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Biological Bases

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Section II
Biological Bases of Behavior

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1. Structure/Function Relationships within Sensory Systems
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    d. Intensity Rating Scale: Sweet & Sour

Lab Title: Structure/Function Relationships within Sensory Systems

Suzanne I. Sollars, Ph.D.

Section 1: Performance Expectations

What will the student be asked to do?

- Select one or more performance standards:
  - Formulate a directional hypothesis regarding the relationship between anatomical structure (number of taste papillae and associated taste buds) and function (taste intensities).
  - Conduct an experiment that contains both anatomical and behavioral data.
  - Analyze correlational data
  - Write a laboratory report that communicates whether your hypothesis was validated
- At the completion of the lab, students will be able to:
  - Create a directional hypothesis
  - Understand the concepts of how anatomical structure can influence perception and behavior

Main Idea/Concept Demonstrated or Taught by Lab:

Students will examine the way in which individual differences in anatomical structures can influence how the system functions. Perception of a sensory stimulus can vary between individuals. Such structure/function relations in physiological systems can change the way an individual behaves.

In this lab, students will test taste intensity of salt and sugar solutions, quantify fungiform papillae (an indirect measure of the number of taste buds), and determine the correlation between the number of fungiform papillae and taste intensity ratings.

Key Terms and Psychologists Associated with Main Idea/Concept:

fungiform papillae, taste buds, structure/function,

Materials:

Mirror
Lamp
Blue food coloring (large bottles can be obtained from vendors such as Amazon)
Cotton-tipped applicators (e.g. Q-tips)
Food scale for measuring salt and sugar
Beakers (plastic beakers are fine, they do not need to extremely precise)
Camera (most cell phone cameras are adequate)
Printer
Salt (any type)
Sugar
Purified water (bottled or large dispenser is fine, tap water is not best) for students to drink and for use in preparing solutions
Plastic cups (unless students get bottles of water to drink)
Vials that hold approximately 10 ml (1 tablespoon) of liquid. Each group of 5 – 8 students will need 16 vials.

Taste preference survey (attached Appendix A)
Rating sheets (attached Appendix B; each student needs all 4 pages accounting for vials 1-16)
Trash bag to place in center of each student groups' table to throw away applicators.
Tissues or paper towels
Recommended: Clock with second hand visible to all students or let students use cell phone clock to watch seconds. Otherwise, you can have students estimate the time mentioned in the instructions.

Instructions:

There are two parts to the experiment. Part 1 will have students taste and rate the intensity of sugar and salt solutions, and they will take a food preference survey. In part 2, students will determine the number of fungiform papillae in a small portion of the front of the tongue.

Prior to part 1, the purpose of the experiment should not be discussed with students. You may tell them that they will taste test sugar and salt solutions and rate the intensity of those solutions.

After the intensity ratings are completed, students may be told the purpose of the experiment and background information. They could be asked to generate hypotheses about the results of the experiment.

Part 2 is best conducted on a day separate from Part 1.

Procedure for Part 1

Preparation (Solutions for Part 1):

The day before the experiment, prepare the following:

Solutions

BEFORE adding solutions to vials, make sure there is lab tape on each vial. You should have multiple sets (depends on class size) each with 16 vials. I recommend each set has 2 different colors of label. 1-8 is one color and 9-16 is another color. Each vial within a set has a unique number from 1 through 16; mark this number on the label with permanent marker.

Follow the filling of vials as listed below. Each vial should be filled about 1/2 to 2/3 full.

Salt series	**Sugar series**
#1 = 1%	#9 = 5%
#2 = water	#10 = 10%
#3 = 0.5%	#11 = 20%

#4 = 2%	#12 = water
#5 = water	#13= 20%
#6 = 1%	#14 = 5%
#7 = 2%	#15 = 10%
#8 = 1%	#16 = water

Make sure to stir the solutions thoroughly prior to putting the solutions in the vials.

Instead of weighing out salt and sugar in grams, you could translate the quantities to teaspoons (there are online converters). This will result in less precision in concentrations, but as long as you give all students solutions that are prepared at the same time, the experiment will be valid.

Salt series

Measure 200 mL water. Add 4 grams salt – this is your 2% solution

To another 200 mL water, add 2 grams salt – this is your 1% solution

To another 200 mL water, add 1 grams salt – this is your 0.5% solution.

Sugar series

Measure 200 mL water. Add 40 grams sugar – this is your 20% solution

To another 200 mL water, add 20 grams sugar – this is your 10% solution

To another 200 mL water, add 10 grams sugar – this is your 5% solution

Keep extra solutions in case there are any spills during class. Make sure the beakers are labeled (using lab tape and sharpie). Wash out each vial after class is finished. Use water, rinsing thoroughly 2-3 times (more for sugar). Leave them to dry before putting away. Make sure the vials are not sticky before putting away. If any are, rinse these again.

Experimental Procedure

Have small groups (approximately 5 to 8) sit at a common table.

Give each group a set of vials (1 – 16)

Have students complete food preference survey (attached Appendix A). Make sure each student puts her or his name (or code name/number) on each element of the study (preference survey, rating sheets and tongue images).

Instructions to Students

In the first part of this experiment, you will be tasting sweet and salty solutions of differing concentrations. After you taste each solution, you will record how intense that taste seems to you. To record the taste intensities, you will choose a number from 0 to 10 on a scale. There will be one scale for each taste solution.

Please make sure to let your teacher know if you are restricted from tasting salt or sugar. You will not be consuming large quantities of these solutions, but if you need to opt out of this experiment, let your teacher know.

1. Remove any gum, candy, etc., from your mouth. During this experiment, do not consume anything other than the materials we provide.

2. Wash your hands well with soap and water.

3. Collect the following materials:

- 16 cotton swabs (cotton-tipped applicators)
- 2 Napkins
- 1 cup of water from dispenser (if you need more water during the experiment, you may get more)
- 1 set of printed taste intensity rating scales. Make sure you have scales with all numbers (to the left of each scale) from 1 to 16.

4. Set down one napkin and place your cotton swabs on it. Set the other napkin a few inches away from the other one. Put your vials in numerical order in front of a person in the group selected as the "start person." Write your first and last name on all the intensity rating sheets.

5. Here is what you will be doing, but do not start yet.

a. Before you start the experiment, take a small sip of the water in your cup, swish it around your mouth and swallow.

b. You will be opening the vials one at a time in order of the number marked on them.

c. For each vial make sure the rating sheet number and the vial number match before you begin the rating.

d. After a vial is open, you will put one end of the cotton applicator into the solution and give it about 10 seconds to absorb the liquid. Remove the swab and close the vial.

e. You will need to stick out your tongue then apply the solution that is on the swab to about the front third of your tongue, rolling the swab over the entire surface on both sides of your tongue. **Do not put the swab back into the solution** at any point. Put the used swab on the second napkin, or in the trash bag on the table.

f. You will keep your tongue out for 10 seconds after the solution is applied, then put your tongue back in and mark the intensity of the solution on the rating sheet, checking again that you are rating on the

scale that corresponds to the solution number you just tasted. If it doesn't match, contact your teacher.

g. After you rate the solution, take a small amount of the water from your cup (or bottle) and swish it around in your mouth, swallowing after about 10 seconds.

h. Wait 60 seconds. If you can still taste the solution you just rated, take another portion of water, swish it around for 10 seconds and swallow. If you consume more water, wait another 60 seconds. Repeat this procedure until the taste is completely gone.

i. Once you can no longer taste the solution you just rated, move on to the next vial. Repeat the above procedures one vial at a time. STOP after you complete vial #8.

j. Once you complete vials 1 through 8, take a 5-minute break and then start at 'a' again in these instructions, but start with the #9 vial.

k. At the end, throw your napkins, swabs and cup into the trash, wipe down the table, and give your ratings sheets to your teacher.

6. Optional

a. Record the date and the name of the module in data book or notebook page
b. As you proceed through the experiment, write any comments or problems you may have about the experiment
c. After you are finished with all the vials, write your overall perception of what you tasted.

Procedure for Part 2

Preparation

Make a 5% - 10% solution of blue food coloring to water. Add sugar to minimize the taste of the dye. Make sure to use fresh solution each experimental session. Place solution in small vials or plastic containers.

Prepare method for taking pictures and determine the method you will use to have students count papillae. A cell phone camera is sufficient, though best clarity will be achieved with a digital camera equipped with a macro lens. You will need to determine if you plan to download the images to a computer, print them (needs to be color printer), or analyze the images on the phone camera. The more precision you can obtain, the higher the validity of the results, but any of these methods can be adapted to work well for the experiment.

You will need to determine the method of counting papillae from images. If you print images, it is easy to establish a blocked area on one side of each tongue to count. For any method used, consistency across

students is key. You can count only one side of the tongue or both. The counts should be made slightly back from the tip. As you work through the method you will use, papillae count areas for each tongue are sufficient if an average number is around 25 (a range of approximately 15 to 65 per side is standard and shows the anatomical variation across tongue tissue).

If you plan to run this experiment repeated times and with many students, a chin rest may be worth purchasing. Good-Lite has product 705011, a chin rest with table clamp that works well, but any similar product would be fine. A chin rest helps to prevent excess head motion while the tongue images are photographed.

In advance of the experiment, test lighting conditions; a small lamp may be necessary.

Experimental Procedure

Students can work in pairs or groups. The camera and lighting should be ready to take a photograph.

One student will take a cotton-tipped applicator and dip it into the blue dye solution. That student will stick out her or his tongue and apply the solution over the front third. If the solution is too dark, a tissue or paper towel can be used to dab some off. The blue dye will stain the areas around the fungiform papillae more intensely than the papillae themselves, which should appear pink.

Once the student has the tongue properly dyed, the student should press down slightly with her or his front teeth onto the outstretched tongue. This will help stabilize the tongue and plump the tongue tissue which will help during the papillae counting procedure.

Another student should take two or three images, making sure that the images are clear. The picture should be close as possible to the tongue that also allows for a clearly focused picture. If the images are not in focus, it will be difficult to obtain data from them. Make sure images are labeled with student names or code used on the intensity ratings, since you will compare the student papillae counts with their taste intensity data.

Have students use the procedure you determined to count papillae.

Analysis

Use the attached Excel sheet format (Appendix C) to obtain correlations and figures for the taste intensity ratings and the papillae counts. Note the initial calculations add the preference numbers on the rating scale for the 1% and 2% salt solutions for each student, and the 10% and 20% solutions of sucrose. For a large class, this is the simplest measure to obtain reliable preference data. You may instead decide to determine the correlations for each taste and concentration for a more precise measure.

The food preference surveys are used for student discussion. Once they determine their papillae counts, the survey can be useful for them to reflect upon their individual anatomical and intensity rating results to see if their preferences correspond to what is predicted.

Information for each question on the food preference survey:

1. Bitter is a primary taste. Caffeine is bitter.

2. Deep green vegetables are often bitter.

3. Fats are considered by some researchers to be a primary taste.

4. Sugar is a primary taste.

5. Although "spiciness" is not a taste, spicy hot peppers have a substance called capsaicin. Capsaicin affects somatosensory receptors in the filiform papillae and in the connective tissue surround of the fungiform papillae (areas around, but not in, the taste buds).

6. Salt is a primary taste.

7. All foods tend to seem more intense to those with more papillae (and thus more taste buds). Lifelong food selection is sometimes guided by individual differences.

If there was strong agreement with these questions, did the student have a high papillae count? Were intensity ratings high? Conversely, if strong disagreement, did the student have a low papillae count and low intensity ratings?

Follow-up and Discussion:

- **Think about the following questions to guide discussion and check for understanding:**
    - There is a common error on the internet which suggests taste buds occur on the lips, cheeks and hard palates of humans, as well as on the tongue. Often, the soft palate of the roof of the mouth, where there are taste buds, is ignored. While there are taste buds in areas other than the tongue and soft palate in other species, this is not the case in humans. What perpetuates the myth? To explore the differences between "popular press" and scientific research, students could explore both avenues of information and see how they differ.
    - The taste map is another myth long perpetuated. The concept of the map is that there are specific locations across the tongue where each individual taste is intensely concentrated. The myth of the taste map resulted from a mistranslation of research published by David Hänig in 1901. The true nature of taste is that all tastes are represented in every area of the tongue (and soft palate), with only moderate variations noted in taste responses profiles. Have students research how taste is conveyed across the tongue and soft palate. Make sure they use peer-reviewed research and not simply internet searches!

Section 2: Crosscutting Concepts

Select at Least One

- Structure and Function, Cause and Effect, Systems and Model
    - Structure/function relations exist in many physiological systems. Have students explore other sensory systems to determine how other systems may reflect individual differences in function based on anatomical differences.
    - How could diet and health be influenced by the individual differences in food selection that results from individual differences in taste buds and perceptual intensity of tastes? Are there other influences that could override food selection?
    - The foundation of the study is based in genetic differences and the concept of "tasters" and "non-tasters." How do the studies and concepts relate?

Section 3: Lab Report (written, verbal, or recorded)

Suggested Content to be Included in Student Report:

- Introduction to include basic information about the taste system (both in structure, e.g. taste buds, innervation of taste and trigeminal nerves, brain pathways) and function (e.g., taste receptor cell functions, perception of taste intensity, food selection).
- Objectives: Understanding how individual differences in anatomical structure can reflect individual differences in perception and behavior.
- Hypotheses formulated after taste intensity tests.
- Study design and materials
- Experimental procedure including all instructions given during the experiment
- Figures of correlations of taste intensities and taste papillae counts
- Results and Discussion
        - Were the results supportive of the hypotheses?
    - What do these findings suggest about the role of genetic influences in the way we perceive sensory stimuli?
    - What are other ideas to build further upon the concepts contained within this laboratory?

References, recommended readings and video:

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Masi, C., Dinnella, C., Monteleone, E. and Prescott, J. (2015). The impact of individual variations in taste sensitivity on coffee perceptions and preferences. *Physiology and Behavior, 138,* 219-226. doi.org/10.1016/j.physbeh.2014.10.031

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Nuessle, T. M., Garneau, N. L., Sloan, M. M., Santorico, S. A. Denver Papillae Protocol for Objective Analysis of Fungiform Papillae. *Journal of Visualized Experiments* (100), e52860, doi:10.3791/52860 (2015). Video: https://www.jove.com/video/52860/denver-papillae-protocol-for-objective-analysis-of-fungiform-papillae

Lab Title: Modification of Sensory Receptor Structure and Function

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Section 1: Performance Expectations

What will the student be asked to do?

- **Conduct and/or participate in a study that examines cellular function.**
- **Analyze response data from modified Likert Scales.**
- **After completing the lab, students will understand:**
  - Sensory receptor cells interact with chemicals in the environment; through these interactions, cellular structure may be modified.
  - Modification of cellular structure may result in changes in neural function and perception.
  - Nonparametric analysis of data.
  - Communication of experimental ideas through a lab report.

Main Idea/Concept Demonstrated or Taught by Lab:

Students will understand how modification of cellular function may lead to changes in sensory perception.

Key Terms and Psychologists Associated with Main Idea/Concept:

sensory receptor; miraculin; Likert scale; blind coding of data

Materials:

Miracle Berry (Miraculin Tablets) 300 mg (can break 600 mg tablets in half)

Available on Amazon, Think Geek, and other vendors (slight preference for Miracle Frooties brand)

Solutions

Fruit

Lemons

Limes

Grapefruit (yellow pulp works best)

Oranges

Salt (any kind is fine)

Bottle of purified (reverse osmosis) water

6 Plastic food containers – 1 cup or greater capacity

Vials (small cups or other small containers that hold about a tablespoon of liquid)

Label tape

Juicer (juice reamer or juicing by hand is fine)

Food coloring (yellow preferred; any color is fine)

Fine mesh strainer

Coffee filter papers

Coding sheets (attached Appendix D)

Q-tips

Napkins/paper towels

Drinking water. Could be individual bottles, or large jug (give students cups). Preferably reverse osmosis water, but any type will be fine. Should be room temperature at time of experiment.

Trash bags for the Q-tips.

Clock with second hand available for students to watch. Alternatively, they can use any type of device that will allow them to observe time in seconds.

Preparation for Lab

Solution preparation

You will need 6 food containers for: lime juice, lemon juice, orange juice, grapefruit juice, salt water, and water. Use label tape to mark each one.

No more than two days in advance of the experiment, juice the citrus fruit into plastic food containers. Place the fine mesh strainer over a plastic food container and juice each citrus fruit into its own container. If mesh is large enough to allow pulp to get through, use coffee filter paper to strain out remaining pulp.

Make a salt solution with 1/8 teaspoon of salt to 1/2 cup of bottled water. This is approximately equivalent to 0.1 Molar (0.1 M) or 0.6% weight per volume.

Put bottled water separately into a food container.

To each container, add approximately 1 - 3 drops of food coloring. The goal of the food coloring is to try to mask the type of solution.

Decide upon an order to present the solutions. Randomization works best. All students will get the solutions in the same order. Solutions will be given in the same randomized order in the pre-miraculin condition and the

post-miraculin condition. Make sure to record the order of solution delivery and keep it hidden from participants until after the experiment is complete.

Vial preparation

For each set of 5 to 6 students, you will need a set of 12 vials (or small food containers).
In advance of adding solutions to the vials, use label tape to number each vial (1 through 12). Different colors of label tape could be used to help distinguish the vials for each set of students. Do *not* record any indication of the solution type on the label.

Fill vials 1 through 6 approximately half full, making sure to match the vial label number to the randomized order you decided for solution presentation. Fill vials 7 through 12 in the same manner, again matching the vial number to the order of solution presentation.
As an example: Vial #1 = lime; #2 = grapefruit; #3 = salt; #4 = orange; #5 = water; #6 = lemon; #7 = lime; #8 = grapefruit; #9 = salt; #10 = orange; #11 = water; #12 = lemon.

Refrigerate solutions if they are prepared in advance of the experiment day. Be sure to remove the vials from the refrigerator in time for the solutions to obtain room temperature prior to the start of the experiment.

Coding sheet preparation

Each student will need one set of Likert-style coding sheets (attached Appendix D; all 6 pages for each student).

Miraculin preparation

If 600 mg miraculin is purchased, break each tablet in half. Pushing on the center of the tablet with a knife works well. Put miraculin tablets in a separate food container that is does not have an identifying label.

Experiment Instructions:

General procedure: During the experiment, students will dip a cotton-tipped applicator into a solution apply it to their tongue, record *both* the sweet intensity and the sour intensity of the solution on the coding sheet, rinse with water, then move to the next solution. After all students have tasted all solutions in vials 1 - 6, they will let a tablet of miraculin dissolve on their tongue, then wait 15 minutes. Afterwards, they will taste the solutions in vials 7 – 12 and record both sweet intensity and sour intensity on their coding sheets.

{At the start of the experiment, and before using the words “cotton-tipped applicator,” hold up a Q-tip and ask students if they know the ‘scientific’ name for it. It is a lesson in learning that brand names are not used in research methods.}

Specific Procedures

Students sit at tables in groups of approximately 4 to 8. They should have a pen.

Ask students to rid their mouths of any gum or food.

Have each student collect supplies:

1 set of coding sheets (each student needs all pages accounting for vials 1 - 12)

12 cotton-tipped applicators (Q-tips)

Bottle (or cup) of water

1-2 napkins (they place Q-tips on the napkins)

Tell students that if they consumed any food or drink within the previous half-hour, they should take a drink of water, swish it around their mouth and swallow.

Place one set of vials (1 through 6) on each table.

Set one trash bag in the middle of each table.

Have students choose a code name/number and have them record this on each page of their coding sheets.

If you plan to have the students write a report based on the experiment, you may tell them to write down all instructions you give them or give them a handout of the instructions.

Ask anyone who has a food allergy or sensitivity to raise his or her hand. Take each student who raises their hand out of hearing range of the group, and ask about the nature of the allergy/sensitivity. If the allergy/sensitivity is to citrus fruit, salt, or potato (potato starch is the base of the Frooties – if you use a different brand, determine the base substance of the tablet), excuse them from the data collection portion of the experiment.

Tell students the following instructions:

Students may, at any time, opt out of the experiment by raising a hand and letting you know she or he would like to opt out.

They will taste several food-based substances. After the experiment is completed, you will tell them what the substances are.

During the experiment, students may talk to members in their group, *except* any discussion about the tastes.

Throughout the experiment, they should be careful to avoid making facial reactions or any other indication about the tastes.

In order to ensure individual experiences are recorded on the rating sheets, looking at the responses to tastes recorded on other students' coding sheets is not allowed.

It is very important to note that for each taste solution, they will be recording BOTH the sweet intensity and the sour intensity perceived. Have them look at the coding sheets and note the scale from "*not at all*" to "*strongest imaginable*."

Make sure coding sheets are in order from 1 to 12. As they are tasting the substances, they should double-check each time that the coding sheet scale and the vial number match before recording ratings. If a mistake is recorded, they should raise a hand and let you know right away. A simple correction in rating can be made by putting an 'X' through the mistake and circling the new intensity selection. Changes in ratings should be made only if the next solution has not yet been tasted.

Each group will choose a start person.

> This person starts with vial 1. Other students wait, such that each student receives the solutions to taste in the same order 1 through 6 (i.e., all students taste the solutions in the same randomized order that you decided in advance of the experiment).

When told to start, everyone will take a sip of water, swish it around in her or his mouth and swallow.

Then, the start person from each group will select vial 1, open it, and insert one cotton-tipped applicator, making sure to absorb the solution.

The start person will hold their tongue out of the mouth with their mouth open.

The student will roll the cotton-tipped applicator with the solution across the front 1/3 or more of the tongue, making sure to contact all of this area of the tongue with the solution.

The tongue should be left out for 5 seconds, getting a sense of the intensity of the taste.

After the 5 seconds, the tongue should be placed fully back in the mouth and then a rating made on the Likert Scale for *both* the sweet intensity and the sour intensity of the solution, checking first to make sure the vial number and the scale number match. Emphasize that both the sweet intensity and the sour intensity should be rated for each solution, even if the solution cannot be characterized as either taste. As an example, suppose they taste something intensely sweet that has no sour taste. They might record a '9' on the sweetness scale and a '0' on the sour scale.

The start person passes the solution vial to the person on the right, who can start the procedure.

After recording intensity responses, she or he takes a drink of water, swishes it around the mouth, swallows, and waits 30 seconds (watching the time on the clock).

If the taste of the solution remains after 30 seconds, the student takes another drink of water and waits an additional 30 seconds. This rinse procedure is repeated until the taste no longer remains.

Once the taste of the solution no longer remains, they open vial 2 and repeat the procedure.

Each student follows the same procedure when receiving the vial passed to her or him.

Students pass the vials in numerical order until all students have tasted and recorded the intensities of all 6 solutions.

After all groups have completed tasting solutions in vials 1 through 6, students each take another sip of water and wait 5 minutes.

Give each student one of the ½ tablets (300 mg) of miraculin. Have them place it on their napkin. Do not tell them what it is; you may explain it is a food-based substance.

Tell them to place the tablet in their mouth and let it dissolve. They should not chew. The tablet should be moved around the mouth especially focusing on letting it dissolve on the areas on the tongue where they applied the solution in the vials.

Have each student raise his or her hand once the tablet is dissolved. This will let you know when all the tablets have dissolved.

Once all tablets are dissolved, tell the students they will get a 15-minute break. [They can get a break up to 30 minutes, but no shorter than 15 minutes.] During this time, they should not eat or drink anything, not even water.

Clear each table of the first sets of vials and place the vials 7 through 12 on the tables. Do not tell them this set is the same as the first set of vials.

Have students repeat the above procedures, recording taste intensity responses for the solutions in vials 7 - 12. Remind them to check the vial number against the recording sheet to make sure they match.

Collect coding sheets.

Prior to debriefing students after the experiment, ask them what they experienced while tasting the solutions in the vials.

For each type of solution, ask their prediction for the direction of ratings after miraculin as compared to those before miraculin.

Debriefing:

Tell students about the solutions they tasted, the order of solutions, and how miraculin affects taste receptor cells.

After you debrief them, ask them if they thought the solutions tasted sweeter after miraculin, and if so, whether all the solutions tasted sweeter.

Let them know that miraculin's effects to the taste system will wear off in approximately 2 hours.

Possible Further Procedures:

Cut pieces of fruit such as lime, lemon and grapefruit into small pieces. After the experiment is completed and you have explained the function of miraculin, offer the students the fruit. Have them describe their experience.

Follow-up and Discussion:

Data Analysis

The procedure for data analysis can involve the students, or the data can be computed, and the results given to them.

Statistical software may be used, though data analysis is easily accomplished without statistical software.

The Wilcoxon Signed-Ranks Test is a nonparametric test. This test is appropriate for ordinal data [the order of the variable is important (e.g., a rank of '2' suggests a *less* intense taste than a value of '6'), but the differences between the rankings are not necessarily equivalent (e.g., the intensity differences between a rank of '2' and '4' is not necessarily equivalent to the difference in intensity between ranks '5' and '7')]. The Wilcoxon is used when the comparison is between two non-independent variables. In the case of the miraculin study, the rating for solution in vial '1' will be compared to the rating for vial '7,' rating for '2' compared to the rating for '8,' etc.

For each student, assign a letter and record the pre-miraculin ratings and post-miraculin ratings for each solution. Place the numbers to be compared next to each other.

Calculate the difference for each participant and each solution as a before-miraculin minus post-miraculin measure as in the following example for lemon sour taste rating.

Participant	Before Miraculin	After Miraculin	Difference Score
A	9	0	9
B	8	1	7
C	8	7	1
D	9	9	0
E	3	2	1
F	7	6	1
G	8	1	7
H	7	8	-1
I	7	2	5
J	2	4	-2
K	10	4	6
L	7	2	5
M	10	2	8

Rank order the participants using the absolute value of the difference score. When the difference scores are the same (e.g., C, E, F, and H), add their rank numbers and calculate the average. The average of the ranks becomes the rank for *each* of those participants. All difference scores of zero get ranked as zero. In other

words, when using this particular way to calculate, you ignore the participants who reported the same rating before miraculin as they did after miraculin.

Participant	Difference Score	Rank	Recalculated Rank
D	0	0	--
C	1	1	2.5
E	1	2	2.5
F	1	3	2.5
H	-1	4	2.5
J	-2	5	5
I	5	6	6.5
L	5	7	6.5
K	6	8	8
B	7	9	9.5
G	7	10	9.5
M	8	11	11
A	9	12	12

The statistic you calculate (W) is a sum of the recalculated ranks. However, you sum the ranks that had negative difference scores separately from the ranks that had positive difference scores.

In the example, add H and J's ranks (W^- = 2.5 + 5 = 7.5). Add C, E, F, I, L, K, B, G, M, and A's ranks (W^+ = 2.5 + 2.5+ 2.5 + 6.5 + 6.5 + 8 + 9.5 + 9.5 + 11 + 12 = 70.5). Take the lowest of the calculated scores (7.5 in this example). This becomes your *T* value in the following formula.

$$z = \frac{T - \frac{n(n+1)}{4}}{\sqrt{n(n+1)(2n+1)/24}}$$

The n equals the total number of participants minus any that had a zero rank (n = 12 in this example).

Thus, in the example for ratings of sour for lemon,

$$z = \frac{7.5 - \frac{12(12+1)}{4}}{\sqrt{12(12+1)(2x12+1)/24}}$$

$$z = \frac{7.5 - \frac{156}{4}}{\sqrt{156x25/24}}$$

$$z = \frac{7.5 - 39}{\sqrt{3900/24}}$$

$$z = \frac{-31.5}{12.74}$$

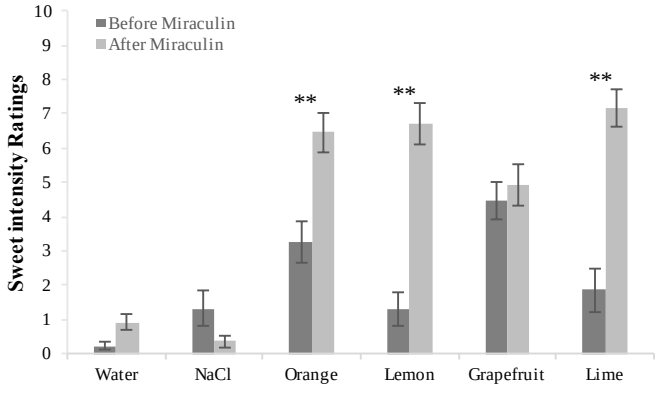
z = -2.47

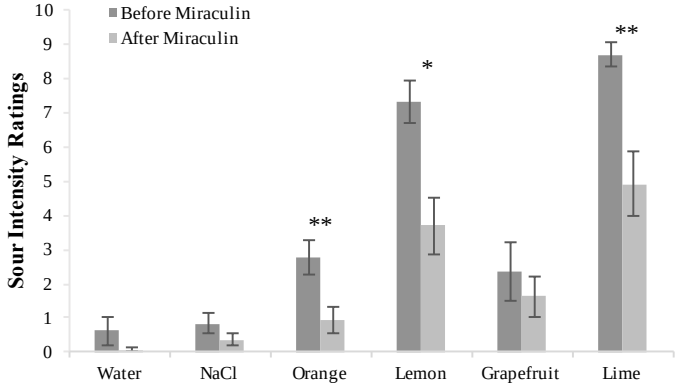
Use z table look-up for *p* value. Here, *p*-value = 0.00676. The alpha level is $p < .05$

Resources: There are online calculators for the Wilcoxon Signed-Ranks Test. One good one can be found here: http://www.socscistatistics.com/tests/signedranks/Default.aspx

Prepare Figures

Prepare 2 figures. One will have the results of the sweetness ratings and the other will have the results of the sour intensity ratings. Figures will have the y-axis representing intensity ratings, and the x-axis representing substances tasted. Data will be plotted as bars. Below is an example.





* $p < .05$; ** $p < .01$

Prior to debriefing students after the experiment, ask them what they experienced while tasting the solutions in the vials.

For each type of solution, what is their prediction for the direction of ratings after miraculin as compared to those before miraculin?

After you debrief them, ask them if they thought the solutions tasted sweeter after miraculin, and if so, whether all the solutions tasted sweeter.

Discussion

Miraculin works to change sweet receptors on taste receptor cells of the taste buds. After miraculin contacts taste buds, the sweet receptors will allow sour substances to bind to them. Since sweet taste receptors convey the perception of "sweet" to the brain, even when the receptors bind sour substances the brain still interprets the information as "sweet." Although sometimes there are noted changes in taste of substances other than sour components, the mechanism of miraculin works primarily with sour components (Kant, 2005; Koizumi et al., 2011; Sato, 1987). Thus, the salt and water solution likely did not taste sweeter.

Acidic substances such as citrus fruit are still perceived as sour after miraculin because sour taste receptors remain functional. The overall perception of sourness may decrease when the sweetness of a substance is perceived as stronger after miraculin. This does not mean that the sour receptors change. Rather, the brain perceives sweet as stronger which can mask some of the brain's interpretation of intensity of the sour taste (Capitanio, Lucci and Tommasi, 2011).

Taste sensory receptors have receptor subtypes on them that are responsive to the basic tastes. When these receptors are activated, they signal information to the brain that encodes our interpretation of whether something is sweet, sour, salty or bitter. Sensory experience is, in part, a product of what sensory receptors are activated and the locations where their circuits activate the brain. Sometimes, like in this study, a stimulus (sour in this example) is able to activate a sensory receptor that it usually does not activate. Since the sensory receptor is of a particular type (sweet receptor in this example), the brain circuits signal to produce a sensation of "sweet" even though humans do not usually consider the chemical components in sour substances sweet (see Breslin, 2013 for review).

Section 2: Crosscutting Concepts

Select at Least One

- Patterns, Structure and Function, Stability and Change
- Students will discuss ways other types of sensory systems encode information and how the information might be modified by experience, drugs, or individual differences.
- Students will explore and report on differences and similarities in sensory system receptor types across species.
- Students will discuss the concept of taste as a construct. We often say a food has a taste quality, such as sweet or sour. Do the results of the experiment suggest otherwise (e.g., is a lime "sour"?).

Section 3: Lab Report (written, verbal, or recorded)

Suggested Content to be Included in Student Report:

The laboratory report should include the following:

- Introduction, including background information on the taste system, taste sensory receptors, and miraculin history and effects on taste receptors
- Experimental questions posed by the study
- Study design and materials
- Experimental procedure including all instructions given during the experiment
- Results of the experiment including the statistical findings
- A figure of the results
- Discussion including
    - Why some of the ratings may have changed pre-miraculin as compared to post-miraculin and why others did not.
    - Other experiments that have used miraculin in different species and their findings
    - Alternate methods to test receptor function and/or how the brain processes that information

References and Suggested Readings

Breslin, P. A. S. (2013). An evolutionary perspective on food review and human taste. *Current Biology, 23*(9), R409—R418. doi.org/10.1016/j.cub.2013.04.010

Capitanio, A., Lucci, G., & Tommasi, L. (2011). Mixing taste illusions: The effect of miraculin on binary and trinary mixtures. *Journal of Sensory Studies*, *26*(1), 54-61. doi:10.1111/j.1745-459x.2010.00321.x

Igarashi, G., Higuchi, R., Yamazaki, T., Ito, N., Ashida, I., & Miyaoka, Y. (2013). Differential sweetness of commercial sour liquids elicited by miracle fruit in healthy young adults. *Food Science and Technology International, 19,* 243-249. doi: 10.1177/1082013212443060

Kant, R. (2005). Sweet proteins- potential replacement for artificial low-calorie sweeteners. *Nutrition Journal*, 4(5). doi:10.1186/1475-2891-4-5

Koizumi, A., Tsuchiya, A., Nakajima, K., Ito, K., Terada, T., Shimizu-Ibuka, A.,…& Abe K. (2011). Human sweet taste receptor mediates acid-induced sweetness of miraculin. *Proceedings of the National Academy of the Sciences,*108(40), 1-6. doi: 10.1073/pnas.1016644108

Sato, M. (1987). Taste receptor proteins. *Chemical Senses,* 12(2), 277-283. doi.org/10.1093/chemse/12.2.277

Sun, H., Kataoka, H., Yano, M., & Ezura, H. (2007). Genetically stable expression of functional miraculin, a new type of alternative sweetener, in transgenic tomato plants. *Plant Biotechnology Journal,* 5(6), 768–777. doi: 10.1111/j.1467-7652.2007.00283.x

Appendix A. Food Preference Survey

PLEASE CIRCLE MOST ACCURATE RESPONSE ON THE LINES BELOW THE QUESTIONS:

Strongly **Disagree** = 1 Disagree = 2 Neutral = 3 Agree = 4 Strongly **Agree** = 5

1. I don't like coffee, or I drink coffee only if there is cream and/or sugar in it.

1	2	3	4	5

2. I avoid foods such as raw broccoli, dark leafy greens or brussels sprouts because they are bitter.

1	2	3	4	5

3. Strictly from a *taste* perspective, I prefer skim milk to the taste of whole or 2% milk.

1	2	3	4	5

4. When I drink pop, I prefer the *tastes* of those with less sugar (diet, clear sodas, etc.).

1	2	3	4	5

5. If a waiter asks me how spicy I would like my dish, I would request it to be mild or unseasoned.

1	2	3	4	5

6. I like chips, fries or popcorn when they are unsalted or have a minimal amount of salt.

1	2	3	4	5

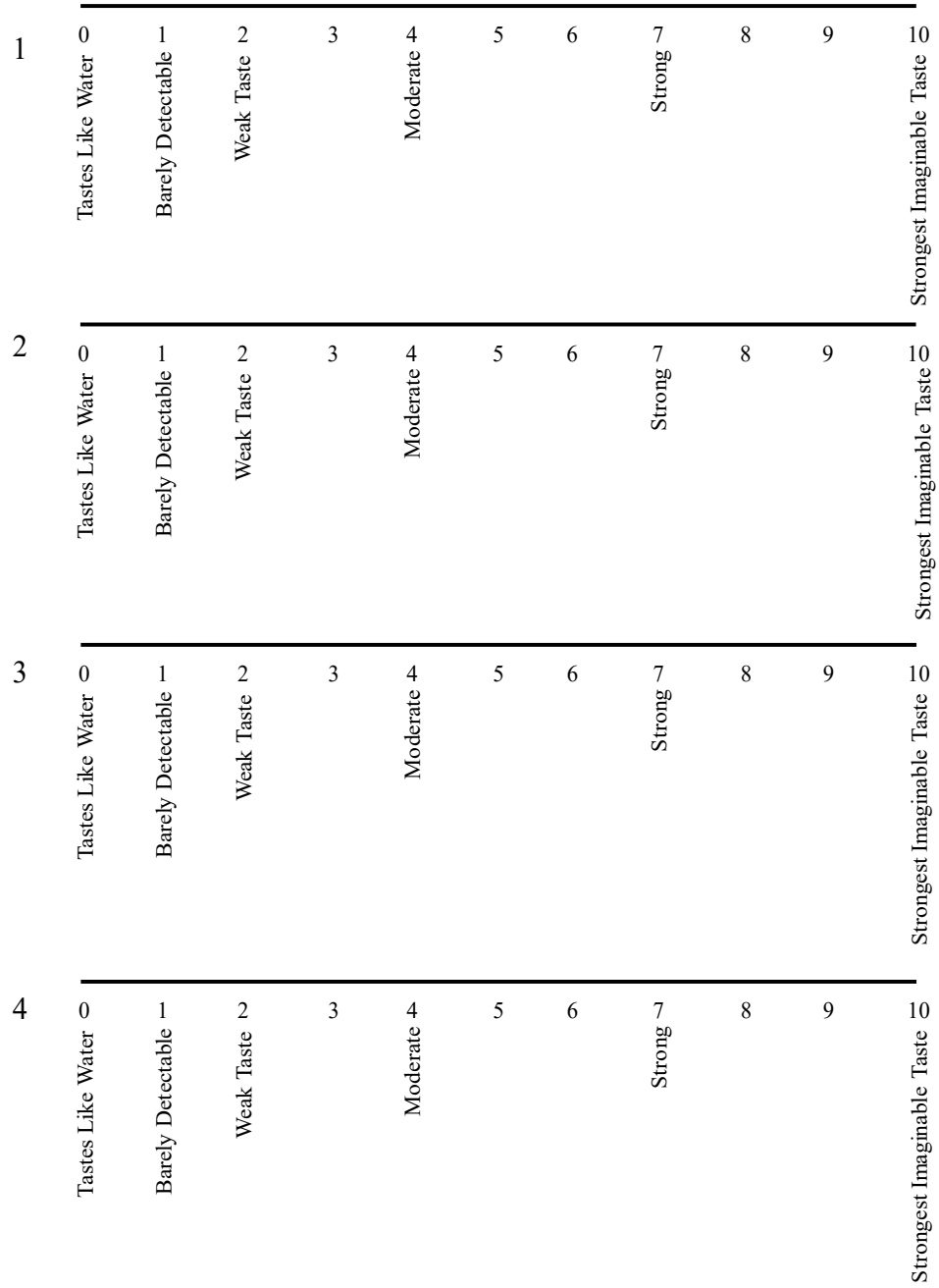
7. I have been called a picky eater and/or I tend to prefer bland foods.

1	2	3	4	5

Appendix B. Intensity Rating Scale

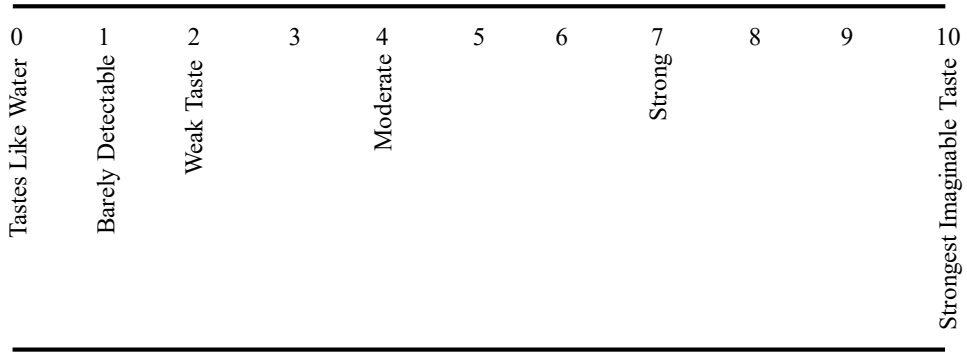
Intensity Rating Scale

Name _____

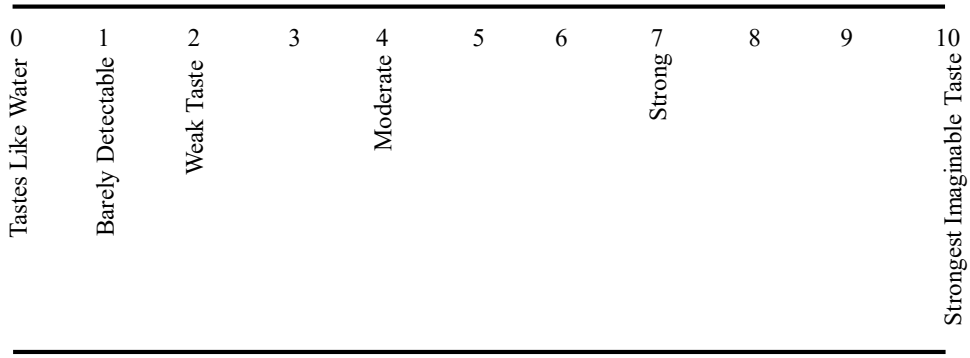


Name _____

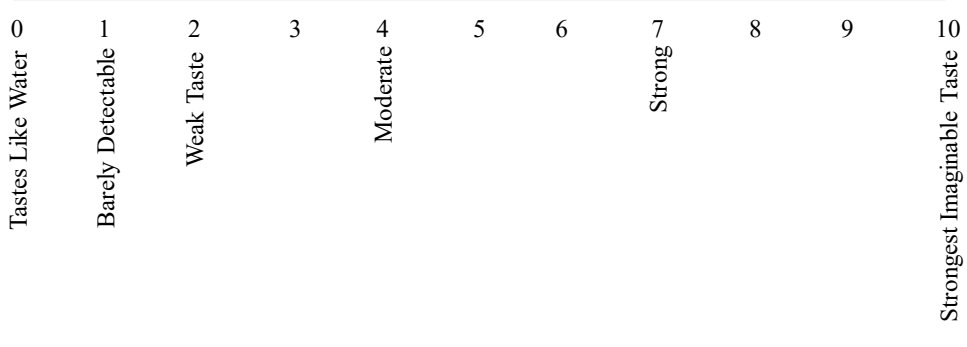
5


0 Tastes Like Water
1 Barely Detectable
2 Weak Taste
3
4 Moderate
5
6
7 Strong
8
9
10 Strongest Imaginable Taste

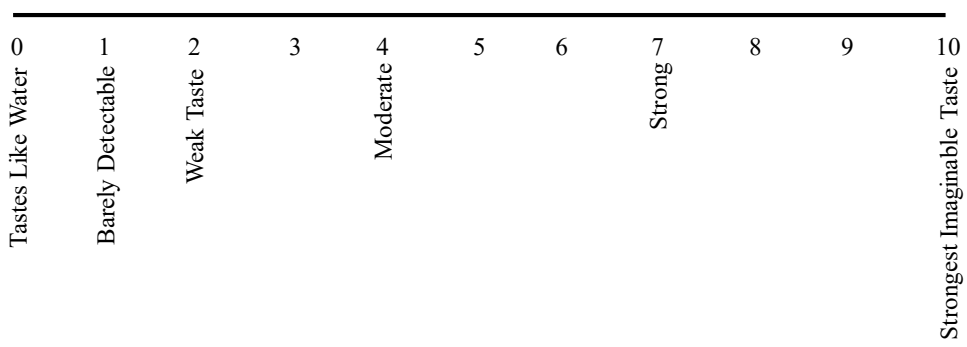
6


0 Tastes Like Water
1 Barely Detectable
2 Weak Taste
3
4 Moderate
5
6
7 Strong
8
9
10 Strongest Imaginable Taste

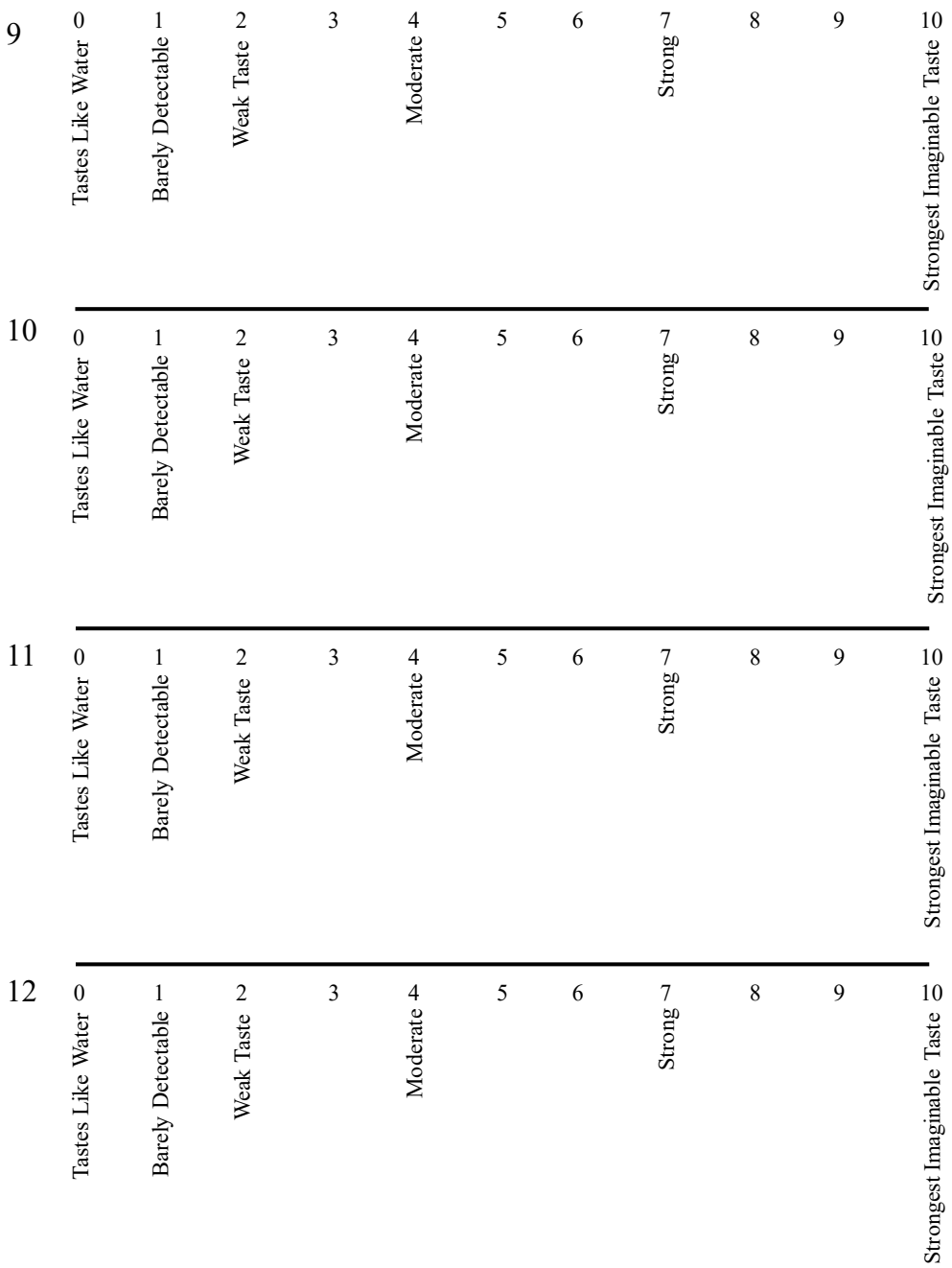
7


0 Tastes Like Water
1 Barely Detectable
2 Weak Taste
3
4 Moderate
5
6
7 Strong
8
9
10 Strongest Imaginable Taste

8


0 Tastes Like Water
1 Barely Detectable
2 Weak Taste
3
4 Moderate
5
6
7 Strong
8
9
10 Strongest Imaginable Taste

Name _____


9
0 Tastes Like Water
1 Barely Detectable
2 Weak Taste
3
4 Moderate
5
6
7 Strong
8
9
10 Strongest Imaginable Taste
10
0 Tastes Like Water
1 Barely Detectable
2 Weak Taste
3
4 Moderate
5
6
7 Strong
8
9
10 Strongest Imaginable Taste
11
0 Tastes Like Water
1 Barely Detectable
2 Weak Taste
3
4 Moderate
5
6
7 Strong
8
9
10 Strongest Imaginable Taste
12
0 Tastes Like Water
1 Barely Detectable
2 Weak Taste
3
4 Moderate
5
6
7 Strong
8
9
10 Strongest Imaginable Taste

Name _____

13

0	1	2	3	4	5	6	7	8	9	10
Tastes Like Water	Barely Detectable	Weak Taste		Moderate			Strong			Strongest Imaginable Taste

14

0	1	2	3	4	5	6	7	8	9	10
Tastes Like Water	Barely Detectable	Weak Taste		Moderate			Strong			Strongest Imaginable Taste

15

0	1	2	3	4	5	6	7	8	9	10
Tastes Like Water	Barely Detectable	Weak Taste		Moderate			Strong			Strongest Imaginable Taste

16

0	1	2	3	4	5	6	7	8	9	10
Tastes Like Water	Barely Detectable	Weak Taste		Moderate			Strong			Strongest Imaginable Taste

Appendix C. Taste Rating Calculations

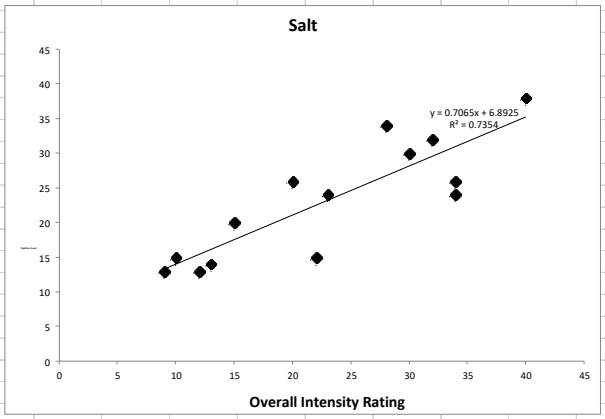
Taste Ratings calculations

Calculate the total of SALT ratings for each student.
Use data from ratings 1. 4. 6, 7 and 8 ONLY == add the numbers for each student

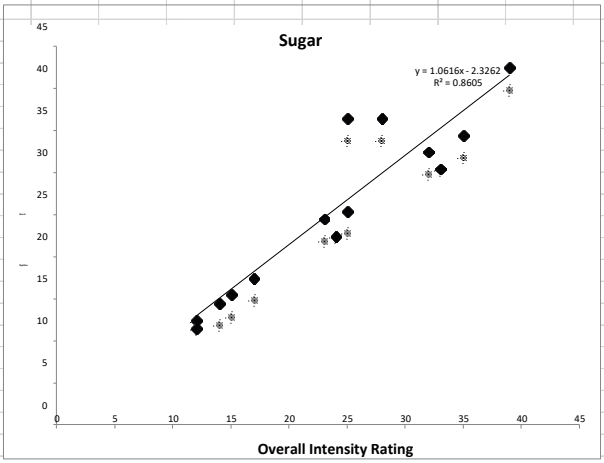
Calculate the total of SUGAR ratings for each student
Use data from ratings 10, 11, 13, and 15 ONLY == add the numbers for each student

Match each student's papillae counts with their overall salt rating & enter below
Match each student's papillae counts with their overall sugar rating and enter below

Student	Papillae	Salt	Sugar
1	23	24	25
2	32	32	35
3	12	13	14
4	15	20	17
5	30	30	32
6	22	15	23
7	34	26	28
8	40	38	39
9	34	24	25
10	13	14	15
11	10	15	12
12	28	34	33
13	9	13	12
14	20	26	24



Select Papillae & Salt Columns

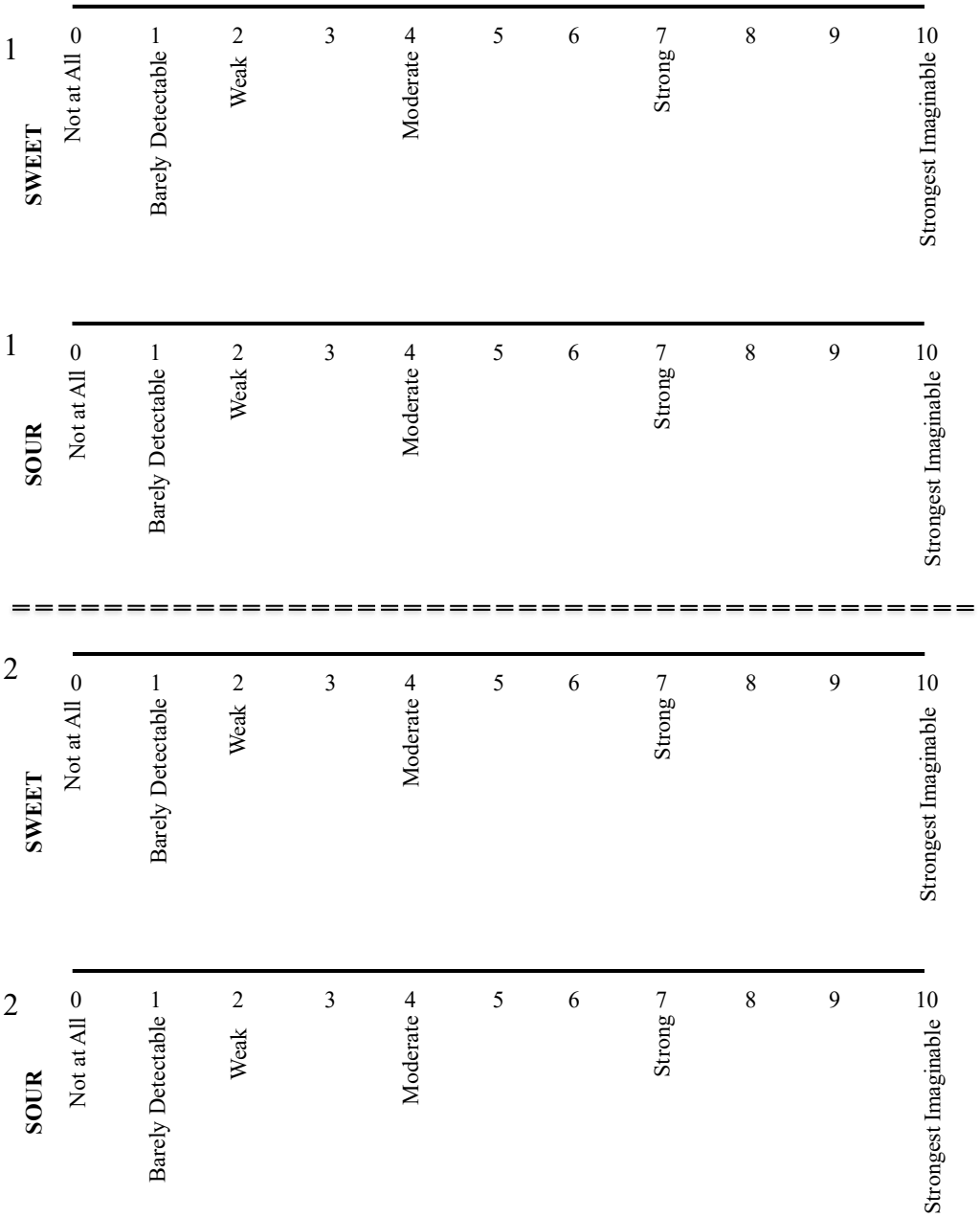


Select Papillae & Sugar Columns

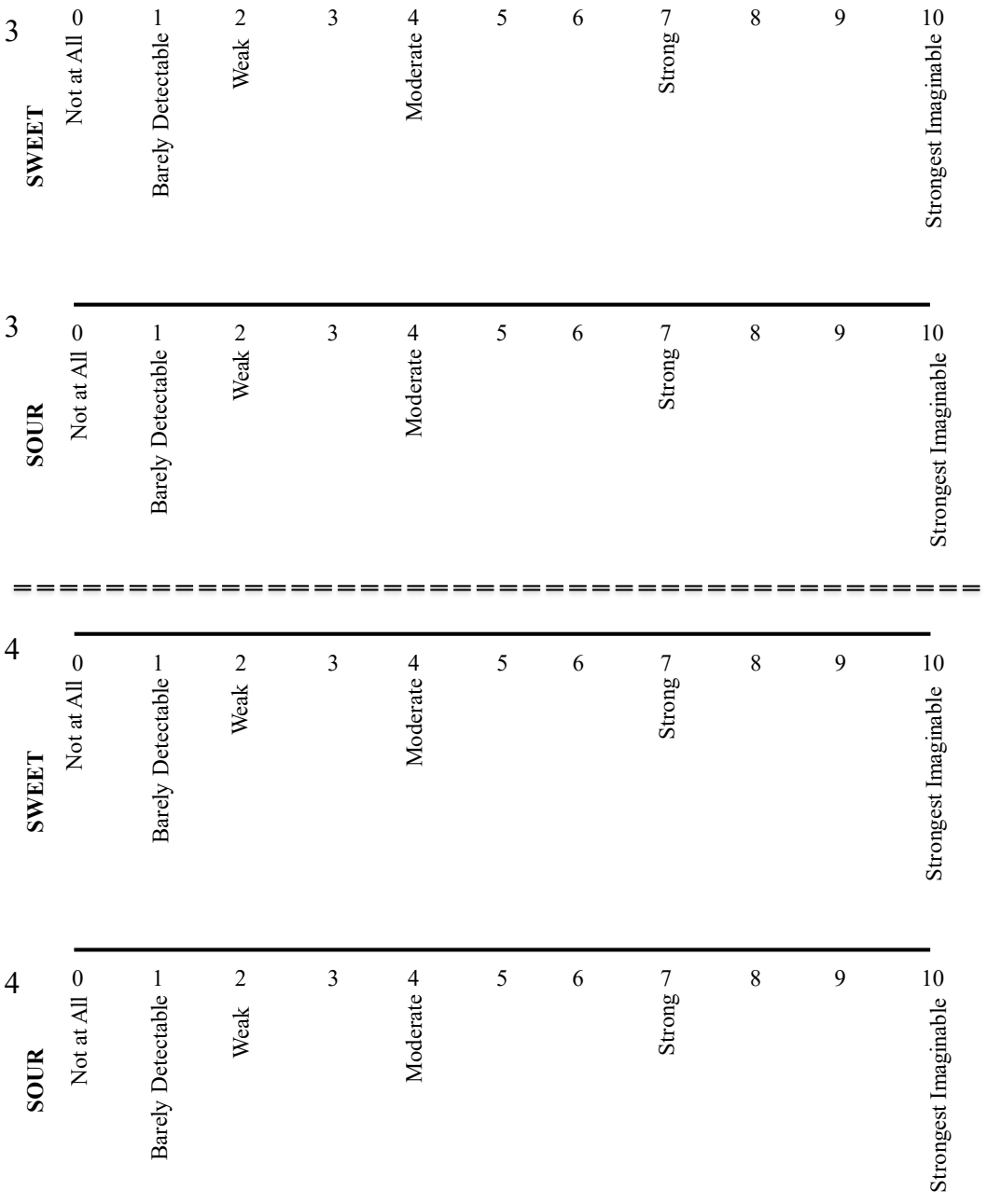
Appendix D. Intensity Rating Scale Sweet & Sour

Intensity Rating Scale Sweet & Sour

CODE #_____

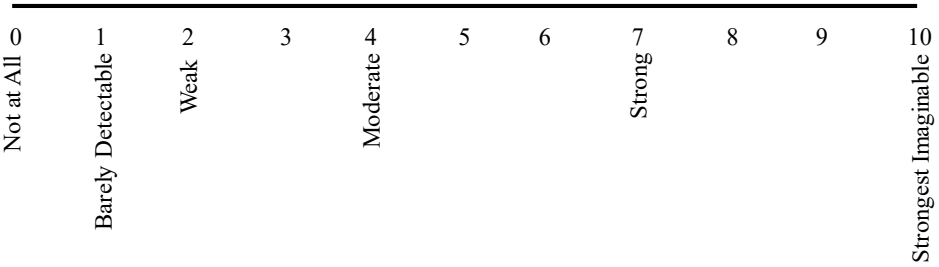


CODE # _____

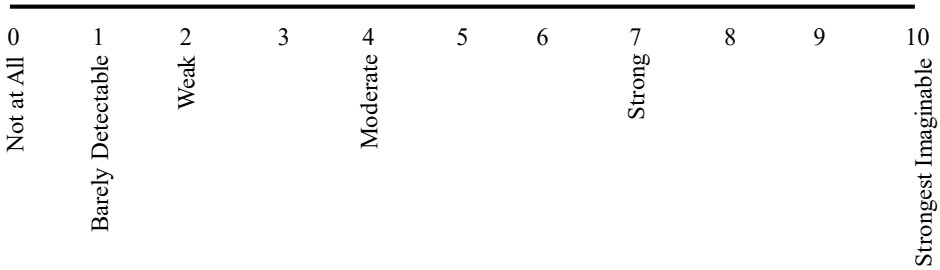

3
SWEET
0 Not at All
1 Barely Detectable
2 Weak
3
4 Moderate
5
6
7 Strong
8
9
10 Strongest Imaginable
3
SOUR
0 Not at All
1 Barely Detectable
2 Weak
3
4 Moderate
5
6
7 Strong
8
9
10 Strongest Imaginable
4
SWEET
0 Not at All
1 Barely Detectable
2 Weak
3
4 Moderate
5
6
7 Strong
8
9
10 Strongest Imaginable
4
SOUR
0 Not at All
1 Barely Detectable
2 Weak
3
4 Moderate
5
6
7 Strong
8
9
10 Strongest Imaginable

CODE #_____

5 **SWEET**



5 **SOUR**



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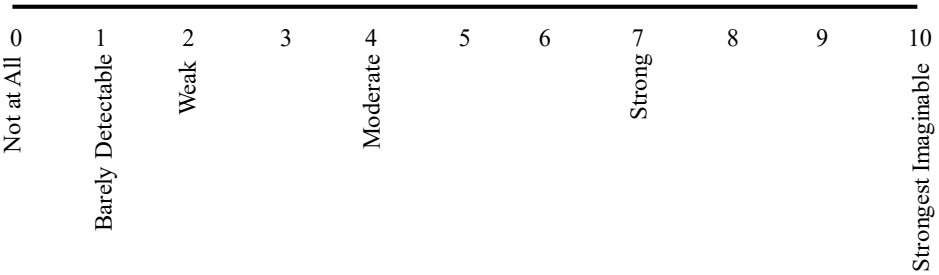
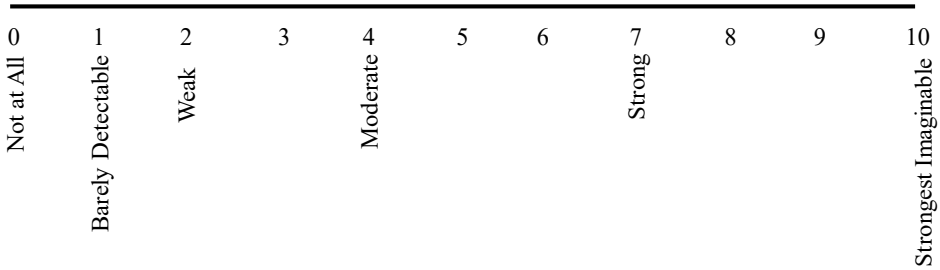
6 **SWEET**

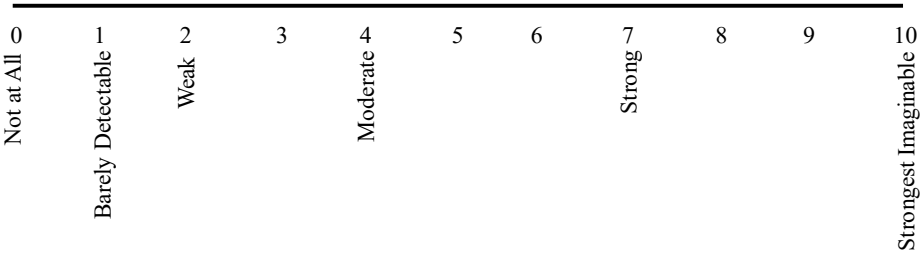


6 **SOUR**

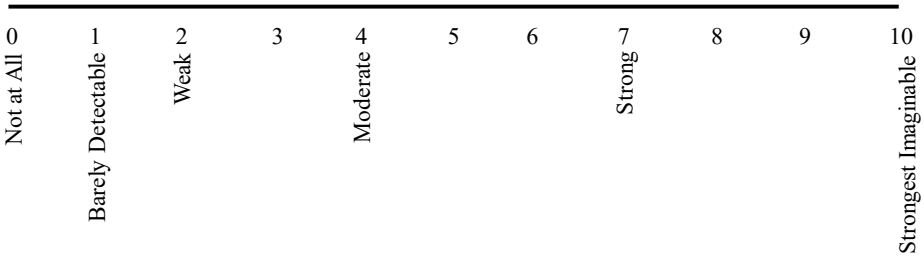


CODE #_____

7 **SWEET**



7 **SOUR**



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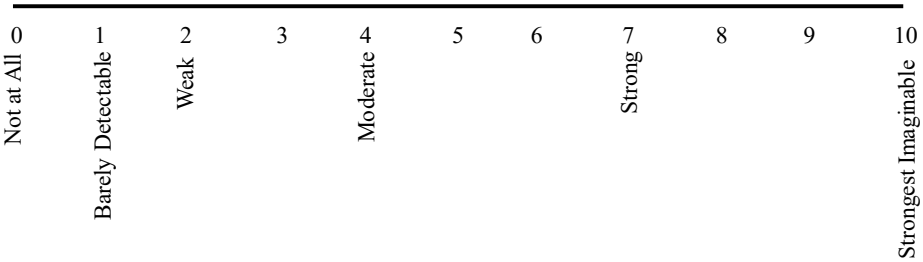
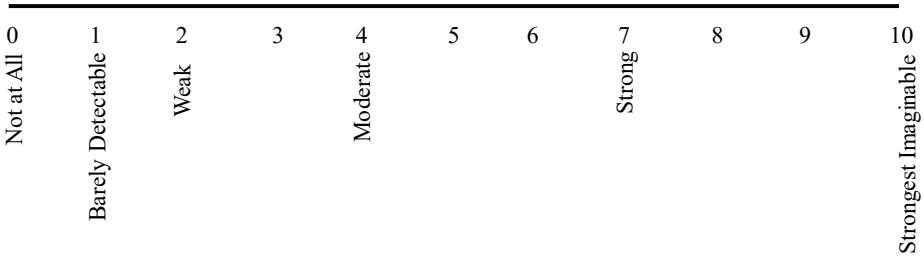
8 **SWEET**



8 **SOUR**



CODE #_____

9 **SWEET**

0 Not at All | 1 Barely Detectable | 2 Weak | 3 | 4 Moderate | 5 | 6 | 7 Strong | 8 | 9 | 10 Strongest Imaginable

9 **SOUR**

0 Not at All | 1 Barely Detectable | 2 Weak | 3 | 4 Moderate | 5 | 6 | 7 Strong | 8 | 9 | 10 Strongest Imaginable

==========

10 **SWEET**

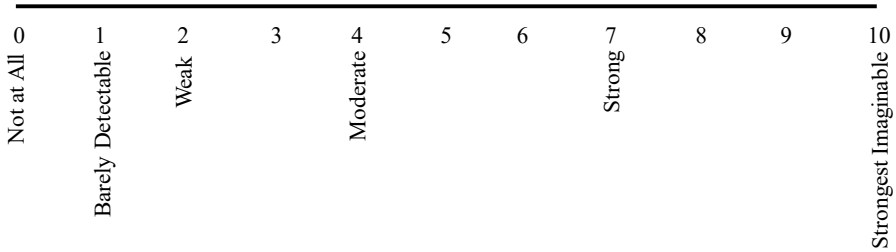
0 Not at All | 1 Barely Detectable | 2 Weak | 3 | 4 Moderate | 5 | 6 | 7 Strong | 8 | 9 | 10 Strongest Imaginable

10 **SOUR**

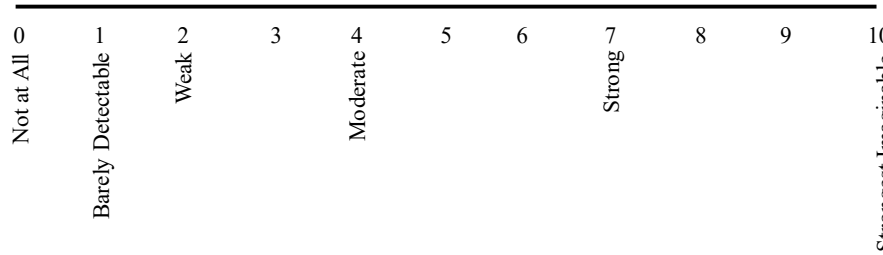
0 Not at All | 1 Barely Detectable | 2 Weak | 3 | 4 Moderate | 5 | 6 | 7 Strong | 8 | 9 | 10 Strongest Imaginable

CODE #_____

11 **SWEET**



11 **SOUR**



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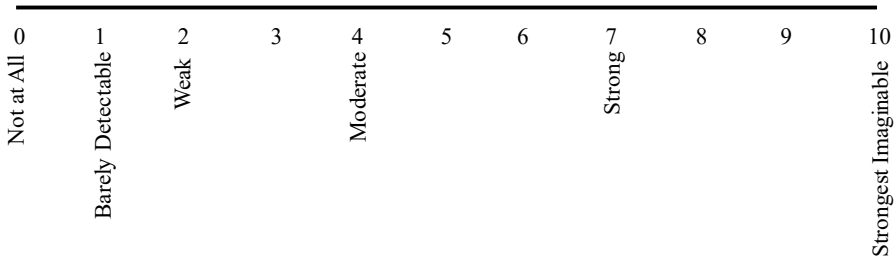
12 **SWEET**



12 **SOUR**