

March 8, 2021

Dr. Nam Nguyen  
Manager of Review  
JoVE

Re: Manuscript ID: JoVE62384

Dear Dr. Nguyen:

Thank you for the thoughtful and constructive evaluations of and compliments on our manuscript entitled "Psychophysical Tracking Method to Assess Taste Detection Thresholds in Children, Adolescents, and Adults: The Taste Detection Threshold (TDT) Test". We appreciate the opportunity to submit a revision and provide point-by-point responses to each of the Reviewer's and Editor's concerns and queries, which are in **bold font**.

### **Reviewer 1**

1. **Minor Concerns: I believe some explanation can be enriched again by providing additional references, such as challenges in implementing this test in children.**

Reply: We expanded the second paragraph of the Discussion and emphasize how many psychophysical taste methods have not been validated for use in children and list the features of this manuscript and others that are critical when testing young children [see also Reviewer 1, Response 16 below].

2. **Line 44...sucrose, sodium chloride, or monosodium glutamate... I surprise that you include salty (NaCl) and umami (monosodium glutamate) taste in children. Do you by chance follow specific training first with the children before the evaluation to be able to discriminate between salty and umami taste?**

Reply: The protocol did not include specific training to identify the basic tastes; that would be more appropriate for recognition psychophysical tasks. We would like to emphasize that we measured detection thresholds from salt on separate days than umami. Participant were never asked to discriminate between salty and umami taste. The task for the participant is simply to discriminate between water and "different than water". We now clarify in the text that "When assessing detection thresholds in pediatric population, we have always limited testing to a single tastant per session. However, adults can complete all three thresholds in a single session".

3. **Also, I am curious why sour and bitter did not include in your study, can you please explain in your manuscript on why the selection is focus on sweet, salty, and umami only?**

Reply: We would like to clarify that we submitted this manuscript to the special issue of JoVE entitled "Current tools and methods in taste science" that aims "to document those tools and methods in one location to help taste scientists find the resources they need".

We provide details on this method to measure detection threshold for sucrose, salt and MSG because of its use in prior research on pediatric and clinical populations but now include that the method has been applied to other food-grade tastants (e.g., citric acid, quinine). This method, which includes an important set of criteria established by Dr. Cowart in clinical practice at the Monell-Jefferson Chemosensory Clinical Research Center to enhance confidence in the validity of individual measures of taste functioning, has never been described with the level of detail provided herein.

4. **Line 48-49: We describe a two-alternative forced-choice, staircase tracking procedure, called the Taste Detection Threshold (TDT) test what is the differences between your proposed method Taste Detection Threshold (TDT) and the usually used detection threshold method?**

5. Reply. The key difference between the TDT and the two-alternative, forced-choice, staircase tracking procedure is in the stopping criteria. Whereas stopping criteria for the latter test consist of one incorrect up and 2 correct down and 4 reversals, the TDT is deemed completed after the occurrence of four reversals, provided there are no more than two dilution steps between two successive reversals and the series of reversals do not form an ascending pattern. This set of criteria, which was established by Dr. Cowart and colleagues at the Monell-Jefferson Taste and Smell Clinic, enhances confidence in the validity of individual measures of taste function. As discussed in the text, "These additional criteria, which were established based on clinical experience, allow for the evaluation of the functioning of the taste system of the individual in part because they control for false positives, especially when the participant is simply guessing.

6. **Line 59-63: The concentration of the stimulus in the subsequent pair increases after a single incorrect response and decreases after two consecutive correct responses. A reversal occurs when the concentration sequence changes direction. The task is deemed completed after the occurrence of four reversals, provided there are no more than two dilution steps between two successive reversals and the series of reversals do not form an ascending pattern. I believe this method has been described previously, please see DOI: 10.1097/NNR.0000000000000138. Is there any differences with the method that you have used now?**

Reply: Aligned with the goal of the special issue (see Response #3), we document and describe herein a method used in prior research (References 2, 4-10).

7. **Line 72-73... we reject a food or liquid I kindly suggest to include liquid as foods**

Reply: Although foods can exist in liquid form, a liquid (e.g., water, medicine) is not necessarily a food.

8. **Line 75-78: Not only are there several basic tastes (sweet, salt, bitter, sour, umami), but each taste quality can be characterized by distinct perceptual dimensions, including how sensitive individuals are in detecting the chemical stimulus or recognizing its taste, and how much they like or dislike the taste sensation; how about the taste sensitivity and genetic?**

Reply: We agree that individual differences in taste perception are due to both genetic and experiential factors. It is our hope that the TDT can help elucidate the important dimension of taste detection threshold for research in the future.

9. **Line 105: highlighting the appropriateness of the method for pediatric populations. could you please be specific the pediatric population is refer to a healthy population or not?**

Reply: We now specify that the method has been validated in healthy pediatric populations.

10. **Line 114-115: Ideally, the diluent should be distilled water (dH<sub>2</sub>O) and not tap water due to taste issues. I agree with the use of distilled water than tap water as tap water will have different filter systems which creates different tastes in different country. regarding this taste issue, I think that would be great if you could provide references in your manuscript.**

Reply: Hoehl et al., 2010 is now included as a reference for the statement that tap water should not be used because of taste issues.

11. **Line 220-222: For the first pair, the tastant paired with dH<sub>2</sub>O is concentration step 10 when determining sucrose thresholds or step 12 when determining NaCl or MSG thresholds; these concentrations are a few steps below the average detection threshold for each tastant. can participant start with other step? I know that you are emphasizing the participant to not taste any samples in the end, but in regards for someone who were really sensitive or not too sensitive they may need different step solutions to begin with? and why it is different step between sweet (step 10) and salty/umami (step 12)? could you please explain it in your manuscript.**
12. **Line 236 – 237: the concentration that testing starts with is step 12 for NaCl or MSG, instead of step 10 for sucrose. why it should start in different step? does each taste have same intensity perception level for each step? for example if step 8 between sweet, salty, and umami were perceived equally in terms of their intensity. Have you investigate that using trained panelist?’**

Reply to #10 and #11: The difference between the initial concentration steps used for thresholds for sucrose and for salt or MSG reflects established differences in taste detection thresholds among these three tastants, while keeping the stimulus preparation series as nearly identical as possible for the sake of simplicity. Ideally, the test should begin with a concentration of the tastant slightly below what the participant can detect. The text has been modified to read “The concentrations of tastant in the first step were chosen because each is a few steps below the average detection threshold for that particular tastant. Nevertheless, the TNT is a reliable tool to measure thresholds regardless if above or below the average.

13. **Line 246-248: b. Two hours before testing, remove bottles containing solutions (steps 0-16) and dH<sub>2</sub>O from the refrigerator and transfer ~120 mL of solution for each step into appropriately labeled 120 mL sterilized glass bottles. I think that would be great if you also could suggest on how long the stock solutions can be stored, and in which condition (storage temperature, etc.)? or its better to always make a fresh samples solution every time you conduct the test?**

Reply: As specified in the methods, the solutions can be stored refrigerated for a maximum of 2 weeks. It is not necessary to make fresh solutions, if solutions are prepared as described in this protocol, which includes the use of clean and sterilized glassware and distilled water and storage at 4°C. We agree that this should be specified in the text which has been modified to read: “The solutions can be stored refrigerated for a maximum of 2 weeks but only if the protocol described below, i.e., sterilized glassware and distilled water diluent, stored refrigerated at 4°C, is adhered to. They are then brought to room temperature 2 hours before testing”.

14. **Line 259-260: and instruct parents to not give their child participant anything to eat or drink, for at least 1 hour before testing. I think you should also write the inclusion criteria (for examples for those who were sick or have flu-related sickness prior to the test which will influence their taste perception.**

Reply: To eliminate the effects of recency of eating or drinking on taste thresholds, our protocol specifies that the participant should not eat or drink anything but water for at least 1 hour before testing. We do not consider this an inclusion criteria per se.

15. **Line 327-329. Circle the steps on the grid where there is a reversal—a change in direction in the accuracy in the participant's response, that is, when the participant becomes either more or less successful in identifying the tastant when tasting the next steps on the staircase: I am curious if you also consider this (participant (especially children) who were simply guessing their choice) as they have a correct chance by 50% in your detection threshold calculation?**

Reply: We would like to clarify that although in each given pair of solutions there is a 50% chance of guessing correctly (assuming the participant is unable to detect a signal), the staircase with a 1-up and 2-down procedure places the signal strength on the psychometric function at the 70.1% correct point (not at 50%) (see reference 13). In addition, simulation studies that assume a participant is guessing at each single pair of solutions demonstrate that the staircase with the 1-up and 2-down procedure has a low probability (~5%) of reaching a detection threshold by chance with run lengths that are within 12 pairs; <https://doi.org/10.3758/s13414-014-0798-9>. We expect this to be further reduced when applying the strict stopping rule criteria used in our method (i.e., no more than two dilution steps occur between two successive reversals and the series of reversals do not form an ascending pattern).

16. **Line 452-454: its use to measure detection thresholds for unpalatable tastants among some young children may be problematic due to their heightened sensitivity to some bitter tastants and their potential unwillingness to continue participation I do agree with this statement, however since you measuring detection threshold and not recognition will you consider to also involve the two another basic tastes (bitter, sour) in your study - children may not taste it (bitter/sour) since they will be asked whether this different with water or not.**

Reply: The reviewer brings up a good point and the method described in our paper has been used to measure detection thresholds for the basic tastes of bitter or sour in adult populations. We now emphasize in the Discussion that “The method has real-world relevance and has been used for other basic tastes (e.g., citric acid and quinine in individuals of varying ages).

17. **Line 456-458 embedded in the context of a game, the method is sensitive to the cognitive and language limitations of children and requires only that the participant point to the cup that contains the taste. I kindly suggest to emphasized this more as there are some challenges in conducting sensory testing in children.**

Reply. The Reviewer brings up an important point in that many psychophysical taste methods until recently were not validated for use in children. The evidence (including the present and past manuscripts published in JoVE and other references cited in the manuscript) reveal that children can perform the psychophysical task if using an age-appropriate method that is short in duration and has critical features. We have expanded the second paragraph of Discussion on this important topic.

## **Reviewer #2**

- 1. There is no any accord of the subjects participating in this study. Please mention that important aspect.**
- 2. You must have the ethic approval and permission for research involving human subjects. Mention that in the article.**

Reply to #1 and #2: We agree with the Reviewer regarding ethical approval and informed consent. The General Considerations section now reads: "This method has been approved by the Office of Regulatory Affairs at the University of Pennsylvania. For the research studies described herein, informed consent was obtained from each adult or parent/legal guardian and informed assent was obtained from each child aged seven years or older prior to participation."

- 3. After discussions please insert the CONCLUSIONS.**

Reply: Per the author instructions, JoVE is a methods journal and does not include a CONCLUSION section.

## **Editor**

- 1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.**

Reply: Done as instructed.

- 2. Please revise the following lines to avoid previously published work: 58-61,62-63,94-97,97-99,258-262,273-280,283-290,303-307,434-436,461-465.**

Reply: The text on the lines referenced above corresponds to description of the TDT test and procedures when referenced in previously published research. We think that changing the text could confuse the reader and could be perceived as inconsistent with the description and advantages of the method in this JoVE manuscript.

- 3. Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).**

Reply: Done as instructed.

- 4. Line 111-132/216-237: Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.**

Reply: Done as instructed

- 5. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets or dashes.**

Reply: Done as instructed.

- 6. Line 156: For SI units, please use standard abbreviations when the unit is preceded by a numeral. Abbreviate liters to L to avoid confusion. Examples: 10 mL, 8  $\mu$ L, 7 cm<sup>2</sup>**

Reply: Done as instructed.

7. **Please include a one-line space between each protocol step and highlight up to 3 pages of the Protocol (including headings and spacing) 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. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.**

Reply: Done as Instructed.

8. **Please include at least one paragraph of text to explain the Representative Results in the context of the technique you have described, e.g., how do these results show the technique, suggestions about how to analyze the outcome, etc. The paragraph text should refer to all of the figures. Data from both successful and sub-optimal experiments can be included.**

Reply: We include paragraphs of text to explain the Representative Results for each of the Figures.

9. **Please include a Figure Legends section. Please include a title and a description of each figure and/or table. All figures and/or tables showing data must include measurement definitions, scale bars, and error bars (if applicable). Please include all the Figure Legends together at the end of the Representative Results in the manuscript text.**

Reply: Done as instructed.

10. **Please remove the embedded Table from the manuscript. All tables should be uploaded separately to your Editorial Manager account in the form of an .xls or .xlsx file. Each table must be accompanied by a title and a description after the Representative Results of the manuscript text.**

Reply: Done as instructed.

11. **Please title case and italicize journal titles and book titles in the Reference section. Do not use any abbreviations. Article titles should start with a capital letter and end with a period and should appear exactly as they were published in the original work, without any abbreviations or truncations.**

Reply: Endnote format has been corrected.

12. **Figure 3: Please remove the titles and Figure Legends from the uploaded figures. The information provided in the Figure Legends after the Representative Results is sufficient**

Reply: Done as instructed.

13. **Please include a Table of Materials and include the list of materials, consumables and equipment used in the study.**

Reply: Everything that is needed is listed in the protocol. We do not think it is appropriate to suggest certain brand names for glassware, stopwatch, or food-grade tastants).

Best regards,



M. Yanina Pepino  
On behalf of all co-authors