ARTICLE The Miracle Fruit: An Undergraduate Laboratory Exercise in Taste Sensation and Perception

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"Miracle Fruit" is a taste-altering berry that causes sour foods to be perceived as sweet. The present paper describes a laboratory exercise that uses Miracle Fruit to educate students about the sensation and perception of taste. This laboratory exercise reinforces course material pertaining to the function of sweet taste receptors covered in a Sensation and Perception course at Christopher Newport University. Here we provide a step-by-step explanation of the methodology, and an example of data collected and analyzed by one group of students who participated in this laboratory exercise. The origins of the Miracle Fruit, the structure and the physiological function of miraculin (the glycoprotein responsible for the tastemodifying effect found in the pulp of the Miracle Fruit) were discussed before the laboratory exercise. Students then sampled foods known to target different types of tastes

(i.e., sweet, sour, bitter and salty) and rated their perception of taste intensity for each food item. Next, students each consumed Miracle Fruit berries, then resampled each original food item and again recorded their perception of taste intensity ratings for these foods. The data confirmed that the sour food items were perceived sweeter after the Miracle Fruit was consumed. The students also completed a written assignment to assess what they learned about the origins, structure, and physiological function of Miracle Fruit. This hands-on laboratory exercise received positive feedback from students. The exercise can be used by other neuroscience educators to teach concepts related to the sensory system of taste.

Keywords: sensory, sweet, sour, receptor, miracle berry, taste modifier

A hands-on approach to learning has enormous value as a pedagogical practice. Engaging students in laboratory exercises is an effective method to enhance student learning (Clough, 2002; Hofstein and Lunetta, 2004), and many undergraduate science courses often include a laboratory component. Sensation and Perception, a commonly offered course at colleges and universities, is rich in neuroscience content and often includes complementary laboratory exercises to teach students the neurophysiological basis of various sensory systems (i.e., vision, audition, olfaction, taste and touch). For example, Schroeder and Flannery-Schroeder (2005) designed a laboratory exercise that provided students with an opportunity to experience an altered perception of taste induced by Gymnema sylvestre, an herb that blocks sweet taste receptors, and thus impairs the typical perception of sweet food items.

The laboratory exercise described in this paper uses a berry from a naturally growing plant, commercially referred to as a "Miracle Fruit" (*Synsepalum dulcificum*), to educate students about taste sensation and perception of sweet and sour foods. This so-called "Miracle Fruit" resembles a fresh cranberry and is nearly tasteless to humans. However, if a person chews on the berry and holds it on his/her tongue for a short time, it causes subsequent sour foods to taste sweet. The taste altering effect lasts approximately thirty minutes.

There is fascinating information about the Miracle Fruit that can be explored by students to help them learn different themes surrounding taste sensation and perception. For example, students read about the historical origin and usage of the Miracle Fruit to discover how human adaptation to an indigenous environment and vegetation shapes one's diet and food culture. Additionally, students learn the means by which miraculin, the taste-altering molecule in the berry, binds to and activates sweet taste receptors. This information reinforces class material pertaining to the neural mechanisms of the sweet taste receptor. The natural function of a sweet receptor is to detect the presence of simple carbohydrates on the tongue and normally evokes the perception of sweetness. The perception of sourness typically occurs when an acidic compound activates sour taste receptors on the tongue. Miraculin binds to the sweet taste receptor and does not induce physical changes in the sour taste receptor, yet acidic substances are perceived as sweet. This phenomenon provides a real world example the psychophysical nature of sensation by for demonstrating that the sensation is not in the stimulus (sour food), but in the psychological interpretation of that Additional information regarding the history, stimulus. molecular structure and miraculin's influence on sensation and perception is described below.

The Miracle Fruit plant was discovered in the 1700s by European explorers traveling to West Africa (Daniell, 1852). In 1852, the first description of the fruit appeared in the literature and it was called the "miraculous berry" (Daniell, 1852; Kurihara and Beidler, 1968). The West African natives chewed the "miraculous berry" prior to food consumption to make acidic foods that were overly sour more palatable (Inglett et al., 1965; Inglett and May, 1968). During the lab exercise, students are asked to speculate why the West African natives cultivated and consumed the "miraculous berry" and how this custom helped shape their dietary practices. For example, natives may have incorporated the Miracle Fruit into their diet solely for the hedonic value of sweet perception. It is also possible that West African natives who routinely used the Miracle Fruit incorporated larger quantities of fermented foods into their cuisine than cultures without access to the Miracle Fruit. Although it originated in West Africa, the flavor-altering Miracle Fruit berry is now internationally available.

Miraculin is a glycoprotein (a protein that has a carbohydrate group attached to the polypeptide chain) that has been isolated as the potent component of the Miracle Fruit that alters taste perception by binding to sweet receptors on the tongue. Miraculin is the largest known macromolecule (Cagan, 1973; Kurihara, 1992) that can influence taste perception. The molecular weight of miraculin is 24,600 Da, which includes 86.1% polypeptide and 13.9% carbohydrate. The complete sequence of the 191 amino acid single polypeptide portion of the molecule has been identified (Theerasilp and Kurihara, 1988; Theerasilp et al., 1989; Yamashita et al., 1990). Interestingly, no particular amino acid homology was found between miraculin and sweet proteins, such as thaumatin and monellin (Theerasilp et al., 1989). Explaining the function of this glycoprotein molecule introduces students to the general mechanics of a sweet taste receptor. The sweet taste receptor is a heterodimer consisting of T1R2 and T1R3, both of which appear to contain sites for sweet ligand binding (Nelson et al., 2001). Small molecules bind to T1R2 sites and large molecules bind to T1R3 sites (Morini et al, 2005). The T1R2-T1R3 taste receptors respond to a variety of sugars, sweeteners and small proteins (Nie et al., 2005). When one of these ligands binds to a sweet taste receptor, it directly activates the gprotein coupled receptor resulting in the perception of sweet taste. The mechanism of sensory transduction of a typical sweet taste receptor function that students learn about in class can be compared with the taste-altering mechanism of miraculin.

The exact mechanism of miraculin action is not entirely However, based on the known information a clear. theoretical model is proposed to explain the power of miraculin to modify taste perception from sour to sweet. This model suggests that miraculin binds directly to the sweet taste receptor T1R2-T1R3 extracellularly within the taste buds of the tongue epithelium's plasma membrane (Misaka, 2013). While it can bind to the receptors under neutral pH conditions, miraculin only activates the receptor in the presence of an acid, thus rendering sour foods to be perceived as sweet. The receptor undergoes a structural change in the presence of protons (H^{+}) , causing the carbohydrate portion of the miraculin molecule to bind to the sweet receptor site, leading to a pH-dependent (between pH 4.8 and 6.5) activation of human sweet taste receptor cells (Misaka, 2013). The most interesting aspect of this mechanism is that unlike sugary substances, Miracle Fruit alone does not evoke a sweet taste, but alters the perception of sweet in the presence of H⁺ ions. In other words, when a person eats a Miracle Fruit and then consumes a sour substance (e.g., a lemon wedge) his/her sour taste receptors are activated, yet the individual does not perceive the expected sourness and instead perceives the sour substance as sweet. This phenomenon demonstrates the psychophysical nature of human senses by highlighting how sensation can be altered without modifying the normal function of sour taste receptors.

The purpose of this laboratory exercise is to reinforce students' understanding about the neural mechanisms of sweet taste receptors and to demonstrate the psychophysical nature of taste perception. During the lab exercise, students experience first-hand that taste receptors can be "tricked" to produce an altered perception. This paper provides a detailed description of the "Miracle Fruit" laboratory exercise currently used in the Sensation and Perception course taught at Christopher Newport University (CNU). The students test the action of miraculin by consuming the miracle fruit prior to tasting foods that activate four different taste receptors (e.g., sweet, salty, sour, and bitter). While miraculin can be acquired commercially in various forms (e.g., tablets, powder, juice extract, etc.), it is most meaningful to have the students experience the actual fruit consumed by the indigenous people with the purpose of making sour foods taste sweet. Following this exercise, students are given an assignment focusing on the mechanisms of sweet receptors and miraculin actions. At the conclusion of the lab, the students are asked to identify practical ways that the Miracle Fruit can be used in society today, such as using the Miracle Fruit as a sugar substitute, as a treatment of negative taste alterations in chemotherapy patients, or for making nutritious but sour foods palatable.

MATERIALS AND METHODS

Informative Introduction to the Lab Exercise

The course instructor provided the students with an oral and written description of the Miracle Fruit and its function as described in the introduction section of this paper. Then the students were briefed about the exercise procedure, purpose and safety information. The following statements were made to the class prior to the experiment: "(1) *If you have any allergies to berries or fruits, you should not eat a Miracle Fruit berry.* (2) Since the berry does not change any chemical compound of any of the foods that are used, the sour food items will retain their normal acidity. Take care not to ingest high quantities of highly acidic foods."

Materials

Miracle Fruit berries (1-2) per subject are required. One berry will produce an effect lasting for approximately thirty minutes; however there are individual differences that can lead to shorter or longer effective durations. Consuming more than one berry will usually result in longer lasting effects. The Miracle Fruit berries were purchased from Miracle Fruit Farm, Inc. (Miami, FL). The berries were freshly picked and shipped overnight to be used in the lab the following day. The berries were stored at refrigerator temperature until the time of the exercise. It is important to preserve the freshness of the berries for maximum effectiveness of miraculin.

Four food-types were used to target different taste

receptors (sweet, sour, salty and bitter). We used jelly beans for the sweet taste receptor, lemon wedges for the sour taste receptor, Goldfish® crackers for the salty taste receptor, and raw broccoli pieces to target the bitter taste receptor. Additional food items (grapefruit, limes, green apple, sour candy and apple cider) were used to further test the effect the Miracle Fruit berry on the taste perception of additional sour foods. The students were also given paper plates, napkins and bottles of water to rinse their palates.

Procedure

The students tasted each of the experimental and additional sour food items and scored their taste perception of each on a 0 - 10 scale (See Supplementary Materials for an example data collection sheet). The students rinsed their mouths with water to clean their palate after each food was tasted.

The participants must thoroughly coat the entire membrane of their tongue with the Miracle Fruit berry in order to produce an effect on taste alteration. To do so, each student put one berry at a time in his/her mouth and chewed the flesh of the fruit for approximately 30 seconds while allowing the pulp of the Miracle Fruit to come in contact with the surface of their tongue. The seed of the fruit should be spat out. Then, the students again ate a piece of each food item. Following each taste, the students rinsed their mouths with water to clean their palate and recorded the perceived taste intensity of each food in the data table prior to tasting the next food. Approximately 45 seconds elapsed between tasting of each food item.

Following the data collection students discussed their results with other lab members, answered lab write-up questions (see Supplementary Materials) and discussed the possible practical uses for the Miracle Fruit.

RESULTS

The present results were obtained from a group of undergraduate students (n = 19) enrolled in a *Sensation and Perception* course at CNU. Interestingly, one student did not experience any change in taste perception following consumption of the Miracle Fruit. Such an outcome is a good example of individual differences in taste perception.

As predicted, the Miracle Fruit did not alter the perception of salty or bitter tastes (Figure 1). Paired t-tests revealed that the bitterness rating of broccoli and saltiness rating of the Goldfish® crackers did not change after the berry was consumed (ps > 0.05). However, paired t-tests showed that the perceived sweetness for each acidic food item (lemon, grapefruit, lime, sour candy and cider vinegar) significantly increased after the berry was eaten (ps < 0.01), whereas sweetness perception of bitter (broccoli), salty (Goldfish® crackers) and sweet (jelly bean) tastes did not change (ps > 0.05; Figure 2).

As part of the lab exercise, students practiced analyzing the data using analysis software (e.g., SPSS). Students were allowed to choose an analysis test that they believed appropriate for their data. The results can be analyzed in multiple ways. For example, the perceived intensity of sweetness before consumption of the Miracle Fruit can be compared to the perceived intensity after fruit consumption for each food item with multiple paired t-tests. Students can also calculate the before-and-after difference scores, followed by a within-subjects repeated measures analysis of variance (ANOVA), in order to determine whether some foods produced a greater sweet intensity alteration than others. Additionally, students may also perform a 2x2 (Lemon vs Broccoli X Before vs After) and/or a 2x2x2 (Sweetness vs Sourness X Lemon vs Broccoli X Before vs After) factorial ANOVA to analyze their data.

In addition to analyzing the change in the intensity of sweet perception, we compared the altered perception of sourness in highly acidic foods. As depicted in Figure 3, all of these foods showed a decreased perception of sour intensity following consumption of the Miracle Fruit when compared to the intensity perception prior to Miracle Fruit consumption. These findings were confirmed with paired t-tests (ps < 0.01). Thus, similar analysis can be performed on the change of sour perception intensity to that described for the perception of sweetness intensity.

DISCUSSION

The "Miracle Fruit" laboratory exercise is an excellent activity to reinforce course material related to the sensation and perception of taste. During this exercise students recorded their perceived intensity of sweet and sour tastes for various food items before and after they consumed a Miracle Fruit berry. The data students collected and analyzed revealed that miracle fruit consumption robustly alters the taste of sour foods to become sweeter and less sour. The results also showed that chewing on the Miracle Fruit had little effect on the perception of non-sour food items. Interestingly, the fact that the perception of sourness is decreased appears to be a central perceptual phenomenon. Miracle Fruit does not have any physical effect on the sour taste receptor (Kurihara and Beidler, 1969). The change in perception of sourness without physical alteration of the receptor demonstrates the psychophysical nature of human senses.



Figure 1. Mean (+SEM) taste rating for the bitterness of broccoli and the saltiness of Goldfish® reported before (blue bars) and after (orange bars) the Miracle Fruit was consumed. Miracle Fruit consumption had no significant effect on the rating of each food item (p > 0.05).

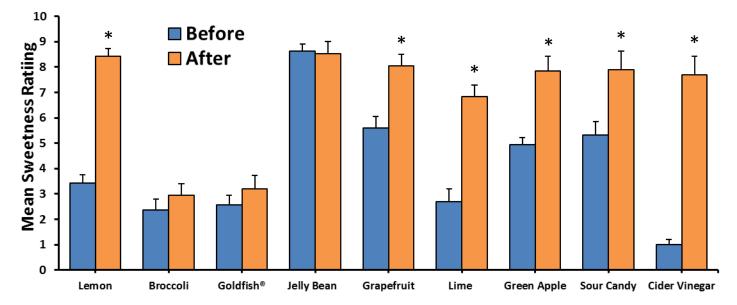


Figure 2. Mean (+SEM) sweetness rating. The blue bars show the sweetness rating reported prior to Miracle Fruit consumption and the orange bars show the sweetness rating reported after consumption of the Miracle Fruit. Note: * indicates that the sweetness rating of a food item significantly increased after Miracle Fruit consumption (p < 0.01).

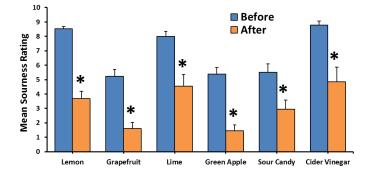


Figure 3. Mean (+SEM) sourness rating. The blue bars show the baseline rating prior to Miracle Fruit consumption and the orange bars show the sweetness rating after consumption of the Miracle Fruit. Note: * indicates that the sourness rating of a food item decreased significantly after Miracle Fruit consumption (p < 0.01).

A set of questions was given to each student to assess what he or she learned about the origins, structure, and physiological function of Miracle Fruit.

The responses to the questions clearly indicated that most students had a firm grasp of each. The students correctly reported the origin and indigenous use of the Miracle Fruit. They also accurately identified miraculin as the molecule responsible for taste altering effect and were able to describe the molecule's structure and function. Finally, the students demonstrated an understanding of the sweet taste receptor physiology as it relates to the taste altering mechanism of the miraculin, which goes beyond the description of the receptor physiology provided in the textbook. Moreover, in-class analysis of the data provided students with an opportunity to review different experimental designs and practice performing appropriate statistical tests. A formal laboratory write-up can be

assigned to provide students with an opportunity to engage in science writing and creating figures.

Additionally, the students discussed possible practical uses for the altering taste perception induced by the Miracle Fruit. During the discussion, students were encouraged to provide their own ideas followed by the instructor's informative review of the current studies that employ the use of Miracle Fruit. Some of the creative examples given by students were to use the berry by parents to entice their kids to eat healthy foods they were not fond of or to mitigate the unpleasantness of medication.

Many laboratories around the world actively conduct research using the Miracle Fruit. One commonly explored use of the Miracle Fruit is to adopt it as a sugar substitute for diabetics and those suffering from obesity (Kant, 2005; Wong and Kern, 2011). The natural sweet taste receptor modulation may establish a safer sugar substitute than artificial low calorie sweeteners currently available on the market (Kant, 2005). Miracle Fruit produces a comparable perceptual effect between sugar and artificial low calorie sweeteners when consumed with acidic foods (Yamamoto et al., 2006). In addition to the use of Miracle Fruit as a sugar substitute, attempts have been made to evaluate the effectiveness of this berry as a treatment for negative taste alterations (dysgeusia) in chemotherapy patients (Soares et al., 2010; Wilken and Satiroff, 2012). These pilot studies indicate that Miracle Fruit improves patients' taste perception, which might ultimately lead to better eating.

In addition to being a useful pedagogical exercise, the "Miracle Fruit" laboratory is financially practical for most budgets. The laboratory requires no specialized equipment or materials and a container of 100 Miracle Fruit berries can be purchased for ~\$45 (USD). One hundred berries are enough for 50 students to participate in the exercise. The other food items and supplies are also relatively

inexpensive and available at most grocery stores. Lastly, this exercise was thoroughly enjoyed by the students. Student responses (n = 19) to a feedback questionnaire showed that they overwhelmingly "strongly agreed" [scale: 1 = strongly agree, 2 = agree, 3 = neutral, 4 = disagree and 5 = strongly disagree] that this laboratory was *"interesting"* (average = 1.1), *"informative"* (average = 1.3), and *"relevant"* (average = 1.3). Moreover, 74% of students indicated this laboratory exercises was their favorite among 11 other laboratory exercises also conducted throughout the semester in the *Sensation and Perception* course.

Additional independent and dependent variables can be used to build upon the laboratory exercise specifically described in this paper. For instance, instructors can introduce novel foods to be tested following berry consumption. They can consider their results in terms of sensory coding of taste sensation in the CNS. Students can also be given an opportunity to measure the duration and intensity of miraculin's taste-altering effect in a dose dependent manner. The Miracle Fruit can also be explored as a sugar substitute in a laboratory setting. For example, the instructor might prepare lemon beverages with and without sugar and have the students compare the sweetness perception between the two beverages before and after Miracle Fruit consumption. Incorporating additional variables into the design of the "Miracle Fruit" laboratory exercise described in this paper may improve upon what is already an educational, engaging and wellreceived hands-on opportunity that students experience as part of their Sensation and Perception course.

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