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Psychophysical Tracking Method to Assess Taste Detection Thresholds in Children, Adolescents, and Adults: The Taste Detection Threshold (TDT) Test

Paule V. Joseph[1], **Julie A. Mennella**[2], **Beverly J. Cowart**[2], **M. Yanina Pepino**[3]

[1] National Institute of Alcohol Abuse and Alcoholism, Section of Sensory Science and Metabolism [2] Monell Chemical Senses Center [3] Department of Food Science and Human Nutrition, University of Illinois at Urbana Champaign

Corresponding Author

M. Yanina Pepino
ypepino@illinois.edu

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Abstract

This paper describes a two-alternative, forced-choice, staircase, tracking procedure, called the Taste Detection Threshold (TDT) test, that provides a reliable measure of sweet, salty, and umami taste detection thresholds from childhood to adulthood. Advantages of the method include procedures that are identical for children and adults, thus allowing the determination of age-related and individual differences in taste perception, if any, and tasks that can be completed in a relatively short time frame, do not rely on continuous attention or require memorization, control for subjective response biases, and minimize the impact of language development. After a 1 hour fast, participants are presented with pairs of solutions; in each pair, one solution is water, and the other solution contains varying concentrations of the tastant.

Using a whole-mouth tasting method, participants taste each solution (without swallowing and with rinsing between tastings) and then point to the solution with a taste or that tastes different from water. 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 a maximum of two dilution steps between two successive reversals, and the series of reversals do not form an ascending pattern. These additional criteria ensure greater reliability in outcomes. The TDT is then calculated as the geometric mean of the concentrations of the four reversals. This method has real-world relevance as it provides information on a dimension of taste perception that is independent of hedonics, and that can change with aging and certain disease states, making it a valuable psychophysical test.

Introduction

The sense of taste functions as a gatekeeper, determining in part whether an individual rejects a food or liquid or accepts it into the oral cavity. **Taste psychophysics**-the study of relationships between distinct chemical stimuli and the sensations and perceptions they produce-provides important information on the functioning of the taste system[1]. Not only are there several basic tastes (sweet, salty, 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.

This article describes a psychophysical method that can be used to reliably measure **taste detection thresholds** (i.e., the lowest concentration of a tastant that can be detected) in individuals as young as 6 years. From childhood to adulthood, detection thresholds have been used in clinical assessments of the effects of trauma or disease states[2,3] and in basic research applications, to study the effects of diet, aging, development, obesity, and smoking on the taste system, as well as genotype-taste phenotype relationships[4,5,6,7,8,9,10,11].

This **taste detection threshold** (TDT) test, which typically takes an average of 15 min per stimulus (range: 4-35 min; median: 13 min) to complete, consists of a two-alternative, forced-choice, staircase, tracking procedure that has been used to measure the lowest concentration of sucrose, sodium chloride (NaCl), or monosodium glutamate (MSG) in solution that can be detected as a taste. As outlined herein, participants are presented with pairs of solutions; in each pair, one solution is water, and the other solution contains varying concentrations of the tastant. Using a whole-mouth-tasting method, participants taste each solution (without swallowing) and then point to the solution with a taste or that tastes different from water. 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 a maximum of two dilution steps between two successive reversals, and the series of reversals do not form an ascending pattern. These additional criteria, which were established in clinical practice by Dr. Cowart and colleagues at the Monell-Jefferson Chemosensory Clinical Research Center[2], ensure greater reliability in outcomes and enhance confidence in the validity of individual measures of taste functioning. Research studies have used this method to determine taste detection thresholds for sucrose, salt, or MSG in hundreds of healthy children as young as 6 years, adolescents, and adults[4,5,6,7,8,9,10,11] and have demonstrated that the majority (>~80%) of children can complete the psychophysical task[4,6,7,8], highlighting the appropriateness of the method for pediatric populations.

Protocol

1. General considerations

NOTE: This protocol for the TDT test describes the procedures for preparing the taste solutions and for determining taste detection thresholds for sucrose, NaCl, or MSG, using sucrose as the example. 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 participant or parent/legal guardian of pediatric participants. Informed assent was obtained from each child aged seven years or older prior to participation.

1. As shown in **Table 1**, prepare 17 solutions, ranging from 1 M to 0.00010 M, that are quarter-log steps apart. Ideally, use ultrapure water such as distilled water (dH_2O) as the diluent and not tap water due to taste issues[12]. Refrigerate the solutions for a maximum of 2 weeks, but only if the protocol described below is adhered to.
2. After informed consent is obtained from the adult participants or parent/legal guardians and, when applicable, assent from the pediatric participants, conduct the tests in a comfortable, private room that ideally has a sink for expectoration. Ensure that the solutions are not swallowed but rather swished in the oral cavity and spat out. If a sink is not available, provide a large cup for spitting.
3. Ensure that testing personnel do not wear heavily scented products and limit conversation to instruction or explanation of methods. Instruct the adult participants and the parents/legal guardians of child participants that the participant should abstain from eating or drinking anything but water, or using tobacco products (adults only) for 1 h prior to testing.

2. Materials and recipes to make taste stimulus solutions

NOTE: Detailed instructions for making the stock solution (1000 mmol/L; hereafter referred to as **stock**) and the 16 serial dilutions of the stock solution (in quarter-log steps) for sucrose, NaCl, or MSG are provided here. **Table 1** lists the concentrations of each dilution step. **Figure 1** illustrates the steps to make stock solution through dilution steps 1-16. The volume of solution made will be sufficient to determine thresholds for at least four participants.

Step	Molar (1/4 log units apart)	Sucrose (g/L)	NaCl (g/L)	MSG (g/L)
0	1 M	342.3	58.44	187.13
1	0.562 M	192.37	32.84	105.17
2	0.316 M	108.17	18.47	59.13
3	0.178 M	60.93	10.4	33.31
4	0.100 M	34.23	5.84	18.71
5	0.056 M	19.17	3.27	10.48
6	0.032 M	10.95	1.87	5.99
7	0.018 M	6.16	1.05	3.37
8	0.010 M	3.42	0.58	1.87

9	0.0056 M	1.92	0.33	1.05
10	0.0032 M	1.09	0.19	0.6
11	0.0018 M	0.62	0.11	0.337
12	0.0010 M	0.34	0.058	0.187
13	0.00056 M	0.19	0.033	0.105
14	0.00032 M	0.11	0.019	0.059
15	0.00018 M	0.06	0.0105	0.034
16	0.00010 M	0.03	0.0058	0.019

Table 1: Concentration steps and corresponding molarity of sucrose, sodium chloride (NaCl), and monosodium glutamate (MSG) solutions needed for Taste Detection Threshold (TDT) testing.

1. Prepare test materials.
    1. Obtain a food-grade source of sucrose, NaCl, or MSG.
2. Clean and sterilize all needed glassware (see **Table of Materials**).

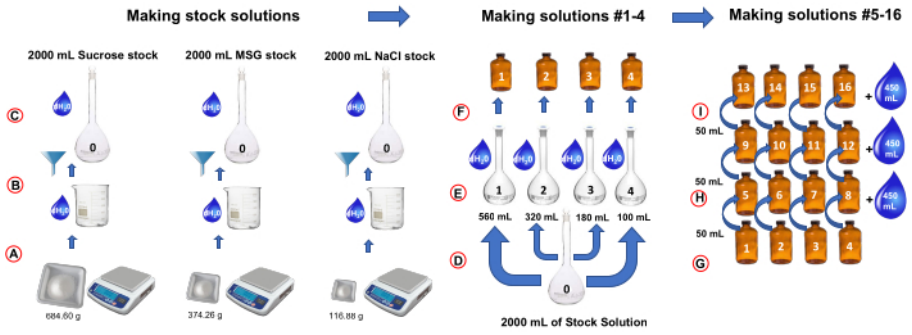

Figure 1: Step-by-step instructions to make stock solutions through dilution steps #1-16. Please click here to view a larger version of this figure.

2. Make stock solution, as depicted in **Figure 1A-C**.
    1. Label all glassware with the date, type of tastant, and **Stock**.
    2. Weigh the tastant into a disposable weigh boat on a scale accurate to 0.01 g, and transfer to the 2000 mL beaker.

NOTE: The amounts needed to prepare the stocks are 684.60 g for sucrose, 374.26 g for MSG, and 116.88 g for NaCl.

3. Rinse with dH_2O any tastant remaining in the weigh boat, and pour into the beaker. Add 1500 mL of dH_2O to dissolve the sample.

4. Transfer the contents of the beaker to the 2000 mL volumetric flask using a funnel, and rinse the beaker and funnel with more dH_2O, pouring the rinse water into the flask. Fill the flask with dH_2O to the 2000 mL mark, and affix the stopper on the flask. Invert to mix until the tastant is dissolved.

3. Make solutions **#1-4**, as depicted in **Figure 1D-F**.

1. Label 1000 mL volumetric flasks with numbers 1 to 4 and corresponding 1000 mL glass bottles with the date, type of tastant, and **Stock** to **Step 4**.

2. Transfer 560 mL, 320 mL, 180 mL, and 100 mL of **stock** into **flasks 1, 2, 3**, and **4**, respectively. Fill flasks **1-4** with dH_2O to the 1000 mL mark, affix with stopper, and mix until the tastant is dissolved. Pour the contents of each flask into its corresponding 1000 mL glass bottle (labeled **Step 1** to **Step 4**) using a funnel if needed.

3. Pour the remaining stock solution into the bottle labeled **Stock**; close the lid tightly, and place in the refrigerator at 4 °C.

4. Make solutions **#5-16**, as depicted in **Figure 1G-I**.

1. Label twelve 1000 mL bottles with the date, type of tastant, and **Step 5** to **Step 16**.

2. Line up the bottles in a 4 x 4 grid with the bottles containing step 1 - 4 solutions in the front row (as shown in **Figure 1G-I**).

NOTE: This positioning allows a simple dilution series, such that it starts with the most diluted step in the row (*e.g.,* **step 4**) and end with the most concentrated step (*e.g.*, **step 1**).

3. Pipet 50 mL of **steps 1, 2, 3**, and **4** into bottles **5, 6, 7**, and **8**, respectively. Add 450 mL of dH_2O to bottles 5-8, affix the stoppers, and invert to mix (**Figure 1 H**).

4. Repeat the process starting with the second row. Pipet 50 mL of **steps 5, 6, 7**, and **8** into bottles **9, 10, 11**, and **12**, respectively. Add 450 mL of dH_2O to bottles **9-12**, affix the stoppers, and invert to mix.

5. Repeat the process starting with the third row (**Figure 1 I**). Pipet 50 mL of **steps 9, 10, 11**, and **12** into bottles **13, 14, 15**, and **16**, respectively. Add 450 mL of dH_2O to bottles **13 - 16**, affix the stoppers, and invert to mix. Place lids on bottles **1 - 16**, close the lids tightly, and store in the refrigerator at 4 °C.

6. Fill several sterilized 120 mL labeled glass bottles with dH_2O, close the lids tightly, and store in the refrigerator at 4 °C.

3. The psychophysical method: TDT

1. Present the participants with medicine cups containing pairs of solutions, one of which is a given concentration of a tastant and the other dH_2O.

NOTE: For the first pair, the tastant paired with dH_2O is concentration **step 10** when determining sucrose thresholds and **step 12** when determining NaCl or MSG thresholds. 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 TDT is a reliable tool to measure

thresholds, regardless of whether these are above or below the average.

2. Ask the participants to taste both solutions without swallowing and rinse their mouth with dH_2O between tastings. Instruct them to point to the medicine cup they think has a taste in it or that tastes different than water.

   NOTE: The concentration of the tastant presented during the subsequent pairs depends on whether or not the participant's response was correct (*i.e.*, the participant pointed to the tastant). The method is a **forced-choice** procedure, which means that participants cannot respond by saying "neither" or "I don't know"; rather, they must pick one of the two solutions. The method is a **staircase** procedure because the taste stimuli are presented in ascending (higher concentrations of tastant) or descending (lower concentrations of tastant) order, depending on the participant's response[13]. For ease of description, instructions have been provided for making the sucrose series and determining sucrose detection thresholds. The methods for MSG and NaCl are identical with two exceptions: (a) concentration of tastant needed to make stock solution differs (**Table 1**), and (b) as noted above, the concentration that testing starts with is step 12 for NaCl or MSG, instead of **step 10** for sucrose.

3. When assessing detection thresholds in pediatric population, limit testing to a single tastant per session.

   NOTE: Adults can complete all three thresholds in a single session.

Time Start: _____ Time End: _____

THRESHOLD TRACKING GRID

Tastant: **Sucrose** **NaCl** **MSG** **(circle)**
Begin at **step 10** for sucrose and **step 12** for NaCl or MSG

Pair#	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
Order*, W or T	W	T	T	W	W	T	T	W	T	T	T	W	T	T	T	W	T	W	T	W	W	W	W	T	T	T	T	T	W	W	T	W	W	T	T	W	T	T	T	W
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Step 16																																								

*Order of Presentation: W=Water First; T=Tastant First

Figure 2: Threshold tracking grid. (**A**) Recording taste detection thresholds. (**B**) Setup of one tray. Please click here to view a larger version of this figure.

4. Preparation of materials prior to testing

1. Generate a randomization sequence for the order of presentation of stimuli within pairs, and fill in the top row of the tracking grid (**Figure 2A**) for each pair by placing W in the box if water comes first, or T if tastant comes first.
2. Remove the bottles containing solutions (**steps 0 - 16**) and dH_2O from the refrigerator, and transfer ~120 mL of the solution for each step into appropriately labeled 120 mL sterilized glass bottles 2 h before testing.
3. Return **step 0 - 16** bottles to the refrigerator, and allow the transferred solutions to equilibrate to room temperature.
4. Label two, 12-cup muffin pans with the pair number, and mark positions that will hold the dH_2O medicine cups with a **W** (**Figure 2B**).

NOTE: Although it is not known how many pairs will be required, fill the medicine cups that are in in the **W** positions with 10 mL of dH_2O for the first 6 pairs.

5. Preparation of participants for testing

1. Instruct adults to abstain from eating, drinking, or using tobacco products, and instruct parents to not give their child participant anything to eat or drink for at least 1 h before testing.
2. Seat one participant at a table in front of a sheet of paper, labeled with the numbers **1** and **2** (**Figure 3**).
   NOTE: Participants should not see the taste stimuli until they are placed in front of them; this can be achieved by having an opaque partition separate the participant from the investigator.
3. Allow the participants to acclimate to the testing room and the tester for at least 10 min.
4. Use a stopwatch to time the 10 s interstimulus intervals (time from expectoration of the first stimulus to sipping the second stimulus).

Figure 3: Child participating in a taste threshold detection test. A pair of solutions is placed on the table in front on the participant in the order that it should be tasted. The participant is asked to taste the solution in **position 1** for 5 s, to expectorate, to rinse her mouth with dH_2O, and to repeat for the solution in **position 2**. After tasting both solutions, the participant is asked to point to the solution that has a taste or tastes different than water. Please click here to view a larger version of this figure.

6. Verbal instructions to participants

1. Pediatric participants
   1. Show the cups to the participant and say: "We're going to play a game with things to taste. Here are two cups. You will taste what is inside the first cup, swish it around your mouth, but don't swallow, and I will tell you when to spit it out in the sink (or cup). You will then rinse with water, and taste what is inside the second cup. I will tell you when to spit it out. Then I want you to point to the one that tastes different than water. If you are not sure, just guess. You will then

rinse your mouth two times with water, and we will do this again. There is no right or wrong answer; we want to know which one you think has a taste."

2. After every response, reward the participant by saying "Thank you. You are doing a good job!"

2. Adolescents and adults

1. Show the cups to the participant and say: "We're going to give you solutions to taste. Here are two cups. You will taste what's inside the first cup, swish it around your mouth, but don't swallow, and I will tell you when to spit it out in the sink (or cup). You will then then rinse with water and taste what's inside the second cup. I will tell you when to spit it out. Then I want you to point to the one that tastes different than water. If you are not sure, just guess. You will then rinse your mouth two times with water, and we will do this again. There are no right or wrong answers; we want to know which one you think has a taste."

2. After every response, reward the participant by saying "Thank you. You are doing a good job!"

7. Investigator instructions: Taste detection thresholds

1. As indicated on the tracking grid, start at **step 10** for sucrose (or **step 12** for NaCl or MSG). Place two medicine cups, one containing 10 mL of **step 10** and the other containing dH_2O on the sheet of paper with **1** and **2** in front of the participant (**Figure 3**).

NOTE: The number the water or taste solution is placed on is determined by the generated, randomized order of stimuli presentation. For example, in **Figure 2**, the randomized order for pair 1 is **W** (water first), so the cup containing water is in position **1**, and the one containing **step 10** is in position **2**.

2. Instruct the participant to taste the solution in position **1** by swishing; after 5 s, instruct the participant to expectorate, to rinse his or her mouth with water, and to expectorate again.

3. Instruct the participant to taste the solution in position **2** by swishing and to expectorate after 5 s.

4. Ask the participant to point to the solution that has a taste or tastes different than water. If the participant says neither, instruct the participant to choose one.

NOTE: Participants cannot go back and retaste either solution and must pick one of the two.

5. After they make their choice, instruct them to rinse their mouths with water, and place a plus sign (**+**) on the grid if the participant picked the cup with the tastant (**correct response**), or a minus sign (**-**) if they picked the cup with water (**incorrect response**).

6. Continue to the second pair, noting that the concentration of the tastant depends on participant's response for first pair. If the participant was incorrect for first pair (the participant chose **W**), then proceed up on the grid, noting that the tastant in the second pair will be the next higher concentration (**step 9**). If the participant was correct (chose **T**), then note that the tastant in the second pair will be the same **step 10**. Refer to the grid for the order of presentation (**W** or **T** first).

7. Repeat this process described in steps 7.2-7.5. If the participant is correct two times in succession at **step 10** (picks **T** both times), then remember that the next pair will contain the next lower concentration (**step 11**). If the participant is incorrect for pair 2 (picks **W**), proceed up on the grid to the next higher concentration (**step 9**).

8. Continue this process, moving up the grid to the next higher concentration with each incorrect answer, or down the grid to the next lower concentration after two correct answers in a row.

9. 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.
   NOTE: Specifically, the participant goes from failing to identify **T** at one step (**-**) to successfully identifying **T** at the next more concentrated step two times in a row (**+ +**), or the participant goes from successfully identifying **T** twice at the same step (**++**) to failing to identify **T** when given the next less concentrated step; this failure can occur during either the first or second presentation of the less concentrated step (**-** or **+-**).

10. Continue with the taste testing until four reversals are achieved, and list the step numbers of these four reversals.

11. Determine that the four reversals met the desired criteria; *i.e.*, successive reversals are no more than two steps apart from each other, and there are two sets of pairs in which the participant correctly identified the **T** twice at the same step. **STOP** and go to 7.13 to calculate the detection threshold.

12. Alternatively, determine that the four reversals did not meet criteria; *i.e.*, successive reversals are more than 2 steps apart from each other, or at least 2 sets of pairs are not present in which the participant correctly identified the **T** twice at the same step. **CONTINUE** with testing until four reversals meet the criteria, or the participant reaches the top of the grid (threshold is 1 M (**stock**)) or continues to provide correct answers and reaches the bottom of the grid, giving correct responses twice at **step 16** (threshold is 0.00010 M (**step 16**)).

13. Determine the participant's detection threshold by calculating the arithmetic mean of the log values of the molarity of those four reversals:
    arithmetic mean = (log concentration step of reversal 1 + log concentration step of reversal 2 + log concentration step of reversal 3 + log concentration step of reversal 4) / 4.
    NOTE: This is equivalent to calculating the geometric mean of the concentrations of the last four reversals:
    $\sqrt[4]{[reversal\ 1] \times [reversal\ 2] \times [reversal\ 3] \times [reversal\ 4]}$

14. Discard the unused taste solutions that were transferred into 120 mL bottles during the preparation of materials for testing.

Representative Results

Figure 4 illustrates the tracking grid results from four representative participants (A-D). Reversals, which are changes in the direction of the participant's responses, are denoted by circles and numbered in order of occurrence to illustrate when the criteria are met. Reversals are color-coded to illustrate when the change in direction goes from incorrect to correct (green) or from correct to incorrect (red).

Figure 4A shows the tracking grid from a participant whose responses met the criteria within the first four reversals. In order of occurrence, reversals for this participant occurred at **steps 8**, **9**, **8**, and **10**. This sequence met the criteria because (a) there were no more than two steps between any two successive reversals (**step 8** *vs* **9**, **9** *vs* **8**, **8** *vs* **10**), and (b) there were two sets of pairs in which the participant correctly identified the **T** twice at the same step (**8**). The detection

threshold for this participant is determined by the geometric mean of the concentrations of those four reversals:

$$Geometric\ mean = \sqrt[4]{[0.010\ M] \times [0.0056\ M] \times [0.010\ M] \times [0.0032\ M]}$$

Geometric mean = 0.0065 *M*

Figure 4B shows the tracking grid from a participant with a relatively high sucrose detection threshold (low sensitivity) whose responses in the first four reversals did not meet the criteria. In order of occurrence, the first four reversals occurred at **steps 9**, **10**, **8**, and **9**. Although these reversals were within two steps of each other (**9** *vs* **10**, **10** *vs* **8**, **8** *vs* **9**), there were not two sets of pairs in which the participant correctly identified the T twice at the same step (**8** *vs* **9**). These reversals formed an ascending pattern; therefore, criteria were not met and testing continued. Reversals **6-9** met the criteria because there were (a) no more than two steps between any two successive reversals (**step 8** *vs* **6**, **6** *vs* **7**, **7** *vs* **6**), and (b) two sets of two correct answers in a row were obtained at the same step (**step 6**). The detection threshold for this participant is determined by the geometric mean of the concentrations of those four reversals:

$$Geometric\ mean = \sqrt[4]{[0.010\ M] \times [0.032\ M] \times [0.018\ M] \times [0.032\ M]}$$

Geometric mean = 0.021 *M*

Figure 4C shows the tracking grid from a participant with a relatively low sucrose detection threshold (high sensitivity) whose responses in the first four reversals did not meet the criteria. Reversals occurred at **steps 9**, **10**, **9**, and **13**. Although in two pairs (pairs **3-4** and **7-8**), the participant correctly identified the tastant twice at the same step (**step 9**), there were more than two steps between reversals **3** and **4** (**step 9** *vs* **13**). Thus, testing continued. The last four reversals (**steps 13**, **12**, **13**, **12**) met the criteria because (a) there were no more than two steps between any two successive reversals (**13** *vs* **12**), and (b) the participant correctly identified the same concentration (**step 12**) when given pairs **17-18** and **20-21**. The detection threshold for this participant is determined by the geometric mean of the concentrations of those four reversals:

$$Geometric\ mean = \sqrt[4]{[0.00056\ M] \times [0.0010\ M] \times [0.00056\ M] \times [0.0010\ M]}$$

Geometric mean = 0.00075 *M*

Figure 4D shows the tracking grid from a participant with a relatively high sucrose detection threshold (low sensitivity) whose responses met the criteria within the first four reversals (**steps 6**, **7**, **5**, **8**). There were no more than two steps between any two successive reversals (**6** *vs* **7**, **7** *vs* **5**, **5** *vs* **8**), and the participant correctly identified the same concentration (**step 6**) when given pairs **7-8** and **13-14**. The detection threshold for this participant is determined by the geometric mean of the concentrations of those four reversals:

$$Geometric\ mean = \sqrt[4]{[0.032\ M] \times [0.018\ M] \times [0.056\ M] \times [0.010\ M]}$$

Geometric mean = 0.024 *M*

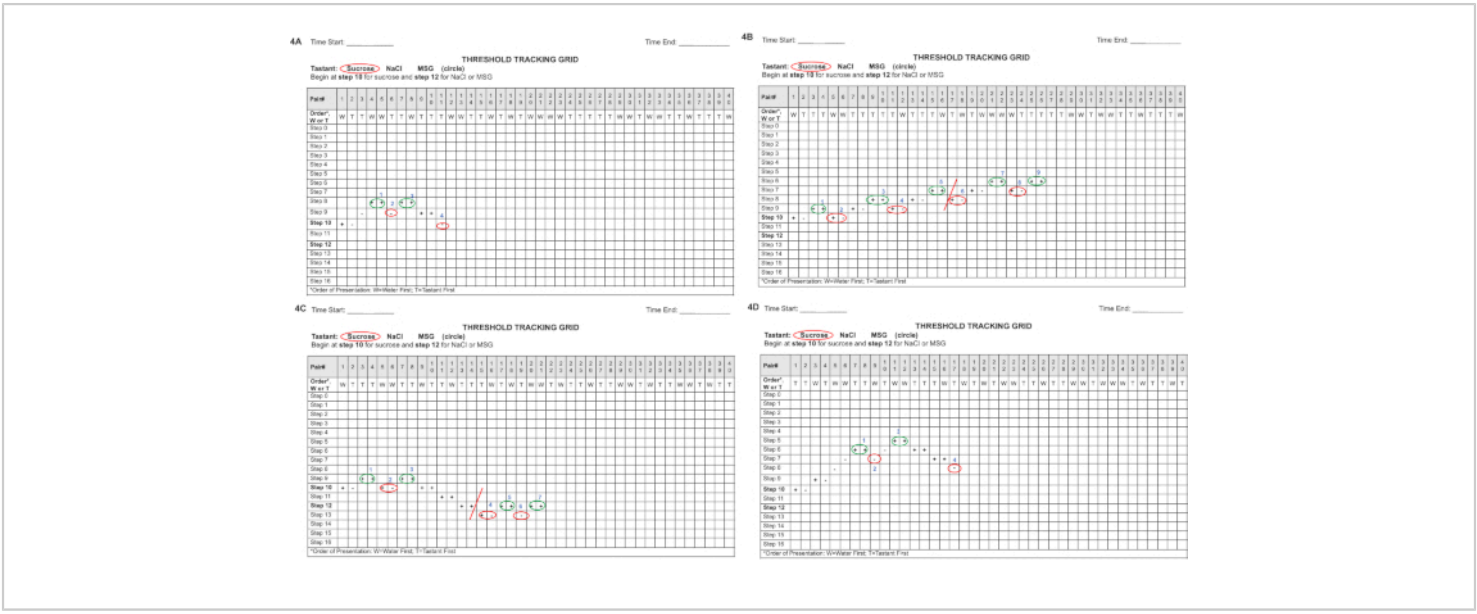

Figure 4: Tracking grids. (**A-D**) Representative data from four subjects. Please click here to view a larger version of this figure.

Discussion

The TDT test is a two-alternative, forced-choice, staircase procedure that uses strict rules to meet criteria than prior methods[12], thus ensuring a more stable outcome measure. Using criteria established at the Monell-Jefferson Chemosensory Clinical Research Center[2], the TDT is a reliable swish-and-spit method that measures the lowest concentration of sucrose, NaCl, or MSG in solution that can be detected by taste among individuals as young as 6 years. If completed as described, including enforcing participants rinsing their mouths before and after each tasting, the results are reliable and quick and provide insight into an important dimension of taste that is independent of hedonics[8].

Although the application of psychophysical tools to measure this dimension of taste is well established in the field, many methods have not been validated for use in children[14]. There are several critical steps in the protocol, some of which apply particularly to children [see also reference[15]]. First, criteria for attaining threshold should not rely solely on the occurrence of any four reversals or vary due to the age of the participant. Rather, there should be a maximum of two dilution steps between two successive reversals, and the series of reversals should not form an ascending pattern, which may be the case when the participant is simply guessing or not attending to the task. These additional criteria, which were established based on clinical experience[2], 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[16].

Second, the procedure is forced-choice, so if participants respond that "neither" or "both" solutions have a taste, that answer is not accepted. Rather, they are told to "guess." During TDT, participants often feel like they are guessing, but that should not be accepted as evidence that they are completely unaware of the taste stimuli[17]. Moreover, individuals may vary in their internal criteria for what

constitutes a taste sensation and hence, their willingness to say that a solution does or does not have a taste. Third, because the recency of eating affects taste perception[18], standardizing the time since the participant last ate or drank anything but water is important to reduce intersubject variability caused by sensory adaptation or enhancement. Fourth, the tastants used herein are palatable and presented in solution, not in a food matrix. When a food matrix is used, longer interstimulus intervals might be required for foods to clear the palate. While this method has been used to measure detection thresholds for sour or bitter tastants among adults[2,11], 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[19].

A forced-choice procedure of presenting up to four pairs of ascending concentrations of bitter-tasting solutions and dH_2O has been successful for pediatric populations[19,20]. Fifth, 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. In a recent study, 80% of the children provided sustained attention for, on average, 15 min and reached criteria[8]. Such information on completion of the tasks should be reported, particularly when pediatric populations are studied.

The present method has real-world relevance and has been used for assessing detection thresholds for the other basic tastes of sour (citric acid) and bitter (quinine)[2] and in adults of varying ages[8]. Because the method does not require verbal responses, the instructions should easily be translated to other languages[21], making it a valuable psychophysical tool for scientists worldwide. However, like any other psychophysical methods, there will likely be limitations in its use, particularly with younger children. The procedure may be more difficult to attain criteria for children than for adults. In one study, 20% of children did not reach criteria, compared to 5% of adults[8]. Reasons for non-completion included unfocused behavior, failure to understand the task, or becoming fatigued and unable to continue.

Findings from studies that used this taste TDT have contributed extensively to the diagnosis of taste ageusia in the clinic and have furthered the understanding of how taste sensitivity changes with age and health status. Clinical evaluation of patients revealed that sucrose detection thresholds ≥ 0.025 M for both sexes and NaCl detection thresholds ≥ 0.012 M for men or ≥ 0.010 M for women are considered abnormal[2]. Among adults, there is a gradual decline in taste sensitivity for sweet, salty, sour, and bitter tastes that continues into the eighth decade[22]. Younger adults typically have lower taste detection thresholds (are more sensitive) than are older adults[22,23,24,25]. However, children and adolescents have taste thresholds for sucrose that are higher (less sensitive)[8] and that are lower (more sensitive) than those of adults for the bitter taste of propylthiouracil, with the adult pattern emerging during adolescence[19,26].

Taste detection thresholds have been shown to be related to indicators of health. For example, salt taste detection thresholds positively correlated with systolic blood pressure among children who were normal weight[7], whereas children with central obesity had lower detection thresholds for sucrose (more sensitive) than those without central obesity[4], with similar findings among adolescents[27]. However, the relationship between obesity and sucrose detection

thresholds was not observed in adult women, and adult women with obesity had higher detection thresholds (were less sensitive) to the savory taste of MSG[9].

While research on the differences in detection thresholds between children and adults are limited, it is known that sucrose taste detection thresholds do not predict sweet taste preferences or suprathreshold intensity ratings from childhood to adulthood[8,28,29], providing further evidence that taste sensitivity represents a distinct dimension of taste that is independent of preferences and thus suggesting different underlying mechanisms. Greater understanding of the complex interplay among age, dietary habits, health status, and the sensitivity of the taste system, and whether such interactions differs among the primary tastants, is an important area for future research.

Disclosures

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References

1. Bartoshuk, L. M. The psychophysics of taste. *The American Journal of Clinical Nutrition.* **31** (6), 1068-1077 (1978).
2. Pribitkin, E., Rosenthal, M. D., Cowart, B. J. Prevalence and causes of severe taste loss in a chemosensory clinic population. *Annals of Otology, Rhinology, and Laryngology.* **112** (11), 971-978 (2003).
3. Kouzuki, M. et al. Detection and recognition thresholds for five basic tastes in patients with mild cognitive impairment and Alzheimer's disease dementia. *BMC Neurology.* **20** (1), 110 (2020).
4. Joseph, P. V., Reed, D. R., Mennella, J. A. Individual differences among children in sucrose detection thresholds: Relationship with age, gender, and bitter taste genotype. *Nursing Research.* **65** (1), 3-12 (2016).
5. Nance, K., Acevedo, M. B., Pepino, M. Y. Changes in taste function and ingestive behavior following bariatric surgery. *Appetite.* **146**, 104423 (2020).
6. Bobowski, N., Mennella, J. A. Repeated exposure to low-sodium cereal affects acceptance but does not shift taste preferences or detection thresholds of children in a randomized clinical trial. *Journal of Nutrition.* **149** (5), 870-876 (2019).
7. Bobowski, N. K., Mennella, J. A. Disruption in the relationship between blood pressure and salty taste thresholds among overweight and obese children.

Journal of the Academy of Nutrition and Dietetics. **115** (8), 1272-1282 (2015).

8. Petty, S., Salame, C., Mennella, J. A., Pepino, M. Y. Relationship between sucrose taste detection thresholds and preferences in children, adolescents, and adults. *Nutrients.* **12** (7), 1918 (2020).
9. Pepino, M. Y., Finkbeiner, S., Beauchamp, G. K., Mennella, J. A. Obese women have lower monosodium glutamate taste sensitivity and prefer higher concentrations than do normal-weight women. *Obesity (Silver Spring).* **18** (5), 959-965 (2010).
10. Pepino, M. Y., Mennella, J. A. Effects of cigarette smoking and family history of alcoholism on sweet taste perception and food cravings in women. *Alcoholism: Clinical and Experimental Research.* **31** (11), 1891-1899 (2007).
11. Cowart, B. J., Yokomukai, Y., Beauchamp, G. K. Bitter taste in aging: compound-specific decline in sensitivity. *Physiology & Behavior.* **56** (6), 1237-1241 (1994).
12. Hoehl, K., Schoenberger, G. U., Busch-Stockfisch, M. Water quality and taste sensitivity for basic tastes and metallic sensation. *Food Quality and Preference.* **21**, 243-249 (2010).
13. Wetherill, G. B., Levitt, H. Sequential estimation of points on a psychometric function. *British Journal of Mathematical and Statistical Psychology.* **18**, 1-10 (1965).
14. Chambers, E. Commentary: conducting sensory research in children. *Journal of Sensory Studies.* **20** (1), 90-92 (2005).
15. Mennella, J. A., Bobowski, N. K. Psychophysical tracking method to measure taste preferences in children and adults. *Journal of Visualized Experiments: JoVE.* 113, e354163 (2016).
16. Running, C. A. High false positive rates in common sensory threshold tests. *Attention, Perception, & Psychophysics.* **77** (2), 692-700 (2015).
17. Kunimoto, C., Miller, J., Pashler, H. Confidence and accuracy of near-threshold discrimination responses. *Consciousness and Cognition.* **10** (3), 294-340 (2001).
18. Puputti, S., Hoppu, U., Sandell, M. Taste sensitivity Is associated with food consumption behavior but not with recalled pleasantness. *Foods.* **8** (10), 444 (2019).
19. Mennella, J. A., Pepino, M. Y., Reed, D. R. Genetic and environmental determinants of bitter perception and sweet preferences. *Pediatrics.* **115** (2), e216-222 (2005).
20. Anliker, J. A, Bartoshuk, L., Ferris, A. M., Hooks, L. D. Children's food preferences and genetic sensitivity to the bitter taste of 6-n-propylthiouracil (PROP). *American Journal of Nutrition.* **54** (2), 316-320 (1991).
21. Okronipa, H. et al. Exposure to a slightly sweet lipid-based nutrient supplement during early life does not increase the level of sweet taste most preferred among 4- to 6-year-old Ghanaian children: follow-up of a randomized controlled trial. *The American Journal of Clinical Nutrition.* **109** (4), 1224-1232 (2019).
22. Murphy, C. The effect of age on taste sensitivity. In: *Special senses in aging: A current biological assessment.* Han, S. S., Coons, D. H. (eds) Ann Arbor, MI: Institute of Gerontology, 21-33 (1979).
23. Moore, L. M., Nielsen, C. R., Mistretta, C. M. Sucrose taste thresholds: age-related differences. *Journal of Gerontology.* **37** (1), 64-69 (1982).

24. Richter, C. P., Campbell, K. H. Sucrose taste thresholds of rats and humans. *American Journal of Physiology.* **128**, 291-297 (1940).

25. Schiffman, S. S., Sattely-Miller, E. A., Zimmerman, I. A., Graham, B. G., Erickson, R. P. Taste perception of monosodium glutamate (MSG) in foods in young and elderly subjects. *Physiology & Behavior.* **56** (2), 265-275 (1994).

26. Mennella, J. A., Pepino, M. Y., Duke, F. F., Reed, D. R. Age modifies the genotype-phenotype relationship for the bitter receptor TAS2R38. *BMC Genetics.* **11**, 60 (2010).

27. Pasquet, P., Frelut, M. L., Simmen, B., Hladik, C. M., Monneuse, M. O. Taste perception in massively obese and in non-obese adolescents. *International Journal of Pediatric Obesity.* **2** (4), 242-248 (2007).

28. Snyder, D. J., Prescott, J., Bartoshuk, L. M. Modern psychophysics and the assessment of human oral sensation. *Advances in Otorhinolaryngology.* **63**, 221-241 (2006).

29. Webb, J., Bolhuis, D. P., Cicerale, S., Hayes, J. E., Keast, R. The relationships between common measurements of taste function. *Chemosensory Perception.* **8** (1), 11-18 (2015).