet foods because carbohydrates are the most preferred and efficient energy source in the body. Additionally, the brain is an obligate glucose user and a lack of glucose will eventually lead to brain failure (Cryer, 2007).

The opioid receptors are responsible for the binding of ligands that produce both pleasure and analgesia. A review by Waldhoer et al. (2004) provides a thorough evaluation of the literature concerning the biological structure and function of the human opioid receptors. Similar to taste receptors, opioid receptors are G-protein coupled receptors (GPCRs) that have an extracellular N-terminal domain (Waldhoer et al., 2004). There are four types of opioid receptors: μ -opioid receptors, δ -opioid receptors, κ -opioid receptors, and nociception receptors. Each of the receptor types has an affinity for a variety of unique endogenous (i.e. endorphins) and exogenous (i.e. morphine) opioids. μ -opioid receptors and δ -opioid receptors are considered "analgesic and

rewarding”, whereas κ -opioid receptors are considered “dysphoric” (Waldhoer et al., 2004).

Mutations resulting in differences in the structure or expression of the opioid receptors can directly affect the efficacy of ligand binding and activation (Waldhoer et al., 2004), which may explain some of the variation in hedonic responses to sweet and palatable foods.

Opiate antagonists and agonists

The relationship between the opioid system in food reward processing has been extensively investigated previously, yet many of the ‘hows’ and ‘whys’ in food reward processing remain elusive. The opiate antagonists naltrexone, naloxone, and nalmephe provide insight into the role of the endogenous opioid system in taste hedonics and consumption behavior. Naloxone and naltrexone are non-selective antagonists that can bind to both the μ and δ -opioid receptors (Waldhoer et al., 2004). Opiate antagonists reduce the pleasantness of rewarding substances and behaviors by competitively and reversibly binding to the opioid receptors, preventing opiate ligands from binding.

It is known that the opiate system is involved in food reward, because opiate antagonists reduce the perceived pleasure of rewarding food stimuli (Fantino et al., 1986; McLaughlin & Baile, 1983; Richardson et al., 2005). The reverse is also true for agonists (i.e. morphine). Although agonists increase the perceived pleasure of rewarding food stimuli, they do not affect the feeling of physiological hunger (Deviche & Wohland, 1984; Yeomans et al., 1990) or intensity perception (Drewnowski et al., 1992).

Agonists, like antagonists, directly affect hedonic reward. Doyle et al. (1993) conducted a study in rats that investigated the effect of opiate agonists on the hedonic responses to palatable (sucrose) and aversive (quinine) solutions. Rats who had been given an opiate agonist (morphine) versus the control had a significantly greater number of hedonically positive facial expressions and behavioral responses. The morphine-induced rats also consumed significantly more food than

the controls, which is consistent with other studies (Olszewski & Levine, 2007; Robert et al., 1989). Interestingly, agonists did not increase the pleasantness of aversive stimuli (i.e. quinine) (Doyle et al., 1993). Similar results have been illustrated with antagonists as they fail to decrease the pleasantness of negative or neutral stimuli (Cooper, 1983; Deviche & Wohland, 1984; Fantino et al., 1986; Richardson et al., 2005) although pleasure and taste aversion are mediated by the same opioid receptors in the brain (Koob et al., 1989; Olszewski et al., 2011).

Drug and alcohol abuse and sweet taste perception

Opiate drug and alcohol abuse alter the function of the brain's reward system and hedonic responses to sweet taste. It has also been shown that excessive consumption of saccharin solutions reduce responsiveness to morphine (D'Anci et al., 1997) and naloxone in rats (Lieblich et al., 1983). This reduced responsiveness to morphine was not found with sucrose consumption (D'Anci et al., 1997) although other studies have found frequent consumption of highly palatable solutions (e.g. sucrose) to affect endogenous opioid activity in rats (Kanarek et al., 1997). Unlike previous work that has concluded that sweet intensity perception and recognition thresholds are not affected by opiate agonists, a study by Amy Green and colleagues (2013) showed that opiate-maintained drug users had significantly higher sucrose recognition thresholds, increased intensity perception, and increased pleasantness ratings than former users, and controls. However, Keast and Roper (2007) suggest that an individual's threshold is not a sufficient predictor of suprathreshold intensity.

Similar to research on sweet perception and opioid dependence, the relationship between alcohol dependence and sweet liking has been studied since the 1940's. Early evidence suggested that heredity played a role in reward processing and individual differences (Williams et al., 1949). Individuals that have a family history of alcohol dependence are more likely to have a preference for sweet foods (Mennella et al., 2010; Pepino & Mennella, 2007). Previous animal studies have

suggested that reward from sugar consumption is concentration dependent (Hajnal et al., 2004) rather than volume dependent (Lieblich et al., 1983). A study by Kampov-Polevoy et al. (1997) compared sweet preferences in 37 non-alcohol dependent men and in 20 alcohol dependent men who had abstained from alcohol for 28 days. In the alcohol dependent group, 65% of men preferred the highest concentration of sucrose (0.83 M) compared to just 16% of the non-dependent men. There was no difference in sweet taste discrimination ability or intensity perception. Another sweet related alcohol study (Garbutt et al., 2009) found that sweet liking phenotype (optimum preference of 0.83 M sucrose) predicted fewer abstinent days in naltrexone treated alcohol dependent patients compared to their 'sweet disliker' phenotype (optimum preference for 0.05, 0.1, 0.21, or 0.42M sucrose). Naltrexone significantly reduced drinking binges in both sweet liker and sweet disliker groups, indicating that alcohol dependence is also mediated by the opiate reward system. There was also a significant interaction between sweet liking phenotype and increased alcohol craving during abstinent days (Garbutt et al., 2009).

In contrast, (Laaksonen et al., 2011) discovered that naltrexone had a reduced effect in preventing alcohol consumption in individuals who preferred lower sucrose concentrations compared to individuals who preferred higher concentrations of sucrose. Other studies that have investigated the effectiveness of naltrexone on treating alcohol dependence have found it to be rather effective (Kranzler & Van Kirk, 2001).

Sucrose analgesia

Further evidence on the involvement of the endogenous opioid system on sweet taste hedonics is apparent in studies that have investigated sucrose analgesia. Analgesia (pain

reduction), like many previously discussed sensations, is mediated by the opioid system in the brain. Sucrose analgesia is a frequently investigated phenomenon that occurs in babies, young children, and infant rats in which intra-oral sucrose reduces unpleasant responses to common painful procedures (Ramenghi et al., 1999) such as circumcision and the lance heel prick (Blass & Hoffmeyer, 1991). Interestingly, sucrose analgesia is naltrexone reversible (Blass et al., 1987) and seems to be age dependent as many studies that have investigated sucrose analgesia in adults have failed to find an effect (Pepino & Mennella, 2005a). There are other additional factors that affect sucrose analgesia other than age and sucrose concentration such as gender (Bhattacharjee et al., 2007) and depression (Mennella et al., 2010). Due to large methodological variability in adult sucrose analgesia studies, there is an opportunity to refine and control for variability to determine if sucrose analgesia occurs in the adult population.

The μ -opioid receptor (OPRM1) and sweet taste perception

Genetic variation, in addition to environmental factors, may account for some of the individual differences in sweet taste hedonics. One of these genes is the human μ -opioid receptor (OPRM1). A common and functional single nucleotide polymorphism (SNP) A118G in the *OPRM1* gene has been associated with alcohol (Bart et al., 2005) and drug dependence. The A118G is a non-synonymous polymorphism, which leads to adenine to guanine nucleotide transition at position 118 in the coding region of the gene. The A118G SNP encodes for an amino acid substitution (asparagine to aspartic acid; Asn40Asp), which removes a glycosylation site from the N-terminal of the μ -opioid receptor and subsequently alters the function (Kroslak et al., 2007). There are three possible genotypes: AA (normal variant); AG (mutation); and GG (mutation). The mutation alters receptor function enabling β -endorphin to bind with greater affinity to the G (Asp40) μ -opioid receptor variant (Bond et al., 1998) than the A (Asn40) variant. In result, individuals with at least one G-allele are considered at 'risk' (Bart et al., 2005) because

they have increased responsiveness and pleasure to endogenous opioids, alcohol, drugs, and desirable visual stimuli (Wiers et al., 2009).

There is variability in the literature concerning biological, physiological, and phenotypic behavioral differences between the AA and AG/GG genotypes which may be due to lack of statistical power and type-I error (Ray et al., 2012; Hardman et al., 2013). Research efforts have focused on the OPRM1 A118G SNP in β -endorphin binding affinity (Beyer et al., 2004; Bond et al., 1998), drug abuse, alcohol consumption, stress (Chong et al., 2006; Hernandez–Avila et al., 2003; Wand et al., 2002), pain thresholds (Fillingim et al., 2005) and smoking (Munafò et al., 2007). An interesting study conducted by (Ray & Hutchison, 2004) investigated and compared the subjective intoxication and self-reported sedation of individuals with the AG and AA genotypes. Individuals with the AG genotype were subjectively more sensitive to the affects of alcohol than individuals with the AA variant. Despite substantial efforts, many studies have failed to find differences between individuals with and without the OPRM1 A118G polymorphism in terms of receptor sensitivity (Beyer et al., 2004), drug responsiveness (Chou et al., 2006), and alcohol dependence (Franke et al., 2001). Additionally, the evidence concerning the extent to which the A118G SNP affects ingestive behavior is lacking (Arias et al., 2006).

The literature concerning the OPRM1 A118G SNP in predicting the effectiveness of opiate antagonists naltrexone and naloxone on alcohol consumption and opiate responsiveness has also been inconsistent. Most studies have reported that individuals that have the ‘risk’ AG genotype respond better to naltrexone than their AA counterparts. Oslin and colleagues (2003) found that self reported heavy drinkers with at least one copy of the G-allele were less likely to relapse than those without the ‘risk’ allele while being treated with naltrexone. Anton et al. (2008) found similar results in alcohol dependent participants with the G-allele. Naltrexone treatment significantly increased the number of abstinent days and decreased the percent of heavy drinking days when compared to participants without the G-allele (Anton et al., 2008). In

contrast, other studies have failed to find an association between the OPRM1 A118G SNP and effectiveness of opiate antagonist treatment in alcohol dependence (Coller et al., 2011; Gelernter et al., 2007).

As previously mentioned, sweet hedonics, drug reward, and alcohol reward, are all mediated by the endogenous opioid system. Although many other environmental and genetic factors are involved in sweet taste perception, it would be expected that individuals with the OPRM1 'risk' G-allele may have increased pleasure from sweet foods because they may have increased responses to appetitive stimuli.

Data on the relationship between the OPRM1 A118G SNP and sweet taste perception and hedonic liking are limited. Two main studies have been interested in the relationship between the OPRM1 A118G SNP and fatty food liking (Davis et al., 2011; Haghghi et al., 2013). (Davis et al., 2011) concluded that only individuals with the rare GG genotype had a significantly higher preference for sweet and fatty foods, yet there was only a trend for high sugar preferences in individuals with the G-allele. Davis et al. also discuss the possibility that there may be two different hypotheses for increased consumption of sweet foods related to opioid function.

- 1.) Increased consumption to compensate for reduced reward responses in opioid signaling.
- 2.) Increased consumption due to heightened reward responses and heightened opioid signaling.

The literature concerning the OPRM1 A118G SNP in sweet taste perception is lacking.

Accordingly, there is an opportunity to investigate the affect of the OPRM1 A118G SNP on sweetness intensity perception and sweet hedonics.

Conclusions

Sweetness and sweet liking are affected by a plethora of environmental, behavioral, and genetic factors. Some of the largest gaps in the literature involve adult sucrose analgesia, the

development of dose-response functions for nutritive and non-nutritive sweeteners, and the identification of sweet liking curves using a full range of sucrose concentrations, and the significance of the OPRM1 A118G SNP in sweet liking.

Studies that have investigated the affects of sucrose analgesia in adults have failed to find an effect and therefore conclude that sucrose analgesia is an age dependent phenomena. Methodological inconsistencies, small sample sizes, and confounding factors (e.g. menstrual cycle) may be responsible for an inability to find an effect. If sucrose analgesia does exist in the adult population, it could provide insight into the role of the endogenous opioid system and the relationship between pain and sweet taste perception in adults.

Few studies have used modern scaling methods to develop dose-response functions for nutritive and non-nutritive sweeteners that enable valid across group comparisons. Most dose-response functions for nutritive sweeteners have been artifactually linearized by the use of time consuming descriptive analysis methods in studies with small numbers of participants (DuBois et al., 1991). The use of modern scaling methods and psychophysical techniques can be used to rapidly generate accurate dose-response functions for non-nutritive and nutritive sweeteners in larger numbers of participants.

Although many studies have attempted to investigate the relationship between sweet liking and sucrose concentration, most have failed to use a representative sample size and a broad range of sucrose concentrations in a medium that does not contain other taste attributes (e.g. coffee or lemonade). Additionally, a wide range of sucrose concentrations is needed to map characterized sweet liking groups and identify optimum liking concentrations.

Furthermore, sweet liking is mediated by the endogenous opioid system. There is little information concerning the OPRM1 A118G SNP in sweet liking status. Understanding the role of the A118G SNP in sweet liking could contribute to the understanding of phenotypic differences associated with the functional OPRM1 A118G SNP.

Here we seek to contribute to the literature concerning adult sucrose analgesia, dose-response functions of nutritive and non-nutritive sweeteners, individual sweet liking curves, and the role of the OPRM1 A118G SNP in sweet liking.

Aims

1. To investigate the effect of sucrose analgesia on cold pain threshold and tolerance during the Cold pressor task in young men.
2. To determine and compare the sweetness intensity dose-response functions of nutritive and non-nutritive sweeteners (NNS) using generalized scaling methods with limited panel training.
3. To identify individual sweet liking curves as a function of sucrose concentration. To determine the relationship between hedonic sucrose curves and intensity, as well as the liking of sweet foods and beverages.
4. To determine the relationship between the OPRM1 A118G SNP on sucrose liking and the liking of sweet foods.

Hypotheses

1. Sucrose will increase cold pain tolerance and thresholds in adult males.
2. Dose-response functions will illustrate that the maximum intensity of NNS will be less than that of nutritive sweeteners. NNS will level off or even decrease in sweetness at high concentrations.
3. Four groups of hedonic sucrose curves will be identified: (1) Slope + (liking increases and concentration increases); (2) Slope - (liking decreases as concentration increases); (3) Inverted-U (concentration increases and decreases after reaching an optimum concentration); (4) Horizontal Line (no change in liking as concentration increases).
4. There will be a significant relationship between hedonic sucrose curves and hedonic liking of sweet foods and beverages.
5. Individuals with the functional OPRM1 A118G SNP will have increased liking of sucrose solutions, and increased liking of sweet foods.

Chapter 2

Sucrose analgesia and the cold pressor task in young men: methodological considerations

Abstract

Sucrose is mildly analgesic in infant rats, human neonates, and prepubertal children. This effect generalizes to non-nutritive sweeteners, indicating the effect is due to sweet taste, not calories. However, consistent sweet analgesia in adults remains elusive: some studies find an effect while others do not. Pain can be safely induced in the laboratory using the cold pressor task (CPT), providing a convenient means to study this phenomenon. It is unresolved whether the inability to consistently observe analgesic effects in adults is due to methodological issues associated with the CPT or an age dependent effect. White and Prescott (AChemS, 2012) reported strong order effects; cold pain tolerance was greater in the second CPT within a session. Here, we describe two studies that attempt to refine an adult sweet analgesia CPT paradigm. In study 1 (36 men), pain was induced by placing the non-dominant hand in circulating water at 8°C. Within a session, tastants were water and 24% sucrose; participants were tested twice (fed/fasted) in a double crossover design. The hand was rewarmed in 35°C water between trials, and hand temperature was confirmed with a laser thermometer. No tastant effect (sugar/water), regardless of hunger state, was observed. However, a weak trend for an order effect within a session was evident, despite rewarming. In study 2, subjects (38 men) received sucrose or water on separate days after a 12h overnight fast. The cold bath was reduced to 4°C, and outcomes included pain threshold (pain onset in seconds) and tolerance (hand withdrawal in seconds). Pain thresholds and tolerance were greater on the second day, but this occurred irrespective of tastant. These data do

not find sucrose to be an effective analgesic in adults even when controlling for gender, hand temperature and hunger state.

Introduction

Sweet taste is a hedonically positive stimulus that is innately liked by humans and most animal species. Also, the inherent pleasantness of sweet taste is mediated by the endogenous opioid system as opiate agonists (Doyle et al., 1993) and antagonists (Richardson et al., 2005) alter the pleasantness of sweet stimuli. One way to further investigate the role of the endogenous opioid system in sweet taste is through sucrose analgesia.

Sucrose analgesia is a phenomenon in which sweet stimuli reduce pain responses in human neonates (Blass & Hoffmeyer, 1991), infant rats (Anseloni et al., 2002), and pre-pubertal children (Miller et al., 1994) when orally provided prior to painful procedures such as the lance heel prick (Bellieni et al., 2001), venipuncture (Acharya et al., 2004; Chu & Joy, 2009), injections (Barr et al., 1995; Hatfield et al., 2008) and circumcision (Blass & Hoffmeyer, 1991). Sucrose analgesia depends on pre-absorptive mechanism in the mouth (Anseloni et al., 2002) as direct stomach loading fails to elicit an effect (Ramenghi et al., 1999). Interestingly, this taste dependent analgesic phenomenon is naloxone reversible (Segato et al., 1997) and is absent in infants born of opiate dependent mothers (Blass & Ciaramitaro, 1994) indicating that sucrose analgesia involves the endogenous opioid system.

The 'effective dose' of sucrose needed to induce analgesia is unknown. Sucrose concentrations in the literature are inconsistent between human studies (Stevens et al., 2013) and can range anywhere between 8% (Mercer & Holder, 2013) to 50% sucrose (w/v) (Barr et al., 1995). Importantly, the analgesic affect of sweet taste is not volume dependent (Lieblich et al., 1983) but is concentration dependent (Blass & Smith, 1992). It should be mentioned that not all sweet substances evoke an analgesic effect (e.g. lactose which is only about 1/5 as sweet as

sucrose) (Blass & Smith, 1992), which may be related to discrimination ability and sweet taste preferences in infants (Desor et al., 1975) and children (Pepino & Mennella, 2005b). Non-nutritive sweeteners (Barr et al., 1999; Ramenghi et al., 1996) and sweet odors (Prescott et al., 2001) have also been shown to induce analgesia. A study by Prescott and Wilkie (2007) found that that odor ‘sweetness’, not pleasantness, was responsible for analgesia in adults. There seems to be an analgesic effect of perceived ‘sweetness’ irrespective of modality and nutritional value. Additional guidelines for the use of sucrose in infants to induce analgesia can be found in a review by Lefrak et al. (2006).

Not all studies have concluded sucrose is an effective analgesic in infants (Johnston et al., 1997; Slater et al., 2010). Slater et al. (2010) looked at nociceptive brain activity in infants using electroencephalography, which is often used to monitor anesthesia. They concluded that sucrose should not be considered a pain reliever as no significant differences were found in nociceptive brain activity between infants that had received 24% sucrose and infants who had received water. Observational pain scores however, were significantly lower in infants that had received sucrose than the water controls. Despite results of electroencephalography, behavioral responses suggest that intraoral sucrose does evoke an analgesic effect (Lago et al., 2014).

Although sucrose analgesia is apparent in infants, evidence suggests that this phenomenon may not carryover into adulthood. Many studies have concluded that sucrose analgesia is an age dependent phenomena that only exists during early stages of life (Allen et al., 1996; Anseloni et al., 2002; Barr et al., 1995; Pepino & Mennella, 2005b; Rogers et al., 2006). Few studies have found sucrose to be an effective analgesic in adults (Eggleston, 2010; Kakeda, 2010), which may be due to methodological variability. Methodological inconsistencies including samples size, gender, hunger state, and stimulus have led to variable conclusions concerning the effectiveness of sucrose analgesia in adults (Lewkowski et al., 2003; Mercer & Holder, 2013) and have made it difficult to objectively compare prior reports.

It is unclear whether the inability to find an effect in adults is due to methodological variability or if it is an age dependent phenomena. The present study explores adult sucrose analgesia in two studies using the cold pressor task (CPT) to safely induce pain in the laboratory. In study 1, we compared fed and fasted hunger states on cold pain tolerance time (time to withdrawal). We hypothesized that there would be a significant difference in cold pain tolerance time between sucrose and water conditions and between fed and fasted hunger states. Prior evidence suggests satiety affects sweet taste pleasantness (Moskowitz et al., 1976), therefore we predicted the pleasantness of sucrose would be greater in the fasted state and would result in a greater analgesic effect. In study 2, we measured both pain threshold (onset of pain) and pain tolerance (time to withdrawal) in sucrose and water conditions in fasted men. We hypothesized that in study 2 pain threshold and tolerance time would be significantly higher in the sucrose condition versus the water condition.

Methods

Study 1

Overview of methods

The cold pressor task (CPT) was used to induce cold pain in participants to measure the effect of sucrose as an analgesic in adult men. The CPT is a common technique used to safely induce pain by submerging the hand in cold water (Pepino & Mennella, 2005b; von Baeyer et al., 2005). CPT procedures used in this study were adapted from the best practice guidelines proposed by von Baeyer et al. (2005). In order to investigate the effect of sucrose analgesia across hunger states, men completed two one-on-one sessions on separate days in a double crossover design (fed vs. fasted across days; both water and sucrose were presented in each session). All sessions were completed between 8:00 am and 12:00 pm.

Participants

Reportedly healthy, non-smoking men (n=36) aged 18-45 years were recruited from the Pennsylvania State University and screened for qualification. Exclusion criteria included history of cardiovascular disorders; fainting or seizures; frostbite; repeated exposure to ice packs or ice baths; open cut or sore on non-dominant hand/wrist; fracture of non-dominant hand/wrist; Reynaud's syndrome; history of a condition involving chronic pain; currently taking prescription medication, antidepressant or anxiolytic pain medication; defects of smell or taste; tongue, cheek or lip piercings; regular use of non-caloric sweeteners; and history of choking or difficulty swallowing. Participants were excused from the session if they had taken any over the counter (OTC) pain medication within twenty-four hours of the session, but were not asked about specific medications. Additionally, women were excluded from this study due to gender differences in pain perception and evidence suggesting variable pain experiences associated with the menstrual cycle (Fillingim & Maixner, 1996; Hellström & Lundberg, 2000; Kest et al., 2000; Wiesenfeld-Hallin, 2005). The study protocol was IRB approved, informed consent was obtained and participants were paid for their time.

Stimuli

Twenty milliliters of 24% (w/v) sucrose and water were presented in 30 mL clear medicine cups at room temperature; presentation order (water/sucrose) was counterbalanced within a session. Participants held the entire sample in their mouth during the task without swallowing. Participants rinsed with water prior to and between each sample.

Water baths

Two water baths (8°C and 35°C) were assembled to conduct the CPT. Temperature displays were concealed to avoid any expectancy effects. Baths were constantly circulated (10 L/min) to minimize boundary layer formation around the hand. Digital thermo-regulators (Techne TE-10D) were used to regulate water bath temperatures $\pm 1^\circ\text{C}$; the thermo-regulator was plumbed in series with a portable chiller for the cold bath.

Hand preparation

Participants washed their hands before each session, and a mark was made 5 cm above the wrist of the non-dominant hand to indicate immersion depth per recommendations (von Baeyer et al., 2005). The hand was warmed in 35°C bath before and after each cold-water immersion to standardize skin temperature. Skin temperature was validated with a laser thermometer. Participants were asked to stand during the task and positioned their hand in the bath with hand unclenched, palm facing upward, and fingers spread. An armrest was not provided. Participants were asked to refrain from moving.

Audience

The experimenter was present in the room throughout the task. The experimenter stood in front of the participant for verbal instruction and when providing liquid samples. The experimenter sat out of sight (behind the participant) during the task. Direct eye contact with the participants was avoided during the CPT itself.

Cold pressor task

Participants were instructed to place the test stimulus in their mouth while their hand was immersed in the warm water bath. Thirty seconds after the sample had been placed in the mouth, the participant was told to transfer their hand from the warm bath to the cold bath. Participants

were instructed to keep their hand in the cold water as long as they could. There was a 240-second time limit for the CPT, and participants were not informed of this ceiling. If the 240-second ceiling was met, participants were instructed to remove their hand. Pain tolerance (time to withdrawal) was measured with a stopwatch (Sportline, EB Sport Group, 2008). Clean cloth towels were provided to panelists to dry hands.

Outcome measure

Pain tolerance (time to withdrawal) was measured in seconds and was defined as the number of seconds until the participant withdrew his hand from the cold-water bath.

Study 2

Overview of methods

To investigate the effect of sucrose analgesia on cold pain threshold (onset of pain) and tolerance (time to withdrawal) men completed two one-on-one sessions on separate days. Study 2 was conducted with the same methods to study 1, with a few important changes listed below.

Participants

A new group of 38 men aged 18-45 years were recruited using the same exclusion criteria and methods as in study 1.

Design

The double crossover design was eliminated. All participants were tested following an overnight fast (12 hours). Participants completed a total of two CPTs on two separate days (one

CPT per day). In contrast to Study 1, participants were required to sit quietly (10 min) and acclimate to the testing room to limit stress responses prior to the CPT.

Bath temperature

The temperature of the cold bath was reduced from 8°C to 4°C.

Test stimuli

Tastant presentation was counterbalanced across 2 separate days. One tastant was presented per day (20 mL 24% (w/v) sucrose on day 1 and water on day 2 or vice versa) in a 30 mL medicine cup.

Outcome measures

Pain threshold (onset of pain in sec) and *pain tolerance* (time to hand withdrawal in sec) were measured. *Pain thresholds* were obtained by asking participants to raise their dominant hand (hand not in the water bath) the moment they began to feel pain.

Results

Study 1

The main effect of stimulus (sugar/water) on tolerance and the interaction between stimulus (sugar/water) and hunger state (fed/fast) were measured. In contrast to what was expected, there was no significant difference between sugar and water on pain tolerance [$F(1,34)=0.5$; $p=0.48$]. Additionally, there was no significant difference in cold pain tolerance time between sucrose and water conditions in fed and fasted hunger states (Figure 2-1).

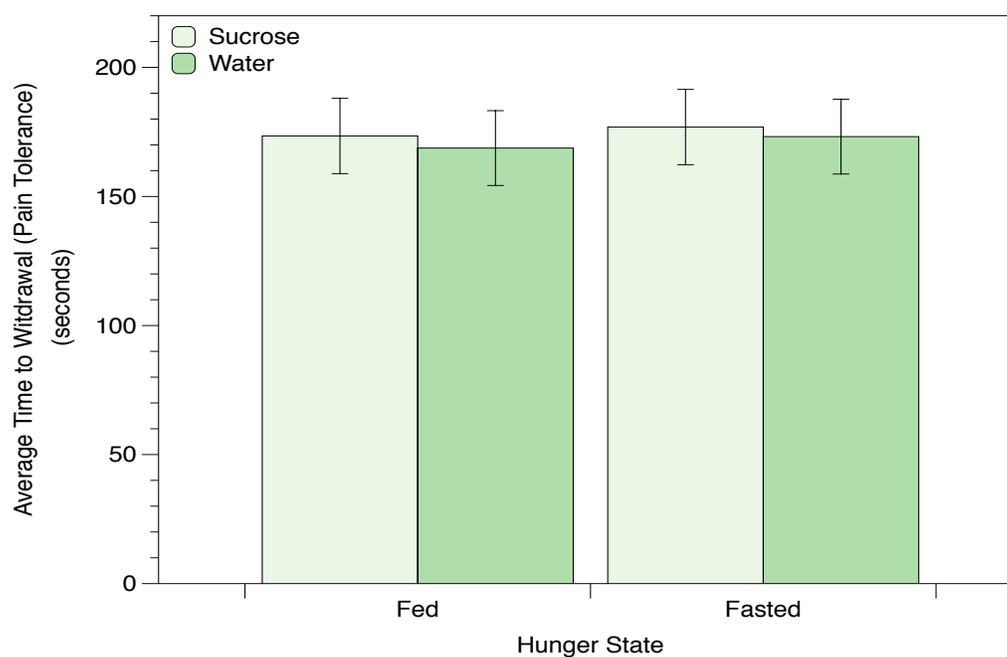


Figure 2-1 Study 1: Average cold pain tolerance times (seconds) between sucrose and water conditions in fed and fasted hunger states. There is no significant difference between sugar and water on pain tolerance [$F(1,34)=0.5$; $p=0.48$]. There is no interaction between stimulus and hunger state [$F(1,34)=0.01$; $p=0.93$].

In Study 1, there also was weak evidence of a slight order effect within a session. Pain tolerance in the second CPT is slightly higher regardless of stimulus (sugar/water) (Figure 2-1) [$F(1,34)=2.32$; $p=0.14$] possibly indicating a learning or practice effect. The trend remained present when accounting for stimulus and hunger state [$F(1,34)=2.15$; $p=0.15$].

Study 2

Similar to Study 1, there was no effect of stimulus (sucrose/water) on pain tolerance [$F(1,18)=1.20$; $p=0.29$] even after altering the testing protocol to control for within session order effects [$F(1,18)=0.00$; $p=0.95$]. Also similar to Study 1, pain tolerance and pain threshold were slightly (not significantly) greater on the second day of testing irrespective of water/sucrose stimulus (Figure 2-2). This once again indicates a learning effect.

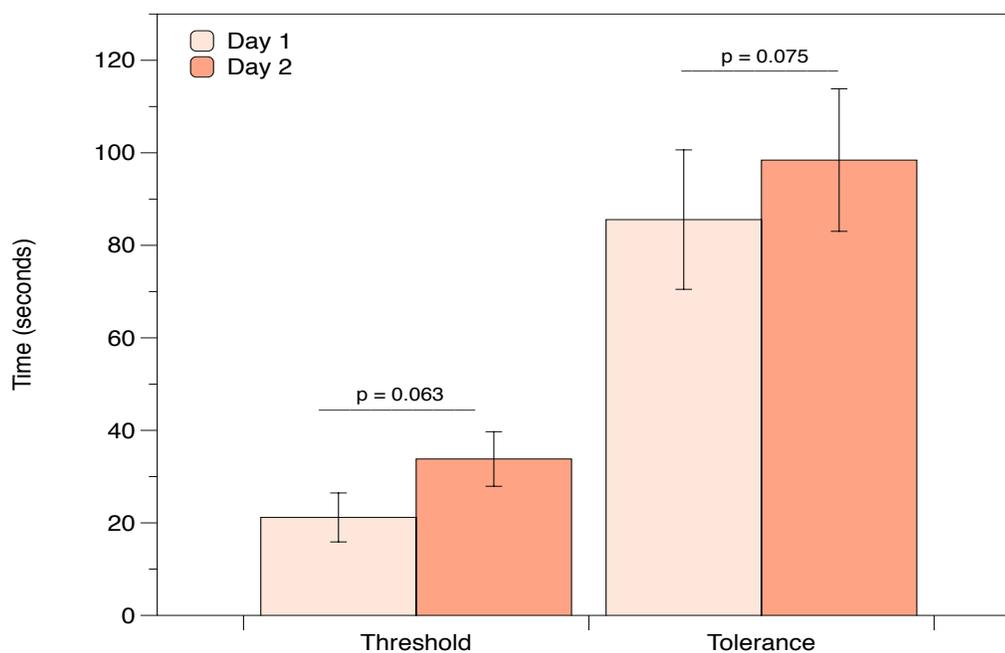


Figure 2-2 Study 2: Comparison of stimulus presentation order on pain tolerance and threshold time (seconds) irrespective of water/sucrose stimulus.

Discussion

In the present study we investigated the effect of sucrose analgesia on cold pain threshold and tolerance. Similar to previous reports, we have been unable to find an effect of sucrose analgesia in the adult male population (Mercer & Holder, 2013). Despite controlling for methodological variability in the CPT including skin temperature (Sawada et al., 2000), gender (Hellström & Lundberg, 2000), water temperature, water circulation, and hunger state (fed/fasted) there was no significant effect of sucrose on pain threshold or tolerance times. The inability to find an effect may be due to a variety of factors including heritability (Birklein et al., 2008), differences in pain receptor pathways, and CPT effectiveness.

Difference in pain pathways may explain some of the variability in sucrose analgesia. Different pathways mediate different pain sensations (cold pain vs. mechanical pain). Cold pain is induced by activation of the TRPM8 receptors, which belong to the transient receptor potential (TRP) family. The pathways of cold pain signal transduction are different from other pain pathways (e.g. heat and mechanical pain (TRPV1 receptors)) (Birklein et al., 2008). This does not however explain results of studies that have found sucrose as an effective analgesic in children using the CPT (Mennella et al., 2010). Additionally, although the CPT is useful in an experimental setting to induce non-invasive pain, cold pain itself may be confounding because it can be analgesic (Proudfoot et al., 2006). Perhaps other methods of inducing pain in adults such as pressure pain (Mercer & Holder, 1997) may be more effective.

The rewarding ‘value’ of sucrose in terms of life experiences may also play a role in the age dependent effect of sucrose analgesia. In this study, we did not select participants based upon sweet liking as Pepino and Mennella (2005) found that sucrose analgesia was only correlated to sweet preferences in children and not in adults. Perhaps the age dependent effect has to do with prior sensory experiences. Children and adults presumably experience a different range of pleasurable sensations. Sweet taste is probably one of the most rewarding stimuli that a child

experiences. In contrast, adults perhaps have been exposed to other more intensely rewarding stimuli (e.g. sex or drugs) possibly dampening the analgesic effect of sucrose. This age dependent phenomenon is also apparent in other analgesic stimuli. Non-nutritive sucking is analgesic in infants (Eriksson & Finnström, 2004; Gibbins et al., 2002; Reis et al., 2003). Interestingly, Lewkowski et al., (2003) investigated the analgesic effect of chewing gum to mimic infant nonnutritive sucking in older children and found that it had no effect (Lewkowski et al., 2003). Non-nutritive sucking may be rewarding to infants, but may also lose its rewarding value with age.

Another plausible reason for sucrose analgesia to be age dependent is that it is a protective mechanism in infants. Sweet taste may only be analgesic until an infant or child is able to physically escape or avoid noxious stimuli (i.e. develop withdrawal reflexes).

Conclusions

The present studies investigated the effect of sucrose analgesia on cold pain threshold and tolerance in adult males using the CPT. In study 1 we were unable to find an effect of stimulus (sucrose/water) or hunger state (fed/fasted) on cold pain tolerance. There did appear to be an order effect from the first CPT experience to the second CPT experience regardless of stimulus. Although changes were made to CPT protocol in study 2 to prevent expectation effects, stimulus had no effect on cold pain tolerance and threshold and there was a marginal effect of day on pain threshold and pain tolerance times. The CPT is a physical self-motivated task and there may be individual differences in motivation and pain expectations (Handley et al., 2013; Hanssen et al., 2013; Hanssen et al., 2012).

The present results fail to find sucrose as an effective analgesic in cold pain tolerance and threshold in young men despite methodological controls. Inability to find an effect is consistent

with other literature investigating this phenomenon the adults. Sucrose analgesia appears to be an age-dependent phenomenon, yet the mechanism and constraints of the phenomenon remain unknown. If other studies have also found null results, there may be a null publication bias and a meta-analysis may be necessary to understand this phenomenon.

Chapter 3

Non-nutritive sweeteners are not super-normal stimuli.

Abstract

It is often claimed that non-nutritive sweeteners (NNS) are ‘*sweeter than sugar*’, with the implicit implication that high potency sweeteners are super-normal stimuli that encourage exaggerated responses. This study aimed to investigate the perceived sweetness intensity of a variety of nutritive (sucrose, maple syrup, and agave nectar) and NNS (acesulfame-K (Ace K), rebaudioside A (RebA), Aspartame, and Sucralose) in a large cohort of untrained participants using psychophysical methods that reduce panel training time and utilize generalized scaling techniques.

Methods: Participants (n=401 total) rated the intensity of sweet, bitter, and metallic sensations for nutritive and NNS in water using the general labeled magnitude scale (gLMS).

Results: Sigmoidal Dose-Response functions were observed for all stimuli except acesulfame K. That is, sucrose follows a sigmoidal function if the data are not artifactually linearized via prior training. More critically, there is no evidence that NNS have a maximal sweetness (intensity) greater than sucrose; indeed, the maximal sweetness for acesulfame K, rebaudioside A and sucralose were significantly lower than for concentrated sucrose. For these sweeteners, mixture suppression due to endogenous dose-dependent bitterness appears to limit maximal perceived sweetness.

Conclusions: In terms of perceived sweetness, non-nutritive sweeteners cannot be considered super-normal stimuli despite their greater potency. These data do not support the view

that non-nutritive sweeteners hijack or over-stimulate sweet receptors to produce excessive sweet sensations.

Introduction

Evolutionarily, sweet taste has enabled humans to make qualitative judgments about the energy density and nutritional quality of their food. Although sweet taste is no longer fundamentally needed for survival, sweet sensations are innately pleasurable across the lifespan (Beauchamp & Mennella, 2011). Non-nutritive sweeteners (NNS) have been utilized since the late 1800's (National Research Council (U.S.). Food Protection Committee., 1955) as alternatives to evoke desirable sweetness in food products without the calories associated with mono- and disaccharides. More recently, consumption of NNS by both adults and children has increased greatly, as NNS are now used by 28% to 85% of the American population (Mattes & Popkin, 2009; Sylvetsky et al., 2012).

Despite increasing use and renewed research interest in NNS, there is no standardized nomenclature for these compounds. The terms artificial, alternative, synthetic, low calorie, non-caloric, sugar-substitute, hyper-intense, high-intensity, and high-potency have all been used roughly synonymously (e.g. Swithers, 2013) to describe NNS despite implicit differences in meaning. We choose to use NNS as a blanket term largely through a process of exclusion. For example, rebaudioside A (RebA), and monkfruit are plant extracts, therefore the terms artificial and synthetic are not appropriate; aspartame is metabolized while sucralose is not, therefore non-caloric and low calorie lack precision.

Critically, the term 'high-intensity' has been repeatedly misinterpreted in the scientific literature and popular press to imply that non-nutritive sweeteners are 'sweeter than sugar' (Hutchinson et al., 1999; Pereira & Odegaard, 2013; Shankar et al., 2013; Zhao & Tepper, 2007) due to the implicit assumption intensity is analogous to the pharmacological concept of activity.

However, there is no evidence to suggest that NNS are ‘super-normal stimuli’ that have greater activity than natural carbohydrate sweeteners, like sucrose. Originally described by Tinbergen, super-normal stimuli are exaggerated stimuli that evoke behavioral responses more effectively than the stimulus for which the response evolved (Tinbergen & Perdeck, 1950). Suggesting that NNS are super-normal stimuli implies that they are able to evoke a sweetness response greater than natural sugars like sucrose. Although it is often claimed NNS over stimulate sweet taste receptors (Alpert et al., 2013; Bloomgarden, 2011; Ludwig, 2009), we fail to find evidence that NNS act as supernormal stimuli. NNS are generally high-potency sweeteners, but potency is *not* synonymous with intensity (activity), and the critical distinction between potency and activity has strong implications for public health and health policy.

The confusion between high-potency and high-intensity is understandable, given common marketing claims like “sucralose is 600 times sweeter than sugar” (McNeil Nutritionals, 2013) that are often repeated uncritically in the scientific literature. While strictly correct in one sense, this phrasing is also a gross oversimplification that is misleading. It may be helpful to recall the critical distinction between potency and activity in pharmacology. Specifically, because NNS have high pharmacological potency with respect to receptor activation, they have very low psychophysical detection thresholds compared to bulk carbohydrate sweeteners. It takes a very small amount of these compounds to activate a receptor and elicit a sensation; accordingly, a metabolized compound like aspartame is able to provide sweetness without contributing a nutritionally meaningful amount of calories to the diet. With regard to their detection threshold, NNS are ‘sweeter’ than disaccharides like sucrose on a weight-to-weight basis. Nonetheless, this does not imply high potency sweeteners are high-intensity stimuli. That is, the psychophysical intensity (i.e. the quantitative magnitude of a given sensation) is roughly equivalent to the pharmacological concept of activity. The important distinction between potency and activity can be illustrated with the opioids buprenorphine and morphine: buprenorphine has much greater

potency than morphine, but the activity of buprenorphine is much less than morphine. Likewise, a sweetener may have a low detection threshold without being intensely sweet. Indeed, it is well known in the food industry that NNS may have low maximal sweetness (e.g. Acesulfame K, Saccharin) (Paulus & Braun, 1988), which limits their utility. DuBois and colleagues (1991) demonstrated this in a dose-response study for numerous nutritive and non-nutritive sweeteners using a small trained panel (n=18). They found sweetness functions for NNS were hyperbolic, as perceived sweetness hit a ceiling and did not increase further as concentration increased. Conversely, monosaccharides, disaccharides, and sugar alcohols showed a linear dose-response (D-R) function in their study, although they noted that this linearity was an artifact of the panelist training (DuBois et al., 1991). Given that sweetness is a G protein-coupled receptor (GPCR) mediated phenomenon (Vigues et al., 2009), we would expect a sigmoidal function if other intensity scaling methods were used.

In terms of measuring sweetness perception, magnitude estimation has been commonly used to collect intensity data in relation to sweet taste stimuli (Cadena & Bolini, 2012; Moskowitz, 1971; Souza et al., 2013). Variation in magnitude estimation data may arise from either true perceptual differences or differences in how the participant uses numbers because individuals do not have the same sensory experiences (e.g. Bartoshuk et al., 2005). The general Labeled Magnitude Scale (gLMS) eliminates this problem because it forces participants to rate outside of the context of taste stimuli with the top anchor being *strongest sensation of any kind* (Snyder & Fast, 2004). Surprisingly, relatively few studies have utilized the gLMS to quantify sweetness intensity perception (e.g. Green et al., 2010; Sartor et al., 2011; Thai et al., 2011), despite the ability of the gLMS to generate ratio level data and allow valid across group comparisons (Snyder & Fast, 2004).

Here, we sought to re-examine and characterize the sweetness intensity dose-response (D-R) functions of NNS (sucralose, acesulfame K, rebaudioside A, aspartame) and ‘natural’

caloric sweeteners (sucrose, maple syrup, agave nectar) using modern psychophysical techniques. The present study aimed to answer the following questions: Is it possible for NNS to elicit a higher absolute perceived sweetness greater than sucrose and what are the estimated D-R functions when they are determined from a large cohort of untrained participants?

Methods

Overview of methods

The purpose of these series of experiments was to investigate the perceived sweetness of NNS (aspartame, acesulfame K, rebaudioside A, and sucralose) and nutritive sweeteners (sucrose, agave nectar, and maple syrup). Data were collected in four experiments conducted on separate days and pooled. For each experiment, the same orientation procedures and testing methods were used. Sucrose concentrations remained constant across experiments to enable comparisons across days. In the fourth experiment, sucrose and aspartame were retested with two additional concentrations to better characterize the D-R functions. Compusense *five* software, version 5.2 (Guelph, Ontario, Canada) was used for data collection. Presentation order of samples was counterbalanced using a Williams design to reduce first order carryover effects. All tests were completed at the Sensory Evaluation Center in the Department of Food Science at the Pennsylvania State University and surrounding area. Participants were provided with an explanation of the experiment in a brief orientation prior to testing in isolated testing booths. The orientation consisted of an overview of the gLMS with a warm-up using both imagined sensations (e.g. Hayes et al., 2013), and presentation of prototypical exemplars of sweet, bitter and metallic stimuli (Kamerud & Delwiche, 2007).

Participants

Reportedly healthy individuals (n=401) were recruited from the Pennsylvania State University campus and surrounding area (State College, PA) via email for their willingness to participate in a taste study. Participants were prescreened for eligibility. Eligibility criteria included: between 18-64 years old; not pregnant or breastfeeding; no known defects of smell or taste; no lip, cheek, or tongue piercings; nonsmoker (had not smoked in last 30 days); no food allergies or sensitivities; no history of choking or difficulty swallowing. Participants were also required to provide 30-35 min of their time for the experiment. Participants provided informed consent and were paid for their time. The Pennsylvania State University Institutional Review Board approved all procedures.

Psychophysical scaling

A generalized Labeled Magnitude Scale (gLMS) (Bartoshuk et al., 2003; Green et al., 1993) was used to measure the perceived intensities of sweetness, bitterness, and metallic sensation (Riera et al., 2007) for all stimuli. The gLMS ranges from 0 (no sensation), 1.4 (barely detectable), 6 (weak), 17 (moderate), 35 (strong), 51 (very strong) and 100 (strongest imaginable sensation of any kind). Data were collected using Compusense *five* software. Prior to rating test stimuli, all participants completed a brief warm-up to familiarize the participants with the gLMS. The warm-up required participants to make overall intensity ratings for 15 imagined and/or remembered sensations that include oral and non-oral sensations (Allen et al., 2013; Hayes et al., 2013). Generalizing the scale outside an oral context allows for increasingly valid comparisons across individuals.

Stimuli

Taste stimuli

All stimuli were presented in 10 mL aliquots in 30 mL medicine cups at room temperature. Solutions were prepared at least 24 hours prior to testing using reverse osmosis (RO) water and were stored at refrigerated temperature for a maximum of five days.

Orientation stimuli

The orientation exemplars (10 mL each) 292 mM sucrose (sweet), 0.032 mM quinine monohydrochloride dehydrate (bitter), a 292 mM sucrose / 0.032 mM quinine mixture (sweet and bitter), and 1.79 mM ferrous sulfate (metallic) solutions, as used by (Kamerud & Delwiche, 2007). Participants were told that they ‘may or may not experience all sensations from the orientation samples during the session in the booth’ and that ‘they may receive samples that have more than one taste quality’. Participants were also instructed to avoid rating how much they ‘liked’ or ‘disliked’ samples and separate intensity from hedonic affect (liking).

Dose-response for non-nutritive sweeteners (Experiments 1, 2, & 4)

Participants rinsed with room temperature reverse osmosis (RO) water before and between each sample. Participants were provided with 45s to rinse before the next sample. Five sucrose concentrations were used as constant stimuli across all experiments. In experiment 1, participants rated the sweetness, bitterness, and metallic intensity for 5 sucrose solutions (109.5, 219.1, 303.8, 409.0, and 818.0 mM), 5 aspartame solutions (0.23, 0.70, 1.0, 1.83, and 1.35 mM), and 5 rebaudioside A solutions (0.04, 0.25, 0.52, 1.03, and 1.55 mM). Concentrations were determined by bench top testing. In experiment 2 participants made the same attribute ratings for 5 sucrose solutions (as above), 5 acesulfame K solutions (1.57, 6.26, 24.9, 99.2, and 394.7 mM), and 5 sucralose solutions (0.20, 0.80, 3.17, 12.6 and 50.18 mM). In experiment 4 participants

made sweet, bitter and metallic ratings for 5 sucrose solutions (as above) and 7 aspartame solutions (0.23, 0.70, 1.0, 1.83, 1.35, 6.79, and 9.0 mM).

Dose-response for nutritive sweeteners (Experiment 3)

In experiment 3 participants made ratings for 5 sucrose solutions, 5 maple syrup (Great Value, Bentonville, AR) solutions (6.25, 12.5, 25, 50, and 100 g/L), and 5 light agave nectar (Madhava, Longmont, CA) solutions (6.25, 12.5, 25, 50, and 100 g/L). In experiment 3 participants wore nose clips to minimize any influence of volatiles on perceived sweetness (e.g. (Bartoshuk & Klee, 2013; Frank et al., 1989; Schifferstein & Verlegh, 1996). Nutritive sweeteners were measured on a weight-to-volume basis (g/L) as they contain a variety of sugars and other components including different ratios of glucose, fructose, and invert sugar (Taga & Kodama, 2012; Willems & Low, 2012). Additionally, moisture content was not taken into account during the analysis.

The sweetener concentrations used in Experiments 1-4 are summarized in Supplemental Table 1.

Procedure

Participants received brief instruction on the gLMS, and taste exemplars in a waiting room. After this orientation, the participants entered isolated computerized sensory testing booths. Once in the booths, participants completed a scaling warm-up procedure on the computer, rating imagined or remembered sensations (e.g. Hayes et al., 2013). Following the scale warm-up, participants received a tray of 15 samples (Test 1, 2 and 3) or 12 samples (Test 4). Participants were instructed to put the entire sample in their mouth, swish for 5 s to obtain total mouth coating, and spit the sample out. Participants then waited 10 s to allow the sensation to peak

before making intensity ratings; 45 s breaks between samples were enforced via software to minimize potential carry over and lingering. *Ad libitum* RO rinse water was also provided.

Statistical analysis

Dose-response functions were fit using GraphPad Prism 5.0C for OSX (GraphPad Software, San Diego CA). Descriptive and inferential statistics were calculated using SPSS statistical software. Because sweetness perception is a receptor-mediated process (Vigues et al., 2009), D-R functions for sucrose, aspartame, rebaudioside A, sucralose, maple syrup and agave nectar were fit *a priori* using the Hill Equation:

$$R = R_{\min} + \left[\frac{(R_{\max} - R_{\min})}{1 + 10^{(\text{LogEC}_{50} - C) \times \text{HillSlope}}} \right]$$

where R is the mean response (perceived intensity) across participants, and C is the stimulus concentration. In this model, R_{\max} is the fit of the top of the curve, R_{\min} is the bottom of the curve, the point halfway between min and max is EC50, and the slope of the linear portion of the model is the HillSlope. Separate functions were fit for sweet, bitter and metallic ratings obtained on the gLMS. The dose response for Acesulfame K could not be fit using this model, so a second-degree polynomial function was used instead:

$$R = b_0 + b_1C + b_2C^2$$

where R is the mean intensity and C is concentration.

Results

Dose-response functions for nutritive sweeteners are not linear

Mean dose-response functions for caloric and non-nutritive sweeteners in a large number of participants are shown in figure 3-1. As expected, all four non-nutritive sweeteners are left shifted compared to sucrose, indicating they have higher potency. Notably, both caloric and non-nutritive sweeteners are well described by a four parameter logistic equation, with the exception of acesulfame K. Present data partially conflict with prior reports: in 18 highly trained assessors, sugars and sugar alcohols showed linear dose-response functions while high potency sweeteners were best described by Hill-type models (DuBois et al., 1991).

Sugars have higher maximal sweetness than non-nutritive sweeteners

The caloric sweeteners (sucrose, agave nectar, and maple syrup) all had higher overall maximal sweetness than the non-nutritive sweeteners, indicating NNS are not supernormal stimuli as compared to sucrose (figure 3-1 and table 3-1).

The absolute maximum intensities (table A-1) for rebaudioside A (20.3) and sucralose (28.5) are significantly less than that of sucrose (36.3) ([F(1,513)= 9.19; p=0.0026] and [F(1,490) = 5.99; p=0.0147] respectively). Also, although the absolute maximum sweetness of aspartame (28.2) is less than sucrose (32.6), it is not significantly different [F(1,501)= 0.81; p=0.37].

The mechanism by which many NNS are unable to elicit a maximal intensity equal to or greater than caloric sweeteners is most likely due to mixture suppression of sweetness by bitterness at higher cognitive levels in the brain (Lawless, 1979). Figure 3-2 shows the mean D-R functions for sweetness and bitterness. For acesulfame K, sucralose, and rebaudioside, bitterness increases with concentration, eventually equaling or surpassing perceived sweetness. In contrast, figure 3-2 shows that aspartame has a similar function to that of sucrose, maple syrup, and agave nectar (see figure A-1): bitterness is minimal and does not increase with concentration.

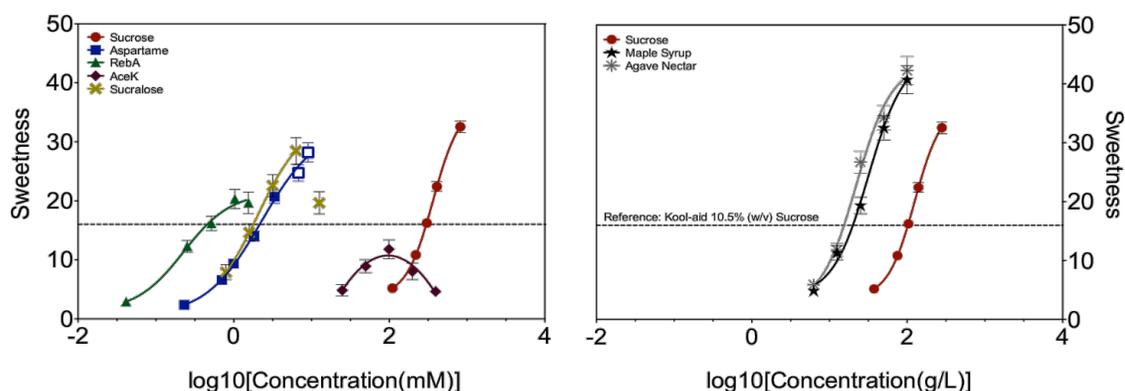


Figure 3-1 Mean Dose-Response Functions for Non-Nutritive (left) and Nutritive (right) Sweeteners. The y-axis indicates perceived sweetness on a generalized Labeled Magnitude Scale (gLMS; see text). Data were pooled across experiments. Total numbers of participants per stimulus were: (Sucrose: $n=401$), (Aspartame, Solid squares $n=216$; Open squares $n=102$); (RebA, $n=114$), (Acesulfame K, $n=91$), (Sucralose, $n=91$), (Maple Syrup, $n=94$), (Agave Nectar, $n=94$). The dashed horizontal line is provided for context: it represents the approximate sweetness of Kool-Aid (10.5% sucrose).

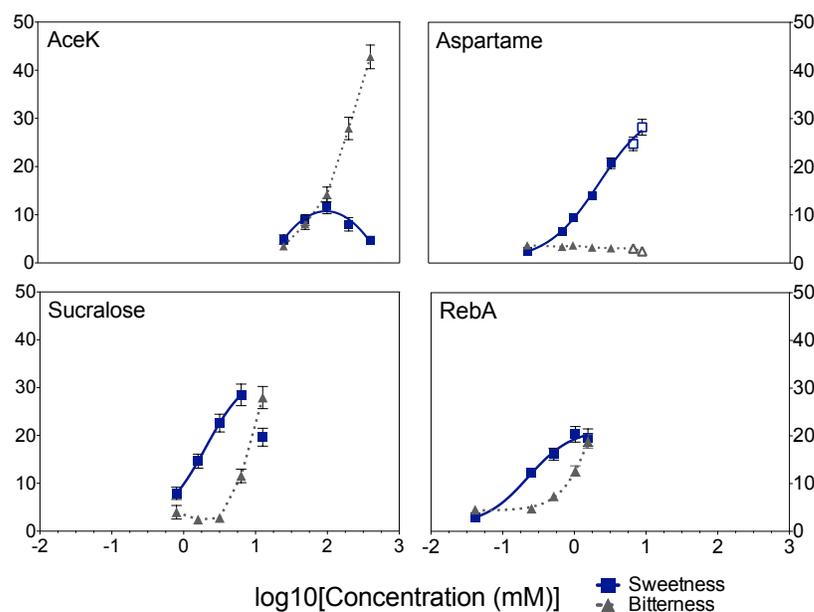


Figure 3-2 Dose-Response Functions for Sweetness and Bitterness in four non-nutritive sweeteners on a gLMS scale. Solid lines (squares) indicate perceived sweetness intensity; the dotted lines (triangles) indicate perceived bitterness. Numbers of participants per stimulus were: (Aspartame, solid squares $n=216$; Open squares $n=102$); (RebA, $n=114$), (Acesulfame K, $n=91$), (Sucralose, $n=91$).

Table 3-1 Hill equation dose-response fit parameters for sucrose, agave nectar, maple syrup, aspartame, rebaudioside A, and sucralose

Sweetener	Rmax	SE	Hill Slope	SE	LogEC₅₀	SE	EC₅₀
Maple Syrup	48.7 ^a	8.93	1.48	0.62	1.52	0.10	33.24
Agave Nectar	45.2 ^a	4.82	1.62	0.62	1.34	0.08	22.02
Sucrose	36.3 ^a	2.39	2.53	0.50	2.11	0.027	124.7
Aspartame	32.6 ^a	3.96	1.21	0.29	0.36	0.09	2.28
Sucralose	33.6 ^b	13.77	1.48	1.88	0.31	0.19	2.03
RebA	21.5 ^{bc}	3.61	1.42	1.18	0.64	0.15	0.23

All values represent means. Means with different letters are significantly different ($p < 0.05$). EC₅₀ is half of the concentration needed to evoke the maximal perceived sweetness rating. Maple Syrup, Agave Nectar, and Sucrose are in (g/L) and Aspartame, Sucralose, and RebA are in (mM).

Discussion

Present data make several important contributions to extant literature. First, these data indicate non-nutritive sweeteners are not super-normal stimuli. That is, they do not evoke sweet sensations that are more intense than concentrated sucrose. Second, we find that sucrose, maple syrup, and agave nectar exhibit sigmoidal dose-response functions, not linear functions, as would be expected given this is a receptor-mediated process.

Although NNS have low psychophysical detection thresholds compared to sugars, it is not valid to use thresholds or the dose over threshold to estimate the perceived intensity of these sweeteners (Bartoshuk & Klee, 2013; Frijters, 1978; Hayes, 2008; Keast & Roper, 2007). Indeed, in 1948, Lichtenstein and colleagues noted that comparing thresholds provides invalid information concerning the relative sweetness of sweet stimuli above threshold levels. The dose-response functions obtained here indicate non-nutritive sweeteners are more potent but less efficacious than sucrose, maple syrup, and agave nectar. The lack of activity in the perceived sweetness intensity of acesulfame K, sucralose, and rebaudioside A is likely a function of increasing bitterness with concentration. Bitterness is a side taste that is associated with many NNS including rebaudioside A and acesulfame K (Allen et al., 2013; Schiffman et al., 1995). In contrast to prior reports that suggest sucralose has minimal bitterness (Schiffman et al., 1995), we find clear evidence that sucralose is bitter, consistent with unpublished data showing sucralose can activate bitter receptors (hT2Rs) *in vitro*. Notably, the bitterness of sucralose and acesulfame K are sufficiently intense to depress sweetness ratings. That is, endogenous bitterness not only provides a ceiling on maximal sweetness, but can actually reverse the slope of the function at the highest concentrations. Similar effects have been shown recently for the stevia glycoside rubusoside (Hellfritsch et al., 2012). Here, the D-R function for rebaudioside A is suggestive of this pattern. Accordingly, we would expect a similar reversal if higher concentrations were used. In contrast, Aspartame lacks any bitterness, showing a pattern like sucrose, maple syrup, and agave nectar. The absence of bitterness in aspartame is well documented in the literature as well as its similar taste qualities to sucrose (Larson-powers & Pangborn, 1978; Mazur, 1984; Schiffman et al., 1995).

Furthermore, the nutritive sweeteners sucrose, maple syrup, and agave nectar follow sigmoidal, not linear, functions. The literature disagrees whether or not nutritive sweeteners like saccharides and sugar alcohols follow linear functions in which intensity increases as a function

of concentration (DuBois et al., 1991; Fujimaru et al., 2012; MacLeod, 1952; McBride, 1983; Stone & Oliver, 1969) or whether sugars diverge from linearity (Moskowitz, 1971). Present data support the existence of sigmoidal dose-response functions, consistent with the underlying biology (DuBois & Prakash, 2012; Vignes et al., 2009).

The previously reported linearity of the sucrose D-R function is likely a result of extensive panel training with reference samples (DuBois et al., 1991). To prevent artifactual linearization in our data, we avoided extensive panel training (e.g. Sensory SpectrumTM universal scaling). Present data shows that using the gLMS is an effective way to collect intensity data in a large number of naïve participants. The gLMS enabled successful sweetener differentiation and non-linear D-R functions that imply that panel training may be unnecessary to collect ratio-level data in a large population.

Conclusions

Although NNS may have greater binding affinity to sweet receptors, this does not imply NNS over-stimulate sweet receptors as has been implied previously (Alpert et al., 2013; Ludwig, 2009). We also show nutritive sweeteners (sucrose, maple syrup, and agave nectar) follow sigmoidal dose response function one would expect from receptor-dependent phenomenon. Present data also clarify the bitterness functions of the NNS as a function of concentration, although we must point out that the use of such high concentrations of NNS in commercial applications would be unlikely. Also, we should note present stimuli were presented in a simple aqueous model system; whether they might behave differently in mixtures with each other or in real foods remains to be tested. Nonetheless, acesulfame K, sucralose, and rebaudioside A are not ‘sweeter than sugar’ in that they do not surpass the perceived sweetness intensities of natural sweeteners like sucrose, maple syrup, and agave nectar. Finally, we do not take a broader position

on the safety of NNS (cf Fitch & Keim, 2012 and Schiffman, 2012), the nutritional consequences of non-nutritive sweetener intake (e.g. the energy signaling/decoupling hypothesis (Swithers, 2013)), or the role of extra oral taste receptors (e.g. Dotson et al., 2010), but the present data suggest that NNS do not result in deleterious health effects by over-stimulating sweet taste receptors to produce hyper-intense sweet sensation.

Chapter 4

Hedonic responses to sucrose solutions and the relationship to sweet food liking

Introduction

Many researchers have been interested in the relationship between the hedonics of sucrose as a function of concentration (Ekman & Åkesson, 1965; Looy et al., 1992; Lundgren et al., 1978; Stone & Pangborn, 1990) and how that relationship translates to sweet food hedonics. Although this topic has been frequently investigated, previous studies have used hedonic ratings from a narrow range of sucrose concentrations to group individuals into sweet liking hedonic groups (Lundgren et al., 1978). Lundgren et al. (1978) investigated hedonic responses to sweet taste and identified four hedonic groups based upon responses to sucrose in coffee on a 17-point line scale. The four groups that Lundgren et al. identified are: Type I (decreasing hedonic response with increasing sucrose concentration), Type II (maximum hedonic response between lowest and highest concentration), Type III (increasing hedonic response with increasing sucrose concentration), and Type IV (no change in hedonic response with increasing concentration). Similar hedonic groups have been found by other studies (Stone & Pangborn, 1990).

There is variability in studies that have investigated the relationship between sucrose liking and sweet food liking. Different conclusions have been made concerning the validity of translating the liking of sucrose solutions into sweet food hedonics (Looy et al., 1992). Here we measure the hedonic liking and intensity perception of sucrose solutions. We attempt to define individual sweet liking curves as a function of sucrose concentration (0.065-2.5M) and to determine the relationship between hedonic sucrose curves and sweet foods and beverages.

Methods

Overview of methods

Present data are part of a larger study (Genetically Informed Analysis of Natural Tastants and Chemesthetic Stimuli, phase 2), on the genetics of oral sensation. All tests were completed at the Sensory Evaluation Center in the Department of Food Science at the Pennsylvania State University. GIANT-CS, phase 2 involves two days of testing; here, we describe methods from the first session on Day 1. On day 1, informed consent was obtained and participants were compensated for their time. After informed consent was obtained, the procedure and the intensity and hedonic scales were explained to participants in a one-on-one setting to ensure a thorough understanding of instructions. Following the study explanation, anthropometric data were collected (height, weight, BMI) followed by digital microscopy of the anterior tongue. Participants then entered isolated sensory testing booths and completed an appetite log, a scale orientation (intensity and hedonic), and made intensity and hedonic ratings for 15 sampled stimuli (14 sucrose solutions, 1 water control). Subsequently, participants completed a generalized degree of liking survey (gDOL) on the computer where they rated their liking or disliking of a list of 70 positive, neutral, and negative food and non-food related items, similar to (Peracchio, et al., 2012; Byrnes and Hayes, 2013). Total time in the laboratory for day 1 was ~1 hour.

Subjects

Reportedly healthy, non-smoking adults (n=154; 46m; aged 22-45) were recruited from the Pennsylvania State University and surrounding campus and prescreened for eligibility. Screening eligibility criteria included: Caucasian (European ancestry); not pregnant or breast feeding; no past or known smell or taste defects; no lip, tongue, or cheek piercings; not smoked in the last 30 days; no history of chronic pain; no prescription pain medication; no difficulty

chewing or swallowing; no history of overactive or underactive thyroid or other thyroid disease.

One participant was excluded from the analysis due to inability to follow the protocol.

Computerized orientation

Prior to rating test stimuli, all participants participated in a computerized orientation on a gLMS (Bartoshuk et al., 2003; Green et al., 1993). The orientation requires participants to practice making overall intensity ratings for 15 imagined and/or remembered oral and non-oral sensations (Allen et al., 2013; Hayes et al., 2013). Participants also participated in a short 5-item hedonic orientation where they practiced rating their liking of five remembered food and non-food related items.

Intensity scaling

A generalized labeled magnitude scale (gLMS) was used to measure the perceived sweetness intensity of test stimuli. The gLMS is a semantically labeled scale that ranges from 0 (no sensation), 1.4 (barely detectable), 6 (weak), 17 (moderate), 35 (strong), 51 (very strong) and 100 (strongest imaginable sensation of any kind). Participants were told *“you can make your ratings anywhere on the scale that you like – the labels are to help you make your ratings”* to prevent participants rating solely on the semantic descriptors. Participants were also reminded to *“avoid rating how much you like or dislike the sample when rating the intensity of the sensation”*.

Hedonic scaling

A generalized bipolar hedonic scale was used to measure the hedonic liking of all sweet test stimuli. This scale ranges from -100 to + 100 with semantic descriptors at -100 (‘strongest disliking of any kind’), +100 (‘strongest liking of any kind’) and at 0 (‘neither like nor dislike’ (e.g. (Byrnes & Hayes, 2013))). All intensity and hedonic rating were collected using Compusense

five software (Guelph, Ontario, Canada). Participants also completed a generalized degree of liking survey (gDOL) over the course of two days. Participants rated their liking of 70 hedonically positive, neutral, and hedonically negative food and non-food related items using the hedonic scale described above.

Test Stimuli

Food grade stimuli were presented in 10 mL aliquots in 30 mL medicine cups at room temperature. Test stimuli in day 1 include seven sucrose concentrations (0.0625, 0.125, 0.25, 0.5, 1.0, 2.0, and 2.5M sucrose) presented in duplicate and one water control (total of 15 samples). All solutions were prepared at least twenty-four hours prior to testing using reverse osmosis (RO) water and stored at refrigerated temperature for a maximum of five days.

Presentation order was a counterbalanced Williams' design. Participants were instructed to put the entire sample in their mouth, swish for 5s to obtain total mouth coating, and spit the sample out prior to making their ratings. Thirty second breaks between samples were enforced via software to minimize potential carry over and lingering. *Ad libitum* RO rinse water was also provided.

Data and statistical analysis

Data were analyzed using SPSS and R statistical software (v3.0.1). The average hedonic and perceived intensity scores were graphed as a function of log sucrose concentration (mM). A linear ($y=ax+b$) or second-degree polynomial ($y = ax^2+bx+c$) equation was fit to individual hedonic scores as a function of concentration. Intensity functions were fit using a linear model and the slope and area under the curve (AUC) were calculated.

Additionally, one-way ANOVAs were conducted to determine if there were significant differences ($p < 0.05$) between sucrose hedonic groups in their hedonic ratings of food items collected in the gDOL survey.

Algorithm: defining hedonic groups

Four hedonic groups, based upon average hedonic ratings of tested sucrose solutions, are defined in the present analysis: Slope -; Slope + ; Horizontal line; and Inverted-U (Figure 4-1).

Groups and optimum hedonic sucrose concentrations are defined as follows:

- **Slope +**: an increasing hedonic response with increasing sucrose concentration (optimum concentration 2.5 M sucrose).
- **Slope -**: a decreasing hedonic response with increasing sucrose concentration (optimum concentration 6.5 M sucrose).
- **Horizontal Line**: no changes in liking with increasing sucrose concentration.
- **Inverted-U**: an increasing hedonic response until an optimum concentration is met and then liking decreases with increasing concentration (optimum between 0.065 and 2.5 M sucrose).

Individual hedonic curve types were defined by an algorithm, implemented in the R language by Moulinier (2013), that determines whether a linear or polynomial model best fits individual hedonic ratings (see Appendix B for algorithm). An ANOVA F-test was used to compare the two models to determine which model best fit the data. The F-test resulted in two outcomes concerning individual hedonic responses: the linear model was the correct fit; or the polynomial was the best fit. If the p-value was significant (< 0.05) it confirmed that the F-test selected model (linear or polynomial) provided a fit better than the other. If the p-value was not

significant ($p > 0.05$) there was no sufficient evidence to support the F-test selected model, so the other model would be accepted.

Parameters for hedonic linear models are defined as follows. ‘Slope +’ was defined as a linear hedonic slope that was > 7.316 . ‘Slope –’ was defined as a linear hedonic slope that was < -4.147 . The ‘Horizontal Line’ group was defined as a linear hedonic slope that was > -4.147 and < 7.316 . ANOVAs were conducted on the slopes of the linear hedonic groups to confirm that the parameters were appropriate to divide them ($[F=74.9; p = 2.2 \times 10^{-16}]$). Proportions for the four hedonic groups are shown in table 4-1.

Results and Discussion

Hedonic groups

The proportions of individuals who were categorized into each group are given in table 4-1. There are no significant gender differences between hedonic groups [$\chi^2=3.39; p=0.336$] or age differences between groups ($[F=0.657; p=8.78]$). The present proportions of individuals who are categorized into each group (table 4-1) differ from previous literature. In coffee, Lundgren et al. (1978) observed the following proportions of sweet hedonic groups based upon ratings ($n=122$) of a range of 0-10% (0-0.34M) sucrose: Slope + (48.4%); Slope – (19.7%); Horizontal Line (9.8%); and Inverted-U (22.1%). There are a variety of reasons that present data may elicit different hedonic group proportions than previous research. The current study used a wide range of sucrose concentrations (0.0625M-2.5M) compared to a smaller range used by (Lundgren et al., 1978). Also, the bitterness from the coffee and scaling method used to measure hedonic liking (17-point category scale) in the Lundgren et al. study may have affected the patterns of the hedonic liking curves.

Table 4-1 Hedonic group frequencies and proportion. Hedonic groups are determined from the average hedonic ratings of sucrose solutions (0.065-2.5M) and fit using an automated algorithm.

Hedonic Group	<i>Frequency</i>	<i>Proportion (%)</i>
Slope +	30	19.6
Slope -	17	11.1
Horizontal Line	42	27.5
Inverted U	64	41.8
TOTAL	153	100

The majority of individuals fall into the ‘Inverted-U’ hedonic liking group. The ‘Slope –’ group contains 11.1% of participants and 19.6% were categorized as ‘Slope +’. It is interesting however, that 27.5% of participants were categorized into the ‘Horizontal Line’ hedonic group. This large proportion of participants in the ‘Horizontal Line’ group can be interpreted two ways: 1.) participants are affectively indifferent to changes in sucrose concentrations or 2.) this group is unable to distinguish between different sucrose concentrations. Closer inspection of the individual ‘Horizontal Line’ hedonic curves indicates both may be true. Many participants in the ‘Horizontal Line’ group made hedonic ratings at ‘0’ which is ‘neither like nor dislike’ on our generalized hedonic scale. This pattern is indicative of central tendency (Moskowitz, 1980) in which participants rate near the middle of the scale. Also, we find evidence that participants in the horizontal line group were not able to distinguish between samples, which is discussed in the intensity perception section.

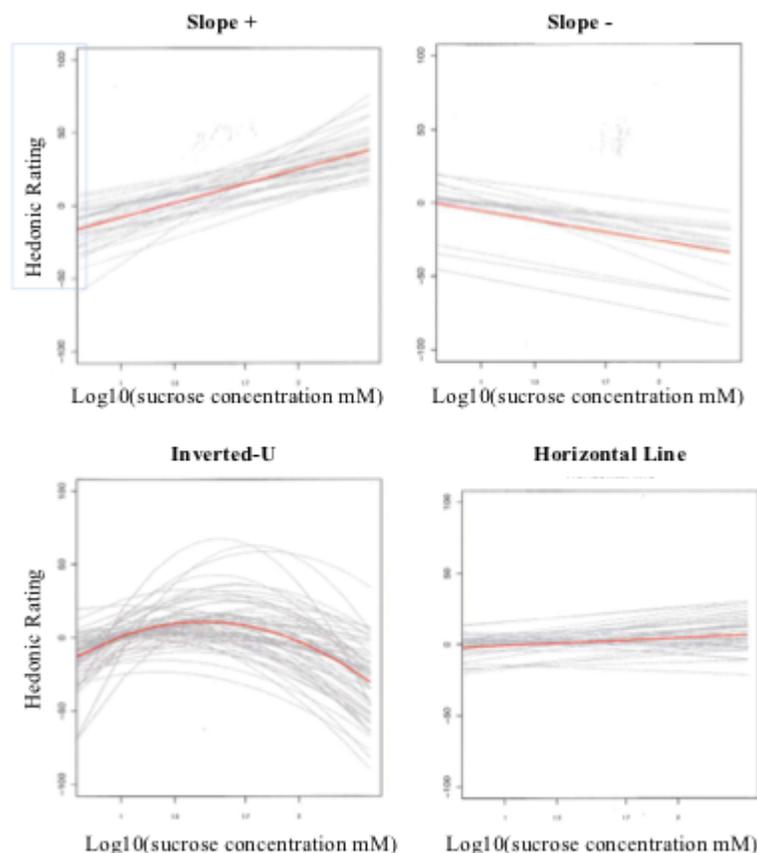


Figure 4-1 Average polynomial and linear hedonic curves: Inverted-U; Slope +; Slope -; and Horizontal line. Curve types are based upon the group average rating across sucrose concentrations.

Intensity Perception

There are significant differences between hedonic groups in the slopes of the sweetness intensity curves ($[F(3,149)=6.64; p < 0.001]$). High slope values indicate greater ability to perceive differences in sweetness between samples. Interestingly, average intensity ratings for ‘inverted-U’ (10.52) and ‘slope +’ (11.22) groups were significantly greater than that of the ‘Slope -’ (6.53) and ‘Horizontal Line’ (7.06) groups. This implies that those who are ‘inverted-U’ and ‘Slope +’ may be better able to discriminate between samples. That is, discrimination ability may affect hedonic liking. For example, if individuals cannot easily discriminate between the intensity of sucrose concentrations, their hedonic ratings should not be expected to change (e.g. ‘Horizontal Line’ group). This does not however, explain the low discrimination ability of the

‘Slope –’ group. It might be expected that the ‘Slope –’ group would have the highest slope of any group, which was not observed. Other factors like viscosity may also play a role.

Generalized degree of liking survey (gDOL)

Information from the gDOL survey indicates that there are a few significant differences among sweet liking groups in their liking of sweet foods, sweet alcoholic beverages, and non-alcoholic beverages. As expected, there were no differences among sweet liking groups in their liking of non-sweet alcoholic beverages. *A priori*, we hypothesized that the ‘Slope +’ group would have significantly higher liking scores for sweet foods than the other three groups because they have increased liking for increasing sucrose concentrations. We also postulated that and ‘Slope –’ would have significantly lower liking scores for sweet foods than the other three groups because they have decreased liking of increasing sucrose concentrations.

For sweet foods (Figure 4-3), there were significant differences in hedonic liking scores among sweet liking hedonic groups for brownies, cookies, and cheesecake, chocolate, and chewy fruity candy. Cigarette smoke is included to provide a negative nonfood contrast to the sweet foods. The ‘Slope +’ hedonic group had a significantly higher average liking score for brownies, cookies, and cheesecake than the ‘Inverted-U’ group ($p=0.010$). There were no significant differences between ‘Slope –’, ‘Horizontal Line’, and ‘Inverted-U’ groups. For chocolate, the ‘Slope +’ hedonic group also had a significantly higher average liking scores than the ‘Horizontal Line’ group ($p=0.029$) and ‘Inverted-U’ group ($p=0.013$). There were no significant differences between ‘Slope +’ and ‘Slope –’ groups ($p=0.150$). The ‘Horizontal Line’ group had a significantly higher average liking score than ‘Inverted-U’ ($p=0.13$), ‘Slope –’ ($p=0.015$), and ‘Slope +’ ($p=0.041$) groups. There were no significant differences between ‘Inverted-U’, ‘Slope –’, and ‘Slope +’.

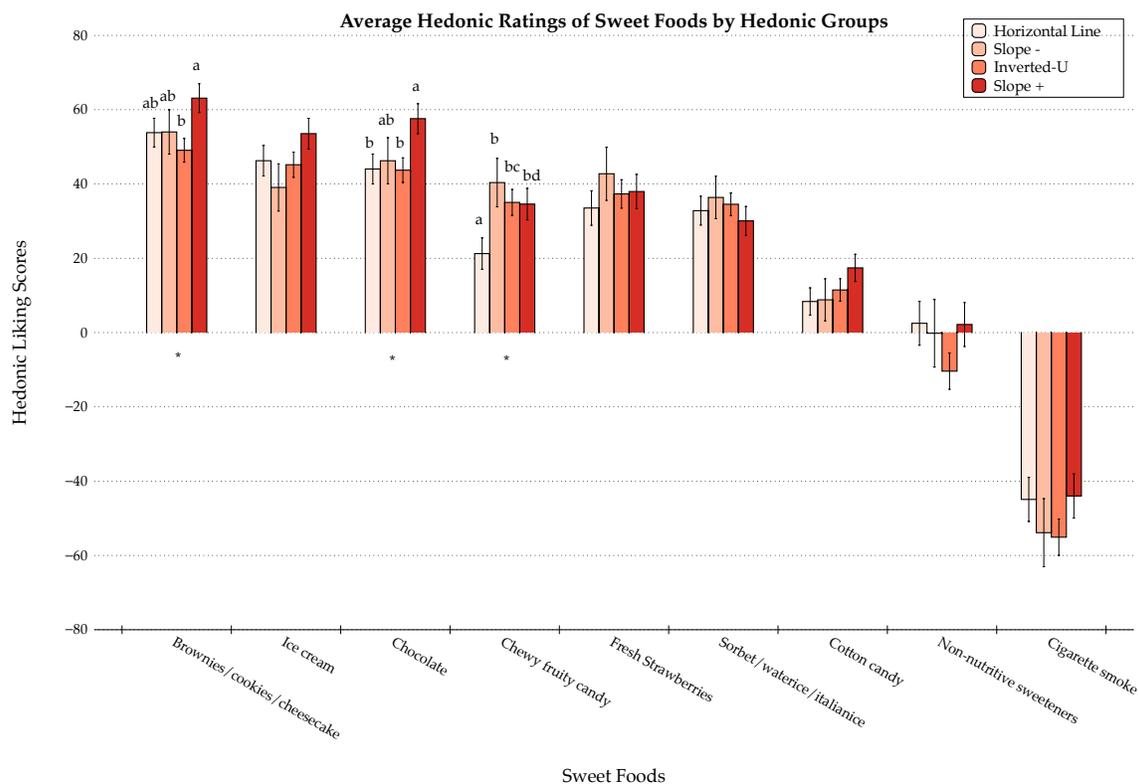


Figure 4-2 Average hedonic ratings of sweet foods by hedonic groups. All bars represent mean values. Bars with different letters are significantly different from each other ($p < 0.05$).

For sweet alcoholic beverages (Figure 4-4), there were also significant differences between sweet liking hedonic groups. For spirits with soda, the ‘Slope -’ group had a significantly higher hedonic liking score than the ‘Horizontal Line’ group ($p = 0.016$), and ‘Inverted-U’ group ($p = 0.046$). There were no significant differences between ‘Slope -’ and ‘Slope +’ groups for liking of spirits with soda ($p = 0.442$). For spirits with fruit juice, the ‘Slope +’ group had a significantly higher hedonic rating than ‘Horizontal Line’ ($p = 0.030$) and ‘Inverted-U’ ($p = 0.008$). For spirits with diet soda, the ‘Slope +’ group had a significantly higher hedonic rating than the ‘Inverted-U’ ($p = 0.017$) group. For malt beverages, the ‘Slope +’ group had a significantly higher average hedonic rating than the ‘Inverted-U’ ($p = 0.037$) group.

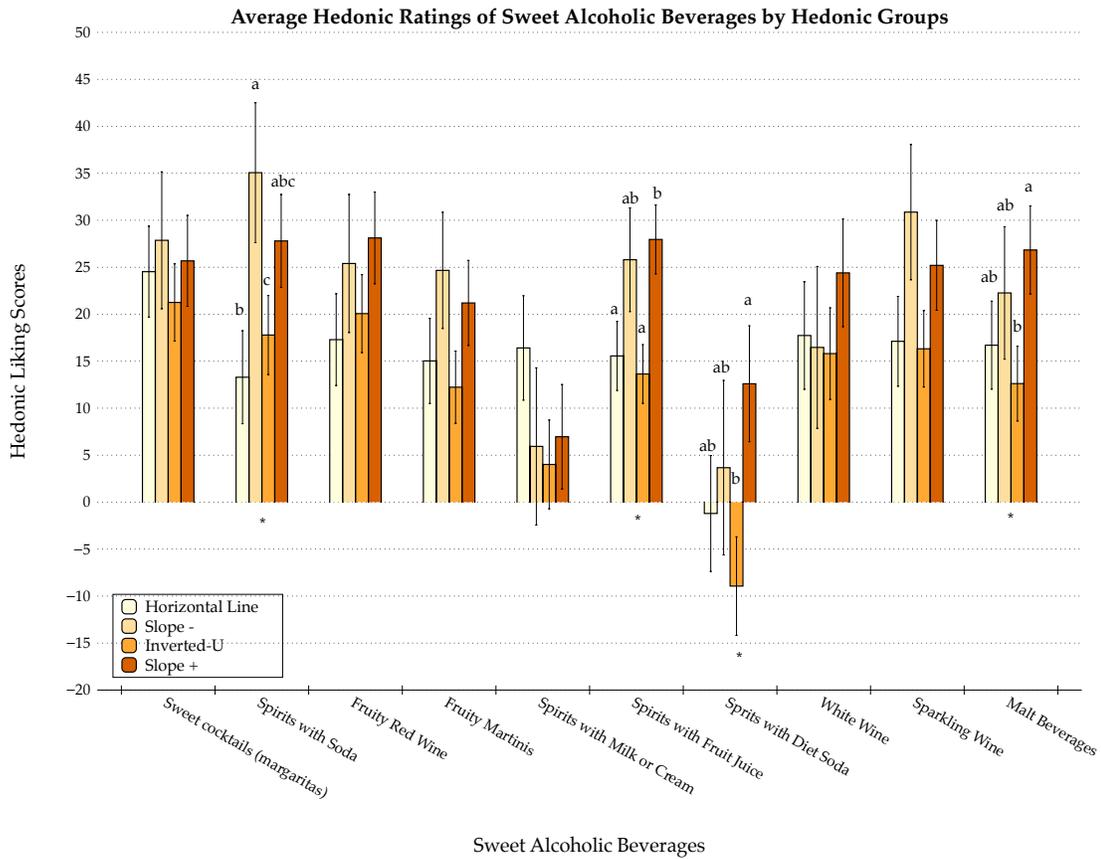


Figure 4-3 Average hedonic ratings of sweet alcoholic beverages by hedonic groups. All bars represent mean values. Bars with different letters are significantly different from each other ($p < 0.05$).

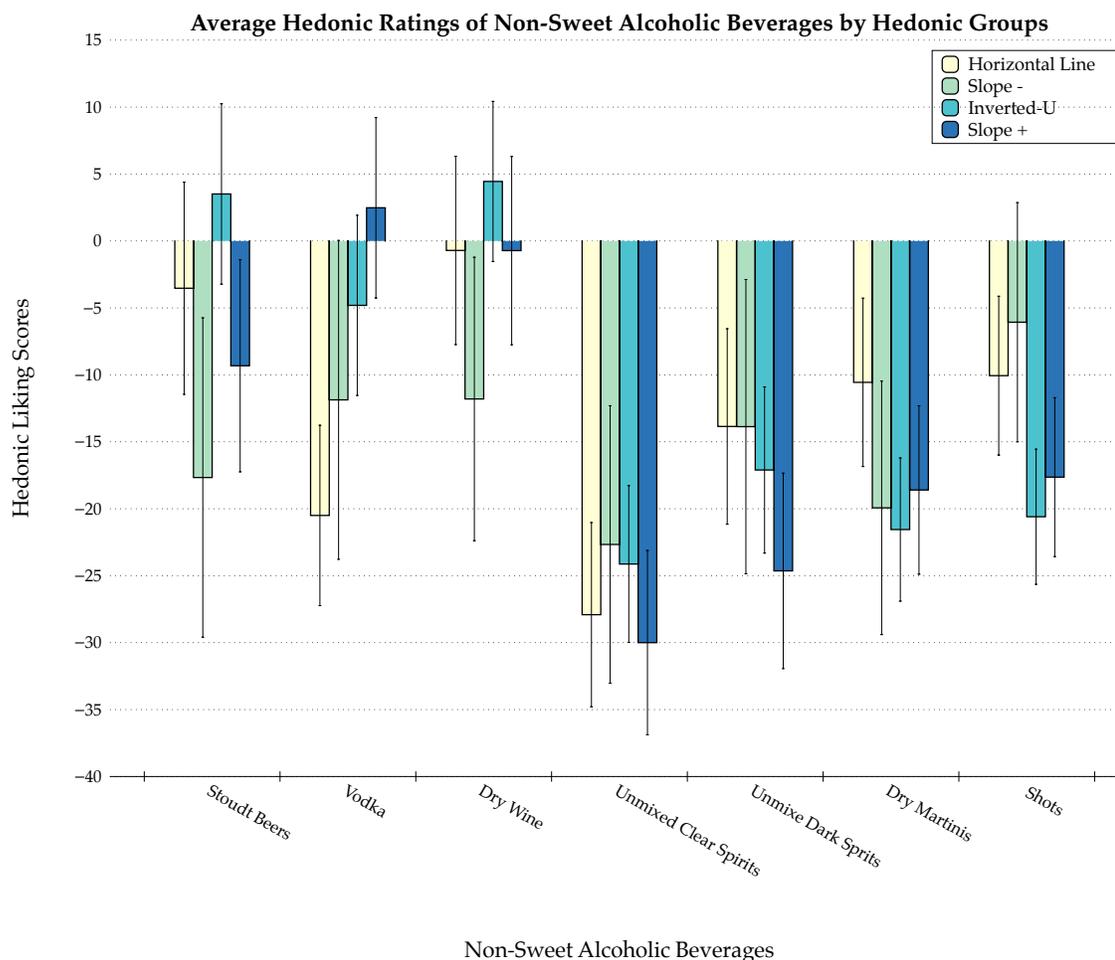


Figure 4-4 Average hedonic ratings of non-sweet alcoholic beverages by hedonic groups. There are no significant differences between sweet liking hedonic groups.

In contrast to sweet alcoholic beverages, there were no differences among sweet liking hedonic groups for non-sweet alcoholic beverages. This indicates that segmentation based upon sweet hedonics significantly affects hedonic liking scores for some sweet alcoholic beverages but not for non-sweet alcoholic beverages, as expected.

For non-alcoholic beverages (Figure 4-6), the only significant difference between sweet hedonic groups was for skim milk [$F(3,148)=4.954$; $p=0.003$]. The ‘Inverted-U’ group had a significantly lower hedonic liking score for skim milk than the ‘Slope +’ ($p=0.021$), ‘Slope -’ ($p=0.001$), and ‘Horizontal Line’ ($p=0.007$) groups.

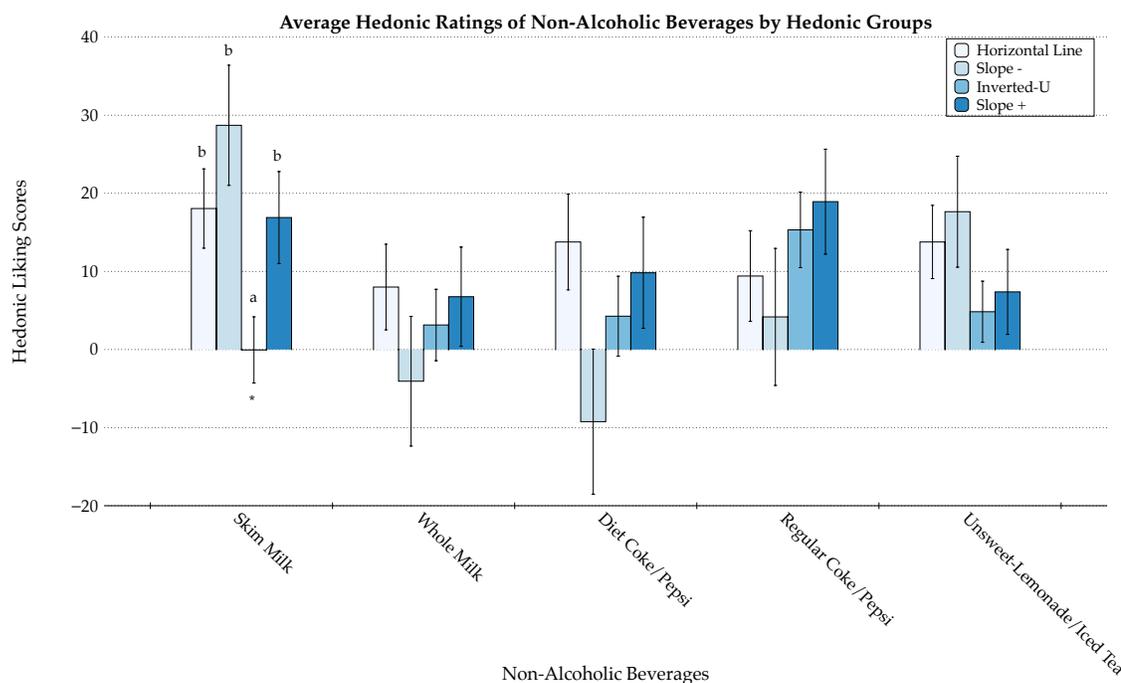


Figure 4-5 Average hedonic ratings of non- alcoholic beverages by hedonic groups. All bars represent mean values. Bars with different letters are significantly different from each other ($p < 0.05$).

Conclusions and limitations

Four types of hedonic groups were defined through hedonic ratings of sucrose concentrations from 0.065 mM to 2.5 M: ‘Slope +’; ‘Slope -’; ‘Horizontal Line’; and ‘Inverted-U’. There were significant differences between the groups in their ability to distinguish among increasing sucrose concentrations. Although there were some significant differences between groups in terms of their liking of sweet and non-sweet foods and beverages, we did not observe strong evidence that sucrose liking hedonic groups can predict the liking of remembered sweet foods. The gDOL, in terms of hedonic food measurement, requires participants to rate hedonic

liking of various 'remembered' food products. Liking when tasting food may be completely different than remembered liking, however previous literature has shown correlations between remembered and sampled food liking (Duffy et al., 2007).

Additionally, food products are complex matrices and although some of the sweet foods measured were sweet, some of them were also high in fat (e.g. ice cream). It is difficult to measure hedonic differences in palatable food products based upon the hedonic liking of sucrose solutions as they differ in complexity. However, considering that there were some significant differences between sweet liking groups in sweet food and alcohol liking and in contrast to Looy et al. (1992), there may be some validity in translating the liking of sucrose solutions to sweet food products. Also, viscosity was not controlled for in this study, which may explain why the 'Slope -' group was not able to distinguish between the sweetness of samples, yet their hedonic ratings continue to decrease as concentration increases (Cardello, 1996) .

Further work is needed to identify hedonic differences between sweet liking phenotype. It would be interesting to investigate the relationship between sweet liking groups and the hedonics of real food products.

Chapter 5

A brief investigation into the functional OPRM1 A118G single nucleotide polymorphism and hedonic responses to sucrose solutions and sweet foods

Introduction

The endogenous opioid system in the brain mediates reward for pleasurable stimuli such as sex, drugs, and* alcohol. It is known that the pleasure from sweet taste is also mediated by endogenous opioid system because it is naloxone reversible (Segato et al., 1997). A functional μ -opioid receptor polymorphism (OPRM1 A118G), that has been correlated with alcohol and heroin abuse (Bart et al., 2005), may affect the rewarding value of sweet taste. The A118G single nucleotide polymorphism (SNP) results in an amino acid substitution (asparagine to aspartic acid), which alters the function of the μ -opioid receptor by eliminating a glycosylation site from the N-terminal of the receptor (Kroslak et al., 2007). Three genotypes are possible: the normal variant homozygote (A118A), the heterozygote (A118G); and the rare homozygote (G118G). The OPRM1 AG genotype has been associated increased β -endorphin binding affinity to the μ -opioid receptor (Bond et al., 1998) suggesting that individuals with the G-allele should have increased pleasure from rewarding stimuli such as sweet taste.

Few studies have been able to provide sufficient evidence that the A118G SNP evokes changes in phenotypic behavior (Arias et al., 2006) especially in terms of sweet food liking. Here we investigate the relationship between the OPRM1 A118G SNP, sucrose liking hedonic curves, and sweet food liking. We hypothesize that there will be a significant difference in genotype frequency among ‘Slope +’, ‘Slope -’, ‘Inverted-U’, and ‘Horizontal Line’ sucrose liking hedonic groups. We further hypothesize that individuals with the G-allele will experience increased reward from sweet stimuli and sweet food and therefore the AG/GG genotypes will have higher hedonic ratings for sweet foods than the AA genotype.

* *rock n' roll*

Methods

Overview of methods

Presented data are part of a larger study (GIANT-CS, phase II) on the genetics of oral sensation. Methods are the same as that of Chapter 4 with a few methodological additions listed below. All tests were completed over the course of two days at the Sensory Evaluation Center in the Department of Food Science at the Pennsylvania State University. Compusense *five*, version 5.2 (Guelph, Ontario, Canada) was used to collect all psychophysical data.

Subjects

Reportedly healthy, non-smoking adults (n=154; 46 m; aged 22-45) were recruited from the Pennsylvania State University and surrounding area and prescreened for eligibility. Only 148 individuals were included in the analyses due to missing genetic data.

Hedonic groups

Hedonic groups were determined from the average hedonic ratings of a range of sucrose solutions (0.065-2.5 M) using an unstructured hedonic scale ranging from -100 (strongest disliking of any kind) to +100 (strongest liking of any kind). An algorithm for R-statistical software, developed by Moulinier (2013) (Figure B-1), determined whether individual hedonic liking functions fit linear or polynomial functions. Participants were divided into four hedonic groups based upon their hedonic ratings of sucrose solutions: ‘Slope +’ (increasing hedonic liking with increased concentration), ‘Slope -’ (decreasing hedonic response with increasing sucrose concentration), ‘Horizontal Line’ (no change in hedonic response with increasing sucrose concentration), and ‘Inverted-U’ (hedonic response increases until an optimum concentration then decreases).

Generalized degree of liking survey (gDOL)

Participants completed a digital generalized degree of liking survey (gDOL) where they rated their liking or disliking of a list of 70 positive, neutral, and negative food and non-food related items.

Genetic analysis

DNA was collected from participant saliva using oragene collection kits on day 2 of GIANT-CS2 testing (Oragene). Saliva was collected according to instructions from the manufacturer (Genotek Inc, Ontario, Canada). TaqMan assay was used to identify the OPRM1 A118G SNP (rs1799971). The rare GG genotype (n=1) was identified that was combined with the AG genotype for analysis (Fillingim et al., 2005).

Statistical analysis

All statistical analyses were completed in SPSS statistical software for Mac in (v. 21). Chi-square tests were used to compare genotype frequencies in hedonic groups and gender frequencies by genotype. ANOVAs were run to identify differences in hedonic liking of sweet foods between AA and AG/GG genotypes.

Results and Discussion

OPRM1 genotype and hedonic group

The majority of participants were genotyped as AA (109 of 148). About one-third of participants were genotyped as AG/GG, which is consistent with previous reports (Fillingim et al., 2005). Unlike what was expected, there were no significant differences in the frequency of genotypes in each hedonic group ($\chi^2=1.491$; $p=0.684$) (Table 5-1). There were also no sex differences by genotype ($\chi^2=0.329$; $p=0.566$).

Table 5-1 Genotype (AA or AA/GG) frequency by hedonic group.

Hedonic Group	Genotype		TOTAL
	AA	AG/GG	
Slope +	19	10	29
Slope -	12	5	17
Horizontal Line	32	10	42
Inverted U	46	14	60
TOTAL	109	39	148

OPRM1 A118G SNP and gDOL sweet food liking

There were no significant differences between genotype and liking of sweet foods (figure 5-1). Brownies, cookies, and cheesecake [$F(1,146)=1.38$; $p=0.242$]; Ice Cream [$F(1,146)=1.049$; $p=0.307$]; Chocolate [$F(1,146)=0.115$; $p=0.735$]; Chewy Fruity Candy [$F(1,146)=0.56$; $p=0.813$]; Fresh strawberries [$F(1,146)=0.01$; $p=0.974$]; Regular Coke/Pepsi [$F(1,146)=2.03$; $p=0.157$]; Cotton Candy [$F(1,146)=0.594$; $p=0.442$]; Sweetened Lemonade [$F(1,146)=0.431$; $p=0.512$];

Cigarette Smoke [$F(1,146)=1.649$; $p=0.201$]. There were also no significant differences ($p>0.05$) between genotype and the liking of other gDOL sweet foods (not included).

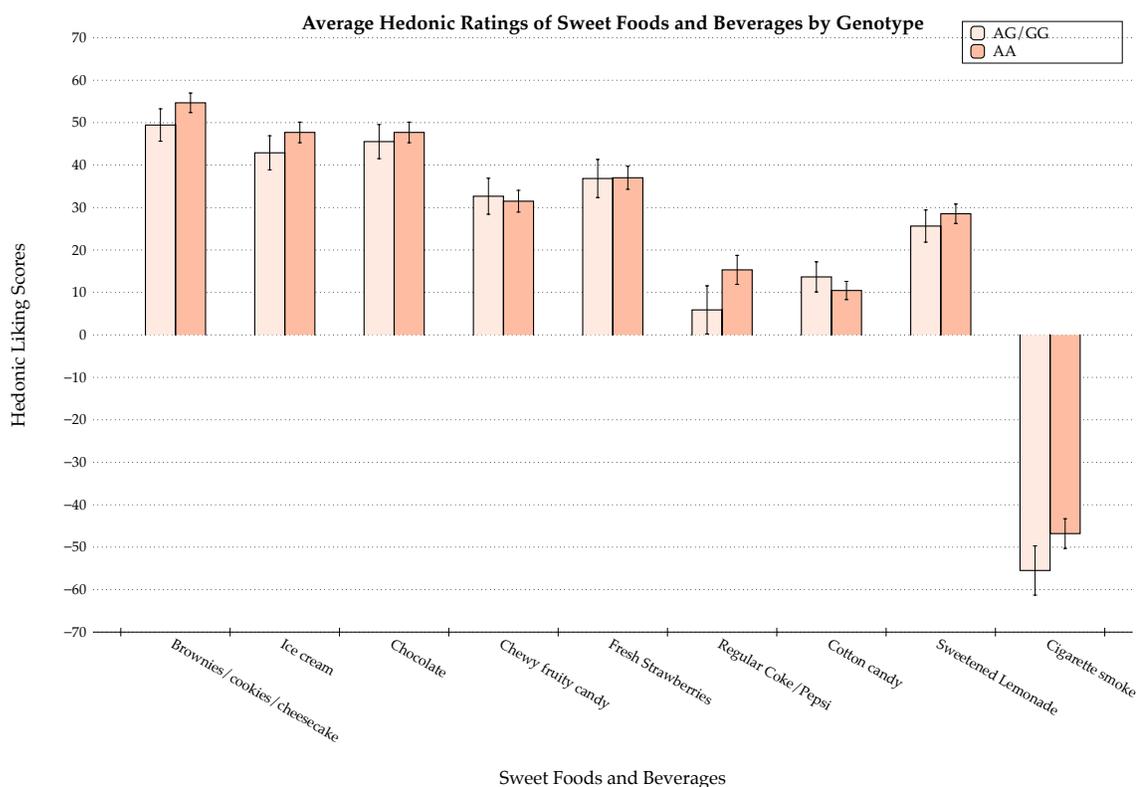


Figure 5-1 Average hedonic ratings of sweet foods and beverages by genotype. There are no significant differences ($p<0.05$) between genotypes for any sweet foods or beverages. Cigarette smoke is included for a hedonically negative non-food related comparison.

Conclusions and limitations

Here we show no evidence of a relationship between sweet liking hedonic groups and the OPRM1 A118G SNP in this pilot study. There were no differences between the AA and AG/GG genotypes in their hedonic ratings of sweet foods. We expected the AG/GG genotypes to have

increased liking of sucrose concentrations and therefore were expected to have a higher frequency in the 'Slope +' and 'Inverted-U' groups. We also expected the AG/GG genotypes to have increased liking of sweet foods and beverages. The OPRM1 A118G SNP is only one of many genetic factors that may affect reward. Also, the sample size (n=148) may not be large enough to see a meaningful effect.

This analysis also ignored other factors such as food restraint, personality factors, and food preferences. In order to see a meaningful effect between genotypes, it may require a participant to eat an actual food product to elicit a response. These data are consistent with (Davis et al., 2011) that found only individuals with the rare GG genotype had a significantly higher preference for sweet foods. The study also and only found a trend for high sugar preferences in individuals with the G-allele. Furthermore, relationships between the OPRM1 A118G SNP and sucrose sweet food hedonics remain to be determined and require further investigation.

Chapter 6

Conclusions and future work

The present findings provide insight into psychophysical and hedonic responses to sweet stimuli. These findings have taken a modern approach to investigating sweet taste perception by using contemporary psychophysical scaling methods. This information can be utilized in future studies that are interested in the individual differences in sweet taste intensity perception and hedonics. Although many of the present findings are null results, they do provide useful information concerning factors that may be contributing the inability to find an effect. The major experimental findings of these studies are:

- 1: Sucrose did not significantly affect cold pain tolerance or threshold times in adult males as hypothesized, even when controlling for methodological variables other studies had not controlled for including: gender, hand temperature and hunger state. Our results are consistent with other studies that suggest that sucrose analgesia is an age dependent phenomenon.
- 2: Sucrose shows a sigmoidal dose-response function, and non-nutritive sweeteners (NNS) elicit a lower maximal sweetness than sucrose and other nutritive sweeteners. A dose-dependent increase in bitterness appears to be responsible for the decrease in NNS sweetness
- 3: Four sucrose hedonic groups were identified from average hedonic responses of sucrose concentrations: 'Slope +', 'Slope -', 'Horizontal Line', and 'Inverted-U'. Some slight differences between the liking of sweet foods and sweet alcoholic beverages were found between four sucrose liking hedonic groups. There were no significant differences in the hedonic ratings of non-sweet alcoholic beverages. In contrast to previous reports,

these results indicate that the hedonic liking of sucrose solutions may translate to the liking of food products.

4: There are no significant differences in OPRM1 A118G SNP genotype frequency between sucrose hedonic groups. There are no significant differences between genotypes in hedonic ratings of sweet foods. Whether the OPRM1 A118G SNP affects sweet taste reward and phenotypic behavior is unknown.

Despite the usefulness of these studies in investigating individual differences in sweet taste intensity perception and hedonic liking, much remains unknown. We were unable to find evidence of sucrose analgesia to exist in adult males. Although this is consistent with other research, there are a few changes that should be refined to further investigate this phenomenon in adults. The method used to induce cold pain, the cold pressor task (CPT), should not be used to induce pain as it introduces a potentially confounding factor as noxious cold can have an analgesic effect. Non-invasive mechanical stimuli (i.e. pressure) should be used to induce pain in adult subjects. Also, there appears to be learning, or practice effects, as pain thresholds increased from the first CPT exposure to later CPT exposures. Therefore, only one CPT should be used to prevent pain threshold inflation across trials.

While many studies have tried to understand the dose-response functions of nutritive and non-nutritive sweeteners, at the receptor level it still remains unknown what mechanism prevents non-nutritive sweeteners from being able to evoke a maximal sweetness response equal to or greater than sucrose or other nutritive sweeteners. Bitterness, an off taste, is clearly responsible for mixture suppression and the decrease in sweetness perception (Lawless, 1979).

There is much promise in translating the hedonic liking of sucrose solutions to the hedonic liking of sweet food products. The usefulness of collecting hedonic information from sucrose solutions are consistently debated. Many argue that individuals do not drink/eat sucrose

solutions, they drink lemonade, or sweetened coffee, or tea. In contrast, our results indicate that the liking of sucrose solutions may generalize into the liking of foods. The generalized degree of liking survey (gDOL) was a useful tool in measuring the general hedonic liking of a variety of food products, however rating remembered foods may be different from actually eating a real food. There has been some question to whether or not the gDOL or other survey-based assessments provide realistic and useful information, although liking surveys have been used in the field and have been found to have validity (Duffy et al., 2009). It would be interesting to do a study validating the gDOL by comparing the hedonic liking of remembered foods and sensations to the hedonic ratings of actual foods and sensations using an unstructured hedonic scale.

The OPRM1 A118G SNP has been correlated with increased reward to highly pleasurable stimuli, however we were unable to find a relationship between this SNP and sweet food liking and sucrose liking. Although the OPRM1 A118G SNP may contribute to the pleasantness of sweet taste, it did not explain the hedonic variation in individual sweet taste here. In adults, environmental and behavioral factors may play a greater role in sweet liking. Similar to sucrose analgesia, it may be worthwhile to investigate the relationship between sweet liking and this polymorphism in children if sucrose analgesia is an age dependent phenomena, perhaps the pleasantness of sweet taste is also age dependent.

Finally, this work provides new information concerning sucrose analgesia in adult men, dose-response functions of nutritive and non-nutritive sweeteners, and the relationship between sucrose liking and sweet food liking. The complex relationship between genetics, intensity and hedonics has yet to be fully understood. These findings contribute to our understanding of individual differences in sweet taste perception and will help to advance knowledge in our field concerning individual differences in food choice and behavior.

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Appendix A: Chapter 3 Supplemental Data

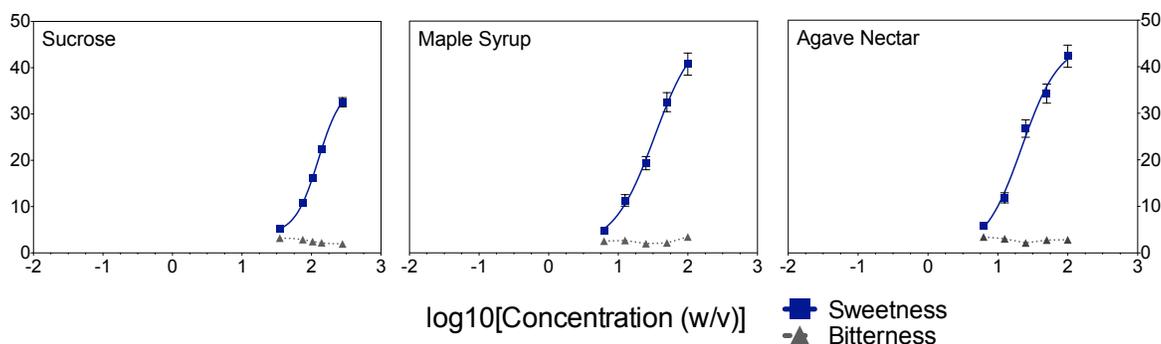


Figure A-1 Dose-Response Functions for Sweetness and Bitterness in three nutritive sweeteners. Solid lines (squares) indicate perceived sweetness intensity; the dotted lines (triangles) indicate perceived bitterness. Numbers of participants per stimulus were: (Sucrose: n=401), (Maple Syrup, n=94), (Agave Nectar, n=94)

Table A-1 Dose-Response Functions for Sucrose, Agave Nectar, Maple Syrup, Aspartame, Reb A, Sucralose, and Ace K

Sweetener	Equation Type	D-R equation
Sucrose	Hill	$R = \frac{36.3}{(1 + 10^{((2.096-C) \times 2.528)})}$
Agave Nectar	Hill	$R = \frac{45.2}{(1 + 10^{((1.343-C) \times 1.624)})}$
Maple Syrup	Hill	$R = \frac{48.7}{(1 + 10^{((1.522-C) \times 1.475)})}$
Aspartame	Hill	$R = \frac{31.8}{(1 + 10^{((-0.1917-C) \times 1.247)})}$
Reb A	Hill	$R = \frac{21.5}{(1 + 10^{((-0.6625-C) \times 1.420)})}$
Sucralose	Hill	$R = \frac{23.89}{(1 + 10^{((-0.1753-C) \times 4.561)})}$
Ace K	Second order polynomial	$R = -17.64 + 44.1(C) + -17.13(C)^2$

Where C= (Concentration (log g/L))

(Hill Equation): $R = \text{Bottom} + (\text{Top}-\text{Bottom}) / (1 + 10^{((\text{LogEC50}-X) * \text{HillSlope})})$

Second Order Polynomial Equation: $R = B_0 + B_1(C) + B_2(C)^2$

Table A-2 Sweetener Concentrations used in Experiments 1-4

Non-Nutritive Sweetener	Concentration (mM)	Experiment
Aspartame	0.23, 0.70, 1.0, 1.83, 1.35, 6.79*, 9.0*	1 & 4
Rebaudioside A	0.04, 0.25, 0.52, 1.03, 1.55	1
Acesulfame K	1.57, 6.26, 24.90, 99.15, 394.71	2
Sucralose	0.20, 0.80, 3.17, 12.6, 50.18	2

Nutritive Sweetener	Concentration (g/L)	Experiment
Sucrose	37.5, 75, 104, 140, 280	1 - 4
Maple Syrup	6.25, 12.5, 25.0, 50.0, 100	3
Agave Nectar (Light)	6.25, 12.5, 25.0, 50.0, 100	3

*Additional concentrations tested in experiment 4 that were not tested in experiment 1.

Table A-3 Absolute Mean Intensity Ratings of Sucrose, Agave Nectar, and Maple Syrup

Sucrose					
Concentration g/L (mM) (n=401)					
	37.5(109.5)	75(219.1)	104(303.8)	140(409.0)	280(818.0)
<i>Mean Intensity Ratings</i>					
Sweetness	5.20	10.85	16.25	22.43	32.56
Bitterness	3.20	2.88	2.46	2.20	1.95
Metallic	2.83	2.28	2.19	1.96	1.40

Agave Nectar					
Concentration (g/L) (n=94)					
	6.25	12.5	25.0	50.0	100
<i>Mean Intensity Ratings</i>					
Sweetness	5.93	11.8	26.7	34.2	42.3
Bitterness	3.45	3.06	2.15	2.77	2.83
Metallic	1.62	2.28	1.51	1.16	2.02

Maple Syrup					
Concentration (g/L) (n=94)					
	6.25	12.5	25.0	50.0	100
<i>Mean Intensity Ratings</i>					
Sweetness	4.87	11.3	19.4	32.5	40.7
Bitterness	2.54	2.68	2.00	2.15	3.44
Metallic	2.77	1.61	2.60	1.76	2.77

Table A-4 Absolute Mean Intensity Ratings of Aspartame, RebA, Acesulfame K, Sucralose

Aspartame							
Concentration (mM) (n=216) (n=102)*							
	0.23	0.70	1.00	1.83	3.35	6.80*	9.04*
<i>Mean Intensity Ratings</i>							
Sweetness	2.37	6.60	9.38	14.0	20.7	24.7*	28.2*
Bitterness	3.69	3.39	3.66	3.24	3.08	3.02*	2.43*
Metallic	3.48	3.07	2.56	2.33	2.41	1.97*	2.06*

RebA					
Concentration (mM) (n=114)					
	0.04	0.25	0.52	1.03	1.55
<i>Mean Intensity Ratings</i>					
Sweetness	2.91	12.3	16.2	20.3	19.7
Bitterness	4.54	4.80	7.35	12.7	18.9
Metallic	5.81	3.96	5.08	2.00	7.57

AceK					
Concentration (mM) (n=91)					
	1.57	6.26	24.90	99.15	394.71
<i>Mean Intensity Ratings</i>					
Sweetness	4.85	8.93	11.8	8.03	4.64
Bitterness	3.49	8.07	14.2	27.9	42.8
Metallic	3.42	4.96	7.45	13.36	17.01

Sucralose					
Concentration (mM) (n=91)					
	0.20	0.80	3.17	12.60	50.18
<i>Mean Intensity Ratings</i>					
Sweetness	7.92	14.6	22.6	28.5	19.6
Bitterness	3.95	2.45	2.77	11.56	27.93
Metallic	2.82	1.55	2.71	5.56	15.55

Appendix B: Chapter 4 Supplemental Data

Figure B-1: Sweet Hedonic Liking and Intensity Curve Algorithm

1. Average the hedonic ratings
2. Fit linear line through hedonic ratings
3. Fit quadratic curve through hedonic ratings
4. Compare linear vs. quadratic fit
5. If quadratic curve is better fit (accept hypothesis) then insert quadratic model with a negative term
6. If hypothesis is rejected then fit data with linear model and define as :
 - a. Slope +
 - b. Slope –
 - c. Horizontal Line

R-Statistical Software Algorithm

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n : number of judge
 ca : number of samples
 l : number of rows
 k : column for the concentration variable
 m : column for the liking rate variable
 n : column for the intensity rate variable

```
library(pracma)
```

```
j <- 153
ca <- 7
l <- j * ca
d <- 7
k <- 3
m <- 4
n <- 5
i <- 1
```

```
optvalues <- numeric() # - create an empty vector to stock the optimum concentration values
(optimum$max)
curvetype <- character()
AUCvalues <- numeric()
slopevalues <- numeric ()
slopehedonic <- numeric()
```

```

while( i<l ) {
  cat(">",i, " ",d,"\n")
  judgenb=(i-1)/ca+1
  titlestr <- paste("participant n°", encodeString(judgenb), sep="") # - use of paste function to
  concatenate text parts
  filename <- paste("participant", encodeString(judgenb), ".pdf", sep="") # - use of encodeString
  function to turn a number into text
  outputname<- paste("output_judge",encodeString(judgenb),sep="")
  x <- sweetliking [ c(i:d),k ]
  y1 <- sweetliking [ c(i:d),m ]
  y2 <- sweetliking [ c(i:d),n ]
  fit <- lm(y1~log(x))
  fit2 <- lm(y1~poly(log(x),2,raw=TRUE))
  fitintens <- lm(y2~log(x))
  fittingfunction<-function(x) {(coef(fit2)[3])*log(x)*log(x)
  +(coef(fit2)[2])*log(x)+(coef(fit2)[1])}
  optimum<-optimize(fittingfunction,interval=c(6.5, 250), maximum=TRUE)
  anova(fit,fit2)
  prtanova <- anova(fit,fit2)
  #str(prtanova)
  pvalue<-prtanova$'Pr(>F)'
  xx <- seq(0,250, length=50)
  xlog<-c(5,10,20,50,100)
  u <- 1:250
  quartz() # Create a new plot at each loop turn
  par(mar=c(5,4,4,6)+0.1)
  plot(x,y1,log="x",pch=4,ylim=c(-
  100,100),xlab="",ylab="",main=titlestr,axes=FALSE,col="chartreuse4")
  axis(2,ylim=c(-100,100),col="blue")
  mtext("Hedonic scores",side=2,line=2.5)
  box()

  if ( is.na(pvalue[1]) & coef(fit2)[3] < -3.158 & 7.07 < optimum$maximum &
  optimum$maximum < 90) {
    lines(xx, predict(fit2, data.frame(x=xx)), col="chartreuse3")
    points(optimum$maximum,optimum$objective,pch=19,col="firebrick1")
    optmax<-optimum$maximum
    capture.output(summary(fit2),file=outputname)
    par(new=TRUE)
    plot(x,y2,log="x",pch=10,ylim=c(-
    100,100),xlab="",ylab="",main=titlestr,axes=FALSE,col="brown4")
    axis(4,ylim=c(-100,100),col="red")
    mtext("Sweetness scores",side=4,line=2.5)
    axis(1,at=xlog,labels=round(log10(xlog),2),cex.axis=0.6)
    mtext("log([sucrose])",side=1,line=2.5)
    lines(xx,predict(fitintens,data.frame(x=xx)),col="brown3")
    dev.print(pdf,file=filename,width=7,height=7,pointsize=12)
    slope <- coef(fitintens)[2]
    fittingfunctionintens <- function(x) {(coef(fitintens)[2])*x+(coef(fitintens)[1])}
  }
}

```

```

v<- fittingfunctionintens(u)
AUC <- trapz(u,v)
slopehed <- coef(fit)[2]
  fittype <- c("inverted U")
    #if (optimum$maximum < 18.33) {
      #fittype <- c("inverted U - less liker")
    #} else {
      #fittype <- c("inverted U - more liker")
    #}

} else {
  lines(xx, predict(fit, data.frame(x=xx)), col="chartreuse3")
  par(new=TRUE)
  plot(x,y2,log="x",pch=10,ylim=c(-
100,100),xlab="",ylab="",main=titlestr,axes=FALSE,col="brown4")
  axis(4,ylim=c(-100,100),col="red")
  mtext("Sweetness scores",side=4,line=2.5)
  axis(1,at=xlog,labels=round(log10(xlog),2),cex.axis=0.6)
  mtext("log([sucrose])",side=1,line=2.5)
  lines(xx,predict(fitintens,data.frame(x=xx)),col="brown3")
  fittingfunction<-function(x) {(coef(fit)[2])*x+(coef(fit)[1])}
  slope <- coef(fitintens)[2]
  fittingfunctionintens <- function(x) {(coef(fitintens)[2])*x+(coef(fitintens)[1])}
  v <- fittingfunctionintens(u)
  AUC <- trapz(u,v)
  slopehed <- coef(fit)[2]

  if (coef(fit)[2]>7.316) {
    optmax <- 250
    #points(250,coef(fit)[2]*250+coef(fit)[1],pch=19,col="firebrick1")
    fittype <- c("slope +")
  } else if(coef(fit)[2] < -4.147) {
    optmax<-6.5
    #points(6.5,coef(fit)[2]*3+coef(fit)[1],pch=19,color="firebrick1")
    fittype <- c("slope -")
  } else {
    optmax<-0
    fittype <- c("horizontal line")
  }

}
capture.output(summary(fit),file=outputname)
#dev.print(pdf,file=filename,width=7,height=7,pointsize=12)
}
i <- i + ca
d <- d + ca
optvalues <- append(optvalues,optmax) #Put the optimum concentration value into the vector.
curvetype <- append(curvetype,fittype)
AUCvalues <- append(AUCvalues,AUC)
slopevalues <- append(slopevalues,slope)

```

```
slopehedonic <- append(slopehedonic,slopedhed)
dev.off() #close the quartz device (R can't keep open more than 60 quartz devices)
}

write.table(optvalues,file="/Users/victor/Desktop/optimum
points.txt",sep="\t",row.names=TRUE)
write.table(curvetype,file="/Users/victor/Desktop/curve type.txt",sep="\t",row.names=TRUE)
write.table(AUCvalues,file="/Users/victor/Desktop/AUC values.txt",sep="\t",row.names=TRUE)
write.table(slopevalues,file="/Users/victor/Desktop/slope values.txt",sep="\t",row.names=TRUE)
write.table(slopehedonic,file="/Users/victor/Desktop/slope
hedonic .txt",sep="\t",row.names=TRUE)
```