Lingual tactile sensitivity: Effect of age, gender, fungiform papillae density, and temperature.

THESIS

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

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2015

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Abstract

The concept of texture greatly contributes to food liking, however it is also one of the least understood components. Textural attributes are multifaceted; and perception of these diverse components can vary from person to person. As a result of this intricate nature, there is no internationally accepted classification scheme for textural attributes. Because of this, the tongue (specifically tactile properties) may be the key component in why textural attributes are discriminated, liked, or preferred.

Despite the amount of research performed on the mechanosensory properties of the skin, lingual tactile sensitivity is understudied. In order to assess one component of tactile sensitivity (edge and point detection), a modified letter identification task was implemented to measure shape and form recognition.

The objective of this study was to identify how threshold and suprathreshold tactile sensitivity varied within the population using this modified letter identification task, and to create a novel device to modulate stimulus temperature in order to investigate how temperature may play a role in influencing lingual tactile sensitivity. In this study, we sought to investigate proximate sources of variation. In particular, we hypothesized that tactile sensitivity of the tongue varied by four parameters: age, gender, fungiform papillae, and temperature.
This study was conducted into two parts. The initial experiment tested 48 panelists using a modified up-down staircase letter identification task to observe lingual tactile thresholds. Suprathreshold sensitivity was measured by having those same panelists estimate letter size using magnitude estimation. The area under the psychophysical curve (AUC) from each panelist was calculated and used as a singular dependent variable for suprathreshold sensitivity. Each panelist’s tongue was painted with blue dye (0.1% w/v) and fungiform papillae, which remained unstained, were counted within a 31.7 mm² area. Statistical analysis for threshold and suprathreshold sensitivity was assessed using ANOVA with age, gender, and fungiform papillae density as the main effects.

The second part of the study utilized a self-built thermode device to modulate stimulus temperature and test lingual tactile thresholds under conditions of cold (8-10°C) and warm (42-45°C). Fifty panelists assessed letters under two thermal conditions using a modified up-down staircase letter identification task. In addition, panelist’s fungiform papillae were counted in the same fashion as the initial study. Differences in tactile sensitivity were assessed using multiway ANOVA with age, gender, fungiform papillae density, and temperature as the main effects.

Results from the initial study showed that lingual tactile thresholds were impacted by age and fungiform papillae density, but not by gender. Suprathreshold analysis indicated that magnitude estimates of letter size were dependent on fungiform papillae density only. It was also shown that no correlation existed between threshold and suprathreshold lingual tactile sensitivity.
The second study showed that tactile thresholds were dependent on age and gender, but not fungiform papillae density or temperature. The effect of age on tactile thresholds was in line with results obtained in Experiment 1, however the impact of gender and fungiform papillae density was inconsistent, suggesting that a new population, the style of letter assessment, or potential letter difficulty may have influenced the result. From these results, it is evident that age plays a strong role in threshold tactile sensitivity, however the effect of gender and fungiform papillae density is less robust and should be further investigated. Further research should involve controlling these variables and link sensitivity to perception of various textural attributes.
Acknowledgements

First and foremost, thank you to my advisor, Chris Simons, for accepting me as his first student to his lab. We have learned a lot together, and I could not have asked for a better advisor to work for. Thanks to my committee members, Dr. John Litchfield and Dr. Dennis Heldman, for their support and knowledge as I traversed the realm of graduate school.

Many thanks to my sensory lab members: Alex Pierce, Gretchen Guttman, Blansh Del-Portal, Rebecca Liu, Brianne Linne, Drew Hathaway, and Melody Leidheiser. In addition, my office colleagues in Howlett 048, for all the laughs and keeping my sanity at times when I really needed it.

Thank you to the undergrads who made my life a lot easier when it came down to executing tasks: Rob Kaufman, Michael Arato, Yun Chen, and Liz Green. Your assistance was integral ensuring that the tests ran smoothly. You guys have a great future ahead of you and I truly appreciate all of your hard work.

Last but not least, my family for having to put up with me, but nevertheless backing me up 100%. Thank you for always finding a way to put a smile on my face when I needed it. Just know that without you guys, none of this would have been possible. I hope I made you all proud.
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Chapter 1: Literature review

1.1 Texture

Texture plays a meaningful role in why we enjoy foods and beverages. Alongside appearance, flavor, and nutrition, food texture is considered a principal quality of food as it is, “the response of tactile senses to physical stimuli that result from contact between some part of the body and the food” (Bourne 2002). In addition to touch sensations, other sensory systems such as sight (by observing rheological properties) or hearing (ie. association with crunchy, crisp textures during mastication) also aid in the assessment of food texture as the overall physical properties of a food product are perceived (Izutsu and Wani 1984). Firmness and crispness in fruits and vegetables convey freshness as opposed to those that are soft or mushy which may show signs of spoilage (Menella & Lukasewycz 2012; Szczesniak & Khan 1971). In addition, characteristics of a food’s structure and its perceived texture are important attributes in the sensory availability of flavor compounds that volatilize during eating (Lubbers and Butler 2010).

Despite the contributions that texture plays in food reward and intake, it is also one of the least understood components. Several reasons account for this. One of the reasons is that the majority of research is conducted classifying specific textural attributes in a specific product (Szczesniak 1962) and therefore does not give the overall scope of this complex quality. Another is that a number of terms are used to describe the same
characteristic (e.g. gritty or grainy) or, alternatively, one term may be used to describe multiple characteristics which could be very confusing (i.e. watery could describe juiciness or wet; Szczesniak 1962; Jowitt 1974). Due to the complexity of this area of study, there has not been a globally accepted classification scheme to define texture in relation to food products, although there have been suggested versions. One suggested version can be defined as:

“The sensory and functional manifestation of the structural, mechanical and surface properties of foods detected through the senses of vision, hearing, touch, and kinesthetics. Texture is derived from the structure of the food (molecular, microscopic, or macroscopic) and is detected by several senses, the most important ones being the senses of touch and pressure” (Szczesniak 2002).

Therefore, texture in itself is a sensory property as only humans are capable of describing textural attributes; it encompasses a variety of textural components; it is based on food structure; and multiple senses are responsible for detection and perception (Szczesniak 2002).

Varieties of definitions regarding texture can be segmented into two groups: one being a “commodity-oriented” group which is focused on a particular attribute of a specific product and the other being a more comprehensive approach to all foods (Bourne 2002). Despite not having a clear definition for food texture, there have been attempts to classify them. Szczesniak (1962) developed a classification of textural characteristics
based on three separable characteristics: mechanical, geometrical, and other (table 1.1) which is still used today. These broad characteristics are further subdivided into primary and secondary parameters that are then described by a common, widely used terminology.

Table 1.1 Textual (mechanical, geometrical, and other) characteristics displaying primary and secondary parameters accompanied by common terminology. (Adapted from Szczesniak 1962)

<table>
<thead>
<tr>
<th>Mechanical Characteristics</th>
<th>Primary Parameters</th>
<th>Secondary Parameters</th>
<th>Popular Terms</th>
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<tbody>
<tr>
<td>Hardness</td>
<td></td>
<td>Soft → Firm → Hard</td>
<td></td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>Brittleness</td>
<td>Brittle → Crunchy → Bristle</td>
<td></td>
</tr>
<tr>
<td>Chewiness</td>
<td>Tender → Chewy → Tough</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gumminess</td>
<td>Short → Mealy → Pasty → Gummy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viscosity</td>
<td></td>
<td>Thin → Viscous</td>
<td></td>
</tr>
<tr>
<td>Elasticity</td>
<td>Plastic → Elastic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adhesiveness</td>
<td>Sticky → Tacky → Gooey</td>
<td></td>
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<table>
<thead>
<tr>
<th>Geometrical Characteristics</th>
<th>Class</th>
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<tr>
<td>Particle size and shape</td>
<td></td>
<td>Gritty, Grainy, Coarse</td>
</tr>
<tr>
<td>Particle shape and orientation</td>
<td></td>
<td>Fibrous, Cellular, Crystalline</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other Characteristics</th>
<th>Primary Parameters</th>
<th>Secondary Parameters</th>
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</tr>
</thead>
<tbody>
<tr>
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<td>Dry → Moist → Wet → Watery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat Content</td>
<td>Oillness</td>
<td>Oily</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Greasiness</td>
<td>Greasy</td>
<td></td>
</tr>
</tbody>
</table>

The importance of texture to food perception and acceptance has been nicely demonstrated in a number of research studies. In a recent investigation, panelists were asked to identify foods solely on the flavor (Wilkinson et al. 2000). Visual cues were eliminated as panelists were blindfolded while textural cues were eliminated by pureeing samples. The main finding of the study was that people were poor in identifying specific foods based on flavor, showing that texture has an integral role in our classification of
foods. In relation to the quality of foods, culture and upbringing is also important in our assessment of texture as people of different ethnicities like specific foods based on personal experiences (Szczesniak and Khan 1971; Menella and Lukasewycz 2012). Evidence of the importance of texture is rich in the field of dentistry as well. Subjects with poor dental health and improper dental care often complain of diets consisting of singular textures and missing the specific textural sensations that occur during mastication (Bourne 2002).

1.2 Tactile sensitivity

Tactile sensitivity relates to the sensation of touch as it is the response of what we feel on the surface of the skin. Its function is due to the multitude of afferent fibers that innervate the glabrous skin and are specific for sub-modalities of touch (e.g. form and texture perception, grip, pressure, stretch, roughness, vibration, friction, temperature; Johnson et al. 2000; Miodownik et al. 2014). It is considered one aspect of a feedback system known as “haptics” with the other aspect being related to kinesthetic perception (Lederman and Klatzky 2009). Together, this system is practical in handling the material characteristics of surfaces and objects (Lederman and Klatzky 2009).

1.2.1 Tactile sensitivity of the skin

The skin can be classified into two categories, glabrous and hairy. Glabrous skin is found on the surfaces of the hands and feet and the hairy skin encompasses the rest of the body. There are four types of mechano-receptive fibers in the glabrous skin which
include the Meissner corpuscle (RA), Merkel cell (SAI), Pacinian corpuscle (PC), and Ruffini ending (SAII), each having very specific characteristics. Meissner corpuscles and Merkel cells react to touch sensations, however the Meissner corpuscles are more involved with grip control while Merkel cells contribute to form and texture perception. Pacinian corpuscles react to vibrational cues, and the Ruffini corpuscles react to stretching of the skin surface (Johnson et. al. 2000).

Mechanoreceptors are located all throughout the skin, however the vast amount of research has been done on the fibers in the glabrous skin of the hand (Lederman and Klatzky 2009, Jones and Lederman 2006), which respond to various types of stimuli (ie. probes, hairs, metal tips, roughness patterns, or wisps of cotton; Lederman and Klatzky 2009).

Interest in tactile sensitivity of the skin for a particular portion of the body can be attributed to Ernst Weber (1834; translated by Ross and Murray 1996), who observed discrimination thresholds using a two point discrimination task. This task involves two probes spaced a certain distance apart and identifies the minimum distance between them that a subject correctly identifies as two points versus a single point. This research identified that the tongue was the most sensitive, while the lips, fingers, toes, and forehead followed. This research provided the foundation to a variety of test assessments in further identifying tactile sensitivity of the skin. Such measures include sensitivity to pressure, point localization, vibration sensitivity, roughness discrimination, shape and form recognition, and spatial resolution (Myles and Binseel 2007).
1.3 Thermal sensitivity

Temperature, besides being an indicator of the degree of cooking, greatly influences our perception of food. Most foods have preferred intake temperatures and are generally more acceptable when served at a temperature range in which that food is normally consumed (Cardello and Maller 1982). Some classic examples would be ice cream, which is best when cold, or coffee which is commonly consumed warm. Serving temperature also has a great influence on our perception of various sensory attributes of different foods (Drake et al. 2005; Engelen et al. 2003; Seo et al. 2013). For example, an increase in temperature was shown to affect the overall odor (Kähkönen et al. 1995) and sour taste intensity (Drake et al. 2005) of cheese products.

There are multiple dimensions related to temperature perception including warm and cold receptors (Green 1986), liking (Ryynänen et al. 2001) taste (Green and Cruz 2000) or age (Stevens and Choo 1998). Warm and cold receptors in particular, affect the perception of skin temperature, however mechanoreceptor and multimodal fibers can also react to thermal stimuli (Engelen et al. 2002).

Warm and cold receptors are innervated by unmyelinated (C) and small-diameter myelinated (A-δ) neurons in the afferent fibers of the dorsal root ganglia, mediating warm and cold sensations respectively (Kandel et al. 2000). Transient receptor potential (TRP) channels are expressed within these afferent fibers and in keratinocytes reacting to broad temperature ranges. The family vanilloid (TRPV) contain six members where TRPV1-4 are heat-activated (Nilius et al. 2007). TRPV1 and TRPV2 activate at noxious temperatures (≥43°C and >53°C respectively) while TRPV3 and TRPV4 activate at non-
noxious temperatures (23-29°C and >24°C respectively; Nilius et al. 2007). In cold temperatures, the ankyrin family (TRPA), TRPA1 reacts to noxious cold temperatures (reported to activate when temperatures fall below 17°C; Patapoutian et al. 2003). In the melastatin family (TRPM), TRPM8 reacts to non-noxious cold temperatures ranging from 8-28°C (Nilius et al. 2007). In addition to activating at this broad range of temperatures, these channels also react to chemical stimuli. For instance, TRPV1 is activated by capsaicin or other vanilloid compounds such as piperine (Nilius et al. 2007; Julius et al. 2006), TRPV3 with camphor (Ramsey et al. 2006), TRPA1 with cinnamaldehyde and isothiocyanates like allicin (from garlic) or allyl isothiocyanate (from mustard oil; Patapoutian 2004), and TRPM8 with menthol (Nilius et al. 2007; Ramsey et al. 2006).

1.4 The tongue

The tongue, besides being the organ commonly associated with the sense of taste, is a very versatile yet complex organ that contains a variety of functions, some of which include aiding in mastication, speech, and respiration (Hiiemae and Palmer 2003). Due to its complexity, there is still much unknown regarding this organ especially with regard to the specialized tongue movements that occurs during specific actions like swallowing or talking (Mu and Sanders 2013).

The tongue can be segmented into two separate regions, the anterior two-thirds and the posterior one-third. Cranial nerves (CN) are responsible for the innervation of the tongue in these regions. Facial (CN VII, chorda typani branch) and the trigeminal
(CN V, lingual nerve) nerves convey sensations of taste and somatosensation (tactile, thermal and pain) respectively in the anterior two-thirds of the tongue. In the posterior third of the tongue, taste and somatosensory sensations are carried by the glossopharyngeal (CN IX) nerve. The hypoglossal nerve (CN XII) innervates the muscles of the tongue to provide fine motor control.

The dorsal surface, or top of the tongue, contains a variety of projections known as papillae. The human tongue contains four different types of papillae (fungiform, filiform, circumvallate, and foliate). These papillae are in general areas of the tongue and not confined to a single, well-defined anatomical location (Arvidson 1979). These papillae, with the exception of the filiform papillae, contain taste buds. Housed within those taste buds are roughly 50-150 taste receptor cells which are involved in the detection of general taste sensations: sweet, sour, salty, bitter, and umami. Filiform papillae, on the other hand, aid in food consumption as they assist in bolus formation and swallowing.

1.4.1 Fungiform papillae

Fungiform papillae (figure 1.1) reside on the dorsal surface of tongue with the majority of the papillae being located on the tongue tip (Miller 1986). More specifically, 87% of the fungiform papillae are located in the anterior 2 cm tip by staining the tongue surface with either an acidic Ponceau S red or basic Alcan blue dye (Cheng and Robinson 1991). These papillae are commonly mushroom shaped, but can also be flat, cylindrical, or conical (Laing et al. 2002).
Figure 1.1 Section of a fungiform papilla with a lone taste bud on the top of the papillae (boxed area). Picture credited to Arvidson and Friberg (1980).

The fungiform papillae are innervated by two cranial nerves: the chorda typani fibers of the facial (CN VII) nerve and the trigeminal fibers of the lingual nerve (CN V3) (Whitehead and Kachele 1994). The chorda typani fibers form synapses with taste receptor cells housed within the taste bud while the trigeminal fibers heavily surround the taste bud within the papillae but do not form synaptic connections with other cell types (Zahm and Munger 1983, Whitehead et al. 1985). The chorda typani fibers are known to respond to taste and general sensory stimulation while the trigeminal fibers react to thermal, tactile, and irritant stimuli (Whitehead and Kachele 1994). Moreover, the trigeminal fibers innervate mechanoreceptors, which is integral in texture perception (Bakke and Vickers 2008).

Each fungiform papillae is described as having “a single pore that serves as a surface applicable for the reception of tastants, transduction of chemical to electrical
signals, and activation of taste cells” (Laing et al. 2002), which indicates that existing pores on the papillae will provide access to functional taste cells (Miller 1986). This suggests that the number of pores in a distinct area of the tongue contributes to taste sensitivity in that region (Zuniga et al. 1993). Also with the majority of papillae being located on the tongue tip, it has been shown that this area was the most highly innervated and contained the most ample somatosensory innervation of all areas of the tongue (Marlow et al. 1965). Therefore, the fungiform papillae serve as a marker for the density of mechanosensory innervation.

1.4.2 Letter identification task

The letter identification task was developed to test spatial acuity of the tongue. It focuses on edge and point sensitivity with those being represented as letters of the alphabet. Other methods that are used for tactile acuity (e.g. grating orientation or two-point discrimination) may cause issues due to the nonspatial cues which may elicit subject responses (Essick et al. 1999). In addition, tongue reflexes cannot be easily suppressed, which also may confound results (Essick et al. 1999; Menella and Lukasewycz 2012).

The idea of the letter identification task is complex, albeit having a simple method. An embossed letter on a Teflon strip is administered to a panelist. Panelists are instructed to assess the stimuli given to them only using the anterior tip of the tongue (figure 1.2).
Figure 1.2 Sketch of how the letter identification task would be performed. Letter stimuli would be presented face down and using the anterior tip of the tongue, subjects would respond on what letter was presented to them. Picture credited to Essick et al. (1999).

In most cases, the presented letter will not be clearly identifiable due to the difficulty of the task. Therefore, a best guess is given from assessment of the edges, points, and shape that was presented to them.

The letter identification task has been used in several studies. This task found that lingual tactile sensitivity covaries with sensitivity to the bitter tastant 6-n-propylthiouracil (PROP) among female subjects and showed that lingual tactile acuity was correlated with those that were bitter sensitive (Essick et al. 2003). However, there was no association of lingual tactile sensitivity and food texture preferences among mothers and their children as the likelihood of preference stems from personal experiences or cultural upbringing (Mennella and Lukasewycz 2012). Tongue strength also does not factor in lingual tactile
acuity as those with poorer tongue strength were able to identify letters to the same capacity to those with stronger tongues (Steele et al. 2014).
2.1 Abstract

Despite contributing to food perception, lingual tactile sensitivity has been understudied. We hypothesized that sensitivity to threshold and suprathreshold tactile stimuli varies in the population and sought to determine proximate sources of variability. Forty-eight adults were tested for threshold sensitivity via a modified letter identification task and magnitude estimation scaling for testing suprathreshold sensitivity. In addition, fungiform papillae density of each panelist was determined.

Lingual tactile thresholds were significantly impacted by age as subjects 40 years or older had higher thresholds (6.25 ± 0.711 mm) than those in their 20’s (3.92 ± 0.208 mm). Moreover, threshold sensitivity increased with increasing fungiform papillae count. Suprathreshold sensitivity was not affected by age, gender, or fungiform papillae count. Finally, as observed for other sensory modalities, there was no correlation among threshold and suprathreshold lingual tactile sensitivity.

Relationships between threshold sensitivity and age and fungiform papillae density were evident, although other variables such as temperature and contact pressure should be considered and taken into further investigation.
2.2 Introduction

Texture plays a major role on why we enjoy and appreciate foods, however it is a complex facet in food reward and intake. What is generally accepted in food texture is that we use it to gauge the food quality. Texture is important for determining food as either fresh, overripe, or spoiled (Menella and Lukasewycz 2012). Although being a general concept, there is little understanding on how specific food textures are perceived, liked, or discriminated. It stems from the amount of detail that one describes in a textural attribute, where multiple attributes describe the same characteristic, or alternatively, where a single attribute is used to describe multiple sensations (Szczesniak 1962). The use of terms in this way can lead to confusion and misunderstanding of textural concepts (Szczesniak 1963; Jowitt 1974).

The sense of touch brings important information regarding the external environment (Bourne 2002) and brings forth interest on how the skin relates to texture perception. Traditional research on texture perception has been done via assessing various types of stimuli with a single finger (Roberts and Humphreys 2009). In addition, with mechanoreceptors located all throughout the cutaneous surface of the body, multiple tests have been conducted comparing tactile sensitivity of different areas of the skin. Such tests include pressure (via punctate stimuli in the form of Von Frey hairs or Semmes-Weinstein monofilaments), roughness discrimination (using sandpaper of different grit), oral stereognosis (via shape and form recognition), and spatial resolution (via two-point discrimination; Boliek et al. 2007; Verrillo et al. 1999).
In the seminal study of cutaneous tactile discrimination, Ernst Weber (1834; translated by Ross and Murray 1996) investigated two-point discrimination thresholds on various areas of the body. It was found that the most touch sensitive part of the body was the tongue, followed by the lips, fingers, and the toes. Interestingly, despite being the most sensitive region, most research has focused on the mechanosensory properties of the skin, leaving tactile sensitivity of the tongue understudied.

A letter identification task was developed to gain further insight on lingual tactile acuity. This method was developed by Greg Essick and his team (1999) to combat limitations associated with the two point discrimination task (ie. subject’s ability to exploit non-spatial cues due to probe or oral surface movements; Goldreich et al. 2013; van Boven and Johnson 1994; Menella and Lukasewycz 2012; Craig and Johnson 2000).

The letter identification task focuses on edge and point sensitivity with these elements depicted in shapes being represented as capitalized letters of varying sizes. Due to having to assess and recognize different letters of varying sizes, this method may also assess facets of oral stereognosis (Boliek et al. 2007; Menella and Lukasewycz 2012). Results from this task showed that lingual tactile sensitivity covaries with sensitivity to the bitter tastant 6-n-propylthiouracil (PROP) among female subjects (Essick et al. 2003), but not with food texture preferences among mothers and their children (Mennella and Lukasewycz 2012) or by tongue strength (Steele et al. 2014).

In order to gain insight on lingual tactile sensitivity, the letter identification task was adopted in our investigation. The aim of this experiment was to observe how lingual tactile sensitivity varied within the population and seek the proximate sources of
variation. We hypothesized that lingual tactile sensitivity was dependent upon age, gender, and mechanosensory innervation. The degree of mechanosensory innervation was estimated by measuring fungiform papillae density; these papillae are of interest due to the network of trigeminal fibers that surround the taste buds within. Since taste performance is dependent on taste bud density (Zuniga et al. 1993), we hypothesize that lingual tactile sensitivity may also depend on papillae density and thus, relationships between anatomy and perception were considered. In addition to observing differences in populations, age and gender were also considered to validate previous research suggesting tactile thresholds increases with age (Thornbury and Mistretta 1981; Woodward 1993), but has not influenced by gender (Woodward 1993; Dahan et al. 2000).

2.3 Methodology

2.3.1 Letter stimuli

Stimuli consisted of 3D printed thermoplastic Polycarbonate Acrylonitrile Butadiene Styrene (PC-ABS) tiles. Dimensions of the tiles were 1 cm x 1 cm x 2 mm. Letters were raised from the center of the tile 0.8 mm (figure 2.1).
Letter sizes were 1.5, 2, 3, 4, 5, 6, 7, and 8 mm which corresponded to font sizes 6, 8, 12, 18, 21, 28, 30, and 34. Letters used (A, I, J, L, O, T, U, W) were all capitalized. The same letters were used as in prior research due to being easily recognizable by the fingertips (Essick et al. 1999). All letters with the exception of “I” were in Arial typeface. To ensure clarity of the capitalized letter (having a clear top and bottom horizontal line), the letter “I” was in Times New Roman typeface. Schematics created for each letter stimulus were made via Sketchup (Trimble Navigation Limited, Sunnyvale, CA), and converted to “.stl” format to be made applicable for 3D printing. Each tile was adhered to a sterile tongue depressor using double stick tape and organized based on letter and size before presenting to each panelist.

2.3.2 Panelists

48 panelists (24 males and 24 females) were recruited from The Ohio State University and surrounding Columbus areas with ages ranging from 18 to 59 years. Panelists (if applicable) were asked to refrain from smoking at least two hours.

Figure 2.1 Computer schematic of an eight millimeter letter tile "A"
prior to their scheduled session. The data and information collected for this study was approved by the local Institutional Review Board under written informed consent of the panelist. Upon completion of the session, a $10 gift card was given to each panelist.

2.3.3 Threshold experimentation

All letter stimuli with the exception of the “O” stimuli were used for this portion of the experiment. The “O” was set aside for examining suprathreshold sensitivity. For each panelist, a sheet of paper containing the English alphabet was provided. This alphabet contained Arial typeface and capitalized letters that resembled the letter stimuli with the exception of the letter “I,” which was in Times New Roman. This sheet was used as a reference to remind panelists of the forms of the letters while testing and/or to point out the letter as their response.

Each letter stimulus was presented to the panelist face down in which the letter tile was situated closest to them on the tongue depressor. Orientation of the letters on each tile was right side up on the tongue depressor. To notify each panelist that each stimuli was not upside-down or in a different orientation other than right-side up, a tongue depressor with a letter tile affixed was directly shown to them as an example prior to testing. During testing, panelists were asked not to look at the letter and advised to give their best guess, even if they had no idea of what letter was presented. Only the first response was accepted, therefore it was advised to each panelist to take their time in assessing each presented stimulus.
Although there were a total of seven different letters used for this part of the test ("O" was excluded), panelists were advised that all letters of the alphabet were possible. They were also told to use the tip of their tongue to assess each stimulus and allowed to use any method they felt comfortable with as long as they did not directly look at the letter. Panelist’s response was either relayed verbally to the investigator or by pointing at the letter template provided (figure 2.2).

![Panelist performing the letter identification task by giving their response via pointing at the letter on the provided template. Panelist consent was provided for use of the photograph.](image)

**Figure 2.2** Panelist performing the letter identification task by giving their response via pointing at the letter on the provided template. Panelist consent was provided for use of the photograph.

An up-down staircase method was used to record responses. Each test started with a randomly selected 3 mm stimulus. If the panelist incorrectly identified the letter, the next stimulus presented would be one size larger (4 mm). If the panelist correctly identified the letter presented, the following stimuli would be one size smaller (2 mm). Stimuli increased
in size until correctly identified or decreased in size until the panelist answered incorrectly. Areas on the panelist’s staircase were documented every time there was a change in direction, which was referred to as a reversal (figure 2.3). Testing concluded once the panelist has reached a total of eight reversals. Threshold for each individual was calculated as the mean of eight reversals.

**Figure 2.3** Threshold documentation sample using an up-down staircase method. Task was completed at the completion of eight reversals (circled). The mean of those eight reversals is the panelist’s threshold average. The threshold average for this panelist is 4.625 mm.

If direction was unable to be changed during testing (i.e. incorrectly identified the largest stimulus or correctly identified the smallest stimulus), a reversal would be counted at that stimulus size and the following stimulus would be another of the same size.
2.3.4 Suprathreshold experimentation

Scaling for suprathreshold sensitivity was performed via magnitude estimation. Magnitude estimation is a scaling procedure where panelists assign numbers to stimuli which reflect a ratio for sensory perception (Moskowitz 1977). For this part of the experiment, the letter “O” was used exclusively. Each panelist was presented with all sizes (1.5, 2, 3, 4, 5, 6, 7, and 8 mm) for this task. All stimuli were randomized before sampled.

To begin, each panelist was given a 4 mm “O” as a reference and advised to use that stimulus as the “modulus” or reference for the remainder of the session. Each panelist was told that the value of the reference stimulus was a 50. A different size “O” was presented face down and the panelist was asked to compare that given stimulus to the reference and asked to give a value for that presented stimulus relative to the reference. For example, if the panelists felt that the stimulus given was twice as big as the reference, they reported a “100,” or if it was perceived as half the size of the modulus, they reported a “25.” Each panelist was prohibited from looking at the test stimuli before testing as well as responding with negative numbers or zero since that would imply there is no letter embossed on the tile.

2.3.5 Fungiform papillae count

A 0.1% (w/v) blue dye solution (Sensient Technologies, Milwaukee, WI) was prepared and a cotton tipped applicator was dipped into the solution and used to paint the anterior portion of the panelist’s tongue. A paper reinforcement label was placed over the dyed area. The area of interest that was circumscribed by the label encompassed a 31.7
mm² area. A photograph using a 16 megapixel F850EXR digital camera (Fujifilm, Minato, Tokyo) was taken of the dyed area (figure 2.4). Two separate investigators counted the number of papillae from the photos of each panelist and averaged.

![Image](image.png)

**Figure 2.4** Dyed area of a tongue with a paper reinforcement label attached. After painting the tongue tip, the fungiform papillae do not stain.

### 2.3.6 Statistical analysis

Statistical analysis was performed using Minitab 16 (Minitab Inc. State College, PA). Descriptive statistics for all variables were reported as mean ± standard error. Threshold analysis was performed using multiway ANOVA measuring threshold tactile sensitivity as a function of age, gender, and papillae count. Post hoc analyses (LSD) were also determined to specifically observe differences in ages by decade (18-29, 30-39, and 40+ years), gender, and fungiform papillae count (>20 papillae, 20-29 papillae, and 30+ papillae).
A repeated measures 2-way ANOVA was performed to determine whether panelists could accurately assess letter size using magnitude estimation. A curve for each panelist was created by plotting magnitude estimates versus letter size. The area under this psychophysical curve (AUC) was calculated on Excel (Microsoft Corporation, Redmond, WA) using the trapezoidal method and used as a singular dependent variable to analyze suprathreshold tactile sensitivity for each panelist (figure 2.5).

**Figure 2.5** Area under the curve (AUC) calculated from the impact of letter sizes on magnitude estimation responses. The AUC for each panelist was used as a singular dependent variable to analyze suprathreshold tactile sensitivity.

Multiway ANOVA, using suprathreshold AUC as a function of age, gender, and papillae count was used with post-hoc LSD tests to identify significant differences within treatments. In addition, to determine if an association exists between threshold and suprathreshold sensitivity, the Coefficient of Determination ($r^2$) was calculated. Graphical representations of the data analyzed were created using GraphPad Prism (GraphPad Software, Inc, La Jolla, CA).
2.4 Results and Discussion

2.4.1 Letter statistics

A total of 679 letter stimuli were presented to panelists during the threshold portion of the experiment. Full descriptive statistics are presented below in table 2.1.

Table 2.1. Letters presented during the threshold portion of the experiment displaying proportion of correct and incorrect responses.

<table>
<thead>
<tr>
<th>Letter</th>
<th>Presented</th>
<th>Incorrect</th>
<th>% Incorrect</th>
<th>Correct</th>
<th>% Correct</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>101</td>
<td>65</td>
<td>64.4%</td>
<td>35</td>
<td>35.6%</td>
</tr>
<tr>
<td>I</td>
<td>86</td>
<td>61</td>
<td>70.9%</td>
<td>25</td>
<td>29.1%</td>
</tr>
<tr>
<td>J</td>
<td>96</td>
<td>34</td>
<td>35.4%</td>
<td>62</td>
<td>64.6%</td>
</tr>
<tr>
<td>L</td>
<td>95</td>
<td>41</td>
<td>43.2%</td>
<td>54</td>
<td>56.8%</td>
</tr>
<tr>
<td>O*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T</td>
<td>100</td>
<td>36</td>
<td>36.0%</td>
<td>64</td>
<td>64.0%</td>
</tr>
<tr>
<td>U</td>
<td>101</td>
<td>58</td>
<td>57.4%</td>
<td>43</td>
<td>42.6%</td>
</tr>
<tr>
<td>W</td>
<td>100</td>
<td>70</td>
<td>70.0%</td>
<td>30</td>
<td>30.0%</td>
</tr>
</tbody>
</table>

Total: 679 365 53.9% 313 46.1%

*O was only used in suprathreshold portion of experiment

Letter stimuli were correctly identified 46.1% of the time. This is significantly better than the 1/26 chance level of detection suggesting, in general, humans have exquisite tactile sensitivity in their tongue. The letter “I” was incorrectly identified the most (70.9%), while “J” had the highest percentage of correct responses (64.6%). Due to the high percentage of incorrect responses, it can be assumed that letter stimuli “A,” “I,” and “W” had a much higher degree of difficulty than the other five letter stimuli.

Through observation, “A” was commonly mistaken for “V,” and “N.” “I’s” were easily mistaken for “T,” “F,” “C,” “L,” “J,” and “S.” “W’s” were commonly mistaken for “V,” “N,” and “M.” In some cases, panelists had the right idea when assessing the letter, but had the letter upside down in their mind. For example, if “W” was presented,
the letter “M” would be the given response. The majority of incorrect responses could have been due to improper or insufficient assessment technique where panelists did not evaluate the entirety of the whole surface area of the presented tile.

2.4.2 Threshold: effect of age

Threshold lingual tactile sensitivity was impacted by age (p=0.008; figure 2.6). Panelists in the 40 years or older age group had significantly higher threshold averages (6.25 ± 0.711 mm) than those in their 20’s (3.92 ± 0.208 mm). These results indicate that those 40 years of age or above exhibited decreased sensitivity and required a larger letter stimuli to be correctly identified. In some instances for those 40 and older, panelists had a difficult time assessing any of the stimuli and ultimately remained guessing even at the 8 millimeter stimulus until the test ended.

Figure 2.6 Threshold averages as a function of age.
It is widely known that sensory systems tend to decline as we age (U.S. National Library of Medicine 2012). One study suggests that the age effect may be the sole factor is sensory loss, specifically taste (Mojet et al. 2001). From this observation, it can be suggested that the loss of tactile sensitivity in the tongue may also be attributed to the age effect and contribute to difficulties in chewing and swallowing as people get older (Sura et al. 2012).

Of all 48 panelists tested, two subjects correctly identified the smallest stimulus (1.5 mm) once. Several others (regardless of age) were able to reach the smallest stimulus point, however incorrectly identified the letter. Prior research that has utilized this same task (Menella and Lukasewycz 2012) reported young children having more success correctly identifying the smallest letter size consistently (which was 2.5 mm in their study). In an attempt to potentially identify more sensitive populations, the present research utilized a 1.5 mm size as the smallest stimulus. However in this case, we were unable to identify persons consistently able to identify the smallest stimulus, suggesting that the 18-29 age group tested presently is no more sensitive than the young children that were tested in a previous study.

2.4.3 Threshold: effect of gender

Gender played no role in influencing lingual tactile thresholds (p=0.204, figure 2.7), as threshold averages were not different between men and women (male=4.36 ± 0.287 mm; female=3.99 ± 0.324 mm), with the difference being 0.37 mm. More likely,
the nearly equal distribution of ages within the male (age range 18-59) and female (18-59) cohorts may have resulted in the almost equal threshold averages.

![Figure 2.7 Threshold averages as a function of gender.](image)

**2.4.4 Threshold: effect of fungiform papillae density**

Papillae density had a highly significant effect on threshold tactile sensitivity (p<0.001; figure 2.8). Figure 18 shows that those who had papillae counts less than 20 required stimulus sizes over 2 mm larger than subjects having more than 20 papillae per area. Those with counts less than 20 exhibited significantly higher thresholds (6.16 ± 0.506 mm) than those who had counts between 20 and 30 (3.76 ± 0.251 mm) and above 30 (3.93 ± 0.314 mm).
It has been shown that the fungiform papillae is integral in detecting and perceiving taste sensations. Taste intensity perception and response to compounds increases with an increasing number of fungiform papillae (Miller and Reedy 1990; Arvidson and Friberg 1980; Zuniga et al. 1993). Our data suggest that with increasing taste papillae density, mechanosensory innervation of the tongue also increases resulting in greater lingual tactile acuity. Interestingly, children may have more papillae or more localized sensitive regions of the tongue compared to adults (Stein et al. 1994). Increased mechanosensory innervation may also contribute to the age effect discussed previously.

These data show that subjects exhibiting fewer papillae required a larger letter size to correctly identify the letter stimulus, suggesting that the number of papillae is integral for detecting lingual tactile sensations.
2.4.5 Impact of letter size

As expected, the actual size of the letter greatly influenced panelist’s estimates of letter size (p<0.001; figure 2.9), showing that panelists were able to properly estimate how much larger or smaller a test stimulus was relative to the reference when using only their tongue. Upon further investigation, there was a strong positive linear relationship between estimated and actual size (p<0.001, r² = 0.991), where the slight deviations arose from under/over estimations primarily at the stimulus extremes.

Figure 2.9 Actual values compared to the estimated responses given for magnitude estimation scaling on each letter size.

2.4.6 Suprathreshold: Effect of age, gender and fungiform papillae density

The psychophysical curve was generated for each panelist and the calculated area under the curve (AUC) was used as a singular dependent variable to test whether suprathreshold sensitivity varied by age, gender, and/or papillae density. Age (p=0.749, figure 2.10), gender (p=0.782, figure 2.11), nor papillae density (p=0.246, figure 2.12)
had an influence on lingual estimates of letter size. In determining a letter size relative to a reference, none of these variables affected the ability to distinguish letter sizes.

Figure 2.10 Suprathreshold AUC as a function of age.

Figure 2.11 Suprathreshold AUC as a function of gender.
The majority of panelists were able to properly estimate the general sizes of the stimuli, as their estimates match the corresponding sizes accordingly. For example, the majority of panelists (27/48, 56.2%) understood that the 2 mm stimulus was indeed larger than the 1.5 mm stimulus, despite such a small difference between the two. Others had difficulty in estimating those two sizes, as they indicated the 2 mm stimulus to be smaller than the 1.5 mm stimulus (9/48, 18.8%). Finally, 25% (12/48) of the panelists felt that the 1.5 and 2 mm stimuli were the same size.

Although most panelists were able to correctly identify stimuli as relatively larger or smaller, their estimates were not accurate. Even though actual letter size had the largest impact on estimated letter size, the panelists did not accurately estimate size ratios. The largest letter stimulus provided was an 8 mm stimulus; therefore an accurate estimation compared to the 4 mm reference would be 100. In most cases, panelists felt
that this 8 mm stimulus was three to almost five times as large as the reference. The majority also believed that the 6 mm stimulus was double the size of the reference, as that stimulus commonly received an estimate of “100.”

Responses that were given for the stimuli smaller than the reference were also not accurate in terms of the correct ratio estimates. For instance, the 1.5 mm stimulus commonly received an estimate of “1” and the next size up, 2 mm, commonly received an estimate of “5” or “10.” More accurate estimates for these stimuli should have been “18” and “25” respectively, since these were 32% and 50% of the size of the reference. This was likely due to panelists having difficulty estimating ratios. Instead, panelists had a tendency to resort to assigning favorite numbers or a specific range that they felt comfortable with (Lawless and Heymann 1999a).

2.4.7 Correlation

A correlation was tested to identify if there was any relationship between suprathreshold and threshold lingual tactile sensitivity by plotting each individual’s AUC against their calculated letter recognition threshold. No correlation existed between the two variables (p=0.869, r²=0.0006, figure 2.13), therefore the ability to estimate letter size was not influenced by a subject’s edge and point sensitivity as measured by the letter recognition task.

It was likely that the letter identification task was more cognitively demanding as panelists took more time and effort in assessing letters. However when estimating sizes, it was more apparent that panelists displayed more confidence (with the exception of the
1.5 and 2 mm stimuli) in their responses, and as a result, responded at a faster rate. The difficulty of the letter identification task compared to magnitude estimation may contribute to the lack of correlation.

This finding is consistent to another study investigating relationships between vibrotactile threshold and suprathreshold sensitivity on the tongue tip (Fucci et al. 1985). In an attempt to identify relationships to gain insight on oral tactile processing and subjects with normal speech versus those with speech disorders, they found that there was no correlation between the two and suggested that the tactile mechanisms operate at peripheral and central processing levels. Although there are some instances where threshold and suprathreshold show a correlation, such as detection thresholds with phenylthiocarbamide (PTC) sensitivity at suprathreshold levels (Drewnowski et al.1997;

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**Figure 2.13** Correlation displaying the relationship between threshold and suprathreshold lingual tactile sensitivity.
Bufe et al. 2005), it is generally known that there is no necessary relationship among the two (Lawless and Heymann 1999b).

2.5 Conclusions

An up-down letter identification task was implemented to observe how lingual tactile sensitivity differs by age, gender, and mechanosensory innervation (via fungiform papillae density). Two concepts of tactile sensitivity were measured: threshold and suprathreshold sensitivity. In addition, a correlation was analyzed between the two to identify any relationships.

Age and fungiform papillae density played a factor in threshold lingual tactile sensitivity, while none of the variables had any effect in suprathreshold lingual tactile sensitivity. However, as expected, suprathreshold sensitivity, as measured by the ability to estimate letter size, was dependent upon the stimulus size. There was no correlation between threshold and suprathreshold sensitivity, suggesting that one does not predict the other.

In conclusion, the letter identification task may provide useful information on the differences in lingual tactile sensitivity within the population. As found in this present study, age and papillae density played a role in these differences. Although several variables were observed throughout this experiment, there are additional factors that should be taken into account. These may include temperature and contact pressure. Temperature is a main factor contributing to food liking and may influence tactile sensations. Also, the temperature of a food may dictate the amount of pressure that is
applied to it when placed in the mouth. Future research should address this area by finding a proper methodology to assess how lingual tactile sensitivity is affected by these two variables.
Chapter 3: Creation of a novel device to further assess lingual tactile sensitivity: Effect of age, gender, fungiform papillae density, and temperature.

3.1 Abstract

The most noticeable aspect of food texture lies primarily in its role as an indicator of food quality. Lingual tactile sensitivity may contribute to food texture perception, however this topic has been understudied. To gain insight on lingual tactile sensitivity, we used a modified letter identification task to show that threshold sensitivity was dependent on age and fungiform papillae density, but not by gender (see chapter 2). Furthermore, one source of variation that was not investigated was the impact of temperature and its role in lingual tactile sensitivity. In order to assess the influence of temperature on lingual threshold acuity, a device to alter the temperature of letter stimuli was created. We hypothesized that sensitivity to threshold tactile stimuli was dependent upon age, gender, fungiform papillae density and stimulus temperature.

A modified up-down staircase letter identification task was implemented to assess lingual threshold tactile sensitivity. Fifty panelists used the anterior tip of their tongue to identify embossed letters ranging from 1.5-8 mm in height. To test the effect of temperature, a novel device was created to modulate stimulus temperature. Panelists were asked to assess letter stimuli that were cooled (~10°C) and warmed (~45°C). Following threshold testing, the anterior tip of each panelist’s tongue was painted with brilliant blue dye (0.1% w/v) to observe and count fungiform papillae in a 31.7 mm² area.
Differences in threshold were assessed using a multiway ANOVA with age, gender, fungiform papillae, and temperature as the main effects.

Threshold tactile sensitivity was dependent on age (p=0.02) and gender (p<0.01), but not by fungiform papillae (p=0.202) nor temperature (p=0.801). Those aged 40 and over exhibited decreased sensitivity and higher identification thresholds (4.49 ± 0.414 mm) compared to those aged 18-29 (4.02 ± 0.486 mm) and 30-39 (3.51 ± 0.211 mm). The male cohort also exhibited decreased sensitivity as threshold averages were significantly higher (4.44 ± 0.339 mm) than the female group (3.41 ± 0.168 mm).

As expected from the initial study, threshold lingual tactile sensitivity was dependent on age. However, lingual tactile sensitivity also differed by gender. In the present study, panelists were required to project their tongue onto a device in which a letter stimulus was attached, compared to Experiment 1 (refer to chapter 2.3.3) wherein panelists placed the stimulus in their mouth and were able to freely manipulate the letter with their tongue. This methodological change may have contributed to the differences observed presently. Additionally, although randomly presented, some panelists may have been exposed to a higher proportion of more difficult letters making the letter identification task more difficult, and therefore, affecting their threshold average. Future studies should investigate these factors to update a more proficient methodology to assess lingual tactile acuity.
3.2 Introduction

Temperature plays a key role in food liking and preference. Its influence on taste perception in particular is normal for our daily experiences (Talavera et al. 2007). Ice cream for example is most pleasurable when eaten cold, and pizza is preferred when served hot. However, the preferred temperature for a given foodstuff may differ. For instance, water is most generally satisfying at temperatures around 5°C, however maximal intake is shown to be at 15°C (Engelen et al. 2002).

In light of the previous findings (refer to Chapter 2), further research was necessary to gain more insight into how tactile sensitivity varies within the population. Research on taste perception and temperature has been reported. For instance, thermally stimulating small portions of the tongue can evoke sweetness or sourness (Green and Cruz 2000). Other studies have observed how oral temperature effects perceived temperature of food products (Engelen et al. 2002). Research regarding the perception and interaction of temperature with other modalities has focused on warm and cold receptors, and the impact of taste, and irritation (Engelen et al. 2002). However to our knowledge, temperature dependence of lingual tactile sensitivity is one aspect where research is either limited or missing. This is surprising given (1) temperature sensitivity is highest in the face and lips compared to other parts of the body and (2) temperature sensitivity declines with age (Stevens and Choo 1998).

A letter identification task was once again implemented to assess lingual tactile sensitivity, however in this study, the stimuli were heated and cooled. Heating and cooling stimuli for psychophysical experiments requires significant control. In order to
accomplish this, a Peltier thermode device was created for experimentation. It has been reported that tactile sensitivity declines for roughness at temperatures below 32°C (Green 1979), punctate thresholds around 10°C (Stevens et al. 1977), and vibrotactile thresholds around 20°C (Green 1977). In addition to those studies, it was found that the effect of warming had a negligible effect on tactile sensitivity. Therefore, we believe that at colder temperatures, lingual tactile sensitivity will decrease.

The objective of this experiment was to further continue the use of a letter identification task to assess lingual tactile sensitivity under conditions of thermal modulation by creating a novel device to modulate stimulus temperature. In addition to potential temperature effects on lingual tactile sensitivity, age, gender, and mechanosensory innervation were analyzed.

3.3 Methodology

3.3.1 Letter stimuli

Stimuli consisted of 3D printed thermoplastic Polycarbonate Acrylonitrile Butadiene Styrene (PC-ABS) tiles. Dimensions of the tiles were 1 cm x 1 cm x 2 mm. Letters were raised from the center of the tile 0.8 mm (refer to figure 2.1). Sizes of the letter were 1.5, 2, 3, 4, 5, 6, 7, and 8 mm which corresponded to font sizes 6, 8, 12, 18, 21, 28, 30, and 34. Letters used (A, I, J, L, O, T, U, W) were all capitalized. The same letters were used as in prior research due to being easily recognizable by the fingertips (Essick et al. 1999). All letters with the exception of “I” were in Arial typeface. To ensure clarity of the capitalized letter (having a clear top and bottom horizontal line), the letter “I”
was in Times New Roman typeface. A schematics created for each letter stimulus was created via Sketchup (Trimble Navigation Limited, Sunnyvale, CA), and converted to “.stl” format to be made applicable for 3D printing. Each tile was adhered to a sterile tongue depressor using double stick tape and organized based on letter and size before presenting to each panelist.

3.3.2 Peltier thermode device to modulate temperature

3.3.2.1 Device schematics

Besides its main purpose to modulate the temperature of the plastic tiles, the instrument was designed with the intention of making access to the stimuli easy and to allow the experiment to run smoothly. Therefore a “benchtop” style design was implemented. The Peltier thermode (Vktech TEC1-12706 Peltier thermode VK Technology & Trading, Muar, Johor, Malaysia; figure 3.2 B) was adhered to a 50x41 mm Socket 7/370 CPU Cooler Fan with Heatsink (Startech, Lockbourne, OH; figure 3.2 A) with double sided tape. Initial trials substituted thermal paste in place of double sided tape, however the Peltier thermode would not affix completely and would have a tendency to slide along the heatsink. On the bottom and back of the device, two 2” x 3” rectangular plates of sheet metal wrapped in electrical tape were attached using hot glue. To maintain a secure foundation for the steel plates, two 0.75” x 0.5” corner braces (National Manufacturing Co., Cobourg ON) were glued behind the plates.

On the bottom of the thermode, a tray was made to hold stimuli grids (refer to section 3.3.2.2.2). This tray was created using a 0.008in x 4in x 10in tin sheeting (K&S
Precision Metals, Chicago, IL) cut into a 2.5” x 0.75” rectangular sheet. From this sheet, a 0.4” x 0.4” square was cut from the two long ends (figure 3.1). The tabs that extended from the square cuts were folded into a box shape and fastened onto the device using hot glue.

**Figure 3.1** Template to cut sheet metal. The red perforated areas were cut off and folded on the solid dark blue line to create a tray.

In order to create a “benchtop” design a white 5 inch floral foam cube (Floracraft, Ludington, MI) was cut on a bias and a center portion from the cut area was sectioned out to house the device (figure 3.2 C&D). Holes were bored on the back of the foam cube to allow for the wiring to be threaded through. From the plates of sheet metal, two rubber bands were wrapped on the top and bottom of the device to ensure that the device was properly secured in place and that the plastic grids were secured and flushed against the surface of the Peltier thermode.
Figure 3.2 The device to modulate temperature from start to finish only displaying the main components. The computer heatsink (A) is attached to a Peltier thermode (B). Along with other supplementary materials, a completed device was created. C and D represent a completed device from an overhead and side view respectively.

A full set up of the device can be seen in figure 3.3. To ensure that the test ran smoothly and efficiently, a second device was created. It served as a standby, which contained a set of stimuli that panelists moved to once they exhausted the available stimuli that would be on the other device. While panelists would assess the stimuli on the second device, the stimuli from the first device would be switched out for a new set of tiles.
Figure 3.3 Investigators view of the testing area. Two devices were wired to a power supply. One channel of the power supply was dedicated to the thermode and the other channel was dedicated to the heatsink fan.

The Peltier thermode is a solid state heat pump. When current is passed through the Peltier, the heat is pumped from the cold surface to the hot surface. To create a cold surface on the thermode, the direction of the current passing through the thermode is reversed. To improve the efficiency of heat transfer and to enable the Peltier to reach colder temperatures, one face of the Peltier was glued to a heatsink using double sided tape and the heatsink fan turned on to remove the heat pumped from the cold surface.

3.3.2 Supplementary accessories

3.3.2.1 Clay molds

Two separate clay molds were created for development of the grid. The first mold was created as an initial component to the final mold product. Clay molds made
with Sculpey Oven Baked Clay (Polyform Products Co., Elk Grove Village, IL) were used to cast each plastic grid. To ensure that each grid encompassed the Peltier thermode surface, a stencil was created and applied to a rolled out 3 mm sheet of unbaked clay. Dimensions of the stencil were 4.4 cm x 4.4 cm with each square being 1.5 cm x 1.5 cm which will make up for the slight expansion of the clay once placed in the oven to dry. Squares were cut along the stencil to create the 3x3 mold using a fine tip utility knife. Prepared clay molds were placed on a baking sheet lined with parchment paper and baked in a preheated 275°F for 15 minutes. After 15 minutes of elapsed time, baked clay grids were removed from the oven and allowed to cool for one hour.

As the initial clay mold was cooling, a 5 mm sheet (5 cm x 5 cm) of unbaked clay was rolled out. The cooled initial clay grid was impressed onto the unbaked sheet ensuring that the whole initial grid was encompassed within the entire unbaked portion. A utility knife was carefully used to pry out the initial grid from the unbaked mold. Edges of the unbaked portion were trimmed to 4.5 cm x 4.5 cm dimensions and molded to smooth out any imperfections. Completed molds were placed in a preheated oven (275°F) for 15 minutes, then allowed to cool for at least one hour (figure 3.4). After cooling, these final molds were used to cast out the plastic grids.
Figure 3.4 Finished clay molds used to cast plastic grids. The left mold was created and impressed on a flat clay sheet. That clay sheet was trimmed into a square formation and baked, which is the final model on the right.

3.3.2.2 Plastic grids

3”x 3” grids were created using Instamorph Moldable Plastic (Happy Wire Dog LLC, Scottsdale, AZ), which is commonly used in arts and crafts. Approximately 1 cup of friendly plastic was placed in a warm water bath (~160°F) and allowed to fully melt. Approximately 8 grams of melted friendly plastic was weighed out, formed into a ball, and set aside. This was done continuously until the full cup of plastic was depleted. A plastic ball was individually placed back into the water bath and allowed to fully melt. Once melted, each ball was rolled out into 5 cm x 6 cm flat rectangular piece, formed and pressed evenly onto the clay mold, ensuring that the plastic fills all crevices in the mold, and allowed to set for one minute. The plastic was then released from the mold and trimmed to a 4 cm x 6 cm rectangular shape. To create 1 cm$^2$ pockets for the letter
stimuli, a utility knife was carefully used, creating five square holes per grid. Grids were then placed aside and allowed to dry for 24 hours prior to detailing. Once 24 hours elapsed, cut grids were placed directly on a hot plate on the first setting and immediately shut off to prevent burning and/or discoloring. The purpose of this was to ensure that each created grid was completely flat when placed onto the device (figure 3.5).

![Figure 3.5 Finished plastic stimuli grids in an “x” and “t” formation.](image)

3.3.2.2.3 Visual impairment glasses

Figure 3.6 (left picture) displays the panelist view of the stimulus apparatus. As seen in this figure, each letter stimulus presented can be observed easily. To avoid having each panelist blindfolded or requiring them to keep their eyes closed (as it would cause complications in assessing stimuli), visual impairment glasses were necessary to proceed. A combination of safety glasses and Parafilm “M” (Bemis Flexible Packaging, Neenah, WI) sheeting was used to create these glasses. A 4 cm x 4 cm sheet was cut from the Parafilm roll and stretched over the glasses. Excess Parafilm was trimmed off using a
utility knife. The completed model, along with the modified view are posted below (figure 3.6), showing that the goggles were effective in distorting panelist vision. Although the vision is impaired, panelists were able to recognize the location of the letter tiles presented to them, but were not able to make out specific letters on each one.

![Side by side panelist view](image)

**Figure 3.6** Side by side panelist view when not wearing the visual impairment goggles (left) compared to having them on (right).

### 3.3.2.2.4 Stimuli blanks

Letter tiles in which the offset letter fell off due to excessive depreciation were recycled and used as “blanks.” Three tile blanks were placed onto finished plastic grids and positioned on the top, middle, and bottom areas of the grid. Equipped grids were placed directly on a preheated hot plate (setting 1) and immediately turned off once placed. Melted grids were formed while on the hot plate ensuring that the blank tiles are fully affixed to the plastic grid.

These blanks served two purposes. One was to check the tile temperature as well as the interfacial temperature once a panelist’s tongue came in contact with the tile.
Temperature was monitored using a SDL200 4-channel thermocouple SD logger (Extech, Nashuam NH) and was placed on the upper surface of the target tile (figure 3.7).

**Figure 3.7** Warmup demonstration for training panelists how to assess each letter tile and to measure temperature when the tongue hits the tile. Panelists were asked to place the tip of their tongue on the tile where the thermocouple was placed.

Panelists were asked to place the tip of their tongue at the same location where the thermocouple probe was placed. The second purpose of the blank tiles was intended to work as a training exercise to help with aim. Vision was impaired for every panelist, therefore the possibility of missing the target tile was likely. This brief training exercise aided with learning how and where to assess each stimulus.

### 3.3.2.3 Device calibration

To check if the thermod and letter tiles adequately maintained temperature of the stimuli, calibration curves were generated. In addition to observing potential temperature
fluctuations, the calibration curves provided information on the time to reach the maximum hot/cold temperatures.

With the setup of the completed device replete with plastic grids and letter stimuli, calibration was performed with the 4-channel thermocouple SD logger attached with four type K probes. Each temperature run lasted 10 minutes with temperatures recorded via the thermocouple every 30 seconds. After 10 minutes elapsed, the device was shut down and allowed to cool for at least 10 minutes before the next trial. Each temperature assessment was completed three times and the mean of those triplicates were recorded. Voltages used per trial were 2.0, 2.5, 3.0, and 3.5 V when generating hot temperatures. For the cold temperature, 4.0 V was added in addition to the other voltages to observe the maximum cooling that was achievable with our device. It is important to note that the 4.0 V was not set when operating on the hot side due to the plastic grids melting onto the thermode. Operating the thermode on the cold side required the use of the heatsink fan to draw heat away from the opposing side of the thermode. The voltage set for the fan was 12.0 V. Amperage for the thermode and heatsink fan were set at one and two amperes respectively.

Four thermocouple probes were strategically placed onto various portions of the device. Two of the probes were placed directly on the thermode slightly off from the center (figure 3.8). This was to observe if the thermode was warming/cooling evenly throughout the surface and placing the probes off center would give a better temperature measurement when letter stimuli are directly on the thermode surface. Therefore, the tape on the thermode surface would not act as an insulator for letter stimuli.
The other two thermocouple probes were placed on a blank letter stimulus grid which was affixed to the thermode surface (figure 3.8). This was to observe the rate of temperature change of the letter stimuli when the device was operational. Operating on the hot side did not require the use of the heatsink and therefore the channel operating the fan was disabled. Figures 3.9-3.12 display the calibration curves for both and each tested portion.

Figure 3.8 Thermocouple placement on the surface of the thermode (A), placement of a probe on a blank stimuli grid (B), and a full model with all probes attached.
**Figure 3.9.** Temperature calibration curves for the top portion of the thermode (A) and the blank letter tile (B) when operating on the cold side.
Figure 3.10 Temperature calibration curves for the bottom portion of the thermode (A) and the blank letter tile (B) when operating on the cold side.
Figure 3.11 Temperature calibration curves for the top portion of the thermode (A) and the blank letter tile (B) when operating on the hot side.
Figure 3.12 Temperature calibration curves for the bottom portion of the thermode (A) and the blank letter tile (B) when operating on the hot side.
Temperature for each thermode reached its peak or nadir after one minute and increased/decreased slightly for the remainder of the ten minute reading. Letter tiles took about 2 minutes to reach their peak or nadir temperatures and also increased/decreased slightly for the remainder of the ten minute reading. The top portion of the thermode was substantially warmer and colder than the bottom portion. Hot and cold temperatures for the top portion averaged 57°C and bottoms at 8°C respectively, whereas for the bottom of the thermode, hot and cold temperatures reached 50°C and 12°C respectively. The temperature difference of the letter stimuli averaged 7-8°C on the warm side and 2-4°C (both regardless of location).

Despite reaching and maintaining a relatively stable holding temperature, the amount of time necessary to reach the target temperature was not conductive to testing panelists. Therefore, other means were necessary to ensure that testing panelists would run smoothly and efficiently.

### 3.3.2.4 Warming and cooling stations

Prepared letter stimuli grids were placed onto warming and cooling stations to keep the desired warm or cold temperature constant prior to panelist arrival. The cooling station consisted of a cooler and several flat frozen ice packs used so that each grid placed on them was flush with the surface of the ice pack. The warming station consisted of a 20 inch electric flat top griddle (National Presto Industries Inc., Eau Claire, WI) set at 200°F and lined with a sheet of aluminum foil. Attached to the griddle plug was a Variac (Standard Electric Product Company, Dayton, OH) set at 23V to control the
surface heat of the griddle to approximately 103-105°F. Along with controlling the heat, this temperature range was adequate in heating the letter stimuli without melting the plastic grids.

3.3.3 Panelists

Fifty panelists (26M, 24F) were recruited from the surrounding Columbus metropolitan area ranging in age from 18 to 65 years. This was two more panelists than used previously in Experiment 1 (n=48, 24M, 24F). Prior to experimentation, each panelist gave their written informed consent approved by The Ohio State University Institutional Review Board. Panelists were advised to refrain from smoking (if applicable) two hours prior to their scheduled time. Each experimental trial lasted roughly 30 minutes. Upon completion of the session, a $10 gift card was given to each panelist.

3.3.4 Threshold experimentation

3.3.4.1 Experimental setup

A total of five random letter stimuli were placed into each grid. Based on the results of the previous chapter, threshold averages ranged from 3-4 mm amongst the majority of the panelists. Therefore, most grids contained sets of 2, 3, and 4 mm letter stimuli, followed by sets of 4, 5, and 6 mm letter stimuli while few grid sets containing 1-2 mm letter stimuli and 7-8 mm stimuli were prepared.
3.3.4.2 Pretest training

A brief practice and temperature check was conducted prior to beginning each part of the letter identification task. Panelists were not required to wear the visual impairment goggles. Using a stimulus blank placed onto the device, panelists were asked to place the tip of their tongue on each blank tile. Each blank grid was preheated or cooled and placed on the device which was switched on via the power supply. The DC power supply was set at 3.3V and 0.2A to maintain stimuli temperature at 8-10°C or 42-45°C for the cold and hot conditions respectively. An extra channel (12.0V, 1.0A) on the power supply was turned on only when testing under cold temperature. This operated the heatsink fan, which drew out heat radiating from the opposing side of the thermode, therefore maintaining a desired cold surface.

On each targeted blank tile was a thermocouple probe, which was then sandwiched between the tile and the panelist’s tongue and allowed for the documentation of temperature at the tongue-tile interface. In addition, this pre-training helped subjects to practice finding each presented letter tile before the beginning of each assessment task. Temperature check/tile practice was completed after six readings.

3.3.4.3 Panelist briefing

Brief information was given to panelists prior to letter assessment. One important bit was that all letters of the alphabet were possible when there were actually eight possible letters. Decreasing the probability from 1/8 to 1/26 helped determine if in fact panelists were able to correctly assess letters. Panelists were also informed that all
presented letter stimuli were capitalized, randomized, and right side up (to limit any confusion of the stimuli orientation). To ease panelists, they were told that the test would not cause any pain or discomfort and the temperatures of the letter stimuli they would be assessing were mentioned. After briefing, panelists were advised to put on the visual impairment glasses to continue testing.

3.3.4.4 Letter identification task

Prepared grids containing five letter stimuli were placed onto the device which was powered on to 3.3V and 0.2A. A second channel (12.0V, 1.0A) to operate the heatsink fan was operational only when assessing stimuli at cold temperatures. Two letter identification tasks to assess stimuli under hot and cold letter conditions were given and these conditions were counterbalanced between each panelist to control for order effects.

Regardless of temperature condition, the procedure for the identification task was the same. As mentioned, five randomized letter stimuli with predetermined sizes were placed onto the device and panelists were directed (top, bottom, left, right, center) on which stimulus to assess.

A modified up-down staircase method was used to document threshold sensitivity. All panelists started with a 3 mm letter stimulus. If the response was incorrect, letter sizes would increase 1 mm. Once a correct letter was identified, letters decrease in size 1 mm until another incorrect response was given. Every time a change in direction in the up-down staircase response sheet occurred, it was documented and
considered a reversal. The task was completed after a total of eight reversals. Threshold average for that temperature assessment was determined as the mean of those eight reversals.

3.3.5 Fungiform papillae count

A 0.1% (w/v) blue dye solution (Sensient Technologies, Milwaukee, WI) was prepared and a cotton-tipped applicator was dipped into the solution and used to paint the anterior portion of the panelist’s tongue. A paper reinforcement label was placed over the dyed area. The area of interest that was circumscribed by the label encompassed a 31.7 mm² area. A photograph using a 16 megapixel F850EXR digital camera (Fujifilm, Minato, Tokyo) was taken of the dyed area. Three separate investigators counted the number of papillae from the photos from each panelist and averaged.

3.3.6 Post-test questionnaire

To gain further insight into the effectiveness of the device, several questions were asked upon completion of the test (figure 3.13).
Figure 3.13 Post-test questionnaire presented to panelists to enquire about the efficacy of the device.

These questions were used to ensure that (1) the device was adequately modulating and holding the target temperature, (2) the panelists did not feel discomfort or pain with the varying temperatures, (3) the letter stimuli were noticeably colder/warmer than they would be at room temperature, and (4) if the temperature of all presented stimuli was evenly distributed throughout the grid (i.e. the top portion being significantly warmer/colder than the bottom portion).

3.3.7 Statistical analysis

Data analysis was performed using SPSS 16 statistical software (IBM, Armonk, NY) and graphical representations were designed using GraphPad Prism (GraphPad Software, INC., La Jolla, CA). A multiway ANOVA was used with threshold as the dependent variable and age, gender, fungiform papillae count, and temperature as the
main effects. Descriptive statistics were displayed as mean ± SE. Significance for all analyses were based on $\alpha=0.05$.

### 3.4 Results and Discussion

#### 3.4.1 Letter statistics

A total of 1,343 letter stimuli were presented to 50 panelists. Full descriptive statistics of all letter stimuli can be seen in Table 3.1

<table>
<thead>
<tr>
<th>Letter</th>
<th># Presented</th>
<th># Incorrect</th>
<th>% Incorrect</th>
<th># Correct</th>
<th>% Correct</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>167</td>
<td>109</td>
<td>65.27</td>
<td>58</td>
<td>34.73</td>
</tr>
<tr>
<td>I</td>
<td>175</td>
<td>138</td>
<td>78.86</td>
<td>37</td>
<td>21.14</td>
</tr>
<tr>
<td>J</td>
<td>173</td>
<td>70</td>
<td>40.46</td>
<td>103</td>
<td>59.54</td>
</tr>
<tr>
<td>L</td>
<td>148</td>
<td>67</td>
<td>45.27</td>
<td>81</td>
<td>54.73</td>
</tr>
<tr>
<td>O</td>
<td>173</td>
<td>79</td>
<td>45.66</td>
<td>94</td>
<td>54.34</td>
</tr>
<tr>
<td>T</td>
<td>200</td>
<td>85</td>
<td>42.50</td>
<td>115</td>
<td>57.50</td>
</tr>
<tr>
<td>U</td>
<td>150</td>
<td>71</td>
<td>47.33</td>
<td>79</td>
<td>52.67</td>
</tr>
<tr>
<td>W</td>
<td>157</td>
<td>112</td>
<td>71.34</td>
<td>45</td>
<td>28.66</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1343</strong></td>
<td><strong>731</strong></td>
<td><strong>54.43</strong></td>
<td><strong>612</strong></td>
<td><strong>45.57</strong></td>
</tr>
</tbody>
</table>

Letter stimuli were correctly identified 45.57% of the time. This is significantly better than the 1/26 chance level of detection suggesting, in general, humans have exquisite tactile sensitivity in their tongue. The letter “I” was incorrectly identified the most (78.86%), while “J” had the highest percentage of correct responses (59.54%). Due to the high percentage of incorrect responses, it can be assumed that letter stimuli “A,” “I,” and “W” had a much higher degree of difficulty than the other five letter stimuli.
Through observation, “A” was commonly mistaken for “V,” and “N.” “I’s” were easily mistaken for “T,” “F,” “C,” “L,” “J,” and “S.” “W’s” were commonly mistaken for “V,” “N,” and “M.” In some cases, panelists had the right idea when assessing the letter, but had the letter upside down in their mind. For example, if “W” was presented, the letter “M” would be the given response. The majority of incorrect responses could have been due to improper or insufficient assessment technique where panelists did not evaluate the entirety of the whole surface area of the presented tile.

Although the letter on each tile was selected randomly, it is possible that the difficulty of the letter itself may have played a role. Some panelists may have been presented the most difficult stimuli “A,” “J,” or “W” at a higher frequency than others therefore affecting their overall threshold average.

3.4.2 Effect of age

Threshold tactile sensitivity was dependent on age (p=0.02, figure 3.14). Consistent trends were identified when compared to study 1 (see chapter 2) as the over 40 group exhibited decreased sensitivity (as displayed by having higher threshold averages) compared to the 18-29 group. This suggests that one of the effects of aging is the loss of lingual tactile sensitivity. Mechanisms underpinning age-related loss of tactile sensitivity in the tongue have not been described. However, this finding is very similar to tactile sensitivity losses in the skin for older populations which has been attributed to biological changes such as thinning of the epidermis and decreases in collagen and elastin (Thornbury and Mistretta 1981).
This is also consistent with the effect of age observed for other sensory systems. Smell, for example, is impaired in older populations which affects taste and flavor perception (Doty et al. 1984; Stevens et al. 1984; Boyce and Shone 2006).

![Figure 3.14](image)

**Figure 3.14** Threshold averages as a function of age resulting from the use of a device to modulate temperature. Note the 40+ age group had thresholds that were significantly higher than the 18-29 age group indicating decreased sensitivity with age.

### 3.4.3 Effect of gender

Gender played a role in threshold tactile sensitivity as the female cohort was more sensitive than the male cohort ($p<0.001$, figure 3.15). Several questions arise from this result as gender had no significant effect on tactile sensitivity in our prior study (see chapter 2). One possibility was that the recruited population was comprised of more sensitive female subjects. Regardless, further investigation with larger experimental sample may give a better idea on tactile sensitivity and gender, despite research showing that gender has no influence on oral stereognosis (Dahan et al. 2000) or temperature sensitivity on various parts of the skin (Stevens and Choo 1998).
3.4.4 Effect of fungiform papillae density

Fungiform papillae density had no effect on threshold tactile sensitivity (p=0.202, figure 3.16) therefore the ability to correctly identify letters was not dependent on the number of papillae counted. This was an interesting outcome considering that the prior study (see chapter 2) showed a significant effect of fungiform papillae as threshold sensitivity was greater in those that had higher papillae counts. Although the results were not significant for this part of the study, it should be noted that the group with the lowest papillae density (<20) exhibited a higher threshold average than the other two groups, consistent to the prior study.

Figure 3.15 Threshold averages as a function of gender resulting from the use of a device to modulate temperature.
This could potentially be the result of letter difficulty, assessing tiles improperly, or the effect of group sample size. For example in the initial study, panelists were given each letter stimulus and were told to assess in any fashion using only the tip. Almost all of the panelists placed the tile on the tip of their tongue and closed their mouth. This method allowed for the panelist to completely encompass the entire stimulus and assess the full surface area of the letter tile.

As opposed to the initial study, the assessment of letter tiles in this investigation did not allow full enclosure of the stimulus and unimpeded searching strategies. Instead, panelists were required to bend over the letter stimuli on the device and extend their tongue to the target stimulus. This potentially did not allow the panelists to fully assess the letter tile, which may have resulted in incorrect responses (see 3.4.1 Letter statistics).
An unbalanced sample size in each group may also have played a role. There were substantially more panelists who had a papillae count in the 20-30 range (36) compared to the <20 group (5) and the 30+ group (9). The considerable difference showed vulnerability to outliers as one panelist in the <20 group had performed quite well on the task and consequently, had a very low threshold. Therefore, the impact of that panelist had a large influence on the group average and may explain the inconsistency between this study and Experiment 1.

3.4.5 Effect of temperature

Temperature had no effect on threshold tactile sensitivity ($p=0.801$, figure 3.17) as threshold averages for the hot and cold sides were 3.90 mm and 3.97 mm respectively. Low thermoconductivity of the letter tiles was likely the issue for this outcome. Since the letter stimuli were made from low thermoconductive plastic, heat transfer to and from the tile were minimal. Thus, once the panelist’s tongue came into contact with the letter tile, temperature at the tongue tile interface remained close to oral tissue temperature (36.8°C).

Documentation of interfacial temperature prior to each identification task averaged 29.5 ± 0.149°C for the cold side and 35.2 ± 0.132°C for the hot side. Although we observed a temperature difference once panelists placed their tongue on the tile to assess letters, the difference was negligible, suggesting that the cooling and heating of the letter stimuli should be enhanced (e.g. modification of device and/or stimuli with better
thermoconductivity) for future studies to determine if threshold tactile sensitivity is affected in more extreme temperature conditions.

![Graph showing threshold averages as a function of temperature using a device to modulate temperature.](image)  

**Figure 3.17** Threshold averages as a function of temperature using a device to modulate temperature.

### 3.4.6 Efficacy of device

A post-test questionnaire was presented to each panelist upon completion of the letter identification task and the fungiform papillae count. This questionnaire was intended to verify that the device was efficient in modulating temperatures of the stimuli. This can inform future research as to what upgrades are necessary to better continue this line of inquiry. Table 3.2 displays the responses to the questionnaire.
Table 3.2 Panelist responses (n=50) to the post-test questionnaire.

<table>
<thead>
<tr>
<th>Question 1</th>
<th>Was pain or discomfort experienced?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes, at the hot temperature</td>
</tr>
<tr>
<td>% of respondents</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Question 2</th>
<th>Were the letter tiles noticeably hotter or colder?</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of respondents</td>
<td>Yes</td>
</tr>
<tr>
<td>84</td>
<td>16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Question 3</th>
<th>What temperature felt easier to assess letters?</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of respondents</td>
<td>Hot temperature</td>
</tr>
<tr>
<td>42</td>
<td>38</td>
</tr>
</tbody>
</table>

All but six panelists experienced no pain or discomfort while performing the letter identification task (question 1), and those subjects expressing discomfort mentioned the cause as fatigue. Therefore, the device as developed was capable of modulating stimulus temperature without harming the panelists.

A follow up question was asked to observe if the letter stimuli had noticeable changes in temperature compared to a room temperature stimulus. Plastic letter stimuli are not highly thermoconductive, however our data show that 84% of the participants felt that the tiles deviated from ambient temperatures (question 2). These results suggest that the development of the device in combination of the precautionary warming and cooling methods was useful in modulating stimulus temperature at least to some degree.

Question 3 was asked to get the panelist’s opinion of which temperature felt easier to assess letter stimuli. Forty-two percent of the panelists felt the hot side was easier, 38% felt the cold side was easier, and 20% felt that both temperatures were equally difficult. The percentages of those who listed a particular side as more difficult
suggests that the temperature did not necessarily make a difference; this is supported by the threshold averages which were not dependent on temperature. Therefore, we can speculate that the total temperature output from the device was inadequate and should be investigated further to observe if more extreme temperatures should be considered for future testing. In addition, the thermoconductivity of the letter tiles was low and resulted in very little heat transfer between the tongue and plastic letter tile. The consequence of this is a temperature sensation that does not vary much from typical oral temperatures. Different letter stimuli with a higher level of thermoconductivity (metal) should be favored to observe its efficiency compared to the letter tiles used in this study.

3.5 Conclusions

A novel device was created in order to modulate temperature of tactile stimuli in a modified up-down letter identification task. Changes to threshold lingual tactile sensitivity were assessed using age, gender, fungiform papillae density, and temperature as variables. The device modulated temperatures of the letter stimuli while panelists assessed them with the anterior tip of their tongue.

Similar to our previous findings, age played a role in lingual tactile sensitivity as older populations exhibited decreased tactile sensitivity. Although this finding was robust and consistent, gender and fungiform papillae density displayed different results in Experiments 1 and 2. In addition, temperature played no role in threshold lingual tactile sensitivity.
Improper or inefficient assessment by way of not utilizing the whole surface area of the tile and letter difficulty may have contributed to the different results. Also, the paradigm of letter assessment was different than in previous studies, which also may have contributed to the differences observed in this study. Due to the multiple possibilities that may have affected those differences, further research should address updating the methodology in terms of the schematics of the device to identify if letter assessment was an issue. Also, identifying the degree of difficulty of the letter stimuli should be investigated. Correcting for this degree of difficulty could identify populations of people having increased sensitivity. For instance, a subject that correctly identifies a “difficult” letter could conceivably be considered more sensitive than another subject who correctly identifies an “easier” letter of the same size.

It was also observed that temperature did not affect lingual tactile sensitivity. The temperature of the stimuli before contact was not representative of tongue-tile interface and suggests that modifications to either the device (via more extreme temperature) or changes to the letter stimuli (increased thermoconductivity) should be considered. It should be noted that temperature sensitivity declines with age, but central areas of the body (e.g. lips) do not greatly lose sensitivity with age (Stevens and Choo 1998). All these factors should be brought to attention before carrying out subsequent studies.

In conclusion, the letter identification task still shows promise in finding age differences in lingual tactile sensitivity, however further modifications should be implemented to gain consistent outcomes among other variables.
Chapter 4: Overall conclusions

The use of a modified letter identification task is shown to adequately measure lingual tactile sensitivity within the population. Two methodologies were used to perform this task. The first adopted a similar tactic to Essick (1999), Menella (2012), and Steele (2014), however we further investigated potential differences in age, gender, and fungiform papillae density on lingual tactile sensitivity. One source of variation that was not considered was the impact of temperature. Therefore, a second methodology modified the letter identification task with a novel device to modulate temperature and observed how this influenced lingual tactile sensitivity.

It was evident, regardless of the methodology used to assess letter stimuli, that age played a role in sensitivity as older populations required larger stimulus sizes to be recognized. This finding was consistent to previous reports of age-evoked decreases in perceptual acuity. However, the finding that gender and fungiform papillae density produced different results should be investigated further to obtain more robust and dependable outcomes. It was also shown that temperature played no role.

Similar to findings from prior research and those performed on the skin, less sensitive populations were linked to older age groups, thus showing that the age effect is a major factor in tactile sensitivity loss. Although there were inconsistencies among gender and fungiform papillae density, the letter identification task can prove to be a
reliable and promising protocol to assess lingual tactile sensitivity. It gives us more information on what parameters can be investigated and can provide better understanding on why some investigated areas turn out different than expected.

The approach to assessing letter stimuli (complete freedom to explore the stimulus surface versus protruding tongue to assess letter tile) or potential letter degree of difficulty may have contributed to the different outcomes observed in Experiments 1 and 2 and should be investigated further. Also, a greater ability to modulate stimulus temperature and/or designing stimuli with better thermoconductivity should also be explored since the tongue-tile contact temperature was not substantially altered in our study.

Overall, this study aims to further investigate factors influencing lingual tactile sensitivity. One limitation to this experiment was that we only investigated one aspect of tactile sensitivity (edge and point sensitivity). Other aspects of tactile sensitivity, for example roughness, involves the use of different mechanoreceptors and neural mechanisms, thus the protocol to assess is different. Because of this, it is likely that these different mechanisms may give insight on understanding texture further. As this topic is understudied, the paucity of research provides potential opportunities to examine connections between lingual tactile sensitivity and descriptors of food texture and liking. Studies of this nature will give way additional insight regarding how and why we discriminate, perceive, and like various food textural attributes.
Reference List


Appendix A: Consent Form
The Ohio State University Consent to Participate in Research

Study Title: Flavor interactions and the impact on texture assessment and oral tactile sensitivity

Researcher: Christopher T. Simons, Ph.D.

Sponsor: None

This is a consent form for research participation. It contains important information about this study and what to expect if you decide to participate.

Your participation is voluntary.

Please consider the information carefully. Feel free to ask questions before making your decision whether or not to participate. If you decide to participate, you will be asked to sign this form and will receive a copy of the form.

Purpose:
We are interested in how flavor information is processed to create the sensations elicited when smelling and eating foods. The overall purpose of this study is to gain insight into how perceptions and liking of food change as taste, smell, spiciness, texture and temperature components are modulated individually or in combination. In addition, we believe tactile sensitivity of the tongue contributes to texture perception. To gain insight as to how oral texture information is processed, different aspects of tactile sensitivity of the tongue will be measured. This information will be used to determine if tactile sensitivity of the tongue and the perception and liking of different food textures are linked.

Procedures/Tasks:
In some cases, you will be asked to evaluate the intensity and liking of tastes, flavors and/or textures from various model food systems or food products. After tasting each sample, you will be asked to rate how strong you perceive the taste, flavor or texture attribute. Similarly, you will also be asked how much you like or disliked the sample.

In some cases, we may be interested in how sensitive your tongue is to different textural attributes. There are several ways we may test this. In some cases, we may blindfold you and touch various locations on your tongue tip with a thin nylon monofilament. You will be asked to identify the side of your tongue touched by the monofilament. We will also ask you to rate the intensity of this stimulus. We will assess your sensitivity before and after we apply a flavor to your tongue. The flavor may have a taste (e.g. sour or bitter) or it may be spicy or cooling. We will tell you what the flavor is before applying it to your tongue with a cotton swab. The second way we may assess your tongue's sensitivity is by having you rate the...
roughness of various surfaces using your tongue tip. The temperature of these surfaces may vary from cold to hot and so we will ask you not to keep your tongue in contact with them for longer than 15 sec at a time. We may also have you rate the roughness of various surfaces after we apply a flavor to your tongue. The flavor may have a taste (e.g. sour or bitter) or it may be spicy or cooling. The final way we may assess your tongue's sensitivity to tactile stimuli is by having you identify raised alphabetical letters affixed to a holder using only your tongue tip. The temperature of the holder may vary from cold to hot and so we will ask you limit contact between your tongue and the letters to 15 sec or less. In some cases we may ask you to identify letters after pre-treating your tongue with a flavor that may have a taste (e.g. sour or bitter) or may be spicy or cooling.

Duration:
Participation in this experiment will take no more than 30 min. In some cases, you may be asked to return to the laboratory at a subsequent time for further testing. In such instances, you will be notified prior to the onset of the first experimental session so you can decide if you want to participate.

You may leave the study at any time. If you decide to stop participating in the study, there will be no penalty to you, and you will not lose any benefits to which you are otherwise entitled. Your decision will not affect your future relationship with The Ohio State University.

Risks and Benefits:
The food and flavor products that you will evaluate are comprised of ingredients that have been approved for use in foods by the United States Food and Drug Administration. In some cases, the products may contain spicy or cooling compounds that elicit burning or cooling sensations, respectively. You may experience mild discomfort associated with these sensations. Typically, these sensations disappear within approximately 10 min. In some cases, we may ask you to place your tongue on a temperature probe that can be heated or cooled. If your tongue remains in contact with the heated probe for longer than 1 min, you may receive a minor burn. We will ask that you keep your tongue in contact with this probe for no longer than 15 sec at any given time.

You will receive no direct benefit for participating in this study. However, the insight gained from your participation will give us a better idea of how various food attributes are processed by the brain to influence food perception and liking.

Confidentiality:
All information will be stored in a secure computerized database. At the onset of the experimental session, you will be asked to provide general demographic information including age, gender and ethnicity. In some cases additional information regarding eating and dietary habits may be obtained. These data will be collected using secured computerized data acquisition software or, on occasion, paper ballot. Data collected from
paper ballots will be input into a secure computer at the earliest convenience and the paper ballot destroyed.

Efforts will be made to keep your study-related information confidential. However, there may be circumstances where this information must be released. For example, personal information regarding your participation in this study may be disclosed if required by state law. Also, your records may be reviewed by the following groups (as applicable to the research):

- Office for Human Research Protections or other federal, state, or international regulatory agencies;
- The Ohio State University Institutional Review Board or Office of Responsible Research Practices;
- The sponsor, if any, or agency (including the Food and Drug Administration for FDA-regulated research) supporting the study.

Incentives:
You will receive either course credit or a gift card in the amount of $10. At the conclusion of data collection, you can choose to be compensated with a gift card or course credit. In the event that you participate in an experiment that requires returning to the laboratory for multiple sessions, you will receive compensation at the end of each session.

Participant Rights:

You may refuse to participate in this study without penalty or loss of benefits to which you are otherwise entitled. If you are a student or employee at Ohio State, your decision will not affect your grades or employment status.

If you choose to participate in the study, you may discontinue participation at any time without penalty or loss of benefits. By signing this form, you do not give up any personal legal rights you may have as a participant in this study.

An Institutional Review Board responsible for human subject research at The Ohio State University reviewed this research project and found it to be acceptable, according to applicable state and federal regulations and University policies designed to protect the rights and welfare of participants in research.

Contacts and Questions:
For questions, concerns, or complaints about the study, or you feel you have been harmed as a result of study participation, you may contact the Principal Investigator, Christopher T. Simons at (614) 688-1489 or simons.103@osu.edu.

For questions about your rights as a participant in this study or to discuss other study-related concerns or complaints with someone who is not part of the research team, you may contact Ms. Sandra Meadows in the Office of Responsible Research Practices at 1-800-678-6251.
Signing the consent form

I have read (or someone has read to me) this form and I am aware that I am being asked to participate in a research study. I have had the opportunity to ask questions and have had them answered to my satisfaction. I voluntarily agree to participate in this study.

I am not giving up any legal rights by signing this form. I will be given a copy of this form.

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