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Assessment of midline lingual point-pressure somatosensation using Von Frey Hair monofilaments

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TITLE:

Assessment of Midline Lingual Point-Pressure Somatosensation Using Von Frey Hair Monofilaments

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KEYWORDS:

somatosensation, lingual, point pressure, touch, tongue, sensory

SUMMARY:

This work describes a standard method to assess tactile sensation at the midline of the tongue tip. Using Von Frey Hair (VFH) monofilaments, this protocol provides estimates of detection and discrimination threshold estimates for oral point pressure (OPP).

ABSTRACT:

Detection and discrimination threshold estimates for oral point pressure are assessed using Von Frey Hair monofilaments. Consistent with previously published protocols, threshold estimates are determined using a two-interval forced choice (2-IFC) paradigm with a three down-1 up approach. Detection threshold estimates determine the mean force in which a participant can identify the presence of pressure. During the detection threshold procedure, the participant is instructed to choose which of two sequentially presented observation intervals contained the tactile test stimulus. If the participant performs three correct detections in a row (i.e., 3 'hits'), the researcher decreases the stimulus to the next lower target force level. With one incorrect detection (a 'miss'), the researcher increases the force delivered to the next higher level. This threshold estimation approach is known as a 3-down/1-up adaptive staircase. Reponses are recorded on a paper ballot, and a participant's estimated threshold is defined as the geometric mean of five reversals. During the discrimination threshold procedure, the participant is asked to make a choice between two serially presented stimuli as to which is the "harder" or "stronger" pressure. The same scoring of 'hits', 'misses', and stopping points are used. Detection and

discrimination testing for oral point pressure at tongue midline takes approximately 20 min to complete. Using these commercially-available clinical tools, individual touch sensation profiles for the midline tongue can be achieved in a relatively time and cost effective means.

INTRODUCTION:

Any time we eat or drink, we determine the acceptability of a food based on multiple sensory percepts like taste, smell, and texture. Texture is not merely a physical property of the food; rather it arises from interactions of the food with the somatosensory (pressure and touch) system in the mouth. Flavor is the integrated percept that arises from multiple neuronal inputs, including taste, smell and oral touch[1]. Perception of food flavor, including pressure and texture information, is a key driver of food choice. Both common sense and data from numerous studies suggest people eat what they like[2]. However, in practice, this relationship between texture and food selection is more nuanced, as individuals avoid what they dislike[3]. Food selection is a behavior based on both cognitive choices and prior sensory experiences. Individual differences in smell and taste have a demonstrated ability to influence ingestive behavior[4] with large variation across individuals to the point of influencing chronic diet choices[5]. Thus, two people may eat the same food but react very differently to it in regard to the degree of liking of that food. However, the degree to which this food preference is driven by individual differences in oral somatosensory function, including tactile and texture appreciation, remains understudied. In fact, the influences and mechanisms of oral point pressure and texture perception are much less understood relative to other oral sensory systems. Recent data suggests there may be important individual differences in oral tactile sensation abilities[6-8]. Because oral touch information is internalized and individual in nature[9], it could drive individual preferences and influence food choices.

Measurements of peripheral nerve function through cutaneous pressure assessments require activation of slowly and rapidly adapting mechanoreceptors in the skin, including Merkel cells, Meissner corpuscles, Ruffini corpuscles and Pacinian corpuscles – with a high representation of slowly adapting mechanoreceptors in the face, lips, and oral mucosa that are responsive to pressure and slow stretch[10,11]. One relatively time and cost effective means of assessing oral tactile sensation is through the use of point-pressure assessments using Von Frey Hair (VFH) monofilaments. VFH monofilaments are widely used to assess peripheral nerve function through point pressure detection across the body, but specifically in glabrous (non-hairy) skin, including fingertip, hands, and feet[12-16]. In fact, testing with VFH monofilaments has demonstrated high test-retest reliability in the lips, tongue, and feet in healthy young, aging, and disordered populations[16,17].

Assessing oral point-pressure detection and discrimination is potentially one part of a full assessment of a person's individual oral touch profile. Better understanding of individual oral touch responses could inform food choice preferences in healthy and disordered populations. A complete assessment of individual oral touch and texture perception could allow clinicians to improve recommendations for healthy aging adults as well as people with specialized (dysphagia) diets to meet their individual nutrition needs necessary for health and wellness. Select clinical populations with dysphagia, as well as typically aging adults, may require altered food textures

to achieve adequate and safe nutrition; however, these clinical populations may also reject foods based on texture and mouth feel preferences[18,19]. Better understanding of the mechanisms underpinning food preferences that drive food choices, eating behavior, and diet compliance may provide novel targets for intervention, both at a systems level and an individual level.

The purpose of this assessment protocol is to characterize individual differences in oral point pressure (OPP) sensitivity by establishing detection and discrimination threshold estimates at midline tongue. This protocol uses Von Frey Hair monofilaments, commercially-available devices, to complete a relatively cost and time efficient assessment. Quantitative assessments of oral point pressure somatosensation using Von Frey hair monofilaments and this protocol were recently shown to be reliable in a cohort of healthy, young adults [17] for lateral edge of the lips and tongue as a prelude to future work in speech and speech disorders. However, the present protocol and recent work has focused on midline tongue due to its involvement with manipulating foods for tasting and safe and efficient swallowing [20].

PROTOCOL:

All procedures were approved by the Institutional Review Board for conducting human research at The Pennsylvania State University and were consistent with the Declaration of Helsinki.

1. Set-up

1.1. Display all monofilaments with target points between 0.008g (lowest factory setting) and 15 g (a priori ceiling determined during pilot testing).

1.1.1. Set out the monofilaments with target forces of 0.008, 0.02, 0.04, 0.07, 0.16, 0.4, 0.6, 1.0, 1.4, 2.0, 4.0, 6.0, 8.0, 10.0, and 15.0 g.

1.2. Set the monofilaments out on the table so each target level can be easily read.

1.3. Seat the participant in a comfortable chair with a glass of water within arm's reach.

1.4. Instruct the participant that when it is time, they will be asked to close their eyes and stick out their tongue.

1.4.1. Identify the approximate testing location at midline tongue. The testing location should be approximately 10 mm posterior to the tongue tip.

1.4.2. Encourage the participant to stop regularly and take a sip of water approximately every 5-10 trials or after the first incorrect response for either testing protocol, detection or discrimination.

CAUTION: The tongue should gently protrude from mouth such that the tongue tip meets the inferior edge of the lower lip. The participant should not be straining to stick their tongue out as far as possible or it will fatigue quickly and could alter testing results.

2. **Detection threshold estimates**

2.1. Instruct participant: Say to the participant: "For this test, you will hear me say 'Trial 1' and 'Trial 2'. You will feel a point of pressure in one of these trials. If you think you feel a point of pressure in trial 1, put up 1 finger. If you think you feel a point of pressure in trial 2, put up 2 fingers. If you're not sure, take your best guess. There will always be one point of pressure in one of the trials."

2.2. Identify the starting point for testing: Determine the starting point for all participants for the detection threshold estimates. Published protocol begins at 1.0 g. Researchers strongly suggest the experimenter begins at a supramaximal point to be sure that the majority of participants can easily identify the first target force.

2.3. Tell participant to close their eyes and start the trials

2.3.1. Say Trial 1 and pause. Say Trial 2 and pause. Be sure to deliver the pressure in one of these trials such that the monofilament is pressed to the tongue surface until the monofilament buckles. Hold for a second and release.

2.3.1.1. Use a random number generator to create a series of 1 or 2. Follow this series to randomly present the stimulus in Trial 1 or Trial 2 (See Supplementary Files 3 & 4). Do not follow a pattern.

2.3.1.2. Be deliberate and intentional when applying the pressure such that the stimulus is always clearly delivered in Trial 1 or Trial 2 with a 1-s pause before and after when the Trial is said and when the stimulus is delivered.

2.4. Record the participant's response (See **Supplementary Files 1-4** for examples).

2.5. Continue testing using a 3-down/1-up decision rule to select the next stimulus.

2.5.1. Move up to the next monofilament in the series after any incorrect or "missed" response.

2.5.2. Ensure that the participant gets three correct responses in a row for the experimenter to move down to the next monofilament in the series.

2.6. Stopping point

2.6.1. Stop testing when a participant has reached his/her stopping point. This is defined as the point when a participant has crossed over or received the test stimulus from the same target monofilament a total of five times.

2.6.2. If the participant reaches the lowest available testing target (0.008g), continue delivering

this target for 5 consecutive sets before stopping.

2.6.3. If the participant reaches the highest testing target (15 g) and cannot correctly identify the target trial, discontinue testing.

2.7. Estimate approximately 5-10 min to complete testing to determine a detection threshold estimate for midline tongue.

NOTE: Stopping points apply regardless of whether the participant gets that last trial correct or incorrect.

3. Discrimination Threshold Estimates

3.1. To complete discrimination threshold testing, follow a similar protocol to detection threshold testing.

3.2. Instruct participant, "For this test, you will still hear me say 'Trial 1' and 'Trial 2' but this time you will feel a point of pressure in both trials. I want you to identify which trial contained the stronger or harder point of pressure. If you think the harder/stronger pressure was in trial 1, put up 1 finger. If you think you the harder/stronger pressure was in trial 2, put up 2 fingers. If you're not sure, take your best guess."

3.3. Identify a starting point

3.3.1. Begin test at three monofilament levels above their detection threshold estimate stopping point. Use the target stimulus and the monofilament immediately below it in force as the two discrimination stimuli.

3.3.2. Follow this example: If the participant reached a stopping point of 0.008 g, start his/her discrimination testing at 0.07g as the target stimulus and the monofilament directly below it (0.04 g) as the second stimulus delivered.

3.4. Tell the participant to close their eyes and start the trials.

3.4.1. Deliver the two stimuli after clearly stating Trial 1 or Trial 2. Again, this should be randomized, to avoid a pattern.

3.5. Record the participant's response (See **Supplementary Files 1-4** for examples).

3.6. Continue testing using the same 3-down/1-up and stopping point as the protocol from Detection testing

3.6.1. Continue testing with 0.008g with no stimulus delivered in the second trial if the participant reaches 0.008 g during discrimination testing (lowest manufactured level).

3.6.2. Offer the participant a drink of water if they miss two target stimuli in a row.

3.7. Estimate approximately 5-10 min to complete a discrimination threshold estimate for midline tongue.

4. Scoring

4.1. Record all target stimuli and participant response data from threshold estimate testing.

4.1.1. Record the stopping point for each individual for both detection and discrimination

4.1.2. Record the number of trials it took for the participant to successfully complete testing

4.1.3. Record the participant's threshold average by finding the first time the participant was given the eventual stopping point stimulus. Add all the target stimuli delivered between the first instance of the stopping point target force and the last and divide by the total number of trials. This gives a threshold estimate average that takes in to account the variability within a participant.

4.2. Set up data recording sheets using one of the two suggested options. Find examples of completed sheets using the same values can be found in the Supplemental Materials. The first option (**Supplementary Files 1 & 2**) provides a data sheet and completed example in which the participant's responses and threshold estimate can be easily visualized and recorded on one sheet. The second option (**Supplementary Files 3 & 4**) provides a data sheet with randomized order for presenting the test stimuli. A separate sheet is needed for each test – detection or discrimination.

5. Cleaning equipment

5.1. Do not close the monofilaments during testing or immediately following testing until they are cleaned.

5.2. Place the nylon end of the monofilament between an alcohol prep pad (70% alcohol) and gently squeeze the alcohol pad together while pulling the monofilament through.

5.3. Leave the monofilament in the open position, repeat the cleaning process on each monofilament used.

5.4. Allow the monofilaments to air dry in the open position.

5.5. Close the monofilaments and store them in a clean and dry environment, away from direct sunlight, as described in the manufacturer's directions.

REPRESENTATIVE RESULTS:

When interpreting the results, it is important to remember that lower threshold estimates indicate greater sensory acuity. The use of a forced choice procedure helps separates criterion response bias (i.e., a willingness to say yes) from underlying sensitivity of the system. A lower threshold indicates an individual can perceive a lighter stimulus than those with a higher threshold estimate. Higher threshold estimates indicate decreased sensory acuity. A higher threshold estimate indicates an individual requires a larger input to cognitively perceive the stimulus. Higher threshold estimates could be due to difficulty in cognitively completing the task[16,21] or due to peripheral damage associated with advancing age or disorder to the cutaneous surface of the tongue or peripheral nerves[22].

Data is still being collected to further develop normative data from a sample of nominally healthy adults. However, a sample of data on individual differences in oral somatosensory function was recently published[7]. Combining previously published data with additional data, a total of 111 healthy participants (34M:77F; mean 32.1 years, range 18 to 68 years) completed at least one portion of the detection and/or discrimination threshold estimate protocol described here.

FIGURE AND TABLE LEGENDS:

Table 1: Detection and discrimination threshold estimates for pressure point sensitivity on midline tongue. The geometric mean, standard deviation and range are provided from our representative sample. Fifty one participants completed detection threshold estimate testing (10M:41F, mean 37.1 years). A total of 107 participants completed discrimination threshold estimate testing (31M: 76F, mean 31.9 years).

Figure 1: This figure presents data for midline lingual point pressure detection threshold estimate (g) by participant age (years). Note the y-axis ranges from 0.0 – 0.25 g. Participants across this healthy age span demonstrated low thresholds for point-pressure detection.

Figure 2: Figure 2 provides data for midline lingual point pressure discrimination threshold estimate (g) by participant age (years). Note the y-axis ranges from 0.0 – 16.0 g. Participants across this healthy age span demonstrated increased variability in discrimination threshold estimates as compared to detection threshold estimates.

SUPPLEMENTARY FILES: Two potential options for setting up data recording sheets are available in the Supplementary Files. Data can easily be recorded and visualized using a one page data collection sheet (**Supplementary files 1 & 2**). On this sheet, both detection and discrimination data can be recorded in their respective sections. The list of Von Frey Hair monofilament targets in grams (g) and the color of the pen are included on the left hand side. An asterisk (*) has been placed next to the 1.000 g target in the 'Detection' section to remind testers of the starting point for testing using this protocol. The trial numbers are listed across the bottom to easily identify the total number of trials needed. The authors suggest following the key listed on the bottom right to mark correct and incorrect participant responses. A second data recording option is provided in **Supplementary Files 3 & 4**. The series number is noted in the column marked #. The target or test stimuli is recorded in grams in the column labeled 'Target (g)". The next two

columns are labeled 'Trial 1' and 'Trial 2' to indicate in which the trial the tester should deliver the test stimuli. This pattern was created using a random number generator. Experimenters will present the test stimuli in the trial with the "X" in it. For example, in the first series, the target stimuli would be delivered in the second trial because the "X" is under the Trial 2 heading. Finally, in the last column, the experimenter can record the participant's response as correct (Y) or incorrect (N). This column can also be used to note the number of presentations of that target to aide in identifying the stopping point.

DISCUSSION:

In previous studies, researchers observed that periodic rewetting of the tongue was an important step. For example, participants who did not regularly rewet the tongue demonstrated poorer sensory acuity. While no attempts were made to systematically determine the optimal rewetting interval, experience suggests experimenters should ask participants to rewet the tongue every few trials by bringing the tongue back into the mouth and/or having a small sip of water. Although work by Verrillo and colleagues found that vibrotactile detection thresholds were not impacted by skin hydration, these studies were completed on the hand, forearm, and cheek – not the tongue[23]. Because the tongue is consistently bathed in saliva, the change from wet to dry could alter sensory acuity. In fact, in work assessing salivary production and flavor appreciation in older adults, researchers found a relationship between decreased salivation and perception of umami flavor[24]. Aside from lingual moisture, changes in point pressure detection and discrimination threshold estimates can be impacted by a number of individual factors, including advancing age[22] or changes in cognition[25] which may impact attention to task. Additionally, some previous studies have either excluded smokers or asked participants to refrain from smoking for a period of time prior to testing[26].

A blindfold was not used in this protocol as prior experience suggested participants find it distracting to be blindfolded with their mouths open and tongue out. In pilot testing of the protocol, participants were startled by the stimuli or repeatedly told researchers they couldn't concentrate. Therefore, participants were simply told to close their eyes. If a participant opened their eyes during a set of stimuli presentations, that set can be repeated. Other studies in the field have chosen to use a blindfold[20]; however, use of a blindfold may not significantly change the results when comparing across studies [11].

During initial trials of this protocol, researchers selected a ceiling of 15.0 g. Although available monofilaments go up to 300 g, a ceiling was selected a priori to prevent the risk of damaging the skin. Additionally, when testing an area like the tongue for which there is no internal skeletal structure, application of monofilaments higher than 15 g were found to move the entire tongue muscle which could activate sensory nerve endings in surrounding locations[17]. In the representative data sample, many healthy young adults were able to sense the 0.008 g target monofilament – the lowest manufactured Von Frey hair monofilament. Alternatively, Cochet-Bonnet contact aesthesiometers have been used to measure sensitivity of the cornea. It is possible this device may provide an opportunity to test at levels less than 0.008 g; however, there are unresolved questions related to calibration of Cochet-Bonnet devices and ability to share findings across studies [27].

This protocol is adapted from recent work by Etter and colleagues[17] with a few key differences. In Etter's original protocol, threshold estimates were not averaged across trials. This may have missed some of the variability in participant responses. Additionally, Etter's original protocol was focused on using the tongue for speech-related movements, and therefore assessed point pressure of tongue at the right and left lateral edges just posterior to tongue tip versus midline tongue locations that may be more important for texture appreciation and swallowing [28,29].

Future applications of this testing protocol may include studies of individuals with a variety of central and/or peripheral nervous system damage, as well as those with damage to the oral mucosa either from trauma, tumors, or post radiation therapy. For example, researchers recently evaluated individuals post-stroke who were experiencing oral dysphagia and noted increase thresholds, or decreased sensation in people with stroke as compared to healthy controls[30]. Additionally, in a review paper by Kaplan and colleagues, patients with xerostomia for a variety of medical reasons reported changes in mastication and swallowing, decreased salivary flow rates, and changes to oral mucosa [31]. Future work assessing the impact of these changes on tactile point pressure assessments would be beneficial to the field.

Finally, using this protocol does not require any mark to be made to the tongue to ensure exact testing location is repeated with each trial. This was originally done to increase clinical application of this protocol to medical and outpatient clinic locations. Reliability testing by Etter colleagues demonstrated high test-retest results without marking the tongue[17]. However, it is possible to mark the tongue using a dye to ensure a consistent target location as illustrated in a recent study by Santagiuliana and colleagues[26]. The appropriateness of using food dye to mark the tongue may vary depending on testing location and research question.

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DISCLOSURES:
The authors Nicole M. Etter, Scott P. Breen, and Maya I.M. Alcala have no conflicts to disclose; Breen and Alcala were students at the time the work was performed and are now employed in the food industry. The authors John E. Hayes and Gregory R. Ziegler have each received speaking or consulting fees from non-profit organizations and corporate clients in the food industry. Additionally, the Sensory Evaluation Center (Hayes, Director) at Penn State routinely conducts taste tests for the food industry to facilitate experiential learning for students. None of these organizations have had any role in study conception, design or interpretation, or the decision to publish these data.

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Figure

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Figure

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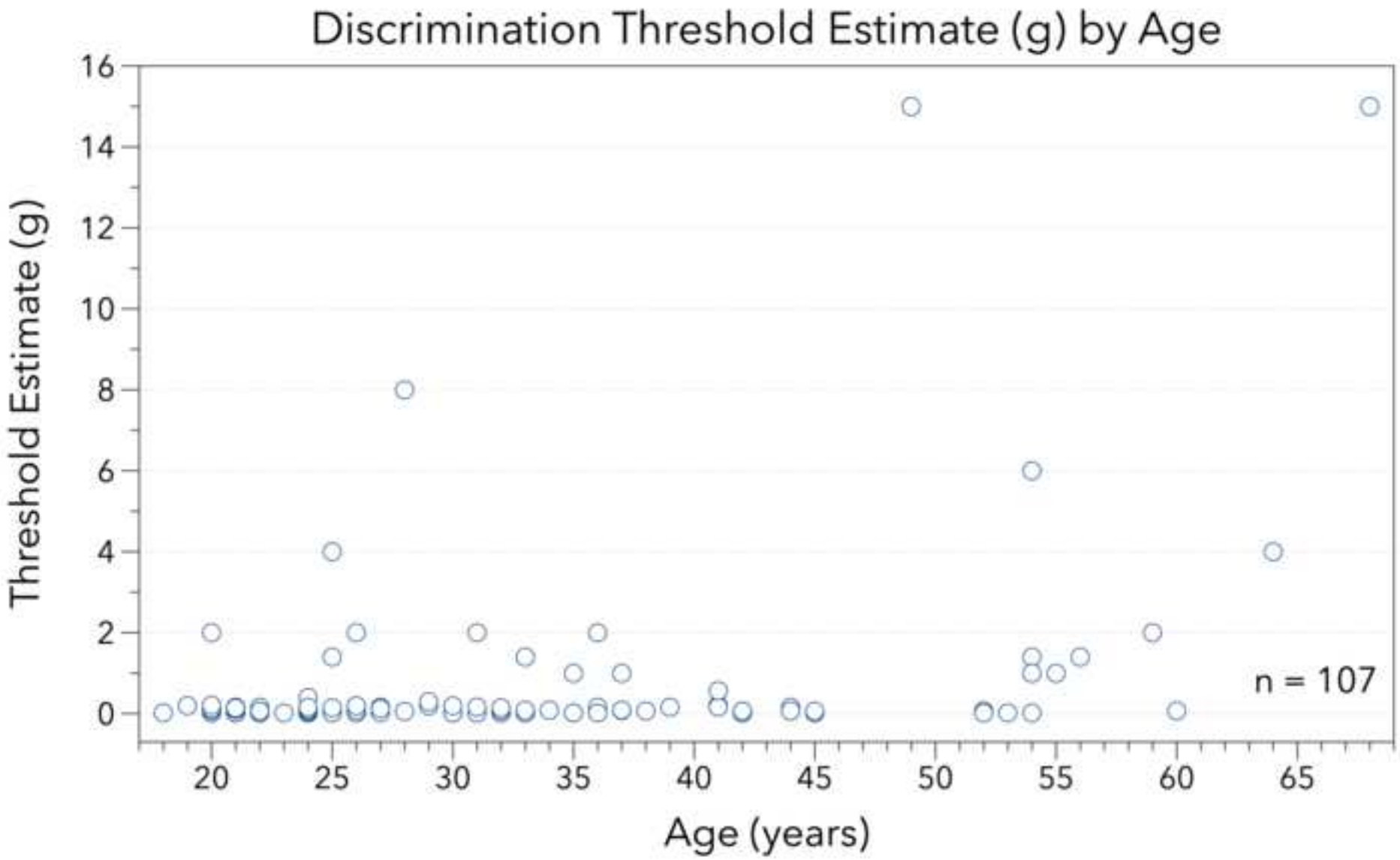


Table

	Detection (n= 51)		Discrimination (n= 107)	
Location	**M (SD)**	**Range**	**M (SD)**	**Range**
Midline Tongue	0.0157 (0.022)	0.008-0.16	0.600 (1.812)	0.02-15

Name of Material/Equipment	Company	Catalog Number	Comments/Description
plastic cup for drinking water	various		
Aesthesio Tactile Sensory Evaluator Kit	DanMic Global LLC, San Jose CA	514000-20C	

26 September 2019
Journal of Visual Experiments

Dr. Wu and Reviewers –

Please find enclosed our revised manuscript (60656_RO): **Assessment of midline lingual point-pressure somatosensation using Von Frey Hair monofilaments.** Please note title change.

Thank you for the thoughtful comments and questions. We have addressed each comment below and feel that your suggestions greatly improve the overall manuscript.

Editorial comments
The manuscript has been modified and the updated manuscript, 60656_R0.docx, is attached and located in your Editorial Manager account. Please use the updated version to make your revisions.

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.
    a. Thank you, prior to submission, we proofread the manuscript closely.
2. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]."
    a. On this revision, we were able to add currently unpublished data to our representative findings and therefore have created new tables and figures that have not been published.
3. Please provide a more specific title for the manuscript.
    a. To increase specificity, the title has been changed to "Assessment of midline lingual point-pressure somatosensation using Von Frey Hair monofilaments"
4. For in-text referencing, the superscripted reference number should be inserted before a comma or period.
    a. This has been corrected throughout the manuscript
5. Please revise the text in Protocol to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).
    a. Corrected in text.
6. Step 1.1.1: Please write this step in the imperative tense.
    a. Corrected in text.
7. Step 1.3: Please write this step in the imperative tense.
    a. Corrected in text.
8. Step 1.4.1: Please write this step in the imperative tense.
    a. This step has been removed and added in the Discussion.
9. Step 2.1.1: Please write this step in the imperative tense.
    a. Corrected in text.
10. Step 2.3.1.1: Please write this step in the imperative tense.
    a. Corrected in text

11. Step 2.5.1: Please write this step in the imperative tense.
    a. Corrected in text.
12. Step 2.6.1: Please write this step in the imperative tense.
    a. Corrected in text.
13. Step 2.7: Please write this step in the imperative tense.
    a. Corrected in text.
14. Step 3.1: Please write this step in the imperative tense.
    a. Corrected in text.
15. Step 3.3.1: Please write this step in the imperative tense.
    a. Corrected in text.
16. Step 3.3.2: Please write this step in the imperative tense.
    a. Corrected in text.
17. Step 3.6.1: Please write this step in the imperative tense.
    a. Corrected in text.
18. Step 3.6.2: Please write this step in the imperative tense.
    a. Corrected in text.
19. Step 3.7: Please write this step in the imperative tense.
    a. Corrected in text.
20. Step 4.1: Please write this step in the imperative tense.
    a. Corrected in text.
21. Figure 1: Please add a short description of the figure in Figure Legend.
    a. Figures for this manuscript were completely redone. New captions have been added under the Figure Legend section in text to provide the reader with more context for interpreting figures.
22. Please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:
    a. Critical steps within the protocol
    b. Any modifications and troubleshooting of the technique
    c. Any limitations of the technique
    d. The significance with respect to existing methods
    e. Any future applications of the technique
        i. Thank you, the Discussion section has been revised to include each of these topics.
23. Please do not abbreviate journal titles for references.
    a. References have been corrected to include the full names of journals, no abbreviations.
24. Please remove trademark (™) and registered (®) symbols from the Table of Equipment and Materials.
    a. The registered symbol has been removed from the text.
25. All tables should be uploaded separately to your Editorial Manager account in the form of an .xls or .xlsx file.
    a. The table has been converted to an Excel file with an .xlsx extension.

Individual Reviewers' comments

Reviewer #1:
Manuscript Summary:
These authors assessed lingual point-pressure somatosensation in healthy subjects, specifically determining thresholds for detection and discrimination pressures on the tongue via monofilaments. The writing is clear. the protocol used to detect and discriminate makes sense and appears appropriate. However, a major concern, per below, is the particular method used to perform the assessment.

Major Concerns:
My major concern is the methodology of assessing detection and discrimination thresholds. The method itself is clear for each. However, on P. 2, line 100 (1.4.2) the authors describe where to place the monofilament: "…just posterior to the tongue tip". This appears rather vague. How do the researchers know that this spot is the same across subjects? Healthy subjects have tongues of varying size. Why was a specific # mm or some other consistent measurement used for the location of sensory testing? Also, how far do the researchers have the subjects "gently" protrude the tongue? Would have been good here to have some consistency as well (i.e., to the outer labial margin, 1cm past lips, etc.). I'm not sure how reproducible this study would be without clearer parameters/descriptions. This should be addressed.

Response: Thank you for your comment. To clarify in text and increase the specificity and reproducibility of this testing procedure, we have added the approximate distance from tongue tip to testing location in millimeters as well as adding the suggested point for which participants should protrude their tongue. Additionally, we have added text to the Discussion regarding the test-retest reliability of this measure assessed in a previous study.

Minor Concerns:

1. What is the gender of the group included in this study? Was gender examined when looking at detection and discrimination thresholds?
    a. The participant breakdown for sex has been added to the Representative Results section. Gender was not examined as part of this study.
2. How are the researchers sterilizing the monofilaments, or are they single use/subject only?
    a. The monofilaments are not single use. The cleaning instructions have been added to the protocol.
3. Figures are a bit grainy and you cannot read the title/text on the y-axis.
    a. The figures have been improved, checked for readability, and uploaded with higher resolution.
4. Perhaps a brief discussion of the implications of this technique to assess detection and discrimination threshold as relates to those populations with impaired lingual anatomy and/or sensation would be in order. This appears to be a very useful tool to assess lingual sensation and could potentially be used with surgically and/or CRT- treated oral cancer patients, stroke patients, etc.
    a. Thank you for this insightful comment – we have briefly touched on this in the revised discussion section.

Reviewer #2:
Journal of Visualised Experiments - Manuscript # JoVE60656
Title: Assessment of lingual point-pressure somatosensation

This article describes a standard method to assess detection and discrimination thresholds for oral point pressure (OPP). Overall the rationale and introduction are clear. The protocol is clear and easy to follow.

General note: The figure are displayed in a very low resolution. Please increase the resolution for better visibility.

New figures with better resolution have been uploaded.

Some minor comments and suggestions for the authors on how the manuscript could be improved.

Abstract:
No comments.

Introduction:

1. The authors could explain in more details why food texture a key role plays in food acceptance and rejection, e.g. texture expectations.
    a. More information on why flavor and texture play a key role in food acceptability has been added to the Introduction.
2. It is unclear what the authors mean with 'two people may react differently', please clarify.
    a. Clarified in text.
3. Lines 74-75 contain twice the word 'recently', please remove one of them.
    a. Corrected in text.

Protocol:

4. Line 82: change 'open' into 'display'
    a. Corrected in text.
5. Lines 96-98 (section 1.4.1.): I am doubtful a blindfold can be a large distraction, I believe this is of very little relevance for the execution of the test. I understand a blindfold might be slightly less comfortable compared to closing of the eyes. However the authors underestimate the risk of subjects opening their eyes in case of uncertainty. I believe this risk is more relevant than the comfortability and possible distraction by the blindfold.
    a. We have removed the step regarding the blindfold from the protocol section and added more to the Discussion to further consider it. Briefly, in our experience, participants were uncomfortable with being blindfolded and having their mouth open to the point of being distracted or startled during testing.
6. Lines 100-101 (section 1.4.2.): Did the authors consider using a marking of the location of 'hitting'? A recent paper of Santaguiliana et al. (DOI: 10.1039/c9fo01211g) used a blue food colouring to mark the location on the tongue.
    a. We did not mark the tongue during this procedure, but it is something that could be done in future studies. This has been added to the Discussion section.

7. Line 129: I would recommend the authors to use one word to describe the bending/buckling of the monofilament.
    a. Corrected in text.

Representative results:
No comments.

Discussion:

8. Lines 240-248: This paragraph seems to fit better in the Introduction than the Discussion. Please consider moving this section to the Introduction.
    a. The first paragraph of the Discussion was moved to the Introduction.
9. General: The authors don't discuss the sensitivity of the lower limit of 0.008g, since the majority of the subjects in the study of Breen et al (2019) reached this lower level for the detection threshold. Is there a solution for this ceiling effect?
    a. In this sample of healthy young individuals, it is not too surprising that participants were able to achieve the lowest testing level of 0.008g at the midline tongue. We will briefly expand upon this in the Discussion.

Reviewer #3:
Manuscript Summary:
Thank you for the opportunity to review this manuscript on midline lingual point pressure testing (discrimination and detection) in healthy adults. The introduction could be revised to focus on background for the specific protocol (oral point perception) instead of acceptance/rejection. Certain portions of the methods require clarification.

Major Concerns:

1. The title should reflect the exact measurements made (1 point on midline of tongue); as it is, it could be interpreted to indicate that several portions of the tongue were tested.
    a. The title has been updated to reflect this.
2. The keyword "swallowing disorders" is not appropriate, as participants did not have swallowing disorders, and the association between swallowing disorders and oral point pressure (or potential implications) was not established. In the introduction, acceptance/rejection of modified textures in patients with dysphagia is discussed (and is not directly relevant to this protocol), but there is no mention of sensory/perceptual deficits present in many of these patients.
    a. Although oral somatosensation is key to managing a food bolus and triggering the reflexive part of a pharyngeal swallow, we agree that the main focus of this protocol is not on swallowing disorders and have removed this from our submission.
3. The first paragraph of the introduction discusses texture, but texture is not addressed by the protocol, and it might be more appropriate to focus on a thorough explanation of oral touch, and to then discuss its potential implications for texture perception.
    a. The Introduction has been heavily revised to incorporate all your suggestions and feedback, including focusing more on oral touch.

4. The introduction seems to conflate acceptability/rejection and other aspects of perception (e.g., detection & discrimination). As this protocol does not test any aspect of acceptability/rejection, I would recommend revising the introduction to focus on perceptual detection & discrimination.
    a. The Introduction has been revised to focus more on assessing peripheral nerve function through cutaneous measures of point pressure.
5. Citations are needed to substantiate claims throughout the introduction.
    a. Citations have been added to strengthen the Introduction.
6. I would strongly recommend including mention of other studies that have utilized Von Frey monofilaments to test oral perception (in addition to those published by the authors themselves).
    a. Additional studies discussing the use of Von Frey Hair monofilaments have been added.
7. Is there any relevant demographic or medical information that might influence testing (& should thus be collected as data)?
    a. Yes, information regarding potential demographic or medical history information that could influence testing has been added to the Discussion section.

Minor Concerns:

1. In the abstract, clarify what is meant by "lowest means" (e.g. mean force).
    a. This correction was made in text.
2. It might be helpful to readers to recommend that the order of trials by random number generator be determined & entered into a template prior to initiation of testing.
    a. Yes, this is recommended in step 2.3.1.2 – however the wording has been updated to be clearer for the reader.
3. Do the authors have recommended practices for setting up data recording sheets?
    a. Yes, during protocol development and practice we have created two means for setting up data recording sheets. Both examples are now provided as supplementary materials.
4. At what times were participants told to close their eyes, and for how long (1.4)?
    a. When to tell the participant to close their eyes has been added to steps under each testing methods: Detection and Discrimination Threshold Estimates.
5. Are there reliability concerns or recommendations with testing the same location (1.4.2)? How was this location selection (in comparison to more anterior or posterior midline)?
    a. More discussion on this has been added to the Discussion section.
6. Provide more rationale for tongue rewetting, including purpose & potential effects on data.
    a. More information has been added to the Discussion section.
7. Do the authors anticipate that this protocol would differ in any way for patients with limited tongue mobility?
    a. Medical history information that could influence testing has been added to the Discussion section.
8. What is the recommended time between trials (2.3.1.2)?
    a. Clarified in text.

9. For stopping point, explain what it means that a participant has "crossed over" a monofilament (2.6.1) (e.g., total number of times the same monofilament is encountered in testing?).
    a. Thank you, this language was added in text.
10. The scoring section seems to be about which data is collected (recording), whereas the first paragraph of the representative results addresses scoring. I would recommend moving the information about the meaning of lower vs. higher thresholds from representative results to the end of the protocol (after 4.1.3).
    a. We have chosen to the leave how to interpret the results in the "Representative Results" Section versus moving it to the protocol.
11. A higher threshold could also be impacted by peripheral, non-cognitive factors (lines 224-225).
    a. You are correct. More information regarding increased threshold estimates due to peripheral, non-cognitive factors has been added in the Discussion.
12. It would be helpful to include commentary on the figures and table.
    a. More commentary on the tables and figures has been added to text.
13. When were monofilaments > 15g determined to cause tongue movement- in a pilot, or in another study?
    a. 15g was set as an a priori ceiling during pilot testing and was published in an earlier protocol using these assessments on tongue lateral edge. We clarified this in text. Briefly, during pilot testing it was observed that monofilaments above 15g would displace the tongue such that we could not be sure if the participant identified the stimulus or felt their tongue move and/or contact other oral structures.
14. Is there evidence or theory to support the statement that the midline tongue may be more important to texture perception (lines 258-259)?
    a. Yes, work by Yackinous & Guinard found that supertasters to the compound 6-n-propylthiouracil (PROP) were more sensitive to stimulation on the center of the tongue with Von Frey filaments. This citation has been added to the Introduction to increase the rationale for testing tongue at midline.

8 October 2019
Journal of Visual Experiments

Dr. Wu and Reviewers –

Please find enclosed our revised manuscript (60656_RO): **Assessment of midline lingual point-pressure somatosensation using Von Frey Hair monofilaments.**

We have made all editorial suggestions and have included brief comments below. Thank you.

Editorial comments:

The manuscript has been modified and the updated manuscript, **60656_R1.docx**, is attached and located in your Editorial Manager account. **Please use the updated version to make your revisions.**

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.
    a. **Completed.**
2. Please include an ethics statement before the numbered protocol steps, indicating that the protocol follows the guidelines of your institution's human research ethics committee.
    a. **This statement has been added to the protocol prior to the numbered steps.**
3. There is a 2.75 page limit for filmable content. Please highlight 2.75 pages or less of the Protocol steps (including headings and spacing) in yellow that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.
    a. **A portion of the protocol (up to ~2.75 pages) has been highlighted.**
4. Step 4.2: Please write this step in the imperative tense.
    a. **The first sentence of step 4.2 has been changed to imperative tense.**
5. Please remove trademark (™) and registered (®) symbols from the Table of Equipment and Materials.
    a. **These symbols have been removed from the manuscript and ToE.**
6. JoVE cannot publish manuscripts containing commercial language. This includes company names of an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. Examples of commercial language in your manuscript include DanMic Global LLC, etc.
    a. **Reference to the company, DanMic Global have been removed from the main manuscript; it has been retained in the ToE.**

Detection Threshold: Location_____ TR Value:_____

Orange	15.000
	10.000
	8.000
	6.000
	4.000
Purple	2.000
	1.400
	*1.000
	0.600
Blue	0.400
	0.160
Green	0.070
	0.040
	0.020
	0.008

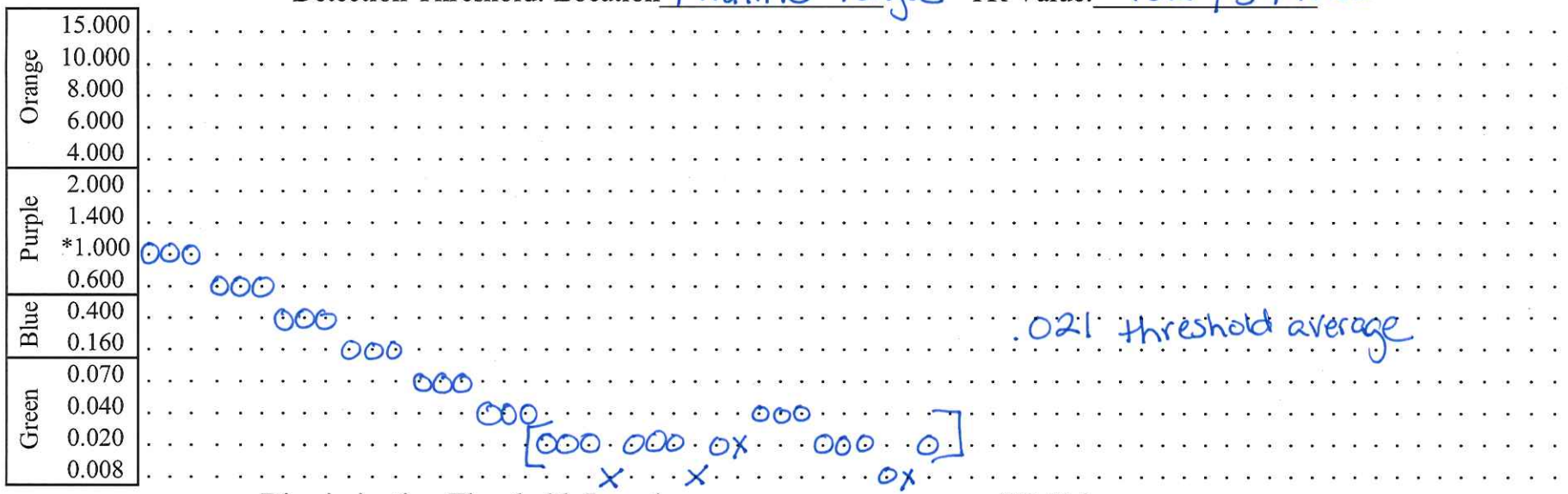
Discrimination Threshold: Location_____ TR Value:_____

Orange	15.000
	10.000
	8.000
	6.000
	4.000
Purple	2.000
	1.400
	1.000
	0.600
Blue	0.400
	0.160
Green	0.070
	0.040
	0.020
	0.008

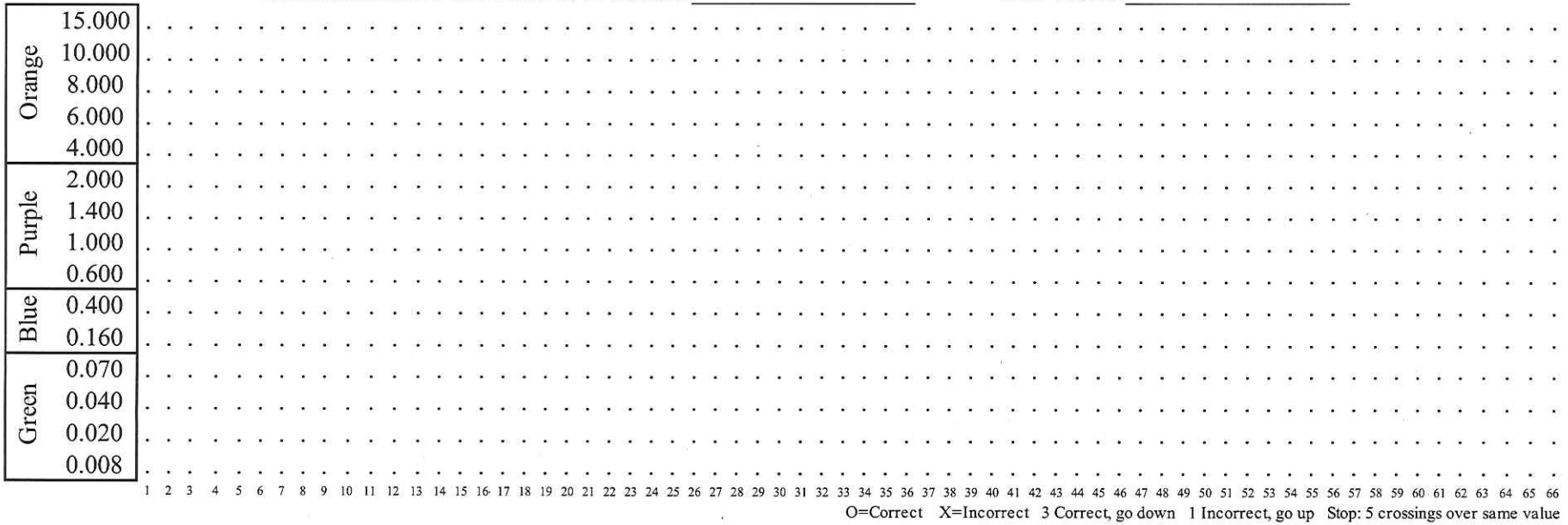
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66

O=Correct X=Incorrect 3 Correct, go down 1 Incorrect, go up Stop: 5 crossings over same value

Detection Threshold: Location Midline Tongue TR Value: .02 | 37 trials total



Discrimination Threshold: Location _____ TR Value: _____



O=Correct X=Incorrect 3 Correct, go down 1 Incorrect, go up Stop: 5 crossings over same value

Supplemental File (Figures, Permissions, etc.)

Click here to access/download;Supplemental File (Figures, Permissions, etc.);EtterSupplementaryFile3-

#	Target (g)	Trial 1	Trial 2	Response Y N #		#	Target (g)	Trial 1	Trial 2	Response Y N #
1			X			52		X		
2		X				53			X	
3		X				54			X	
4			X			55		X		
5			X			56		X		
6		X				57			X	
7			X			58		X		
8			X			59			X	
9		X				60		X		
10		X				61		X		
11			X			62		X		
12			X			63		X		
13		X				64		X		
14			X			65		X		
15			X			66		X		
16		X				67		X		
17		X				68		X		
18			X			69		X		
19			X			70			X	
20			X			71		X		
21			X			72			X	
22		X				73			X	
23			X			74		X		
24			X			75		X		
25		X				76			X	
26			X			77			X	
27		X				78			X	
28		X				79			X	
29			X			80			X	
30		X				81			X	
31			X			82			X	
32		X				83		X		
33			X			84		X		
34			X			85			X	
35			X			86		X		
36		X				87		X		
37			X			88		X		
38			X			89		X		
39			X			90		X		
40		X				91		X		
41		X				92		X		
42			X			93		X		
43			X			94		X		
44		X				95		X		
45		X				96			X	
46			X			97			X	
47		X				98		X		
48			X			99		X		
49		X								
50		X								
51		X								

#	Target (g)	Trial 1	Trial 2	Response Y N #		#	Target (g)	Trial 1	Trial 2	Response Y N #
1	1.0		X	Y		52		X		
2	1.0	X		Y		53			X	
3	1.0	X		Y		54			X	
4	.6		X	Y		55		X		
5	.6		X	Y		56		X		
6	.6	X		Y		57			X	
7	.4		X	Y		58		X		
8	.4		X	Y		59			X	
9	.4	X		Y		60		X		
10	.16	X		Y		61		X		
11	.16		X	Y		62		X		
12	.16		X	Y		63		X		
13	.07	X		Y		64		X		
14	.07		X	Y		65		X		
15	.07		X	Y		66		X		
16	.04	X		Y		67		X		
17	.04	X		Y		68		X		
18	.04		X	Y		69		X		
19	.02		X	Y 1		70			X	
20	.02		X	Y		71		X		
21	.02		X	Y		72			X	
22	.008	X		1 N		73			X	
23	.02		X	Y 2		74		X		
24	.02		X	Y		75		X		
25	.02	X		Y		76			X	
26	.008		X	2 N		77			X	
27	.02	X		Y 3		78			X	
28	.02	X		N		79			X	
29	.04		X	Y 2		80			X	
30	.04	X		Y		81			X	
31	.04		X	Y		82			X	
32	.02	X		Y 4		83		X		
33	.02		X	Y		84		X		
34	.02		X	Y		85			X	
35	.008		X	Y		86		X		
36	.008	X		N		87		X		
37	.02		X	Y 5		88		X		
38			X			89		X		
39			X			90		X		
40		X				91		X		
41		X				92		X		
42			X			93		X		
43			X			94		X		
44		X				95		X		
45		X				96			X	
46			X			97			X	
47		X				98		X		
48			X			99		X		
49		X								
50		X								
51		X								

.02 threshold

.021 threshold average