Journal of Visualized Experiments

A rapid food-preference assay in Drosophila

--Manuscript Draft--

Article Type:	Invited Methods Collection - JoVE Produced Video
Manuscript Number:	JoVE62051R1
Full Title:	A rapid food-preference assay in Drosophila
Corresponding Author:	Yali Zhang UNITED STATES
Corresponding Author's Institution:	
Corresponding Author E-Mail:	yzhang@monell.org
Order of Authors:	Yali Zhang
	John Mack
Additional Information:	
Question	**Response**
Please specify the section of the submitted manuscript.	Neuroscience
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$2,400)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	Philadelphia, PA USA 19104
Please confirm that you have read and agree to the terms and conditions of the author license agreement that applies below:	I agree to the Author License Agreement
Please provide any comments to the journal here.	

TITLE:

A Rapid Food-Preference Assay in *Drosophila*

AUTHORS AND AFFILIATIONS:

John O. Mack[1], Yali V. Zhang[1,2*]

[1]Monell Chemical Senses Center, Philadelphia, PA 19104, USA
[2]Department of Physiology, The Diabetes Research Center, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104, USA

E-mail addresses of authors:

John O. Mack (jmack@monell.org)
Yali V. Zhang (yzhang@monell.org)

Corresponding author:

Yali V. Zhang (yzhang@monell.org)

SUMMARY:

We present a protocol for a two-choice feeding assay for flies. This feeding assay is fast and easy to run and is suitable not only for small-scale laboratory research, but also for high-throughput behavioral screens in flies.

ABSTRACT:

To select food with nutritional value while avoiding the consumption of harmful agents, animals need a sophisticated and robust taste system to evaluate their food environment. The fruit fly, *Drosophila melanogaster*, is a genetically tractable model organism that is widely used to decipher the molecular, cellular, and neural underpinnings of food preference. To analyze fly food preference, a robust feeding method is needed. Described here is a two-choice feeding assay, which is rigorous, cost-saving, and fast. The assay is Petri-dish-based and involves the addition of two different foods supplemented with blue or red dye to the two halves of the dish. Then, ~70 prestarved, 2–4-day-old flies placed in the dish are allowed to choose between blue and red foods in the dark for about 90 min. Examination of the abdomen of each fly is followed by the calculation of the preference index. In contrast to multiwell plates, each Petri dish takes only ~20 s to fill and saves time and effort. This feeding assay can be employed to quickly determine whether flies like or dislike a particular food.

INTRODUCTION:

Despite dramatic differences in the anatomical structure of taste organs between flies and mammals, the flies' behavioral responses to many tastant substances are strikingly similar to those of mammals. For example, flies prefer sugar[1–8], amino acids[9,10], and low salt[11], which indicate nutrients, but reject bitter foods[12–15] that are unpalatable or toxic. Over the past two decades, flies have proven to be a highly valuable model organism for advancing the understanding of many fundamental questions related to taste sensation and food consumption, including tastant detection, taste transduction, taste plasticity, and feeding

regulation[16–20]. Remarkably, a number of studies have demonstrated that the taste transduction and neural circuit mechanisms underlying taste perception are analogous between fruit flies and mammals. Therefore, the fruit fly serves as an ideal experimental organism, enabling researchers to uncover evolutionarily conserved concepts and principles that govern food detection and consumption in the animal kingdom.

To investigate taste sensation in flies, it is critical to establish a fast and rigorous assay to objectively measure food preference for fruit flies. Over the years, various feeding methods, such as dye-based assays[11–13,21–23], the fly proboscis extension response assay[24], the Capillary Feeder (CAFE) assay[25,26], the Fly Liquid-Food Interaction Counter (FLIC) assay[27], and other combinatorial methods have been developed to quantitatively measure food preference and/or food intake for fruit flies[28–31]. One of the popular feeding paradigms is the dye-based two-choice feeding assay using either a multiwell microtiter plate[12,21,32] or, as described here, a small Petri dish[11,22] as the feeding chamber. This assay is designed based on the transparency of the fly's abdomen. During this assay, flies are placed into the feeding chamber and presented with two food options mixed with either red dye or blue dye. Once the assay is complete, fly abdomens appear red or blue depending on which food they have consumed.

Both the Petri-dish and the multiwell-plate dye-based feeding assays are highly robust and yield approximately the same results. Using these two assays, numerous important discoveries and breakthroughs have been made toward deciphering the highly diversified receptors and cells responsible for sensing food tastes and food texture[11,12,21,22,32,33]. In the dye-based assay, one experimental step requiring considerable time and effort is preparing and loading food into the feeding chamber. To reduce the food preparation and loading time, this assay was modified by replacing the multiwell microtiter plate with a small Petri dish, which is divided into two equal compartments. In the Petri-dish-based assay, two different foods supplemented with blue or red dye are added to the two halves of the dish. Then, ~70 prestarved, 2–4-day-old flies are placed in the dish and allowed to choose between blue and red foods in the dark for about 90 min. The abdomen of each fly is then examined, and the preference index (PI) is calculated.

This Petri-dish-based two-choice feeding assay is affordable, simple, and fast. One multiwell plate requires approximately 110 s to fill, whereas each Petri dish takes only ~20 s. In addition, the multiwell plate requires pipetting small volumes of food into a large number of small wells (e.g., 60 or more wells per plate), which demands considerable precision and attention. Conversely, the Petri-dish-based assay requires only two actions per plate. As the feeding assay can involve a large number of replicates, the Petri-dish-based assay saves a nontrivial amount of time and effort. This assay gives results equivalent to those from the multiwell-based assay and has proven successful in addressing many fundamental questions in taste sensation, including salt taste coding[11], taste plasticity modified by food experience[22], and the molecular basis of food texture sensation[33]. In summary, this Petri-dish-based two-choice assay is a powerful tool to investigate how flies perceive external and internal nutrient milieus to elicit appropriate feeding behavior.

PROTOCOL:

1. Assembling the assay chambers

NOTE: While this protocol describes the use of a 35 mm Petri dish (**Figure 1A**), the desired effect can be achieved using any watertight, smooth-bottomed vessel that can be bisected and covered.

1.1. First, bisect a lidded 35 mm Petri dish by fixing a length of plastic (5 mm in width and 3 mm in height) down the midline with waterproof adhesive, forming two watertight compartments. Confirm that the seal is complete to avoid leakage that can lead to mixing of the two food substrates being assayed.

NOTE: After assembly, reuse this apparatus as long as the seal holds.

2. Preparing starvation vials

2.1. Prepare a sufficient number of empty plastic fly vials; then, loosely compact a piece of tissue paper at the bottom. Compress the tissue paper enough that it fills the space, but not so much that it forms a dense mass.

NOTE: Make sure that there are no deep crevices or folds in the tissue, as this can lead to flies getting trapped.

2.2. Add ~3 mL pure water to the vial so that the tissue is completely saturated, but there is no standing water. Ensure that there are no large droplets of excess water on the wall of the vial. Alternatively, substitute agarose for the soaked paper by preparing a 1% w/v agar solution (without sucrose) by adding 5 mL of 1% agarose to each empty vial and allowing the agarose to solidify at room temperature.

3. Wet starvation of flies prior to the experiment

3.1. Initiate starvation 24 h before the time of the experiment. Under CO_2 anesthesia, sort groups of ~70, 2–4-day-old flies into the prepared starvation vials, labeling each vial with the genotype and time of starvation.

4. Reagent setup

4.1. Preparation of dyes

NOTE: Prior to performing any experiments, it is important to perform a preliminary control assay to determine the correct concentrations of red and blue dyes to use.

4.1.1. To do this, prepare a range of dilutions for each dye, and perform the feeding assay with the same food with a different dye color. Use the results to identify two-dye concentrations

(one red, one blue) that yield a PI of ~0 when no experimental compound is added (see section 7).

NOTE: For example, the final blue dye concentration was fixed at 50 µM and tested against a series of red dye concentrations. Based on the red dye dosage curve, the optimal red dye concentration was 210 µM, which gave minimal dye bias (**Figure 1B**). A higher red dye concentration drives flies to prefer red food, whereas a lower concentration drives flies to prefer blue food. Carefully refine blue or red dye concentrations in increments of 1 µM, as differences of this magnitude and greater can affect experimental outcomes.

4.2. Preparation of 1% agarose

4.2.1. Combine 0.5 g agarose and 50 mL of pure water (or some ratio thereof) in a microwave-safe vessel. Microwave the agarose solution until dissolved, stirring it as needed.

4.3. Preparation of other food components

4.3.1. Dissolve each food component, including sucrose and any experimental compounds, in water at a 100-fold or higher concentration of the final tested concentration.

NOTE: The total volume of each food ingredient added to 1% agar should not exceed 1 mL per 10 mL molten agar. Otherwise, the agarose may be too dilute and will not solidify appropriately.

4.4. Preparation of food media

4.4.1. Mix agar, dye, and the desired experimental compound in conical polypropylene centrifuge tubes (15 or 50 mL); use water instead of the experimental tastant in the control food. Do this while the agar is still completely liquid and mix thoroughly using a vortex mixer. Keep the tubes in a 60 °C water bath while not in use to prevent the agarose from hardening before being distributed into dishes.

4.5. Preparing dishes for the experiment

NOTE: Ensure that all dishes are completely dry before starting.

4.5.1. Pipette 1 mL of red experimental food medium into one side of the assay dish (**Figure 1A**); repeat for the desired number of dishes. Allow the agarose to cool until firm (3–5 min), and then pipette 1 mL of blue control food into the other side of the dishes (**Figure 1A**). Repeat this process with the control red/experimental blue pair.

NOTE: Make sure all dishes are fully set before beginning the experiment. Use the dishes within 30 min.

5. Initiating the two-way feeding assay

5.1. Temporarily paralyze experimental fly lines on ice until no obvious motor activities such as flying and climbing are observed. Once the flies are immobilized, gently invert the vial, and tap to transfer all the flies into the assay chamber.

NOTE: Cold shock takes ~3–5 min. Prolonged exposure to cold may affect the fly's physiology and health and should therefore be avoided.

5.2. Quickly place the cover on the chamber and set it aside. Once all the flies have been transferred, move all chambers to a dark, enclosed space. Allow the assay to run for 90 min.

NOTE: A dark environment minimizes the influence of the fly's visual pathway on feeding behavior and removes any environmental cues from outside the dish.

6. Terminating the two-way feeding assay

6.1. After 90 min have elapsed, transfer the chambers to a -20 °C freezer to sacrifice the flies. After ~1 h, count the flies.

NOTE: Invert each Petri dish before placing the dish in the freezer to ensure that no flies will be frozen onto the food.

7. Assigning a preference index (PI) to determine food preference

7.1. Under a standard dissection microscope, examine the flies' abdominal color in each individual dish. Count the flies as either red, blue, or purple according to the color of their abdomen (**Figure 2A**). Count the fly if its abdomen is more than 50% colored, indicating robust feeding (**Figure 2B)**. Exclude the fly if its abdomen contains only a tiny food spot, indicating poor eating (**Figure 2C**).

7.2. After the numbers of flies eating blue, red, or both blue and red foods have been counted, use the following equation to assign each Petri dish a preference index (PI):

$$PI = \frac{(Number\ of\ flies\ eating\ experimental\ food)-(Number\ of\ flies\ eating\ control\ food)}{(Number\ of\ flies\ eating\ experimental\ food)+(Number\ of\ flies\ eating\ control\ food)+(Number\ of\ flies\ eating\ both)}$$

PI > 0 indicates a preference for the experimental compound, PI < 0 indicates an aversion to the experimental compound, and PI = 0 indicates no effect of the compound on feeding behavior.

8. Cleaning the assay chambers

8.1. Promptly clean the Petri dishes by scraping out the food substrate and rinsing them with unscented soap and water. Soak the Petri dishes overnight in distilled water. Check that the dividing seal in each dish is still watertight, then let the dish air dry.

NOTE: After ensuring that there is no residual agarose or dye staining, the Petri dishes are ready to use again.

REPRESENTATIVE RESULTS:

In this assay, a 35 mm dish was divided into two equal feeding compartments, with each half of the dish containing agarose food coupled with either blue or red dye (**Figure 1A**). To exclude dye bias, the blue and red dye concentrations were carefully refined to yield an approximate "0" PI when only these two dyes were added (**Figure 1B**). Once the Petri dish was loaded with tested food, ~70 wet-starved, 2–4-day-old adult flies were transferred to the dish, allowing them to choose between the two food options in the dark. After 90 min, the flies' abdominal color was examined with a dissection microscope. Typically, the fly abdomen appears blue or red if the animal predominantly consumes blue or red food (**Figure 2A**), respectively. If the fly consumes both blue and red, its abdomen turns purple (**Figure 2A**).

The flies ingesting considerable amounts of food were scored (**Figure 2B**), while skipping the flies with insufficient food intake (**Figure 2C**). This Petri-dish-based assay was compared to the multiwell-plate-based assay. The results show that these two feeding methods give essentially the same results in assaying feeding responses to sweet, bitter, or salty food in wild-type flies (**Figure 3A–C**). Notably, it is much faster to prepare and distribute food in the Petri dish than in the multiwell plate containing 60 wells (**Figure 3D**). Altogether, the Petri-dish-based assay is a robust and fast feeding method that can be used to quickly determine the food preference for flies.

FIGURE LEGENDS:

Figure 1: Two-choice assay device and dye dosage curve. (**A**) Two halves of a Petri dish are used to present two different food options. One half of the dish contains blue-dyed food, and the other half contains red-dyed food. Prestarved flies are placed into the dish to allow them to consume whichever food they prefer. (**B**) Food preference for wild-type flies choosing between 1% agarose plus 2 mM sucrose containing either 50 µM blue dye or varying concentrations of red dye. The optimal red dye concentration is 210 µM. Data represent mean ± standard error of the mean. For each data point, n = 6 trials. Approximately 70 flies were tested in each trial.

Figure 2: Fly abdominal color after eating blue, red, or both blue and red foods. (**A**) Representative images of flies after having ingested blue food (top right), red food (top left), or both, making the abdomen appear purple (bottom). (**B**) A fly showing sufficient consumption of blue food. (**C**) A fly after ingesting a small amount of blue food.

Figure 3: Feeding responses to different tastants in wild-type flies, and the food-loading time for the 60-well-plate vs Petri-dish-based feeding device. (**A**) Food preference for wild-type flies choosing between 2 mM sucrose and 10 mM sucrose. n = 12 trials, unpaired Student's *t*-tests. (**B**) Food preference in wild-type flies for food containing 2 mM sucrose with or without 6 mM caffeine. n = 10 trials, unpaired Student's *t*-tests. (**C**) Food preference in wild-type flies for food

containing 2 mM sucrose with or without 20 mM NaCl. n = 10 trials, unpaired Student's *t*-tests. (**D**) Time spent filling food into a 60-well plate and a Petri dish. n = 12 plates or dishes, *$p <$ 0.0001, unpaired Student's *t*-tests. Data represent mean ± SEM. Abbreviations: n.s. = not statistically significant; SEM = standard error of the mean; NaCl = sodium chloride.

DISCUSSION:

This method involves several crucial steps where problems can occur. First, make sure flies ingest a sufficient amount of food to provide stable data. If flies eat poorly, ensure that the flies have been wet-starved for at least 24 h, and that the experimental media contains at least a minimal sucrose concentration (2 mM). To further stimulate food consumption, prolong the wet-starvation period beyond 24 h, depending on the flies' physiological condition. If too many flies fail to survive the prolonged starvation, ensure that enough water is added to the tissue paper when performing wet-starvation in vials. Avoid excessive water that may drown the flies. Second, flies tend to show feeding bias toward either blue or red dye if their concentrations are not carefully balanced. Small variations in dye concentration can have profound feeding effects (**Figure 1B**). Thus, to prevent dye bias, dye concentration should be precise. If flies are influenced by the dye, carefully refine the dye concentration at an increment of 1 µM, and then test different dye combinations to identify the red/blue dye concentration pair that yields a PI = 0 when no experimental compound except a low concentration of sucrose (e.g., 2 mM) is added. The optimal red or blue dye concertation should be readjusted when testing new fly lines or after making new dye stocks. Third, make sure the assay is constrained to 90 min. According to a previous study[22], prolonged feeding can lead to taste adaptation or desensitization.

Compared with other feeding techniques, such as FLIC[27] or CAFE[25] assays, this Petri-dish-based two-choice assay has the following features and advantages: (1) Simplicity: this device comprises only a small Petri dish bisected with a plastic divider. Because the dishes and plastic dividers are inexpensive and easy to assemble, an entire experiment requires only minimal investment. (2) Expediency: the Petri-dish-based device considerably speeds up the feeding assay (**Figure 3D**). The color-scoring process is also fast and straightforward using a regular dissection microscope. With this method, the flies' taste preference toward a particular food ingredient can be quickly tested. Thus, it is suitable for both small-scale research and large-scale genetic screens. (3) Stability: in contrast to other feeding methods that analyze only a few flies in each device, this method allows the quantification of feeding responses for a large number of adult flies at one time, which significantly minimizes the effects of feeding variations among individual flies. This dye-based two-choice feeding assay has proven to be rigorous and reproducible and has been used to isolate important fly mutants with defects in perceiving food tastes and textures[11,22,33].

As demonstrated by these results, the Petri-dish-based assay produces essentially the same results as the multiwell-based feeding assay for sweet, bitter, and salty taste responses, although the Petri-dish-based assay tends to have smaller variations (**Figure 3A–C**). One time-consuming step of the dye-based feeding assay is the discharge of food into the feeding chamber. The multiwell plate, which contains 60 or more wells, can be laborious to set up due

to the requirement of precisely loading melted agarose food into 60 or more wells per plate. It is much faster to prepare and load food in the Petri dish than the multiwell plate, as the Petri dish contains only two separate compartments (**Figure 3D**). Thus, this Petri-dish-based method not only maintains the robustness of the dye-based assay, but also significantly reduces the time and effort spent in assay preparation, thereby significantly scaling up the capacity and speed of the feeding assay. Consequently, it can be readily employed to analyze a large number of fly lines, such as in a genetic screen project.

While dye-based assays provide a high-throughput avenue of study due to their simplicity and speed, they cannot capture information about more detailed quantitative aspects of feeding such as duration or volume. To overcome this issue, a high-speed camera can be installed above the dish, which reveals more detailed information of the feeding process, such as the feeding duration and frequency in each chamber. Moreover, several other feeding paradigms can be used to supplement data gathered from the dye-based experiments. Automatic feeding devices, such as the FLIC[27] and the fly proboscis and activity detector (FlyPAD)[34], can record the temporal dynamics of feeding. The CAFE assay[25] or manual feeding assays[35] can measure the volume of food consumed. Nevertheless, these approaches have their own caveats. For example, compared with the Petri dish or the multiwell plate, automatic feeding devices are very expensive to set up in the lab. Additionally, each device assays only a few flies at a time, making it more vulnerable to variability in individual animals.

As the CAFE assay relies on the flies' ability to maneuver their bodies up to the end of the capillary tube hanging inside the feeding chamber, the results can be confounded by motor impairments unrelated to taste sensation. Although other approaches are powerful in their own right, dye-based assays can be a more efficient tool to rapidly discover and analyze food preference in flies. Furthermore, the two-choice setup can be integrated with cutting-edge techniques such as optogenetics[36] to selectively and acutely manipulate the fly's feeding behavior. This can be done using one half of the dish for light activation and the other half as a light-inactive control. Direct activation or inactivation of specific neurons helps determine whether they have a role in regulating feeding behaviors. In summary, these results show that the Petri-dish-based two-choice feeding assay is a rapid and robust feeding method that can help researchers analyze feeding behavior under different physiological and metabolic states.

ACKNOWLEDGMENTS:

The authors would like to thank Dr. Tingwei Mi for helping them optimize the two-choice feeding assay. They would also like to thank Samuel Chan and Wyatt Koolmees for their comments on the manuscript. This project was funded by the National Institutes of Health grants R03 DC014787 (Y.V.Z.) and R01 DC018592 (Y.V.Z.) and by the Ambrose Monell Foundation.

DISCLOSURES:

The authors declare no conflicts of interest or competing financial interests.

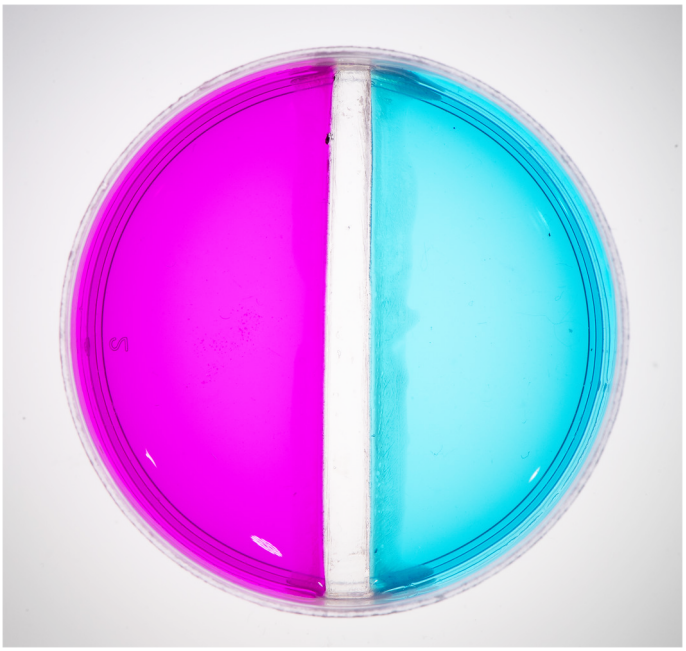
REFERENCES:

1. Jiao, Y., Moon, S. J., Montell, C. A Drosophila gustatory receptor required for the responses to sucrose, glucose, and maltose identified by mRNA tagging. *Proceedings of the National Academy of Sciences of the United States of America*. **104** (35), 14110–14115 (2007).
2. Dahanukar, A., Foster, K., van der Goes van Naters, W. M., Carlson, J. R. A Gr receptor is required for response to the sugar trehalose in taste neurons of Drosophila. *Nature Neuroscience*. **4** (12), 1182–1186 (2001).
3. Ueno, K. et al. Trehalose sensitivity in Drosophila correlates with mutations in and expression of the gustatory receptor gene Gr5a. *Current Biology*. **11** (18), 1451–1455 (2001).
4. Fujii, S. et al. Drosophila sugar receptors in sweet taste perception, olfaction, and internal nutrient sensing. *Current Biology*. **25** (5), 621–627 (2015).
5. Wang, Z., Singhvi, A., Kong, P., Scott, K. Taste representations in the Drosophila brain. *Cell*. **117** (7), 981–991 (2004).
6. Thorne, N., Chromey, C., Bray, S., Amrein, H. Taste perception and coding in Drosophila. *Current Biology*. **14** (12), 1065–1079 (2004).
7. Slone, J., Daniels, J., Amrein, H. Sugar receptors in Drosophila. *Current Biology*. **17** (20), 1809–1816 (2007).
8. Dus, M. et al. Nutrient sensor in the brain directs the action of the brain-gut axis in Drosophila. *Neuron*. **87** (1), 139–151 (2015).
9. Toshima, N., Tanimura, T. Taste preference for amino acids is dependent on internal nutritional state in Drosophila melanogaster. *Journal of Experimental Biology*. **215** (16), 2827–2832 (2012).
10. Melcher, C., Bader, R., Pankratz, M. J. Amino acids, taste circuits, and feeding behavior in Drosophila: towards understanding the psychology of feeding in flies and man. *Journal of Endocrinology*. **192** (3), 467–472 (2007).
11. Zhang, Y. V., Ni, J., Montell, C. The molecular basis for attractive salt-taste coding in Drosophila. *Science*. **340** (6138), 1334–1338 (2013).
12. Weiss, L. A., Dahanukar, A., Kwon, J. Y., Banerjee, D., Carlson, J. R. The molecular and cellular basis of bitter taste in Drosophila. *Neuron*. **69** (2), 258–272 (2011).
13. Moon, S. J., Kottgen, M., Jiao, Y., Xu, H., Montell, C. A taste receptor required for the caffeine response in vivo. *Current Biology*. **16** (18), 1812–1817 (2006).
14. Dweck, H. K. M., Carlson, J. R. Molecular logic and evolution of bitter taste in Drosophila. *Current Biology*. **30** (1), 17–30 (2020).
15. Lee, Y. et al. Gustatory receptors required for avoiding the insecticide L-canavanine. *Journal of Neuroscience*. **32** (4), 1429–1435 (2012).
16. Montell, C. A taste of the Drosophila gustatory receptors. *Current Opinion in Neurobiology*. **19** (4), 345–353 (2009).
17. Clyne, P. J., Warr, C. G., Carlson, J. R. Candidate taste receptors in Drosophila. *Science*. **287** (5459), 1830–1834 (2000).
18. Liman, E. R., Zhang, Y. V., Montell, C. Peripheral coding of taste. *Neuron*. **81** (5), 984–1000 (2014).
19. Scott, K. Gustatory processing in *Drosophila melanogaster*. *Annual Review of Entomology*. **63**, 15–30 (2018).
20. Freeman, E. G., Dahanukar, A. Molecular neurobiology of Drosophila taste. *Current Opinion in Neurobiology*. **34**, 140–148 (2015).

21. Tanimura, T., Isono, K., Yamamoto, M. T. Taste sensitivity to trehalose and its alteration by gene dosage in Drosophila melanogaster. *Genetics*. **119** (2), 399–406 (1988).
22. Zhang, Y. V., Raghuwanshi, R. P., Shen, W. L., Montell, C. Food experience-induced taste desensitization modulated by the Drosophila TRPL channel. *Nature Neuroscience*. **16** (10), 1468–1476 (2013).
23. Bantel, A. P., Tessier, C. R. Taste preference assay for adult Drosophila. *Journal of Visualized Experiments: JoVE*. (115), 54403 (2016).
24. Shiraiwa, T., Carlson, J. R. Proboscis extension response (PER) assay in Drosophila. *Journal of Visualized Experiments: JoVE*. (3), 193 (2007).
25. Ja, W. W. et al. Prandiology of Drosophila and the CAFE assay. *Proceedings of the National Academy of Sciences of the United States of America*. **104** (20), 8253–8256 (2007).
26. Diegelmann, S. et al. The CApillary FEeder assay measures food intake in *Drosophila melanogaster*. *Journal of Visualized Experiments: JoVE*. (121), 55024 (2017).
27. Ro, J., Harvanek, Z. M., Pletcher, S. D. FLIC: high-throughput, continuous analysis of feeding behaviors in Drosophila. *PLoS One*. **9** (6), e101107 (2014).
28. Yoshihara, M. Simultaneous recording of calcium signals from identified neurons and feeding behavior of Drosophila melanogaster. *Journal of Visualized Experiments: JoVE*. (62), 3625 (2012).
29. Deshpande, S. A. et al. Quantifying Drosophila food intake: comparative analysis of current methodology. *Nature Methods*. **11** (5), 535–540 (2014).
30. Yapici, N., Cohn, R., Schusterreiter, C., Ruta, V., Vosshall, L. B. A taste circuit that regulates ingestion by integrating food and hunger signals. *Cell*. **165** (3), 715–729 (2016).
31. Jiang, L., Zhan, Y., Zhu, Y. Combining quantitative food-intake assays and forcibly activating neurons to study appetite in Drosophila. *Journal of Visualized Experiments: JoVE*. (134), 56900 (2018).
32. Moon, S. J., Lee, Y., Jiao, Y., Montell, C. A Drosophila gustatory receptor essential for aversive taste and inhibiting male-to-male courtship. *Current Biology*. **19** (19), 1623–1627 (2009).
33. Zhang, Y. V., Aikin, T. J., Li, Z., Montell, C. The basis of food texture sensation in Drosophila. *Neuron*. **91** (4), 863–877 (2016).
34. Itskov, P. M. et al. Automated monitoring and quantitative analysis of feeding behaviour in Drosophila. *Nature Communications*. **5**, 4560 (2014).
35. Qi, W. et al. A quantitative feeding assay in adult Drosophila reveals rapid modulation of food ingestion by its nutritional value. *Molecular Brain*. **8**, 87 (2015).
36. Simpson, J. H., Looger, L. L. Functional imaging and optogenetics in Drosophila. *Genetics*. **208** (4), 1291–1309 (2018).

Figure 1

A



B

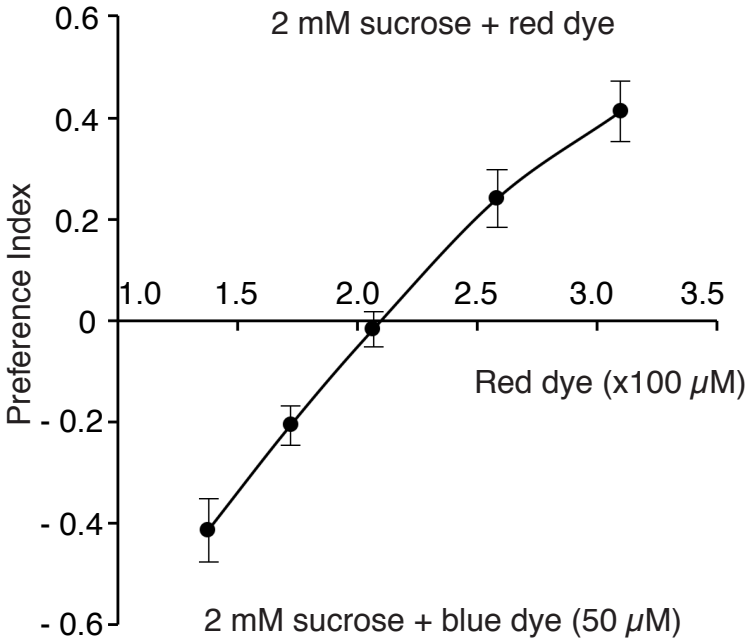


Figure 2

Figure 3

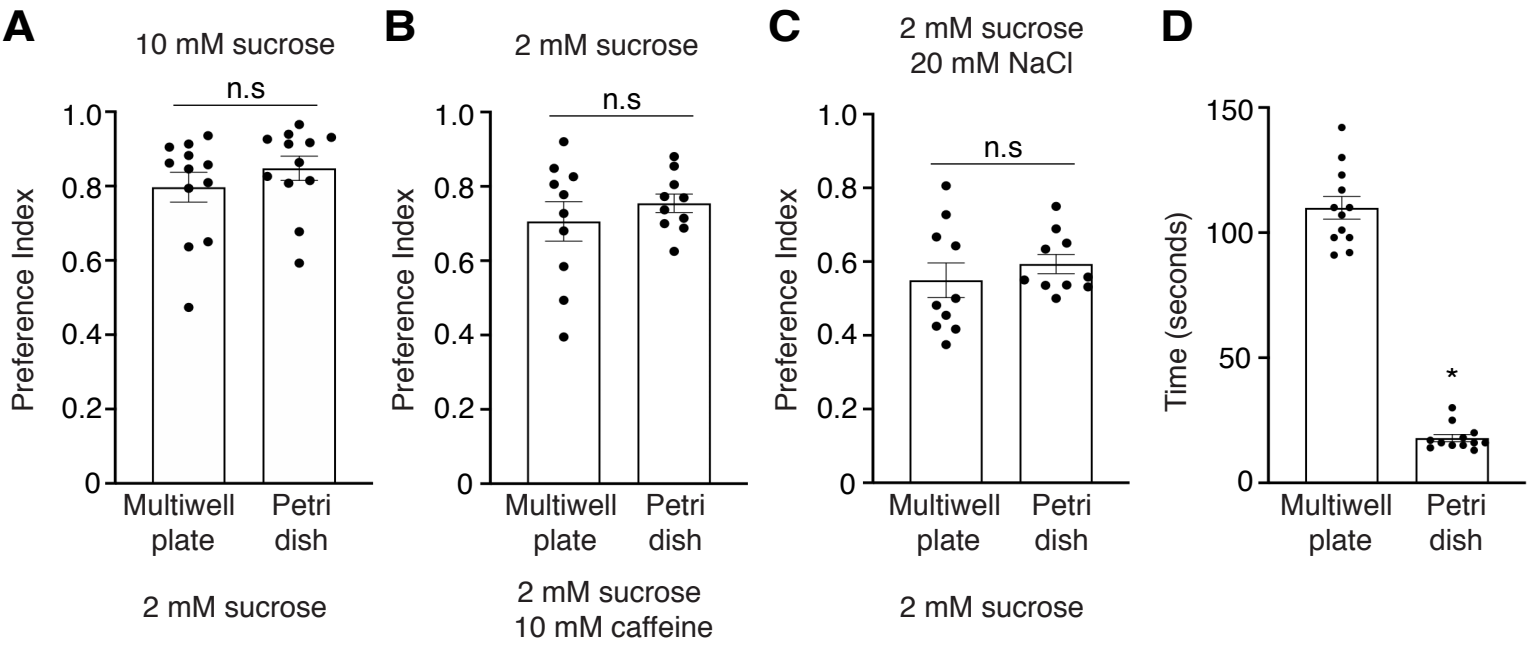


Table of Materials

Click here to access/download;Table of Materials;Fly Taste Method_JoVE_Materials_YZ_12042020.xlsx

Name of Material/ Equipment	Company	Catalog Number
35 mm Petri dish	Fisher Scientific	08-772E
Agarose	Thomas Scientific	C756P56
Clear adhesive	Fisher Scientific	NC9884114
Conical centrifuge tubes	Fisher Scientific	05-527-90
Dissection microscope	Amscope	SM-2T-6WB-V331
FCF Brilliant Blue	Wako Chemical	3844-45-9
Fly CO_2 anesthesia setup	Genesee Scientfic	59-114/54-104M
Fly incubator with programmable day/night cycle	Powers Scientific Inc.	IS33SD
Fly lines		
Glass dish (microwave-safe)		
Kimwipes	Fisher Scientific	06-666A
Media storage bottle	Fisher Scientific	50-192-9998
Plastic divider cut to fit the dish from a sheet no thicker than 5 mm		
Plastic fly vials	Genesee Scientific	32-116
Sucrose	Millipore Sigma	S9378
Sulforhodamine B	Millipore Sigma	S9012
Tastant compound of interest		
Vortex mixer	Benchmark Scientific	BV1000
Water bath	Fisher Scientific	FSGPD05

MONELL CENTER
ADVANCING DISCOVERY IN TASTE AND SMELL

Perelman School of Medicine
University of Pennsylvania

Yali V. Zhang, Principal Investigator
Monell Chemical Senses Center
Department of Physiology, the Diabetes Research Center
University of Pennsylvania Perelman School of Medicine
Philadelphia, PA 19104 USA
Phone: 267-519-4945 Email: yzhang@monell.org
https://www.monell.org/faculty/people/yali_zhang

December 2, 2020

To the editors and reviewers of the *Journal of Visualized Experiments*,

We thank you for providing us with insightful comments on our manuscript titled "A streamlined food-preference assay in *Drosophila*". We are delighted that you expressed positive opinions on our first submission. To address your comments, we added new data, including new **Figures 1B**, **2B, 2C,** and **3A-D,** to the revised manuscript. Below we summarize our responses to each comment or concern:

A. Editorial comments

Comment 1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please define all abbreviations at first use.

Answer 1: This manuscript has been thoroughly reviewed.

Comment 2. Please provide an email address for each author.

Answer 2: Email address has been added for all authors.

Comment 3. Please include a Summary to clearly describe the protocol and its applications in complete sentences between 10-50 words: "Here, we present a protocol to …"

Answer 3: This summary has been added.

Comment 4. For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s), but before punctuation.

Answer 4: This formatting change for references has been made.

Comment 5. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.

Answer 5: All trademarks and proprietary names have been removed.

Comment 6. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the

imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.

Answer 6: The instructions have been changed to the imperative and Notes have been added where appropriate.

Comment 7. How old are the flies?

Answer 7: The flies are 2-4 days old. This information has been added.

Comment 8. How are the flies photoentrained? Any specific light source? Humidity and temperature conditions?

Answer 8: We addressed the concern about the photoentrainment in our response to Concern 6 of Reviewer 3. The humidity level is 50-60%, and the temperature is 25 °C.

Comment 9. What concentrations should be tested in the preliminary assay?

Answer 9: Our feeding assay can test a wide range of experimental food concentrations and the preliminary assay is not necessary.

Comment 10. Do you first establish that flies feed in the dark and then transfer the dishes to the dark?

Answer 10: The flies are transferred immediately to a dark place after being added to the Petri dish, and the entire time course of feeding is done in the dark. We have made this clear in the protocol.

Comment 11. After including a one line space between each protocol step, highlight up to 3 pages of protocol text for inclusion in the protocol section of the video. This will clarify what needs to be filmed.

Answer 11: Highlighting and spacing have been added.

Comment 12. After protocol, please include at least one paragraph of text to explain the representative results in the context of the technique you have described, e.g., how do these results show the technique, suggestions about how to analyze the outcome, etc. The paragraph text should refer to all of the figures. Data from both successful and sub-optimal experiments can be included.

Answer 12: We have included the requested representative results.

Comment 13. After the representative results, list the figure legend. Please submit each figure individually as a vector image file to ensure high resolution throughout production: (.psd, ai, .eps.) and remove the title and figure legend from the uploaded figure.

Answer 13: The figure legends have been added, and the high-resolution psd versions of three main figures have been submitted.

Comment 14. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:
a) Critical steps within the protocol (please move the problems and solutions into the discussion)
b) Any modifications and troubleshooting of the technique (please move the problems and solutions into the discussion)

c) Any limitations of the technique
d) The significance with respect to existing methods

Answer 14: We have added this information to the Discussion.

Comment 15. Please include a Disclosures section, providing information regarding the authors' competing financial interests or other conflicts of interest. If authors have no competing financial interests, then a statement indicating no competing financial interests must be included.

Answer 15: The Disclosure has been included.

Comment 16. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage–LastPage (YEAR).] For more than 6 authors, list only the first author then et al.

Answer 16: These changes have been made to the references.

Comment 17. Please include volume and issue numbers for all references. Please do not abbreviate journal names.

Answer 17: These changes have been made to the references.

Comment 18. Please sort the Materials Table alphabetically by the name of the material.

Answer 18: This change has been made.

B. Reviewers' comments

Reviewer 1:

Minor concern 1: Overall the description of the method and text is written well and with enough detail to describe the assay. However, I strongly suggest removing the less scientific descriptors or comparisons of their method. The authors don't need to convince anyone to use the assay; just describe the pros and cons as succinctly as possible. Statements like "the field lacks a robust, simple, and high-throughput feeding assay" (line 31), and even the title ("streamlined" food-preference assay) are unnecessary and don't really describe the assay well.

Answer 1: We have removed these words from the text and replaced them with purely descriptive language. Also, we changed the title to "A **rapid** food preference assay in Drosophila".

Minor concern 2: Although it's fine to compare their dye-based food-preference assay with other publications that have done something similar, I suggest not going overboard trying to find advantages for their assay. The authors suggest that the food dries out in multiwell plates. Is this really a problem when the assay is over such a brief time period? I have not had this issue and, more importantly, I think I actually see more food drying when the surface area to volume ratio is greater, as in the larger plates that the authors are using. The authors also suggest that their method is "highly reproducible, simple, and fast" compared to other feeding methods. Unless they rigorously show this comparison, this seems like an overstatement. I like their method, but the multiwell format has advantages as well. Multiwell plates are typically used in larger cages that permit larger numbers of flies. I'd rather not speculate which method would actually provide higher throughput, but if the authors want to show this experimentally rather than claim it, that's fine. The total surface area for fly feeding may actually be less in the authors' petri dish format compared to the multiwell plate, when you sum up all of the wells. I don't think this makes the multiwell plate system better! But again the authors should be careful in presenting differences between the methods.

Answer 2: Both the multiwell-plate and Petri-dish-based feeding assays are highly robust and are widely used to examine the taste preference and food intake in flies. We have now added a comparison of these two assays, using them both to examine sweet, bitter, and salty food preferences. Our results demonstrate that both methods yield the same results (**Figures 3A-C**). One big advantage of the Petri dish over the multiwell plate is that it is much faster to prepare and load the food into the feeding device (**Figure 3D**). Therefore, our Petri-dish-based assay significantly reduces the labor and expedites the entire feeding procedure.

Based on our own experience, we have observed that the food in the Petri dish tends to retain moisture for a longer time than the multiwell plate. Since this may vary between labs depending upon the humidity and temperature of the room, we have removed this claim.

Minor concern 3: Rather than persuade others to use their particular technique, my preference is for the paper to help readers make an informed decision on the best assay to use for their experiments. Hence, in addition to a more balanced comparison with other iterations of the dye-based preference technique, it would be useful to, briefly, mention the pros and cons of the dye-based assay in general, and perhaps point out the alternative approaches. Some disadvantages to the assay that I can think of include: 1) scoring of the red and blue fly bellies may be subjective, or at the very least it is not a quantitative assay (i.e. there's no quantification of consumption; 2) the assay can only be run for brief periods (<90 min); and 3) to get flies to eat, they must be starved (at least in rodent studies, this has proven to add significant artifacts to behavioral studies). Alternatives that I can think of include FLIC/FlyPAD approaches with food choices, CAFE with different foods in different capillaries, and a recent study showed quantitative food intake preference using a radioactive tracer (Absolute ethanol intake... DOI: 10.1242/jeb.224121).

Answer 3: We added further discussions regarding the pros and cons of dye-based assays as a whole (Pages 13-14). Also, we added discussions on other feeding methods such as FLIC and CAFE assays that can complement the dye-based assay to resolve the temporal feeding dynamics (Pages 14-15).

Reviewer 2:

Concern 1: For the starvation treatment, authors use a damp kimwipe inside of a fly vial. We have trialed this before in the lab and have found that the kimwipe tend to dry out rather quickly and flies get stuck (this was discussed in the paper). An alternative that we are currently using is to starve the flies in fly vials that have ~5mL of 1% agar solution. This provides a good surface for the fly, whilst preventing desiccation.

Answer 1: With regards to the suggestion about starvation using a vial with 1% agar instead of soaked paper, this is a great suggestion and something we have explored. Both methods of starvation yield equivalent results, so we have included the 1% agarose option in the protocol.

Concern 2: I was wondering why the assays were performed in the dark? Could authors explain in the manuscript?

Answer 2: The assays should be conducted in the dark to minimize any potential external stimuli such as shadows passing over the dish or colors and shapes outside the dish. Thus, it prevents visual stimulus from affecting feeding behavior.

Concern 3: Figure 1B shows what a fly should look like following the experiment, but I would suggest that authors show what flies with "minor spots" look like as well. This will avoid confusion on which flies to exclude/include in dataset.

Answer 3: We have added images to demonstrate what is acceptable vs minor food consumption for each fly (**new Figures 2B and 2C**).

Concern 4: It would be good to include some data, maybe a plot on dye bias. I think it would give readers a better sense on the quantities of dye to add to the agar mixture, and it will also provide some clarity on the boundaries of the dye bias.

Answer 4: We have added data that provides an example for what dye optimization should look like (**new Figure 1B**). Our dosage curve shows that the optimal concentration for red dye is 210 μM when paired with 50 μM blue dye.

Reviewer 3:

Major Concerns: No data are provided to show that the current protocol gives the same results than Tanimura et al (1982) nor that it is improved as respect to the original test.

Answers: We show that the Petri-dish assay produces essentially the same results as the multiwell-based feeding assay for sweet, bitter, and salty taste responses, although the Petri-dish based assay tends to have smaller variations (**Figures 3A-C**). One time-consuming step of the dye-based feeding assay is the discharge of food into the feeding chamber. The multiwell plate, which contains 60 or more wells per plate, can be laborious to set up due to the requirement of precisely loading melted agarose food into 60 or more wells per plate. It is much faster to prepare and load food for the Petri dish than the multiwell plate, since the Petri dish contains only two separate compartments (**Figure 3D**). Thus, our Petri-dish method not only maintains the robustness of the dye-based assay, but also significantly reduces the time and effort spent in assay preparation, thereby significantly scaling up the capacity and speed of the feeding assay. Consequently, it can be readily employed to analyze a large number of fly lines, such as in a genetic screen project.

Minor Concerns

Minor Concern 1: Several more established methods need to be cited.

Answer 1: We have added these citations to our revised manuscript.

Minor Concern 2: "Petri" dish (with capital) - Petri is the name of a researcher.

Answer 2: We have changed to “Petri” throughout the manuscript.

Minor Concern 3: "Because the food volume loaded in each well is quite small, the food tends to dry during testing causing changes in food texture, such as hardness and viscosity. This problem may affect food preference and consumption. Moreover, it is laborious to distribute melted agarose food into 54 or more wells at a time." - show data for this. None of these remarks have been made previously by other researchers.

Answer 3: Compared with the multiwell plate, the Petri dish significantly reduces the time and effort spent on discharging agarose food into multiwell plate (see **new Figure 3D**). Regarding the food drying problem, this largely depends on lab conditions such as humidity and temperature. In our hands, we feel that our data are less affected while using the outlined protocol, though of course this will depend upon lab setting. Thus, we removed this point on food drying from this protocol.

Minor Concern 4: 1 Petri dish = 70 flies; how many repetitions (dishes) are usually needed to get statistically significant results?

Answer 4: At least 4 dishes or 4 trials with ~ 70 flies in each trial are needed.

Minor Concern 5: "the flies should be photoentrained in a 12/12h day/night/cycle": is there a reference supporting this claim? does it really affect the results of such experiments?

Answer 5: This is not an important part of the protocol, and we meant to present it as a suggestion instead of a strict guideline. We have not seen significant differences between photoentrained and arrhythmic flies, and we have removed this point from the protocol to avoid confusion.

Minor Concern 6: "concentrations should be refined in 0.5 mg/mL increments" - usually, pharmacological effects are described using Moles/l (not weight) and the effects usually follow a log10 scale: 1 - 1.333 -10 - 13.333 - 100 etc.

Answer 6: We changed the dye concentration unit to micromolar (μM), and noted that the concentrations should be refined in 1 μM increments.

Minor Concern 7: "Prolonged exposure to cold can affect the feeding" - how strong is this effect?

Answer 7: Keeping flies on ice too long can lead to lethality. We have clarified the time period beyond which cold shock is a concern in the protocol.

In summary, we thank you again for taking the time to review our submission and helping us improve our manuscript. We hope you are satisfied with our revision. We would greatly appreciate your accepting our manuscript for publication.

Sincerely

Yali V. Zhang, Ph.D.