Elucidation of a potential detection mechanism for high-viscosity solutions in the oral cavity using tissue isolation and anatomical characterization

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Abstract

Studies on viscosity and its oral perception are limited to relatively thin viscosities (η<3000cP), thinner than many relevant food products. Moreover, multiple modelling studies indicate perception of high-viscosity solutions is linked to filiform papillae deformation, but this has yet to be tested psychophysically. This study sought to characterize the detection mechanism underpinning perception of high-viscosity (η>3000cP) solutions. We hypothesized the tongue would be chiefly responsible for viscosity perception, due to its active role in product manipulation, and that discrimination ability of high-viscosity solutions would be linked to filiform papillary attributes. Just-noticeable difference (JNDs) thresholds for viscosity were determined for participants (n=59) using the forced-choice staircase method for glycerin/water/carboxymethyl cellulose solutions (η=4798-12260cP). Participants evaluated solutions by pressing them against the hard palate in "palate-blocked", "tongue-blocked", and "unblocked" conditions. Optical profiling was then used to characterize papillary structures in tongue biopsies of a subset of participants (n=45). Papillary attributes were related to "palate-blocked" performance using causal analysis. Participants' differentiations were significantly better ($p<0.001$, both) using the whole oral cavity (JND=1041.71cP±50.36cP) than using tongue (JND=1671.26cP±142.77cP) or palate (JND=1791.42cP±117.42cP) alone. There was no significant difference between the blocked conditions ($p=0.724$), and performance in those conditions was not significantly correlated ($p=0.773$). "Palate-blocked" data significantly correlated to filiform papillae length and density ($p<0.001$, and $p=0.005$, respectively). Variation

The author would like to thank her advisor, Christopher T. Simons, for his contributions and guidance on the development, execution, and analysis of this work, and Kelly Kennedy for her assistance with the oral biopsies.

in these attributes alone explained variation in “palate-blocked” JND ($p<0.001$). Results from psychophysical tests run counter to the original hypothesis, suggesting the tongue and palate play equal roles in viscosity perception, but individuals may differentially utilize these tissues when discriminating between solutions. Similarly, anatomical characterization of filiform papillae provides insight into the role these structures play in high-viscosity perception.

Introduction

Viscosity is a relevant attribute in liquid and semisolid product categories. It has been linked to a variety of textural attributes including positive attributes such a creaminess, and negative attributes such as sliminess and mushiness [1–3]. Yet, the perception of viscosity, like perception of many other textural cues, is not well understood. Oral sensation and perception research has historically focused on the study of chemoception and nociception, with food scientists prioritizing flavor, while dentists and other oral biologists instead concentrate on the study of pain and disease. It is only in the past twenty years that oral somatosensation research and the study of the psychophysiology of texture perception have begun to consistently grow and diversify beyond product specific applications. Companies and scientists are now trying to understand how people interact with their food, why they choose the products they do, and more importantly here, what systems, structures, and underlying processing mechanisms might be driving product-related decisions.

Some preliminary work has been done to characterize oral viscosity perception in humans. These studies can be broken into two main categories: those focused on determining the power function relating physical increases in viscosity to perceived increases in thickness[4–6] and those comparing oral viscosity evaluation to other evaluation methods (i.e. stirring, pouring, etc.) [2,7]. Rarely did studies focus on the mechanisms driving viscosity perception, or how

individual variation may be impacting the process. However, more recently, mathematical modeling studies have investigated a potential mechanism involved in oral viscosity perception, the directional deformation of the filiform papillae. First proposed by van Aken (2010) and further investigated by Lauga, Pipe, and Révérend (2016), these studies suggest the idea of filiform papillae as strain amplifiers on the tongue capable of interacting with viscous solutions on the tongue's surface [8,9]. The structures may be of such importance that their presence causes an increase of strain in response to viscous solution by an order of magnitude [9]. This directional deformation is postulated to then transduce a signal to the brain either via specialized mechanoreceptors inside the papilla structure or via a sublingual receptor responding to strain on the surface [8–10].

Filiform papillae are the non-tasting papillae that cover the majority of the front two-third of the human tongue. They are comprised of cornified projections made of keratin [11]. While these cells do undergo keratin-type differentiation, they do not harden but instead squamatize and plaque on the surface of the structure, leading to a roughened appearance. The structures themselves are a complex, comprised of up to 30 hair-like projections off of one papillary body, although the body and hairs are thought to act as a single unit [11–13]. They have been proposed to play a variety of roles from helping to aid in bolus formation and swallowing to providing optimal salivary wetting of the tongue [10].Unfortunately, while studies appear to be in agreement about the general structural layout on the tongue, reports of the papillae's length (anywhere from 250μm to 2-3mm), width (100μm to over 450μm), and assumptions on their elasticity (2.6-25kPa) vary greatly [8–10,12,13]. This in turn leads to multiple proposed viscosity cut-offs for when these structures may become relevant for perception. For thinner solutions ($\eta<100$), it is universally accepted the structures are likely irrelevant. These solutions do not

generate enough force to lead to deformation. With such variations in proposed structure, however, there is not agreement as to what exact minimum viscosity leads to deformation. Most conservatively, van Aken (2010) proposed that a minimum viscosity of 1000cP was needed. As the authors were unable to find a conclusive set of average dimensions prior to the study, this more stringent cutoff was used to ensure all solutions could be evaluated via the proposed papillary deformation mechanism.

Unfortunately, the bulk of the solutions evaluated in the psychophysical studies on viscosity mentioned previously fall below the 1000cP cutoff [2,4–7,14]. Assuming these solutions could potentially be being detected via a different mechanism or pathway, the findings from these studies have limited applicability here. Moreover, of the limited studies that do exist with solutions in the higher-viscosity range, they tend to utilize confounded systems such as sucrose syrups, which can be also identified on the basis sweetness [4]. Additionally, the primary method used in these studies, magnitude estimation, is prone to memory effects leading to participants comparing the tested solutions to the previous sample instead of the reference unless frequent representation of the reference occurs[2,4,6]. Thus, we elected to utilize the forced-choice, staircase method, which presents the reference as part of every comparison, with the aim of limiting memory effects here.

Fortunately, extensive work has been done to characterize the mechanical evaluation process of high-viscosity fluids in the oral cavity. In a seminal study by Shama and Sherman (1973) researchers characterized the average shear rate utilized by participants to evaluate a series of different liquid food items ranging from water to glucose syrup. Solutions in the high-viscosity range, were consistently evaluated at shear rates between 10 s^{-1} and 50 s^{-1} [3]. Further studies have concentrated of the evaluation of these high-viscosity solutions, and data suggests

that the evaluation rate is likely toward the lower end of the reported values, near 10 s^{-1}[15]. Thus, it can be assumed that for any stimuli used here, the solutions should be devoid of any taste or aroma cues that might aid in identification, and recorded viscosity should be above 1000cP over the 10-50s^{-1} range.

Another challenge that has likely limited previous evaluation of filiform papillae in somatosensation is their small size, which can make them challenging to visualize completely. In order to identify structural variance in the population to relate it to performance, it was necessary to find a method for characterizing filiform papillae in humans. While the non-invasive Denver Papillae Protocol is often used to quantify fungiform papillae size and density, the method lacks the consistency and detail necessary for quantifying variations in attributes of the filiform papillae such as average papilla height, width, and curvature [16]. Moreover, utilizing a hand microscope for *in vivo* visualization of the structures was also challenging due to difficulty completely immobilizing the tongue to allow for focusing. While the very accurate scanning electron microscopy (SEM) has been successfully used to accurately characterize variation in papillae previously, this method is time consuming, requiring a minimum of 48 hour to prep tissue, and can be destructive[13,17,18]. Instead, a novel method for the evaluation of papillary variance, optical profilometry using a Keyence VK-X microscope (KEYENCE, Osaka, Japan), was utilized.

Optical profilometry is a two-part process during which a laser scans the surface of an object, analyzing the distance from the source. This data is then overlaid on a picture of the surface, generating a visual “roughness profile” for each sample, as well as a height heat map. This data is then integrated into a three-dimensional model of the surface. The microscope possesses nanometer level resolution and produces images of similar quality to those seen in

SEM. Unlike SEM, however, optical profilometry requires minimal prep, without complete desiccation required. This process allowed for faster visualization without tissue loss. Once characterized, anatomical variation could be related to psychophysical ability, providing a concrete way to investigate the role of the filiform papillae in viscosity perception.

Taking this information together, we arrive at two primary objectives for this study. First, characterize the perception of high-viscosity solutions in the oral cavity via psychophysical testing. Utilizing tissue isolation, the relative contributions of both the tongue and the palate can be assessed. Second, determine what role, if any, the filiform papillae may be playing in viscosity perception. By correlating papillary traits to psychophysical performance, we can assess not only the role the structures may generally play, but also how individual variation in these structures may influence viscosity perception. We hypothesize that the tongue will show a greater acuity for viscosity perception due to its role in product manipulation and possession of papillary strain amplifiers, and that the perception of viscosity on the tongue will be related to the filiform papillary attributes of length, width, and density.

Methods

Subjects

59 participants (age 19-40, 18M/41F) were recruited via the Ohio State University Consumer Sensory Database. Individuals completed a pre-test screener (Qualtrics, Provo, UT) including questions on age, physical limitations or back problems, food allergies or sensitivities, smoking status, presence of oral diseases, and willingness to undergo the biopsy procedure. As with previous studies in this lab, the age range for recruitment was restricted to the ages of 18-40 to limit any potential decreases in acuity attributable to age [19,20]. Individuals with a self-

reported history of an oral disease or condition, food allergies or sensitives, back or mobility problems that would prevent them from transferring to the dental chair or completing the physical task, as well as smokers, were excluded. Subjects were instructed not to eat food or drink anything other than water one hour prior to their testing sessions. Each subject completed the psychophysical testing individually during two, 1-hour sessions one week apart. After completion of psychophysical testing, subjects underwent the biopsy protocol. All biopsies were completed on the same day of the week in batches of 15 by a practicing oral surgeon. Biopsies were taken between 4-14 days after the last psychophysical testing session. Subjects were compensated $70 for their participation in the study, $20 after each of the psychophysical sessions, and $30 following completion of the biopsy procedure. All parts of this study were approved by the Ohio State University Instructional Review Board (2013B0277, psychophysical protocol and 2018H0509, biopsy protocol). Data was collected under the written and informed consent of each subject.

Viscosity Discrimination

Viscosity discrimination ability for high-viscosity solutions in the oral cavity was determined using the forced-choice, up-down staircase method to assess participants' Just-Noticeable-Difference (JND) threshold for viscosity [21,22]. As previously stated, "high-viscosity" solutions are typically evaluated in the mouth at a shear rate between 10-50s^{-1} [3]. Thus, stimuli were developed that had apparent viscosities above 1000cP across this range of shear rates when measured with a parallel plate rheometer (Anton Paar, Graz, Austria). Stimuli consisted of nine glycerol/water/carboxymethyl cellulose (CMC) solutions, one reference solution ([CMC]=1.125% w/v, $\bar{\eta}$ =5068cP ± 270cP) and eight variable solutions ([CMC] = 1.1538%-1.5% w/v, $\bar{\eta}$ =5507cP-11840cP ± 321cP). Variable solutions increased in concentration

of CMC in 0.03125% w/v increments. All solutions were made with a 55:45 reverse osmosis (RO) water/glycerol solution. Glycerol (Essential Depot, Inc., Sebring, FL) was utilized in this mixture due to its known synergistic thickening with CMC (Sigma-Aldrich Corp., St. Louis, MO) [23]. Solutions were stored in sealed, plastic containers at ambient temperature (20-23°C) before being portioned out into 10mL slip-tip syringes outfitted with 2cm of flexible, plastic tubing, for the duration of testing.

Viscosity discrimination ability was evaluated in three different blocking conditions: an Unblocked (UB) condition wherein individuals evaluated solutions using their entire oral cavity, a Palate-Blocked (PB) condition wherein individuals evaluated solutions with only their tongue while wearing a custom-molded mouth piece to block their hard palate (Figure 1A and B), and a Tongue-Blocked (TB) condition wherein individuals evaluated solutions using only the surface of their palate while wearing a flexible, silicone mouthpiece to block the tongue's surface (Figure 1C and D). Physical barriers, as opposed to topical numbing agents, were used due to the challenges of achieving complete numbing seen previously [24]. Two of these evaluations would occur during one of the two sessions, while one evaluation was completed during the other session. The order of these conditions and number of conditions per session was randomized and counterbalanced across participants.

During the first psychophysical session, participants would complete the informed consent and a brief demographic questionnaire before molding their PB mouthpiece (Figure 1A and B). The mouthpiece was created by pouring 71°C water over 30mL of InstaMorph moldable plastic beads (Happy Wire Dog, LLC, Scottsdale, AZ) and allowing the beads to sit for 105 seconds. The beads were then removed from the water by the researcher and squeezed to remove any remaining liquid. The participant was instructed to bite down into the plastic and spread it

across the palate using their thumbs, making sure to cover the palate completely, until at least the third set of molars. Upon completion, the mouthpiece visually inspected by the researcher to ensure suitable coverage from the piece, and the mouthpiece was then placed into ambient temperature water until it became opaque, indicating the mouthpiece had hardened. Once hardened, the participant would test fit the mouthpiece again for comfort and to ensure that the piece did not cause gagging. In the event the piece was uncomfortable, it would be remolded using the same procedure as above.

TB mouthpieces were cut from 1mm thick silicone baking sheet (Figure 1C and D). The mouth pieces consisted of a rounded portion that was inserted into the mouth to cover the tongue, as well as two “handles”, extra pieces of mat that would sit against either cheek for the participant to grasp (Figure 1C). Participants held the handles during the evaluation and pulled slightly outward to prevent the mouthpiece from sliding during the evaluation (Figure 1D). Mouthpieces were available in two sizes that varied in the width of the insertable section. Based on visual assessment, researchers would provide participants with a mouthpiece of a given size. In the event the mouthpiece was uncomfortable or did not completely cover the front 2/3 of the tongue, the other size was used.

As the final step before both testing sessions, participants would rinse with a concentrated Gymnema leaf extract solution (Bio-Botanica, Inc., Hauppauge, NY) made with 2mL of extract (~1200mg of gymnemic acid) and 6mL of RO water for one minute before expectorating the solution. Gymnemic acids from the Gymnema plant have been shown to inhibit the perception of sweetness by binding to taste cells involved in sweetness perception [25].This rinse was done to help block any sweetness coming from the glycerol in the test solutions. While the solutions should be isointense in terms of sweetness (as all solutions contained the same proportion of

glycerol to water), rinsing helped to guarantee that the only perceivable difference between the solutions was viscosity.

Once blocking was completed participants were seated in a dental chair and reclined to allow researchers access to the oral cavity. A trashcan was placed between the participant's knees, ensuring that one leg was in contact with the edge of the container. Participants were then instructed on the evaluation procedure and allowed to ask questions if they had any. They were provided with paper towel to clean their hands and the mouthpiece while testing before being blindfolded to prevent solution discrimination based on visual cues. During the study, participants evaluated pairs of solutions one of which was always the reference solution ($\bar{\eta}$ =5068cP ± 270cP), and another, variable solution. One at a time, 0.5mL of the solution was applied to the dorsal medial tongue. Once the solution was fully applied, the researcher would give a verbal signal, and the participant would press the applied solution against the rugae of the palate three times before the solution was removed by the participant using a KimWipe (Kimberly-Clark, Irving, TX). In the event there was any residual solution, more wipes were provided as needed. KimWipes were disposed of in the provided trashcan. The short, three-press evaluation was chosen to help standardize oral movement during evaluation and limit salivary incorporation. Wiping was used instead of expectorating or swallow to help minimize cues comping from the lips or esophagus, respectively. The hands, particularly with the KimWipe buffer were thought to be less sensitive than either of these tissues [2].

Once the first solution was removed, the second solution was applied in a similar fashion. However, before participants removed solution two from the mouth, they were asked to provide an answer – either one or two – as to which solution they felt was thicker. If they felt the solutions were of equal thickness, they were informed that they would still need to choose one of

the two solutions. In the event they chose the thicker solution, the variable solution in the next trial decreased in viscosity, becoming closer to the reference solution. If they chose the thinner solution, the two solutions got farther apart. This process was repeated until eight reversals were achieved. A reversal consisted of a change in the participant's direction on the staircase, either an incorrect response followed by a correct response or a correct response followed by an incorrect one. In the event a participant correctly identified the thinnest variable solution (ceiling) or incorrectly identified the thickest one (floor) this was also counted as a reversal as no thinner or thicker solutions were available. In the trials following these events, participants would be presented with the same comparison as in the previous trial. Average JND was calculated by subtracting the apparent viscosity of the reference solution ($\bar{\eta}$ =5068cP ± 270cP) from each of the reversals and averaging the resulting values. Solution presentation order was randomized and counterbalanced across the trials.

In sessions with two evaluation conditions, participants were given the option to take a brief break (< 2min) before returning to the chair for the second condition. The participants also completed a 30s maintenance rinse using the gymnemic acid solution as needed. In the blocked evaluation conditions the evaluation protocol was the same as in the UB condition, however, participants were instructed to either press the solutions against the plastic mouthpiece in the PB condition, or to press the silicone mouthpiece (which the solution was applied to) against the palate in the TB condition. In the PB trials, participants were instructed to wear the mouthpieces continuously during each paired comparison, wiping the mouthpiece and oral cavity between trials, while in the TB trials participants were allowed to remove the mouthpiece for cleaning between solutions. This decision was made due to the difficulty some participants had removing

the PB mouthpieces, which was viewed as a potential distractor if done between compared solutions.

Lingual Biopsy

Biopsies were completed in the Department of Oral and Maxillofacial Surgery at the Ohio State University's College of Dentistry by a trained oral surgeon. After obtaining surgical consent and acceptable vital signs, participants' tongues were isolated and retracted by a surgical assistant while local anesthesia was administered by the surgeon. 1.8ml of 2% lidocaine with 1:100,000 epinephrine was locally infiltrated at the surgical site. After 5 minutes had passed and vasoconstriction was visualized, the tongue was again isolated and retracted for the biopsy procedure. A 5mm biopsy punch (Figure 2) was used to incise through mucosa and submucosa in the middle third of the dorsal tongue to the right of the midline, and tissue was removed using forceps (Figure 2). Once removed the biopsied tissue was placed in a neutral phosphate-buffered 20% formalin solution at 20°C and sealed while the wound was closed. The samples were then stored in the fixing solution at 4°C for one week, prior to anatomical characterization.

Anatomical Characterization

Anatomical characterization was completed for a subset of participants (n=45) using optical profiling (Figure 2). Only a subset of participants were analyzed either due to a failure to attend the biopsy session (n=4), or due to blood present on the surface of the sample during fixing (n=11) preventing the surface structures from being sufficiently visualized. For this study, a Keyence VK-X250 (Keyence Corporation, Itasca, IL) 3D laser scanning microscope was used. Biopsied samples were removed from the fixative, rinsed with DI water, and placed on a disposable, glass microscope slide for the evaluation. The slide was placed on the stage of the

analyzer with the papillae oriented toward the top of the stage when possible. Eight images were taken using the associated imaging software from across the sample using 10x optical zoom, being careful to minimize overlap between the images. While the majority of the samples contained only filiform papillae, a small subset of the samples (n=5) did have at least one fungiform papilla present. To ensure accurate density measurements, all images taken excluded these structures. Following imaging, samples were placed in cryoprotectant (glucose and 1% Phosphate-Buffered Saline) and stored at -80°C.

Papillary data was analyzed using Keyence Multifile Analyzer software (Keyence Corporation, Itasca, IL). Prior to measuring, all samples were corrected for any surface tilt or general curvature. Papillary length was determined using the profile tool to draw segments from the base to tip of papillae, parallel to the direction of the papillary body using either the 2pt line tool or circle tool (Figure 2A and D). Curves were then smoothed by average weight to minimize noise in the curve coming from the papillary surface. Finally, the Continuous Segment (CS) length tool was used to measure the length of the curve, measuring from the local minimum at the base of the papilla to the inflection point at the tip (Figure 2D). As papillae are not flat structures, taking the segment length (as opposed to the absolute distance along the X and Y planes), factored in distance in the Z plane. Papillary width was measured similarly, drawing a 2pt segment perpendicular to the base of the papillae inside the interpapillary furrow (Figure 2B and E). However, as diameter did not depend on the surface profile, width was quantified using the 2pt segment tool, measuring at the inflection point on either side of the papilla (Figure 2E). Three length and width measurements were taken per image.

Papillary density was calculated by visually identifying the number of papillae present in an image (Figure 2C and F). Papillae were considered separate if there was a clearly identifiable

base wherein hair structures attached or if structures were clearly separated by an interpapillary furrow. Using the plane tool, count data was collected, marking each papilla with an x to prevent double counting. In the event there was an incomplete papilla, the structure was only counted if the base was in the frame. In the event only the hair structures were present, the papilla was not counted. Density was determined by dividing the count data by the area as calculated by the XY-measure function (Figure 2F).

Data Analysis

Average viscosity JND was analyzed using a one-way analysis of variance (ANOVA) in SPSS Statistical Analysis Software (IBM, Armonk, NY) with JND as the dependent factor and a Tukey post-hoc to determine if any of the blocking conditions showed a significant difference in acuity (α=0.05). Blocking condition and participant were used as the main effects. To analyze papillary data, values were averaged across images to determine an average papillary length, width, and density for each individual. Papillary attributes were then correlated to PB JND using a linear correlation (α=0.05). Additionally, causal analysis was run using the linear regression tool to determine the impact of the average measured papillary attributes of length, width, and density, as well as demographic information including age and gender. The model was executed in a stepwise fashion, removing attributes that did not meet the criteria for inclusion ($p>=0.100$).

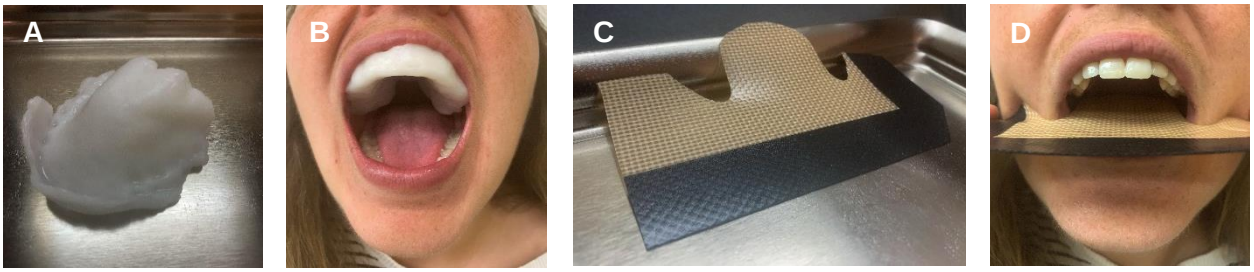

Figure 1: Example mouthpieces for the Palate-Blocked (PB) and Tongue-Blocked (TB) evaluation conditions. PB mouthpieces were made of InstaMorph moldable plastic and custom-molded to the participants' oral cavity prior to testing (A and B). All mouthpieces covered the hard palate past at least the third molar. TB mouthpieces were cut from a reusable silicone baking sheet (C and D). Mouthpieces were available in "Large" and "Small" sizes, and sizing was determined by visually ensuring adequate tongue coverage while maintaining participant comfort.

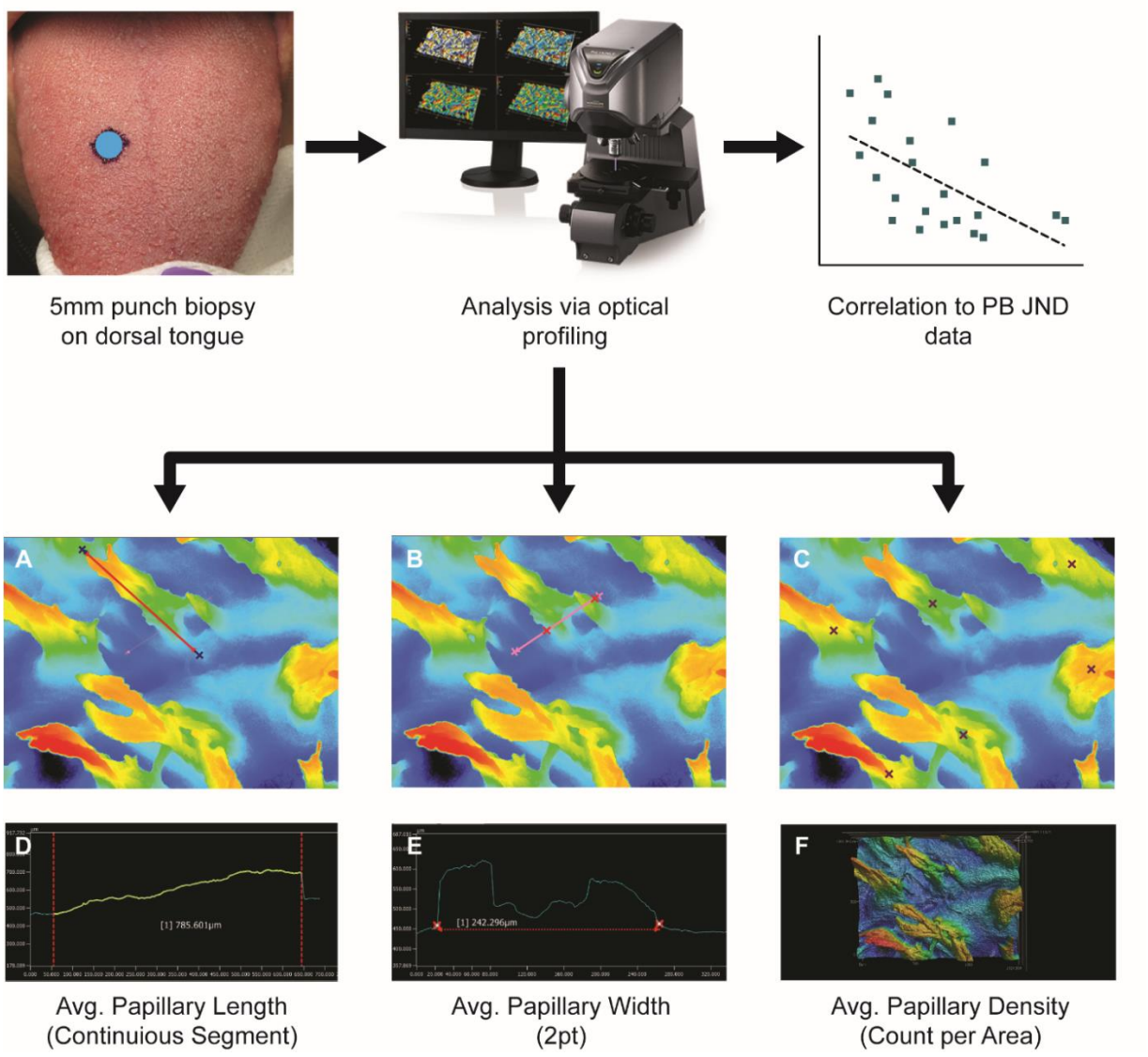

Figure 2A-F: Technique for biopsy analysis via optical profiling. Samples were obtained via a 5mm punch biopsy of the medial, dorsal tongue epithelium adjacent to the midline and fixed in 20% formalin for one week at 4°C. Samples were then analyzed using optical profiling to obtain three-dimensional surface models of the tongue surface. Eight images were taken per sample at 10x optical zoom (example images: A-C). Average papillary length was determined using the continuous segment tool (A and D), measuring for the local minimum at the base of the papillae to the inflection point, where a sudden drop in height was noted. Average papillary width was

determined using the two-point distance tool to calculate the separation between the inflection points on either side of the base of the papillae (B and D). Length and width measurements were taken in triplicate on each image for all eight images and the mathematical average was taken to determine the average length and width for each individual. Average papillary density was measured using the count tool to count the papillae in each image. Partial papillae were included only if the papillary base was visible in the image. Area was standardized across counts and then the mathematical average was taken across images to determine an individual's average density. Average values from all three measurements were then correlated to PB JND data to determine relation.

Results

Viscosity Discrimination

There was a significant difference found between the JNDs in viscosity for the different psychophysical conditions ($F= 13.521$, $p<0.001$). On average, individuals' UB JNDs ($\overline{\Delta\eta}$ = 1041.71cP ± 50.36cP) were significantly lower ($p<0.001$, both) than their JNDs in the PB ($\overline{\Delta\eta}$ = 1671.26cP ± 142.77cP) or TB ($\overline{\Delta\eta}$ = 1791.42cP ± 117.42cP) conditions; however, there was no significant difference between the JNDs in the blocked treatments ($p=0.724$, Figure 3). Additionally, there was no significant correlation ($R^2=0.0014$, $p=0.773$) between performance in the PB and TB conditions. That is to say that just because an individual was sensitive to differences in viscosity with one tissue did not necessarily mean they were sensitive to differences with the other. These findings are not in agreement with the initial hypothesis that the tongue would be chiefly responsible for viscosity perception in the oral cavity and instead suggest a larger contribution from the hard palate than first postulated.

Anatomical Characterization

Characterization of filiform papillary length, width, and density indicated a greater diversity in size and shape than originally anticipated (Figure 4A-D, Figure 5A-C). While average papillary length was 774.74μm ± 29.15μm, values ranged from 462.94μm to 1177.21μm with four individuals with averages below 500μm and four individuals with values over 1100μm. Average length did not correlate with average width (R^2= 0.017, p=0.385) or average density (R^2=0.012, p=0.469), however the two categories were slightly related (R^2= 0.089, p=0.047). When correlated to PB JND, significant relationships were seen between length and PB JND (R^2=0.337, p<0.001) and density and PB JND (R^2=0.178, p=0.005) (Figure 6A and B). There was an inverse relationship present for both attributes with individuals with more, longer, papillae showing lower JNDs, translating to a higher acuity. Width was not significantly correlated to psychophysical performance (R^2=0.001, p=0.996) (Figure 6C).

Further investigation via causal analysis indicated that the significant filiform papillary attributes of length and density were not only strongly correlated to PB JND, but also had a causal relationship with an individual's acuity (Adj. R^2=0.412, p<0.001, Table 1). Papillary length appeared to play the most significant role in predicting an individual's PB JND (β= -0.496, p<0.001), while density was also highly significant (β= -0.385, p=0.004). The other attributes tested in the model, papillary width, age, and gender, were not found to be significant. Moreover, when all non-significant terms were removed, there was an increase in model-fit (Adj. R^2=0.431, p<0.001), the highest of any of any of the models built using the five terms. Data here are in agreement with the second hypothesis, suggesting viscosity perception on the tongue is significantly related to filiform papillary attributes.

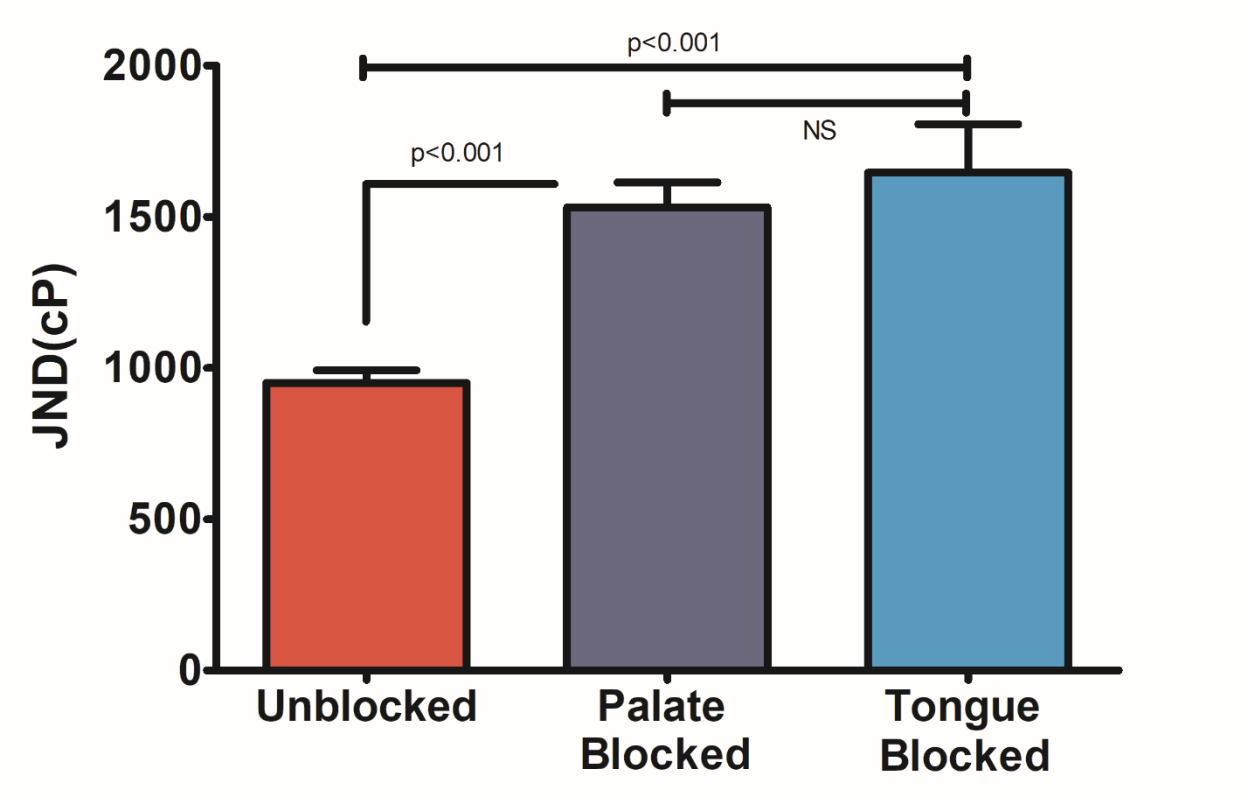

Figure 3: Average Viscosity JNDs for the oral cavity and isolated tissues. Just-Noticeable-Difference (JND) thresholds were calculated by subtracting the apparent viscosity of the reference stimuli from each of the eight reversals and then taking the mathematical average of those values. Values were compared using a one-way ANOVA with JND as the dependent variable (α=0.05, Tukey post-hoc). Individuals were significantly better ($p<0.001$, both) at discriminating between viscosities with both tissues than in either blocked condition, but there was no significant difference between the two blocked conditions ($p=0.724$). Error bars represent standard error of the mean.

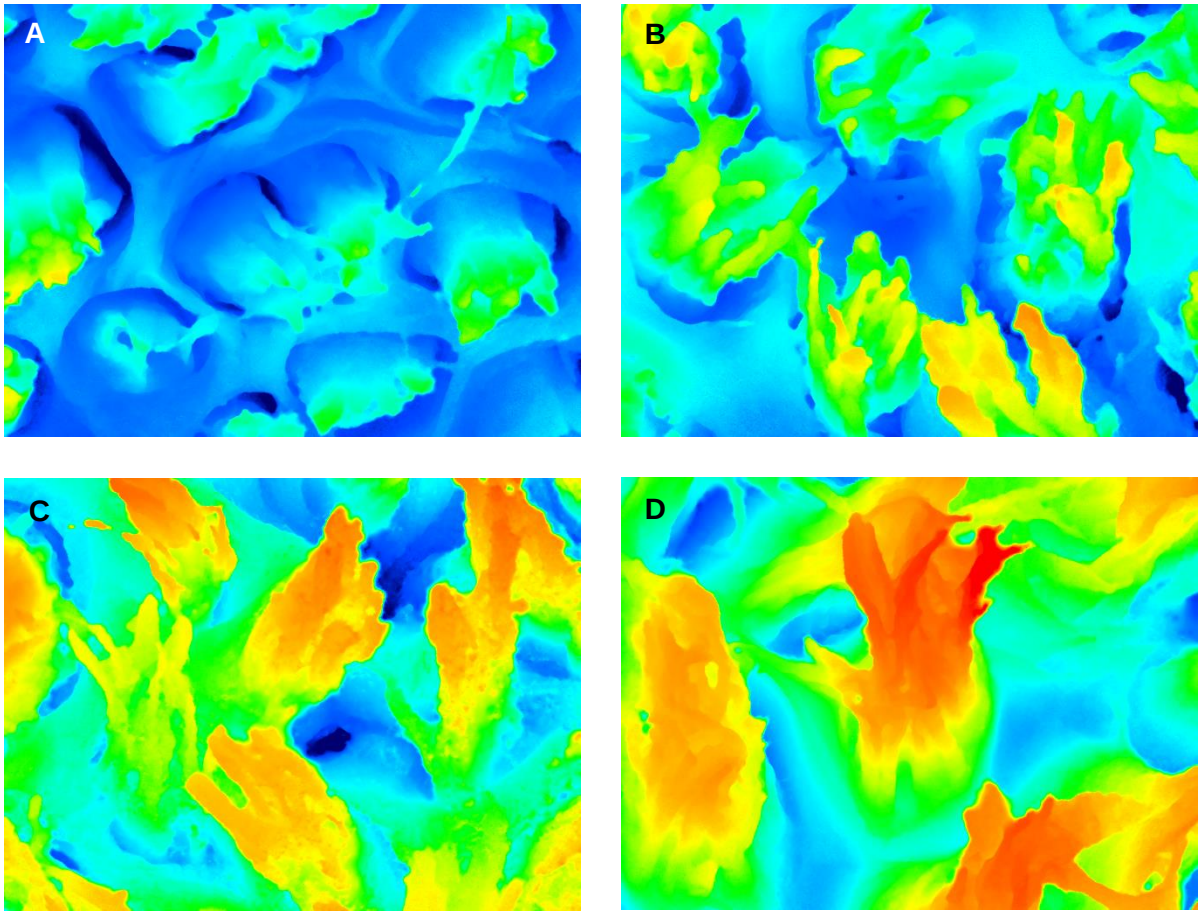

Figure 4(A-D): Visualization of representative papillary length variation across participants. Images include height heat maps from the participants with the shortest papillae (A, $\bar{h}$=462.95μm), the longest papillae (D, $\bar{h}$=1177.21μm), and representative samples participants from the lower and upper quartiles (B, $\bar{h}$=546.24μm and C, $\bar{h}$=818.71μm, respectively). Above images standardized to uniform height scale ranging from dark blue to red for clarity (89.359μm-1086.593μm).

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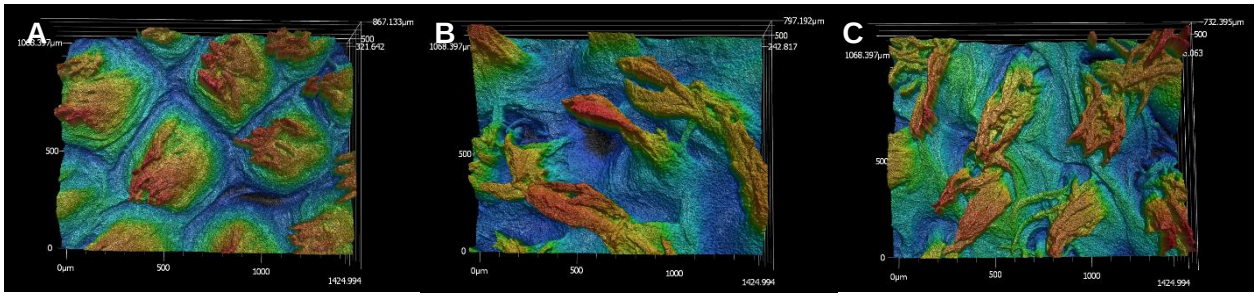

Figure 5A-C: Surface models of tongues of individuals with equivalent PB JNDs. While pictured individuals show a high degree of diversity in structure shape, length, and density all individuals show a similar and high level of acuity (Avg. JND = 714.95cP±158.75cP). Multiple different phenotypes appear to be equally as successful at discriminating between high-viscosity fluids.

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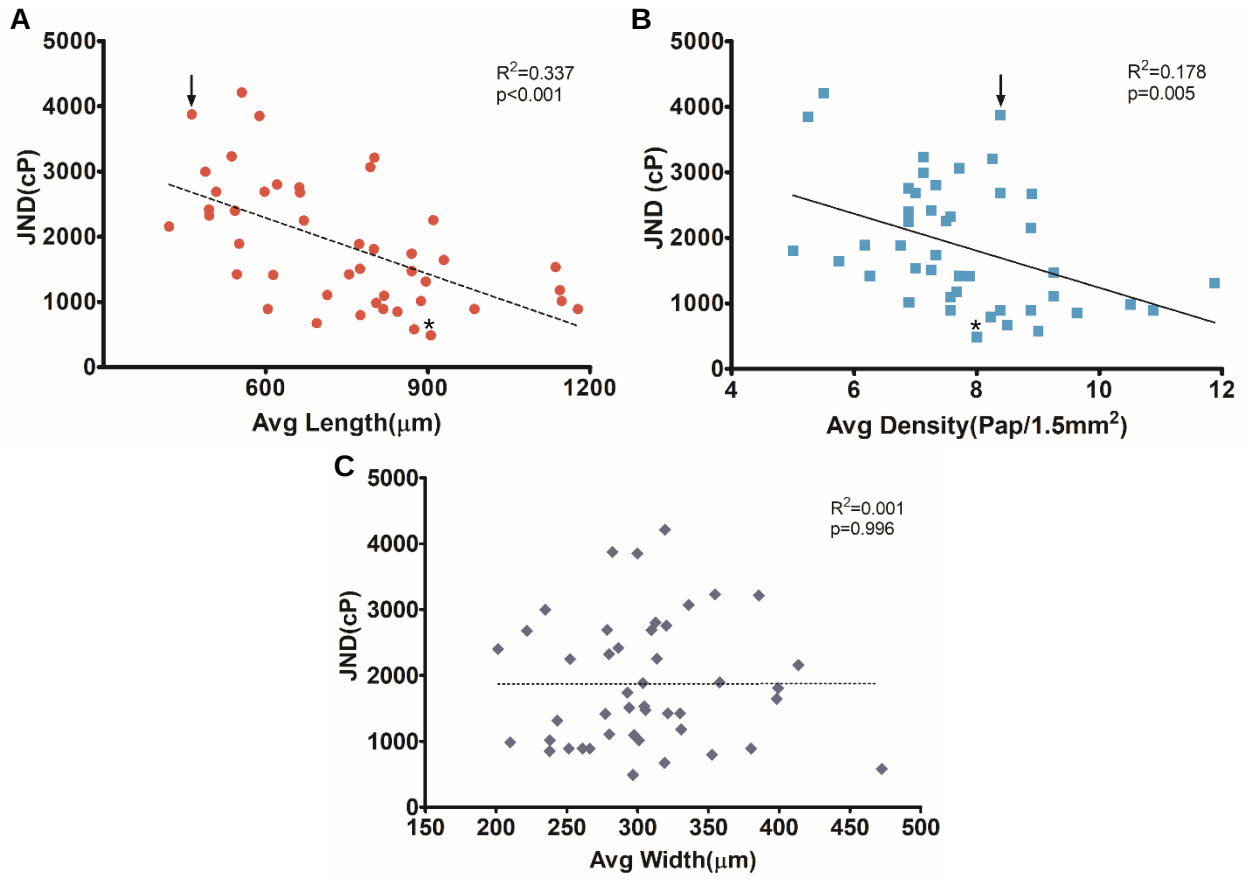

Figure 6A-C: Linear correlations of key papillary attributes and average Palate-Blocked JND. Palate-blocked JND was correlated to papillary attributes of length, width, and density using a linear correlation. Average papillary length and density were significantly correlated to JND ($p<0.001$ and $p=0.005$, A&B, respectively), but papillary width showed no significant correlation ($p=0.996$, C). Arrow (↓) in A and B denotes the same subject, relevant for much higher JND than estimated by density. Asterisk (*) in A and B denotes the same subject, relevant for much lower JND than estimated by density.

Table 1:Causal analysis of variation in Palate-Blocked JND. A linear regression was completed utilizing all collected variables to assess their relative contributions to variation in PB JND. The proposed model was highly significant ($p<0.001$). Average papillary length and papillary density were both significant drivers of variation ($p<0.001$ and $p=0.004$, respectively). Average papillary width, age, and gender were not significant.

Model*	Unstandardized Coefficients		Standardized Coefficients	t	p-value
	B	Std. Error	β		
(Constant)	6963.216	1532.168		4.545	<0.001
Pap. Length	-2.447	0.605	-0.496	-4.043	<0.001
Pap. Density	-266.673	86.158	-0.385	-3.095	0.004
Pap. Width	-0.704	2.172	0.041	-0.324	0.748
Age	-17.573	27.462	-0.083	-0.640	0.526
Gender	-325.515	264.949	-0.163	-1.229	0.227

*$p<0.001$, Adj. $R^2=0.412$

Discussion

Viscosity Discrimination

Based on the JNDs of the three psychophysical conditions it becomes immediately clear that the tongue is not the sole or even the main structure involved in viscosity perception in the

mouth (Figure 3). Instead, it appears that, on average, the tongue and palate may play an equal role in viscosity perception. At the individual level, however, the lack of correlation between the TB and PB JNDs, as well as a lack of any correlation of the blocking conditions to each other suggests that there may be multiple, different, tissue-specific mechanisms at play. This may allow individuals to compensate for a lack of sensitivity of one tissue with sensitivity of the other. Only in the blocked conditions, wherein the compensatory or insensitive tissue is removed, can a structure-specific mechanism be evaluated. A similar phenomenon has been observed when assessing size using these tissues. Engelen, et al. (2002) found that the palate hindered the estimation of the size of large (4-9mm) spheres (attributed to a limited contact area between the sphere and the hard palate) when compared to evaluation with the tongue alone [26]. This finding further underscored the decision to compare papillary traits to the PB JND values, not UB JND ones. Additionally, it is challenging to compare JND values obtained here to values reported previously for two reasons. First, most reported values have been obtained via magnitude estimation, not paired comparison, and second with such low viscosities used in other studies, it is unlikely that the perceptual mechanism proposed here is being utilized. Thus, the psychophysical curves generated in previous studies likely do not apply here.

The relatively equivalent contribution from the palate also highlights an important area for future study. Immunohistochemical studies in rats found that the palatal rugae are innervated with both Merkel discs (SAI) and Messiener corpuscles (RAI), two kinds of specialized, mechanoreceptive nerve endings [27]. While similar innervation studies have not been carried out in human palatal tissue, it is possible the equivalent structures are similarly innervated. Thus, a future study into what drives viscosity perception on the palate focusing on the rugae, similar to the one undertaken here on the filiform papillae on the tongue, could provide insight into

another mechanism of oral viscosity perception. Attributes such as rugal size, shape, density, coverage, and overall tissue keratinization could prove to be informative.

Anatomical Characterization

While viscosity perception in the oral cavity is more multifaceted than originally hypothesized, anatomical characterization does appear to suggest that the perception of high-viscosity solutions on the tongue is related to the filiform papillary attributes of length and density (Figure 6A and B). Moreover, causal analysis finds that variation in PB JND is sufficiently explained by these papillary attributes alone (Table 1). Papillary length appears to be the most relevant attribute and can also serve to explain individuals with unusually high or low JNDs for their density (See ↓ and * in Figure 6A and B). Though these individuals had approximately equivalent papillary densities (8, * vs 8.38, ↓), ↓ had a much higher JND than anticipated (JND = 3874.63cP), while * had a much lower one (JND = 488.00cP). When looking at the length for these individuals the cause of their extreme nature becomes apparent. ↓ had a very short average papillary length (Avg. length = 462.95μm), while * had a much higher average (Avg. length = 905.24μm). This points to the idea that density may not be relevant when structures are too squat to deform or, if innervation is below and not within the papillae, potentially too small to generate sufficient strain on the tongue's surface for signal transduction. Similarly, if structures are long enough, the converse is likely true. This confirmation of the mechanosensory relevance of these structures in viscosity points to their potential relevance in other textural percepts.

It is also important to note that sample analysis via optical profiling also indicated a larger degree of variance in papillary shape and size than originally suggested (Figure 4A-D and Figure 5A-C). While values were not as low as the 250μm proposed in the modelling papers,

structures ranged in average length by over 600μm (Figure 4A-D) [8,9]. This degree of variation present may explain the inconsistencies in the values previously reported in anatomical studies [12,13]. Additionally, there does not appear to be a specific, optimal papillary geometry that is more or less sensitive to these viscosity differences. However, future investigation into additional papillary attributes including hair-like projection length and number, as well as overall papillary rigidity may provide further insight into this mechanism.

Conclusions

Findings from this study serve to confirm an idea previously only suggested theoretically: the perception of high-viscosity solutions on the tongue involves the deformation of the filiform papillae. However, findings also serve to highlight the fact that the tongue is not the sole structure involved in oral viscosity perception. Instead, the palate and tongue are playing approximately equal roles generally, while at an individual level there is a differential utilization of the tongue and palate when discriminating between high-viscosity solutions. It is even possible that individuals may be able to overcome a lack of sensitivity in one tissue by compensating with the other.

Acknowledgements

The author would like to thank Tabitha Herron from the Department of Oral and Maxillofacial Surgery for her assistance coordinating and scheduling biopsies for test participants. Research support was provided by state and federal funds appropriated to The Ohio State University, Ohio Agricultural Research and Development Center.

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