

Journal of Visualized Experiments

Electrophysiological recording from *Drosophila* labellar taste sensilla

--Manuscript Draft--

Manuscript Number:	JoVE51355R2
Full Title:	Electrophysiological recording from <i>Drosophila</i> labellar taste sensilla
Article Type:	Methods Article - JoVE Produced Video
Keywords:	Neuroscience; drosophila; insect; taste; neuron; electrophysiology; labellum; extracellular recording
Manuscript Classifications:	2.1.50.500.131.617.289.310.250.500: <i>Drosophila melanogaster</i> ; 7.11.561.600.810.724: Taste; 8.1.158.782.236: Electrophysiology
Corresponding Author:	Rebecca Delventhal Yale University New Haven, CT UNITED STATES
Corresponding Author Secondary Information:	
Corresponding Author E-Mail:	rebecca.delventhal@yale.edu
Corresponding Author's Institution:	Yale University
Corresponding Author's Secondary Institution:	
First Author:	Rebecca Delventhal
First Author Secondary Information:	
Other Authors:	Aidan Kiely, PhD John Carlson, PhD
Order of Authors Secondary Information:	
Abstract:	<p>The peripheral taste response of insects can be powerfully investigated with electrophysiological techniques. The method described here allows the researcher to measure gustatory responses directly and quantitatively, reflecting the sensory input that the insect nervous system receives from taste stimuli in its environment. This protocol outlines all key steps in performing this technique. The critical steps in assembling an electrophysiology rig, such as selection of necessary equipment and a suitable environment for recording, are delineated. We also describe how to prepare for recording by making appropriate reference and recording electrodes, and tastant solutions. We describe in detail the method used for preparing the insect by insertion of a glass reference electrode into the fly in order to immobilize the proboscis. We show traces of the electrical impulses fired by taste neurons in response to a sugar and a bitter compound. Aspects of the protocol are technically challenging and we include an extensive description of some common technical challenges that may be encountered, such as lack of signal or excessive noise in the system, and potential solutions. The technique has limitations, such as the inability to deliver temporally complex stimuli, observe background firing, or use water-insoluble taste compounds conveniently. Despite these limitations, this technique (including minor variations referenced in the protocol) is a standard, broadly accepted procedure for recording <i>Drosophila</i> neuronal responses to taste compounds.</p>
Author Comments:	<p>Please note that the images in Figure 4 need to be retaken with better photography equipment. The angle of approach between the recording electrode and the sensillum is not accurate because the photos were taken using a microscope and setup other than that normally used for the technique, due to an unavailability of a camera attachment. We ordered an adapter that would hopefully work with the electrophysiology microscope, but it did not arrive in time and Nandita requested that the manuscript be submitted by the end of June. My hope is that we can try retaking the photos on our own at a later date, or in the event that the manuscript is accepted, the JoVE videographer can assist in this.</p>

Additional Information:	
Question	Response

Yale University

Department of Molecular, Cellular
and Developmental Biology
Yale University
P. O. Box 208103
New Haven, Connecticut 06520-8103

Campus address:
Kline Biology Tower
219 Prospect Street
Telephone: 203 432-3460
Fax: 203 432-6161

6-28-13

Editor, *JOVE*

Dear Sir/Madam,

I'd be very grateful if you would consider for possible publication the attached ms. by Delventhal, Kiely and Carlson, "Electrophysiological recording from *Drosophila* labellar taste sensilla". We are grateful to you for having invited submission of this ms.

The manuscript describes how we carry out electrophysiological recordings from taste sensilla on the principal taste organ of the *Drosophila* head, the labellum. We explain our procedures in detail, including description of the equipment and the preparation of the fly. We show some representative data and provide an extensive troubleshooting guide.

We agree with Nandita Singh, Senior Science Editor at JoVE, who invited this submission that publication of such a ms. is likely to be useful to a substantial audience – the use of *Drosophila* as an experimental system to study gustation is expanding, and the procedures we describe are sufficiently challenging as to warrant the kind of visual and textual description that *JOVE* provides so well.

Delventhal, Kiely, and Carlson co-wrote the manuscript. RD and AK assembled figures. RD performed recordings displayed in representative results. RD and AK contributed equally to this work, and we request they be listed as co-first authors.

You may wish to consider the following individuals as reviewers:

1. Dr. Alan Grant Grant@hsph.harvard.edu Department of Immunology and Infectious Diseases, Harvard School of Public Health, Harvard University

2. Dr. Thomas Baker tcb10@psu.edu, Dept. Entomology, Penn State University

3. Dr. Jean-Pierre Rospars jean-pierre.rospar@versailles.inra.fr, INRA Station de Phytopharmacie et Médiateurs Chimiques, 78026 Versailles Cedex, France

4. Dr. Nicolas Meunier Nicolas.Meunier@jouy.inra.fr, NOeMI Neurobiologie de l'Olfaction et Modélisation en Imagerie Jouy-en-Josas 78352 Jouy-En-Josas

5. Dr. John Glendinning jglendin@barnard.edu, Dept. of Biology, Barnard College, Columbia University

6. Dr. Zain Syed zsyed@nd.edu, Dept. of Biological Sciences, University of Notre Dame

Thanks very much for your consideration.

Sincerely yours,

A handwritten signature in blue ink that reads "John Carlson". The signature is fluid and cursive, with the first name "John" and last name "Carlson" clearly distinguishable.

John Carlson
Higgins Professor
Department of Molecular, Cellular and Developmental Biology
Yale University

TITLE: Electrophysiological recording from *Drosophila* labellar taste sensilla

AUTHORS:

Delventhal, Rebecca

MCDB

Yale University

New Haven, CT

Rebecca.delventhal@yale.edu

Kiely, Aidan

MCDB

Yale University

New Haven, CT

aidan.kiely@yale.edu

Carlson, John R.

MCDB

Yale University

New Haven, CT, USA

john.carlson@yale.edu

CORRESPONDING AUTHOR:

Carlson, John R.

MCDB

Yale University

New Haven, CT, USA

john.carlson@yale.edu

KEYWORDS:

Neuroscience, *Drosophila*, insect, taste, neuron, electrophysiology, labellum, extracellular recording

SHORT ABSTRACT:

This protocol describes extracellular recording of the action potential responses fired by labellar taste neurons in *Drosophila*.

LONG ABSTRACT:

The peripheral taste response of insects can be powerfully investigated with electrophysiological techniques. The method described here allows the researcher to measure gustatory responses directly and quantitatively, reflecting the sensory input that the insect nervous system receives from taste stimuli in its environment. This protocol outlines all key steps in performing this technique. The critical steps in assembling an electrophysiology rig, such as selection of necessary equipment and a suitable environment for recording, are

delineated. We also describe how to prepare for recording by making appropriate reference and recording electrodes, and tastant solutions. We describe in detail the method used for preparing the insect by insertion of a glass reference electrode into the fly in order to immobilize the proboscis. We show traces of the electrical impulses fired by taste neurons in response to a sugar and a bitter compound. Aspects of the protocol are technically challenging and we include an extensive description of some common technical challenges that may be encountered, such as lack of signal or excessive noise in the system, and potential solutions. The technique has limitations, such as the inability to deliver temporally complex stimuli, observe background firing immediately prior to stimulus delivery, or use water-insoluble taste compounds conveniently. Despite these limitations, this technique (including minor variations referenced in the protocol) is a standard, broadly accepted procedure for recording *Drosophila* neuronal responses to taste compounds.

INTRODUCTION:

The sense of taste allows an insect to detect a vast range of soluble chemicals and plays an important role in the acceptance of a nutritious substance, or the rejection of a noxious or toxic one. Taste is also thought to play a role in mate selection, through the detection of pheromones.¹⁻⁵ These important and diverse functions have made the insect taste system a compelling target of investigation into how sensory systems translate environmental cues into relevant behavioral outputs.

The primary unit of the *Drosophila melanogaster* taste system is the taste hair, or sensillum. Molecules enter the sensillum via a pore at its tip.^{2,6} Sensilla are found on the labellum, the legs, the wing margin, and the pharynx.⁶ On the labellum, the number and location of sensilla is stereotyped. There are three morphological classes of sensilla based on length: the long (L), intermediate (I), and short (S) sensilla.^{7,8} Each sensillum contains either two (I-type) or four (L- and S- type) gustatory receptor neurons (GRNs).⁹ Different GRNs respond to different categories of taste stimuli: bitter, sugar, salt and osmolarity^{7,10} and express different subsets of gustatory receptors.^{8,11-13} Only I and S-type sensilla contain bitter-responsive GRNs.^{8,10} The GRNs project to the subesophageal ganglion (SOG) and their activation by taste molecules is relayed to the higher central nervous system for decoding, resulting in a behavioral response.⁶ The relatively small number of neurons and the amenability to molecular and behavioral analysis make the *Drosophila* taste system an excellent model for the investigation of gustatory systems in general. The relative ease with which the system can be manipulated via genetic mutation or the GAL4-UAS expression system also serves as a valuable tool.^{14,15}

Because these sensilla protrude from the surface of the labellum, they make excellent targets for electrophysiology. The firing of the GRNs can be monitored using extracellular recording. Historically, the side-wall recording method, which uses a glass electrode inserted into the sensillum to record neuronal activity,²⁶ has been used. However, this method is technically challenging to perform, and it is difficult to record for long from each preparation. The tip-recording method, which measures the response of the neurons with an electrode that

simultaneously delivers a tastant, has since become the method of choice.^{9,16} It has been utilized to investigate the taste system of *Drosophila melanogaster*^{8,10,17,18} as well as a number of other insect species¹⁹⁻²³. It has been greatly facilitated by the development of the tastePROBE amplifier, which overcame one of the major drawbacks of the tip-recording method by compensating for the large potential difference between the reference electrode and the insect sensillum, allowing the GRN action potentials to be recorded without excessive amplification or filtering.²⁴ Another important development was the use of tricholine citrate as the recording electrolyte.²⁵ TCC suppresses responses from the osmolarity-sensitive GRN and does not stimulate the salt-sensitive GRN, making responses generated by bitter and sugar tastants much easier to analyze.²⁵

Here we describe how tip recording of *Drosophila* labellar sensilla is currently performed in the Carlson laboratory. This protocol will explain how to establish a suitable electrophysiology rig, how to prepare the fly, and how to perform taste recordings. We also present some representative data obtained by recording from subsets of *Drosophila* sensilla, as well as some common issues and potential solutions that may be encountered when using this technique.

PROTOCOL:

The following protocol complies with all the animal care guidelines of Yale University.

1. Reagents and Equipment Preparation

1.1) Recording equipment setup (Figure 1A)

[place Figure 1 here]

1.1.1) Choose a room for rig setup that is free of large variations in temperature or humidity and also isolated from sources of electrical and mechanical noise, such as refrigerators and centrifuges.

1.1.2) Mount stereo microscope to center of anti-vibration table or platform.

1.1.3) Attach micromanipulators for the reference electrode/insect preparation and headstage/recording electrode to the left and right of the microscope, respectively, using magnetic stands.

1.1.4) Mount outlet plastic tube in a third micromanipulator to the rear of the microscope, oriented such that the tube opening is pointed toward location of fly prep (see Figure 1B).

1.1.5) Using flexible plastic tubing, attach outlet plastic tube to a vacuum flask partially filled with water. Connect a small aquarium pump to bubble air through the water in the flask, generating a humidified air stream through the outlet plastic tube towards the fly.

1.1.6) Mount fiber optic light source off the vibration table, orienting the outputs to illuminate the preparation by reflecting light via a piece of white card paper directly below the preparation. Ensure that the light source does not rest on the table. Note: the benefit of reflecting the light source on a paper disc is two-fold: it improves the contrast, making sensilla easier to visualize, and it prevents heating of the preparation that would result from direct light.

1.1.7) Plug tastePROBE amplifier into the digital acquisition system (DAS), and the DAS into a personal computer, according to supplier manual. Plug foot pedal trigger in and arrange under workspace. Note: Electrically isolated wall sockets for the amplifier and DAS are highly desirable.

1.1.8) Electrically ground microscope, micromanipulators, and light source by connecting metal components to table using alligator clips and lengths of insulated electrical wire and electrical tape. Electrically ground metal platform by connecting to building ground or DAS, which is grounded through power supply plug.

1.1.9) Install appropriate acquisition software for the DAS of choice on the personal computer. Note: Ensure that the digital acquisition drivers are compatible with the operating system on the PC.

1.1.10) Configure software amplification (10-100x), signal filtering (typically a Bessel bandpass filter set from 100 Hz – 3000 Hz), and sampling rate (at least 10 KHz). Note: Signal amplitudes from gustatory neurons are typically in the 0.5-2 mV range, so the display scale is set to facilitate their visualization. Note: The 100 Hz filter helps to exclude extraneous electrical noise; however, it changes the shape of spikes and can make advanced spike sorting more challenging. Alternatively, a 1 Hz filter can be used.

1.1.11) Optionally, a Faraday cage can be set up around the whole vibration table. However, small sheets of aluminum foil are usually sufficient to reduce any noise generated by the external environment or investigator.

1.2) Glass Electrode Preparation

[place Figure 2 here]

1.2.1) Pull the reference electrode from a glass capillary using a pipette puller instrument. Note: The exact settings of the pipette puller program will vary from instrument to instrument. Try to achieve a very long gradual taper. The pore size at the tip is not crucial because the tip will be broken before fly preparation (Figure 2A,B); however, make sure that the diameter of the tapered length of the electrode is neither too thin, which will not allow for sufficient immobilization of the labellum, nor too large, which could damage the gustatory neurons or rupture the salivary glands.

1.2.2) Pull recording electrode from a borosilicate glass capillary with filament using a pipette puller instrument. Try to achieve a taper that is shallower than that of the reference electrode, and a pore diameter of approximately 10-15 μm (Figure 2C).²⁸

1.3) Tastant solutions preparation

1.3.1) Use Beadle-Ephrussi Ringer solution (B&E) as the reference electrode electrolyte. To make one liter of B&E, dissolve 7.5 g NaCl, 0.35 g KCl and 0.279 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in one liter of ultrapure water. Store smaller aliquots at -20 °C.

1.3.2) Use 30 mM tricholine citrate solution (TCC) as the recording electrode electrolyte and solvent for tastant solutions,²⁵ if bitter or sugar GRN responses are to be measured. Alternatively, 1-3 mM potassium chloride solution can be used if responses of the water cell are to be measured.

1.3.3) To make tastant solutions, weigh appropriate amount of tastant in powder form and add to TCC to make an initial stock concentration. Use this to make serial dilutions from this initial stock to yield the desired concentration for testing. Note: If tastants do not readily dissolve in water, another solvent, such as ethanol, can be used to make initial stock concentration. An appropriate control solution of TCC and solvent without tastant should be used in this case.

1.3.4) Store aliquots long term at -20 °C. Store one working aliquot of a tastant solution at 4 °C for recording use for up to a week, depending on chemical properties of tastant.

2. *Drosophila* preparation

[place Figure 3 here]

2.1) Collect newly eclosed flies for recording from well-maintained fly cultures, grown under temperature- and humidity-controlled conditions, and age them 5-10 days in fresh culture vials before recording.

2.2) Chill microscope plate on ice for 15-30 min before preparing fly.

2.3) Backfill glass reference electrode with B&E solution using a long, thin plastic needle of 0.5 mm diameter, such as a spinal needle and 1 ml syringe and gently tap out any bubbles. Break small amount of tip off using forceps and use capillary action to draw out all remaining bubbles with a tissue, observing under dissecting microscope.

2.4) Slide B&E-filled reference electrode onto wire of reference electrode holder, taking care not to introduce air bubbles.

2.5) Aspirate fly into a P200 pipette tip, using fly aspirator built from tubing, mesh, and pipette tip;²⁹ place in ice bucket and chill for 30 sec – 1 min.

2.6) Remove microscope plate from ice, wipe off any moisture, and position underneath microscope. Gently tap fly out of pipette tip onto microscope plate.
Note: the fly should be sufficiently immobilized to manipulate easily.

2.7) Under low magnification, gently remove the forelegs with one pair of forceps, while holding the thorax stable with the other pair of forceps. Position the fly on its ventral side, dorsal side facing up. Note: Always be careful to avoid touching the labellum with the forceps at all times during the preparation process to minimize mechanical damage.

2.8) While holding the fly in place with one pair of forceps, insert the reference electrode at the midline of the posterior dorsal thorax. A suggested angle of entry is approximately forty-five degrees, in the direction of the head (Figure 3A).

2.9) Secure the reference electrode holder with modeling clay such that the fly is visible underneath the microscope at high magnification. Maneuver and angle the glass electrode through the neck and head, by sliding the fly towards the reference electrode holder using two pairs of forceps. Note: Work quickly but smoothly; it is easier to complete this step while the fly is still immobilized from the cold (Figure 3B).

2.10) Gently extend the proboscis with one pair of forceps, while sliding the fly further down the glass reference electrode, until the tip of the electrode is inside the labellum and the proboscis is fully extended (Figure 3C,D). Note: Take care not to puncture any part of the proboscis tissue or distend the edge of the labellum with the reference electrode, as this may damage the fly and/or taste neurons and affect the recording quality.

3. Recording from labellar sensilla

[place Figure 4 here]

3.1) Always ground yourself by touching the metal surface of the anti-vibration table or platform prior to touching any equipment during recording process! Note: It is extremely important not to deliver a static charge to the headstage as that can damage the circuitry.

3.2) Secure reference electrode holder to micromanipulator mounted on air table of recording rig. Position one lobe of labellum in microscope field of view, under high magnification (typically at least 140x), and in line with humidified air stream.

3.3) Turn on humidified air stream, computer, DAS, and amplifier. Open acquisition software.

3.4) Rinse and fill glass recording electrode with desired tastant.

3.4.1) Rinse glass recording electrode with ultrapure water by using a syringe and plastic tubing²⁸ to pull small amounts of water through the tube at least ten times.

3.4.2) Rinse recording electrode with tastant at least five times. Fill recording electrode approximately one-third to halfway full with tastant and remove from tubing. If there are air bubbles, tap to release or simply refill the electrode.

3.4.3) Slide electrode onto silver wire of the headstage quickly and smoothly so as not to introduce air bubbles.

3.5) Stimulate single sensillum with tastant-filled recording electrode.

3.5.1) Use the micromanipulator to bring the recording electrode aligned with sensillum of interest.

3.5.2) Press foot pedal to trigger acquisition mode of the amplifier.

3.5.3) Advance the recording electrode with the fine control knob of micromanipulator carefully until it makes contact with tip of sensillum and recording commences.

3.5.4) Remove electrode after 1-2 seconds.

3.5.5) Repeat Step 3.5 with other sensilla, if desired. Note: Wait at least one minute in between presentations to the same sensillum. If recording with a single tastant for a prolonged period of time, the tastant solution may dry out and the solution in the tip may become more concentrated. This can be remedied by gently contacting the tip of the glass electrode with smooth paper to remove a small amount of liquid by capillary action.

3.6) To record responses to another tastant, rinse and load recording electrode with new tastant and repeat step 3.4. Note: Thoroughly rinsing the electrode between tastants is absolutely crucial to avoid cross-contamination.

3.7) Save data files periodically with identifying information, such as date, genotype, and tastants. Note: It is important to keep a written record of the tastant and sensillum identity of each presentation during recording session for data analysis.

REPRESENTATIVE RESULTS:

Figure 5A shows the response of an L sensillum to a sugar, sucrose. The same sensillum does not respond to a bitter compound, berberine. Figure 5B shows that an I type sensillum, which contains a bitter responsive neuron, displays larger amplitude spikes in response to berberine, and smaller amplitude spikes in response to sucrose. L sensilla display a minimal background response to the solvent control, TCC, while I sensilla display virtually no response to TCC (Figure

5). For more information on salt and water responses of labellar GRNs, please refer to Hiroi, 2004.¹⁰

Figure Legends:

Figure 1: (A) Overview of recording rig setup. Stereomicroscope (a) is mounted on anti-vibration platform (b). Reference electrode holder (c) is mounted on platform opposite the headstage (d), via micromanipulators. An outlet plastic tube (e) delivering humidified air stream directed at the fly preparation is also mounted on the platform. The headstage is connected to the amplifier (f), which is connected to the digital acquisition system (DAS) (g), which is connected to a PC (h). (B) Configuration of electrodes and outlet tube: reference electrode on the left, recording electrode on the right, and air stream outlet tube directed at fly preparation.

Figure 2: Reference and recording electrodes. Photograph under magnification of glass capillaries pulled into reference electrode, with (A) and without (B) tip broken, and recording electrode (C). White bar represents 2 mm.

Figure 3: Preparation of fly for recording. (A) Insertion position of reference electrode into dorsal thorax of fly. The white arrow indicates the reference electrode. (B) Intermediate position of reference electrode: advanced through neck and head, proboscis not yet extended. (C,D) Fly with reference electrode in final position with tip of electrode inside labellum, and proboscis fully extended.

Figure 4: Recording from fly. (A) labellum of fly preparation on left with recording electrode aligned for contact on right, under high magnification. (B) recording electrode and single sensillum on labellum in contact, under high magnification.

Figure 5: Representative traces of wild-type *Drosophila* labellar responses (A) L sensillum response to 100 mM sucrose (SUC), 1 mM berberine (BER), and 30 mM TCC. (B) I sensillar response to SUC, BER, and TCC. The arrowhead indicates the contact artifact that occurs at the beginning of each recording.

Figure 6: Representative suboptimal electrophysiological results. (A) complete lack of signal (B) 50/60 Hz “noise” (C) stochastic noise (D) mechanosensory neuron firing alone (E) bitter GRN (open triangles) and mechanosensory neuron (filled triangles) both firing

DISCUSSION:

Labellar sensilla vary in the ease of recording due to differences in morphology and anatomical organization. Sometimes a sensillum does not respond to any tastants, even one that is known to elicit a positive response. The frequency with which this occurs varies depending on sensillum type. L sensilla are most consistently responsive and are relatively easy to access due to their length. In general, S sensilla are consistently responsive, but their short length and

position on the labellum make good contact challenging. I sensilla can be accessed more readily, depending on the angle of the preparation; however, they are more frequently unresponsive. On any given fly preparation, a greater proportion of I sensilla may be unresponsive than L or S sensilla. Genetic background can affect the consistency of taste responses as well. For example, some transgenic flies may display less consistent responses than wild-type, presumably because the transgenes affect the general health of the fly. We have observed that *w¹¹¹⁸* mutant flies are particularly challenging to record from.

[place Figure 6 here]

One common technical problem is a lack of signal, i.e., no spikes are observed (Figure 6A). First, sometimes one particular sensillum may be unresponsive, while others of that same class on the same fly may respond. Second, there may be an air bubble in the recording electrode or the reference electrode. If the recording electrode is suspected, this can be fixed by simply removing and refilling the glass electrode, tapping gently and inspecting under magnification to ensure no bubbles are present. If the reference electrode is suspected to contain an air bubble, remaking the prep with a new fly is the easiest way to resolve this issue. Third, sometimes the wires carrying the electrical signal may not be securely connected. Fourth, occasionally the voltage signal being received may be either higher or lower than the range the amplifier can measure. If using the tastePROBE amplifier, check to see if either the clip up or clip down indicator light is on. If the clip up indicator light is on, often removing and refilling the glass reference electrode, while taking care to fill not more than halfway and wiping down the outside to remove moisture will resolve the problem. Moisture on the outside of the glass electrode can make an electrical connection between the metal case of the electrode and the wire, sending the signal out of range of the amplifier. If that fails to solve the issue, or the clip down indicator light is on, consider suggestions in the following paragraph to combat electrical noise in the system. Fifth, sometimes a fly may die during preparation or is otherwise unresponsive despite the preparation's healthy appearance. Growth conditions, such as humidity, temperature, age, food quality, and microbiota, as well as a less healthy genetic background could contribute to a higher proportion of "unresponsive" flies. Lastly, rarely, a piece of equipment may be non-functional. If signal is consistently not being achieved and all other possibilities have been exhausted, it may be necessary to investigate the functionality of each piece of equipment: headstage, amplifier, and digitizer. The easiest way to do this is to replace a piece of equipment with another from a rig that is known to be functional. If only one rig is present in a lab, a signal generator can be used to test functionality of the electronic components.

Another common technical issue is that of "noise," which is an observed signal that does not appear to represent neuronal action potentials fired in response to a gustatory stimulus (Figure 6B-E). First, the signal may result from 50/60 Hz electrical noise from recording equipment or other equipment nearby (Figure 6B). With no fly on the reference electrode, directly connect the recording and reference electrodes through a drop of Ringer's solution and enter the passthrough mode on the amplifier by pressing the up button. If noise is observable on the passthrough signal, this likely means that the noise is external to the fly preparation. Ensure that all rig equipment is properly grounded and that tin foil shields are in place. Try unplugging

nearby equipment to see if the noise is eliminated, or shield additional components. Second, the noise may appear stochastic (Figure 6C). In this case, the steps detailed for 50/60 Hz noise should still be undertaken. Additionally, try unplugging or replacing different components of the recording equipment, particularly the headstage and/or amplifier. If no noise is observed when the electrodes are directly connected, the source is likely the fly preparation itself. It is usually simplest to prepare a new fly for recording, taking care to minimize damage to the fly. Third, activation of the mechanosensory neuron contained within the sensillum (Figure 6D,E) may be observed. The mechanosensory neuron can be activated if the sensillum is deflected or bent upon application of the recording electrode, or bumped during contact. The spikes are usually distinguishable from chemosensory spikes by their irregular pattern, which usually appears coordinated with the mechanical disruption, not the application of a gustatory stimulus. Mechanosensory firing can be minimized by aligning the recording electrode with the sensillum and advancing gently only as far as is necessary to make contact with the tip of the sensillum. Fourth, stochastic spike “bursting” may be observed; this appears similar to neuronal firing, but is of high frequency and amplitude, not coordinated in response to a stimulus. This usually results from the fly prep itself, not from the equipment, and may be due to a nerve disrupted by the reference electrode.

A third common technical issue is that the preparation is mobile, causing the labellum to move, which makes connection with a sensillum difficult. First, the fly preparation may be unstable. Check that the reference electrode is correctly positioned, and readjust if necessary. Second, the reference electrode may be too thin at the tip to hold the proboscis and labellum immobile. Try breaking off a longer amount of the tip before preparing the fly. If that is not sufficient, readjust the pipette puller settings as needed to change the shape of reference electrode such that the taper is more gradual and the diameter is slightly increased. Third, the fly may be unusually active. Remake the preparation with a new fly.

For general electrophysiology information and more troubleshooting guidance, refer to Axon Guide.³⁰

There are a few limitations to the tip-recording method outlined in this publication. One limitation is that the tastant must be water soluble, as it is delivered in the recording electrode along with the electrolyte. This increases the difficulty of recording with hydrocarbon compounds, though use of a solvent like DMSO has made some recording with pheromones possible.⁴ Alternative approaches are to use a sharpened tungsten electrode to perform the recordings from the socket base of the sensillum, or use a glass electrode to perform recordings from the side wall of the sensillum, in both of which the tastant is delivered independently of the recording electrode.^{26,27} However, these techniques are challenging and side-wall recordings are more injurious to the taste organ. Another limitation is the amount of time required to exchange the tastant solution (Protocol Step 3.3), which reduces throughput, and limits the use of complicated stimulus paradigms often seen in olfactory recordings. Gustatory receptor neurons exhibit some variability in amplitude that is dependent on spike frequency. This feature can complicate assessment of neuronal identity and make advanced spike sorting

more difficult.^{25,31-33} In addition, because of the nature of the tip-recording method one cannot record the basal firing immediately prior to the delivery of a stimulus, as is commonly done in olfactory recordings. Despite these drawbacks, the tip-recording method has been successfully used to elucidate many of the principles of taste coding in *Drosophila* and other species.^{8,10,17,19,21-23}

The fly preparation technique outlined here is just one possible approach. In this preparation method the proboscis is fixed in an extended position to facilitate contact of the recording electrode with the sensillum of interest, and the reference electrode is inserted into the animal. Other preparation methods include the mounting of the animal to a ball of modeling clay and the use of thin strips of tape to fix the proboscis.³⁴ Indeed, as long as the basic parameters of tissue stabilization and reference electrode placement are met, sensilla in other locations or from different species can be recorded from in much the same way. For example, leg sensilla can be recorded from by fixing the body of a fly to a sylgard-coated microscope slide with fine insect pins, splaying the legs off the edge of the glass slightly.³⁵ It is possible to deliver pharmacological agents to the sensilla via the recording electrode to investigate signal transduction in the gustatory receptor neurons. It is simply a task of experimentation to determine which approach works best for the desired outcome.

ACKNOWLEDGMENTS:

This work was supported by an NRSA pre-doctoral grant 1F31DC012985 (to R.D.) and by NIH grants to J.C.

We would like to thank Dr. Linnea Weiss for helpful comments on the manuscript, Dr. Ryan Joseph for help compiling figures, and Dr. Frederic Marion-Poll for helpful technical advice. We would also like to acknowledge the helpful comments of four reviewers.

DISCLOSURES:

The authors have nothing to disclose

REFERENCES

- 1 Glendinning, J. I., Jerud, A. & Reinherz, A. T. The hungry caterpillar: an analysis of how carbohydrates stimulate feeding in *Manduca sexta*. *The Journal of experimental biology* **210**, 3054-3067, doi:10.1242/jeb.004671 (2007).
- 2 Yarmolinsky, D. A., Zuker, C. S. & Ryba, N. J. Common sense about taste: from mammals to insects. *Cell* **139**, 234-244, doi:10.1016/j.cell.2009.10.001 (2009).
- 3 Thistle, R., Cameron, P., Ghorayshi, A., Dennison, L. & Scott, K. Contact chemoreceptors mediate male-male repulsion and male-female attraction during *Drosophila* courtship. *Cell* **149**, 1140-1151, doi:10.1016/j.cell.2012.03.045 (2012).
- 4 Toda, H., Zhao, X. & Dickson, B. J. The *Drosophila* female aphrodisiac pheromone activates ppk23(+) sensory neurons to elicit male courtship behavior. *Cell reports* **1**, 599-607, doi:10.1016/j.celrep.2012.05.007 (2012).

- 5 Lu, B., LaMora, A., Sun, Y., Welsh, M. J. & Ben-Shahar, Y. ppk23-Dependent chemosensory functions contribute to courtship behavior in *Drosophila melanogaster*. *PLoS Genet* **8**, e1002587, doi:10.1371/journal.pgen.1002587 (2012).
- 6 Stocker, R. F. The organization of the chemosensory system in *Drosophila melanogaster*: a review. *Cell and tissue research* **275**, 3-26 (1994).
- 7 Hiroi, M., Marion-Poll, F. & Tanimura, T. Differentiated response to sugars among labellar chemosensilla in *Drosophila*. *Zoological Science* **19**, 1009-1018 (2002).
- 8 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**, 258-272, doi:10.1016/j.neuron.2011.01.001 (2011).
- 9 Falk, R., Bleiser-Avivi, N. & Atidia, J. Labellar taste organs of *Drosophila melanogaster*. *Journal of Morphology* **150**, 327-341, doi:10.1002/jmor.1051500206 (1976).
- 10 Hiroi, M., Meunier, N., Marion-Poll, F. & Tanimura, T. Two antagonistic gustatory receptor neurons responding to sweet-salty and bitter taste in *Drosophila*. *Journal of neurobiology* **61**, 333-342, doi:10.1002/neu.20063 (2004).
- 11 Clyne, P. J., Warr, C. G. & Carlson, J. R. Candidate taste receptors in *Drosophila*. *Science (New York, N.Y.)* **287**, 1830-1834 (2000).
- 12 Cameron, P., Hiroi, M., Ngai, J. & Scott, K. The molecular basis for water taste in *Drosophila*. *Nature* **465**, 91-95 (2010).
- 13 Croset, V. *et al.* Ancient protostome origin of chemosensory ionotropic glutamate receptors and the evolution of insect taste and olfaction. *PLoS Genet* **6**, e1001064, doi:10.1371/journal.pgen.1001064 (2010).
- 14 Brand, A. H. & Perrimon, N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development (Cambridge, England)* **118**, 401-415 (1993).
- 15 Parks, A. L. *et al.* Systematic generation of high-resolution deletion coverage of the *Drosophila melanogaster* genome. *Nature genetics* **36**, 288-292, doi:10.1038/ng1312 (2004).
- 16 Hodgson, E. S., Lettvin, J. Y. & Roeder, K. D. Physiology of a primary chemoreceptor unit. *Science (New York, N.Y.)* **122**, 417-418 (1955).
- 17 Dahanukar, A., Lei, Y. T., Kwon, J. Y. & Carlson, J. R. Two Gr genes underlie sugar reception in *Drosophila*. *Neuron* **56**, 503-516, doi:10.1016/j.neuron.2007.10.024 (2007).
- 18 Lee, Y., Kim, S. H. & Montell, C. Avoiding DEET through insect gustatory receptors. *Neuron* **67**, 555-561, doi:10.1016/j.neuron.2010.07.006 (2010).
- 19 Descoins, C. & Marion-Poll, F. Electrophysiological responses of gustatory sensilla of *Mamestra brassicae* (Lepidoptera, Noctuidae) larvae to three ecdysteroids: ecdysone, 20-hydroxyecdysone and ponasterone A. *J Insect Physiol* **45**, 871-876 (1999).
- 20 Glendinning, J. I., Davis, A. & Ramaswamy, S. Contribution of different taste cells and signaling pathways to the discrimination of "bitter" taste stimuli by an insect. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **22**, 7281-7287, doi:20026695 (2002).

- 21 Sanford, J. L., Shields, V. D. & Dickens, J. C. Gustatory receptor neuron responds to DEET and other insect repellents in the yellow-fever mosquito, *Aedes aegypti*. *Die Naturwissenschaften* **100**, 269-273, doi:10.1007/s00114-013-1021-x (2013).
- 22 Merivee, E., Must, A., Milius, M. & Luik, A. Electrophysiological identification of the sugar cell in antennal taste sensilla of the predatory ground beetle *Pterostichus aethiops*. *J Insect Physiol* **53**, 377-384, doi:10.1016/j.jinsphys.2006.12.012 (2007).
- 23 Popescu, A. *et al.* Function and central projections of gustatory receptor neurons on the antenna of the noctuid moth *Spodoptera littoralis*. *Journal of comparative physiology. A, Neuroethology, sensory, neural, and behavioral physiology* **199**, 403-416, doi:10.1007/s00359-013-0803-0 (2013).
- 24 Marion-Poll, F. & Van Der Pers, J. Un-filtered recordings from insect taste sensilla. *Entomologia Experimentalis et Applicata* **80**, 113-115, doi:10.1111/j.1570-7458.1996.tb00899.x (1996).
- 25 Wieczorek, H. & Wolff, G. The labellar sugar receptor of *Drosophila*. *J.Comp.Physiol.A.Neuroethol Sens.Neural Behav.Physiol.* **164**, 825-834 (1989).
- 26 Morita, H. Initiation of spike potentials in contact chemosensory hairs of insects. III. D.C. stimulation and generator potential of labellar chemoreceptor of calliphora. *Journal of cellular and comparative physiology* **54**, 189-204 (1959).
- 27 Lacaille, F. *et al.* An inhibitory sex pheromone tastes bitter for *Drosophila* males. *PLoS One* **2**, e661, doi:10.1371/journal.pone.0000661 (2007).
- 28 Benton, R. & Dahanukar, A. Electrophysiological recording from *Drosophila* taste sensilla. *Cold Spring Harbor protocols* **2011**, 839-850, doi:10.1101/pdb.prot5631 (2011).
- 29 Pellegrino, M., Nakagawa, T. & Vosshall, L. B. Single sensillum recordings in the insects *Drosophila melanogaster* and *Anopheles gambiae*. *Journal of visualized experiments : JoVE*, 1-5, doi:10.3791/1725 (2010).
- 30 Axon Instruments, I. *The Axon Guide for Electrophysiology & Biophysics Laboratory Techniques*. (1993).
- 31 Fujishiro, N., Kijima, H. & Morita, H. Impulse frequency and action potential amplitude in labellar chemosensory neurones of *Drosophila melanogaster*. *Journal of insect physiology* **30**, 317-325, doi:10.1016/0022-1910(84)90133-1 (1984).
- 32 Marion-Poll, F. & Tobin, T. R. Software filter for detecting spikes superimposed on a fluctuating baseline. *Journal of neuroscience methods* **37**, 1-6 (1991).
- 33 Meunier, N., Marion-Poll, F., Lansky, P. & Rospars, J. P. Estimation of the individual firing frequencies of two neurons recorded with a single electrode. *Chem Senses* **28**, 671-679 (2003).
- 34 Marion-Poll, F. *Marion-Poll Lab Website*, <<http://taste.versailles.inra.fr/fred>> (2013).
- 35 Meunier, N., Marion-Poll, F., Rospars, J. P. & Tanimura, T. Peripheral coding of bitter taste in *Drosophila*. *Journal of neurobiology* **56**, 139-152, doi:10.1002/neu.10235 (2003).

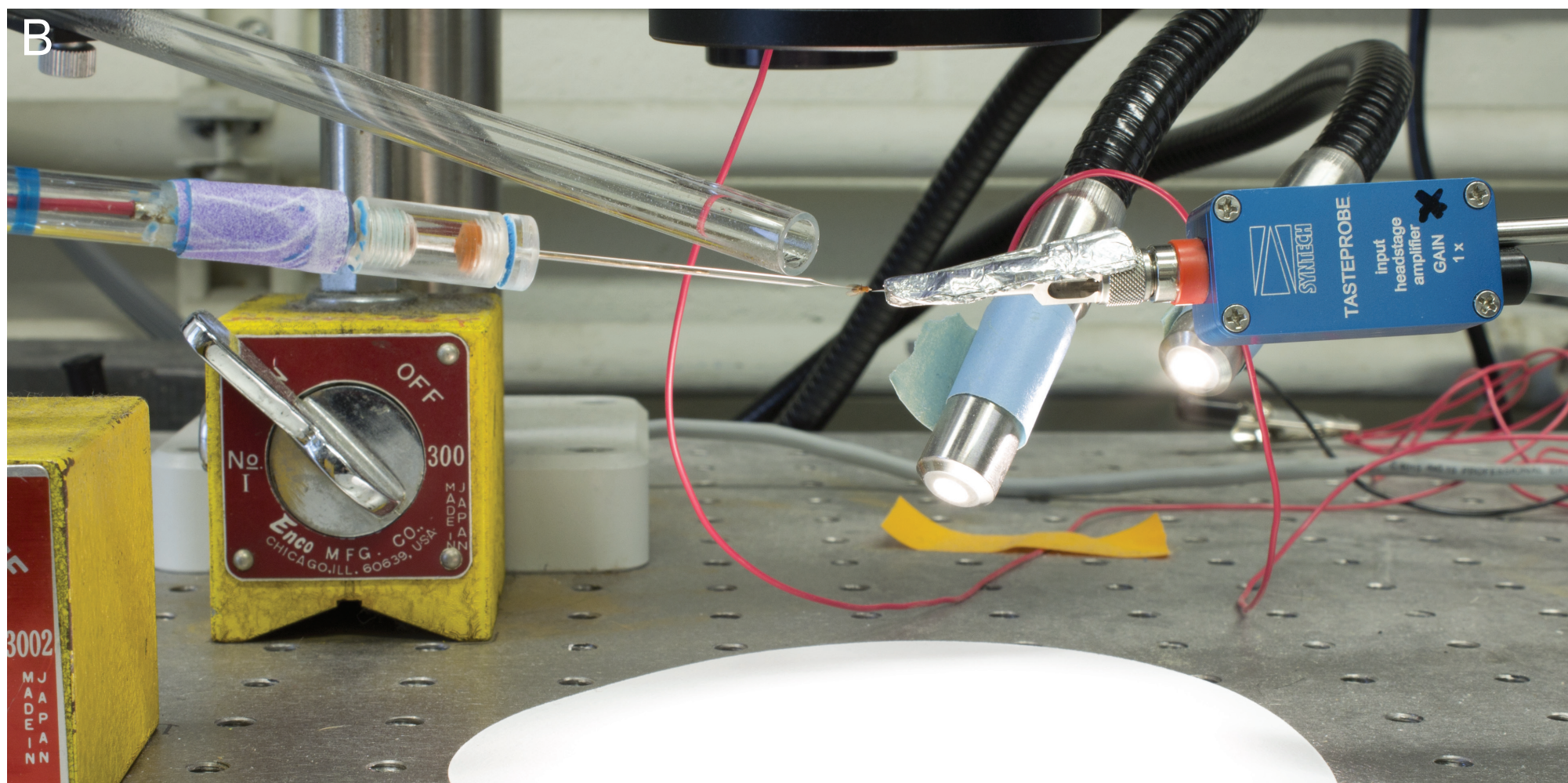
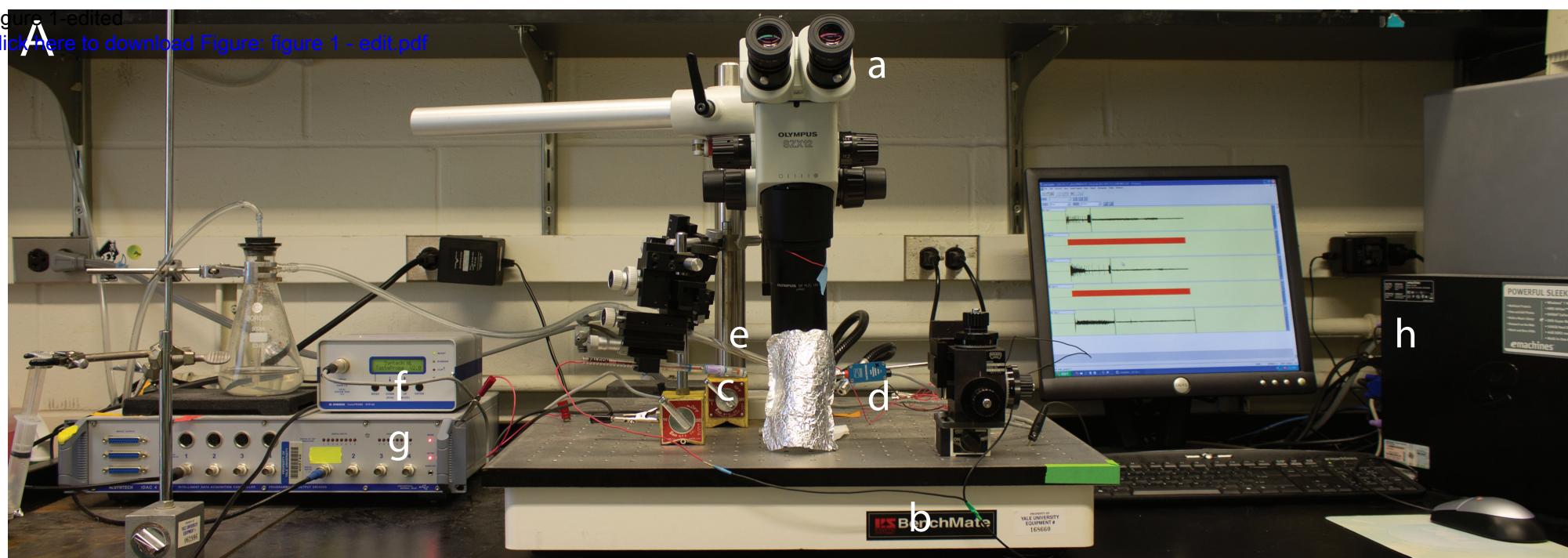


Figure 2

[Click here to download Figure: figure 2.pdf](#)

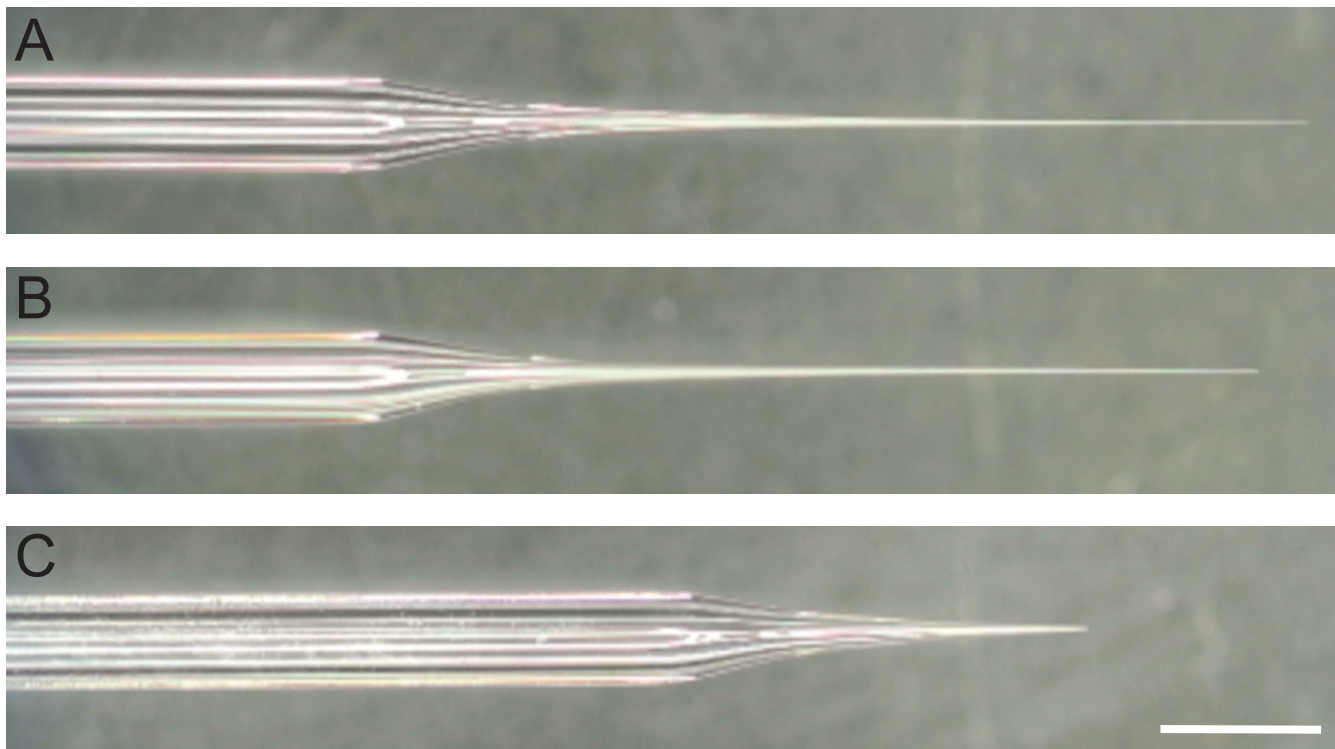


Figure 3
[Click here to download Figure: figure 3.pdf](#)

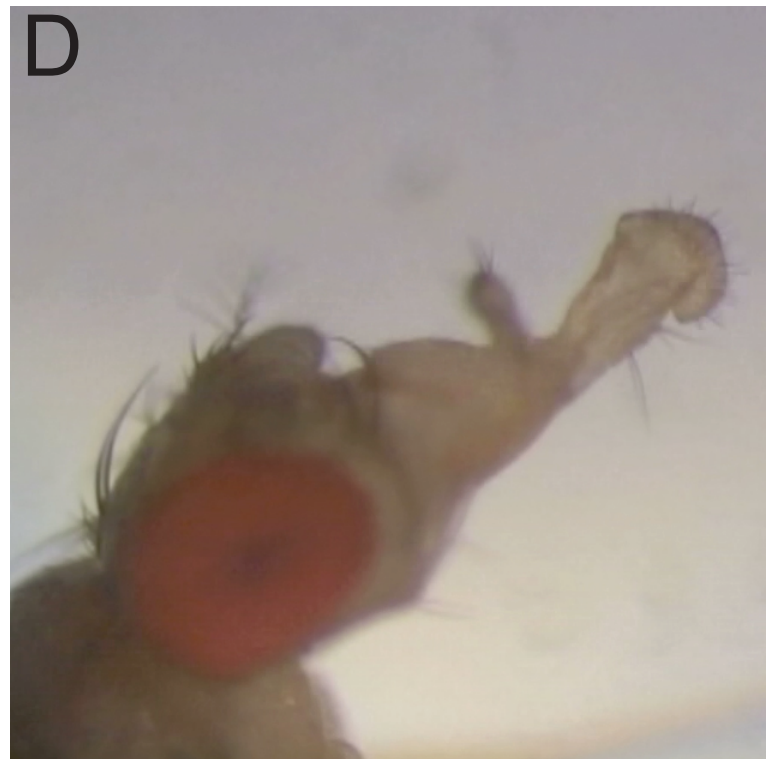
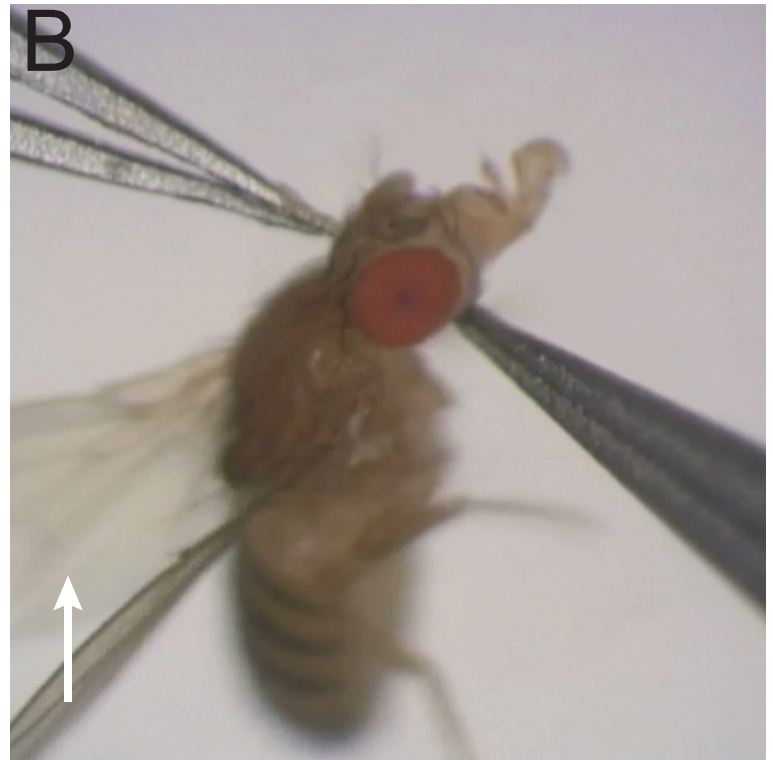
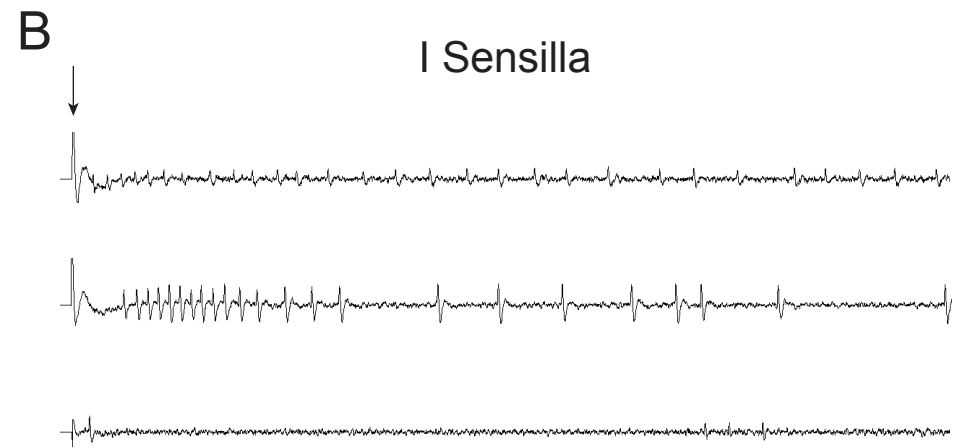
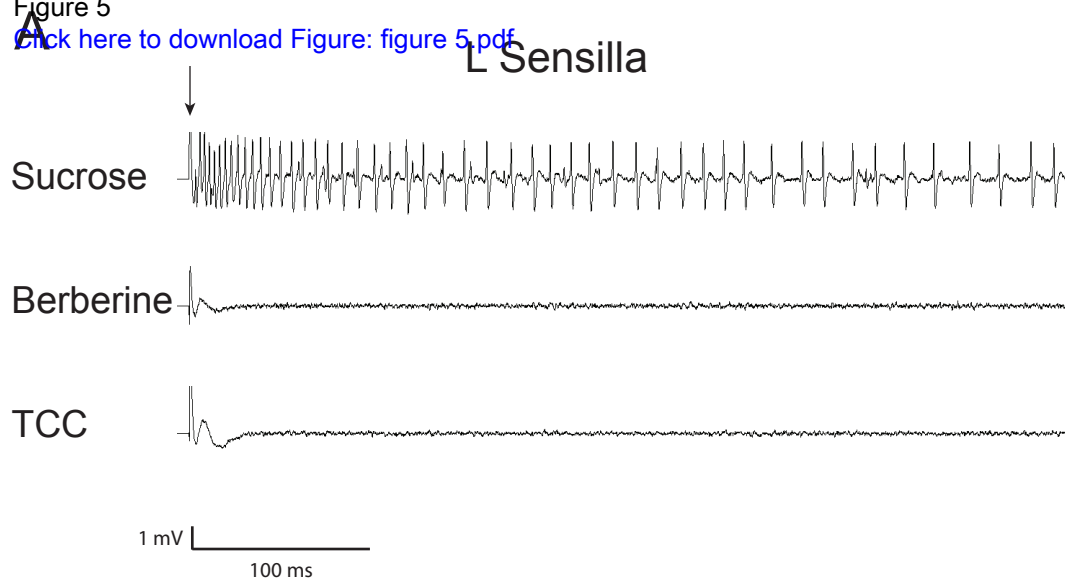


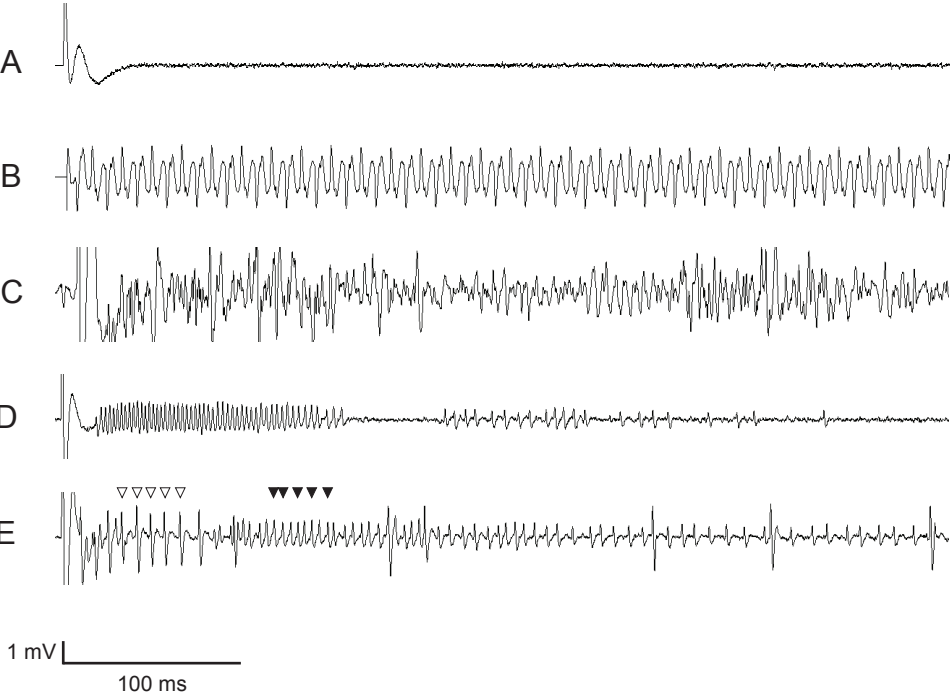
Figure 4
[Click here to download Figure: figure 4.pdf](#)



Figure 5

[Click here to download Figure: figure 5.pdf](#)





Name of Reagent/	Company	Catalog Number	Comments/Description
Stereo Zoom Microscope	Olympus	SZX12 DFPLFL1.6x PF eyepieces: WHN10x-H/22	capable of ~150x magnification with
Anti-vibration Table	Kinetic Systems	BenchMate2210	
Micromanipulators	Narishige	NMN-21	
Magnetic stands	ENCO	Model #625-0930	
Reference Electrode Holder	Harvard Apparatus	ESP/W-F10N	Can be mounted on 5ml serological
Silver Wire	World Precision Instruments	AGW1510	0.3-0.5mm diameter
Retort Stand			generic
Outlet Plastic Tube			generic, 1cm diameter
Flexible Plastic Tubing	Nalgene	8000-0060	VI grade 1/4 in internal diameter
500 ml Conical Flask			generic, with side arm
Aquarium Pump	Aquatic Gardens	Airpump 2000	
Fiber Optic Light Source	Dolan-Jenner Industries	Fiber-Lite 2100	
White Card/Paper	Whatman	1001-110	
Digital Acquisition System	Syntech	IDAC-4	Alternative: National Instruments N
Headstage	Syntech	DTP-1	Tasteprobe
Tasteprobe Amplifier	Syntech	DTP-1	Tasteprobe
Alligator Clips	Grainger	1XWN7	Any brand is fine
Insulated Electrical Wire			Generic
Gold Connector Pins	World Precision Instruments		5482
Personal Computer	Dell	Vostro	Check for compatibility with digital r
Acquisition Software	Syntech	Autospike	Autospike works with IDAC-4; altern

Aluminum Foil and/or Faraday Cage			Electro-magnetic noise shielding
Borosilicate Glass Capillaries	World Precision Instruments	1B100F-4	
		Model P-87 Flaming/Brown	
Pipette Puller	Sutter Instrument Company	Micropipette Puller	
Beadle and Ephrussi Ringer Solution			See recipe in protocol section
Tricholine citrate, 65%	Sigma	T0252-100G	
Stereo Microscope	Olympus	VMZ 1x-4x	Capable of 10x-40x magnification
Ice Bucket			Generic
p200 Pipette Tips			Generic
Spinal Needle	Terumo	SN*2590	
1ml Syringe	Beckton-Dickenson		301025
Fly Aspirator			Assembled from P1000 pipette tips,
Modeling Clay			Generic
Forceps	Fine Science Tools By Dumont	11252-00	#5SF (super-fine tips)
10ml Syringe	Beckton-Dickinson		301029
Plastic Tubing	Tygon	R-3603	

1 long working distance table mount stand

pipette for extended range

NI-6251

acquisition system and software
Alternatively, use Labview with NI-6251

. flexible plastic tubing, and mesh



JOVE
The Journal of Visualized Experiments
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article:

Electrophysiological Recording from *Drosophila labellae* for Sensation

Author(s):

Rebecca D. Wernthall, Andrew Kucy, John C. Nielsen

Item 1 (check one box): The Author elects to have the Materials be made available (as described at

<http://www.jove.com/publish>) via: ☒ Standard Access ☐ Open Access

Item 2 (check one box):

☒ The Author is NOT a United States government employee.

☐ The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

☐ The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. **Defined Terms.** As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: <http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode>; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JOVE**" means MyJOVE Corporation, a Massachusetts corporation and the publisher of *The Journal of Visualized Experiments*; "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JOVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JOVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. **Background.** The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JOVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. **Grant of Rights in Article.** In consideration of JOVE agreeing to publish the Article, the Author hereby grants to JOVE, subject to Sections 4 and 7 below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in Item 1 above, JOVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

4. **Retention of Rights in Article.** Notwithstanding the exclusive license granted to JOVE in Section 3 above, the

Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. Grant of Rights in Video – Standard Access. This Section 5 applies if the "Standard Access" box has been checked in Item 1 above or if no box has been checked in Item 1 above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to Section 7 below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. Grant of Rights in Video – Open Access. This Section 6 applies only if the "Open Access" box has been checked in Item 1 above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to Section 7 below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this Section 6 is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. Government Employees. If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in Item 2 above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict

shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. Likeness, Privacy, Personality. The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

9. Author Warranties. The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, societal, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

10. JoVE Discretion. If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including,

without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

11. Indemnification. The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in JoVE or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or


damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. Fees. To cover the cost incurred for publication, JoVE must receive payment before production and publication of the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US\$1,200 fee to cover pre-production expenses incurred by JoVE. Payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. Transfer, Governing Law. This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to be one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

AUTHOR:

Name: Rebecca Delventhal
Department: Molecular, Cellular, Developmental Biology (MCDB)
Institution: Yale University
Article Title: Electrophysiological Recording from *Drosophila* labellar taste sensilla
Signature:  Date: 6/28/13

Please submit a signed and dated copy of this license by one of the following three methods:

- 1) Upload a scanned copy as a PDF to the JoVE submission site upon manuscript submission (preferred);
- 2) Fax the document to +1.866.381.2236; or
- 3) Mail the document to JoVE / Attn: JoVE Editorial / 17 Setters St / Cambridge, MA 02139

For questions, please email editorial@jove.com or call +1.617.945.9051.

MS # (internal use):

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article:

Electrophysiological Recording from *Drosophila* labellar taste sensilla

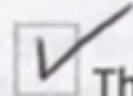
Author(s):

Rebecca Delventhal, Aidan Kiely, John Carlson

Item 1 (check one box): The Author elects to have the Materials be made available (as described at

<http://www.jove.com/publish>) via: ☒ Standard Access ☐ Open Access

Item 2 (check one box):



The Author is NOT a United States government employee.



The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.



The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. **Defined Terms.** As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: <http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode>; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJoVE Corporation, a Massachusetts corporation and the publisher of *The Journal of Visualized Experiments*; "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. **Background.** The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. **Grant of Rights in Article.** In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4 and 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

4. **Retention of Rights in Article.** Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the

ARTICLE AND VIDEO LICENSE AGREEMENT

Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. Grant of Rights in Video – Standard Access. This Section 5 applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. Grant of Rights in Video – Open Access. This Section 6 applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this Section 6 is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. Government Employees. If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict

shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. Likeness, Privacy, Personality. The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

9. Author Warranties. The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

10. JoVE Discretion. If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including,

ARTICLE AND VIDEO LICENSE AGREEMENT

without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

11. Indemnification. The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in JoVE or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or

damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. Fees. To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US\$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. Transfer, Governing Law. This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to be one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

AUTHOR:

Name:

Rebecca Delventhal

Department:

Molecular, Cellular, Developmental Biology (MCDR)

Institution:

Yale University

Article Title:

Electrophysiological Recording from *Drosophila* labellar taste sensilla

Signature:

Rebecca Delventhal

Date:

6/28/13

Please submit a signed and dated copy of this license by one of the following three methods:

- 1) Upload a scanned copy as a PDF to the JoVE submission site upon manuscript submission (preferred);
- 2) Fax the document to +1.866.381.2236; or
- 3) Mail the document to JoVE / Attn: JoVE Editorial / 17 Sellers St / Cambridge, MA 02139

For questions, please email editorial@jove.com or call +1.617.945.9051.

MS # (internal use):

Response to Peer-Reviewer Comments

JoVE51355R1 'Electrophysiological recording from Drosophila labellar taste sensilla'

Summary of updated documents submitted:

-updated manuscript—all changes have been recorded with “Track Changes” function, including some minor revisions to text, not in response to the reviewers’ comments.

-updated Figure 1

-updated Figure 6

Detailed list of changes in response to Reviewers’ Comments:

Reviewer #1:

Manuscript Summary:

The authors have provided a excellent description of how to perform tip recordings on sensilla of the proboscis of adult Drosophila.

Major Concerns:

My only general concern is that the paper's value will be limited to people interested in the adult life stage of this one genus of fly. There is no discussion of how their technique could need to be modified for larval Drosophila, other Dipterans or even other orders of insect. Further, the methodological description is limited to recording from sensilla on the proboscis. There is no discussion of how this technique could be modified to record from sensilla on other body parts (e.g, legs and wings).

Editorial Comment: JoVE agrees that a brief discussion concerning the above mentioned modifications to your protocol would benefit a wider audience. Please add any modifications to the Discussion section of your manuscript.

-We addressed how the protocol can be modified for different tissues or species by adding a brief discussion of these points to the last paragraph of the discussion (noted with Track Changes).

line 69-70: Some caution is needed here. The authors state that the flies experience at least three taste modalities, which were derived from human psychophysical experiments (i.e., bitter, sweet, salty). To my knowledge, there is no evidence that insects actually perceive different taste modalities (or qualities). At this point, all we know is that insects are attracted, deterred or unresponsive to taste stimuli. The most parsimonious explanation for this observation is that insects simply categorize taste inputs along a hedonic dimension (i.e. pleasant to unpleasant). We don't know, however, whether they subdivide pleasant (or unpleasant) into different taste qualities; e.g., whether two aversive concentrations of NaCl and caffeine actually taste differently. Accordingly, even though a high concentration of NaCl would stimulate different populations of receptors and GRNs than a high concentration of caffeine, both stimuli could nevertheless generate the same aversive taste sensation centrally.

-We agree with the reviewer and have clarified the language to “different categories of taste stimuli: bitter, sugar, salt and osmolarity” in order to illustrate that we’re discussing different classes of taste compounds, not the perception of the fly.

line 290: The authors show clear and crisp responses to sucrose and berberine, each of which contain spikes from a single GRN. However, they do not show more typical multi-unit responses. Further, there is no mention of how to analyze these multi-unit responses.

Editorial Comment: While JoVE does not require that you add this additional information, please carefully consider this reviewer's comment and include these results if they will significantly improve the presentation and analysis of your method.

-We have performed very few multiunit recordings in our lab. Most of our recordings, and most recordings performed by others in the field, are from sensilla in which a single neuron fires, so we prefer to focus on such recordings.

line 365: In most insects, there is a mechanosensitive neuron in each taste sensillum. When the wall of the recording electrode strikes the taste sensillum and causes it to bend, this will generate electrical noise that contaminates taste responses. Is this a problem in *Drosophila*? If so, the authors should cite this as another technical issue.

-We have added a description of mechanosensory firing and how to avoid it in the third paragraph of the discussion section. To accommodate this description, we made minor changes to the introductory sentence to that paragraph, as well as the beginning of the following sentence (changing "Third," to "Fourth,").

-We have also edited Figure 6 (and the Figure 6 legend) to include two representative traces of mechanosensory firing (alone and with bitter GRN firing) as panels D and E.

Minor Concerns:

line 65: The pore at the tip of the sensillum is actually quite small. I recommend deleting the word "large".

-Deleted "large"

line 74-75: The authors state that the organization of the *Drosophila* taste system is similar to that of higher organisms, but neglect to state the similarities. They should do so.

-The language we initially used was unclear. We have clarified the reasons for using *Drosophila* as a model system.

line 200: What is a spinal needle? Please elaborate.

-We added "long, thin plastic needle of 0.5 mm diameter, such as a" as a clarifying description

line 286: "contain" should be "contains"

-Corrected

Additional Comments to Authors:

N/A

Reviewer #2:

Manuscript Summary:

While taste sensilla recordings in *Drosophila* were rather confidential 10 years ago, more and more laboratories are interested in developing this technique to support their results based on genetic manipulations. The detail of taste sensilla recordings will thus be of great interest for

many scientists of this field.

Concerning the manuscript, the aims of the technique are well presented and there is no major critic to the manuscript. Everything is clearly explained. Find below a list of minor recommendations to improve the manuscript. Concerning the discussion, the author may consider the possibility of adding drugs in the tip recording glass capillary. Indeed, tip recordings are based on covering the tip of sensilla with a recording electrode which can contain different pharmacological agent to study a specific transduction pathway.

-We added “Additionally, it is possible to deliver pharmacological agents to the sensilla via the recording electrode to investigate signal transduction” to the last paragraph of discussion

Major Concerns:

N/A

Minor Concerns:

Line 86 : tastePROBE

-Corrected

Line 89 : TTC may be useful to improve the signal/noise ratio but as the authors mentioned, it removes the activity from the W cell. A very interesting study from the same group suggests that the inhibition of one cell may affect the activity of the others, so it may be worth to discuss briefly this part. Numerous studies already mentioned elsewhere in the manuscript are using KCl at low concentration (1-3 mM) for tip recordings as it allows a good conductance of the solution without stimulation of the L1 cell or inhibiting the W cell.

-We have now included mention of KCl as an alternative electrolyte in protocol step 1.3.2.

-We would prefer not to comment on the possibility of inhibition of one cell by others, because our preliminary data have not yet provided evidence for such ephaptic coupling in gustatory sensilla.

Line 146: Filtering as high as 100 Hz will alter the shape spikes and make harder spike sorting. A bandpass set from 1Hz to 3000 Hz will preserve the original signal.

-The filter settings we recommend are those recommended by the company that produces the TastePROBE. Please refer to Figure 2 in <http://www.syntech.nl/documents/TastePROBE.pdf>

-We have added a note to Protocol step 1.1.10, clarifying the effect of the filter settings on spike shape.

Line 147: KHz not k Hz

-Corrected

Line 178: The protocol to dilute TTC is not necessary; any scientist should be able to prepare 30 mM of any molecule soluble in water.

-Deleted sentence

Line 183: Many tastants are used at concentration outside the 1-100 mM range. I am not sure these indications are necessary.

-Removed both references to concentration range of tastants

Line 212: The fly is not manually manipulated but with forceps.

-Removed “manually”

Line 271: Even with a humidified air flow, in some case, the tip of the electrode may slowly dry and thus the tastant becomes more and more concentrated. Some people contact the glass containing the tastant with smooth paper to remove by capillarity a small drop of the tastant. This way, the solution at the tip remains at the desired concentration.

-Added note to Protocol step 3.5.5, describing this method

Line 316: The concentration of sucrose and berberine used should be indicated.

-Concentrations have been added to Figure 5 legend

Line 348: "Check to see if either the clip up or clip down indicator light is on." I guess the authors are referring to an overload of the amplifier. It should be explained accordingly and not directly referred to the specific amplifier that they are using.

-Added qualifying language of “If using the tastePROBE amplifier,” for the description of troubleshooting overloaded amplifier.

-We would like to note however, that the protocol is written predicated on the use of the tastePROBE system. If a reader will be using some alternative electronics, they would have to be responsible for knowing what signals overload of the amplifier.

Line 367: Not everyone use 60Hz alternative current sources in the world, please correct accordingly

-Corrected mention of 60 Hz to 50/60 Hz in discussion paragraph, Figure 6 legend.

Line 368: To test the level of surrounding noise, keeping the recording electrode in the air may not be the best way. Indeed, first you may have way more noise than when connecting sensilla (and thus removing most of the noise thanks to the reference electrode which will be next to the recording electrode). Worst, according to the amplifier, you may not be able to record noise at all because the signal is out of amplifier range. Another method to test the noise consists of directly connecting the reference and recording electrode through a drop of ringer solution. If the signal is noisy then the surrounding of the setup has to be improved, if not, this is the fly preparation.

-We have added a description of this procedure for troubleshooting noise to the third paragraph of the discussion, in response to the reviewer’s very helpful comment.

Additional Comments to Authors:

N/A

Reviewer #3:

This m/s is pretty straight forward in describing a very useful method of recording single unit responses from gustatory neurons in fruit flies. With powerful genetic tools at disposal, and easily accessible sensilla/neurons, flies do offer a unique advantage and this m/s definitely will serve a large community of researchers interested in chemosensory research. It might have been useful to add a note on recording from other organs, such as legs, but this technique can be extended to other systems with slight modifications. I would suggest that authors can add a line towards the end, simply stating as such. Only major modification will be to place the reference electrode somewhere else (in thorax etc) and restrain the fly differently.

-We have now addressed how the protocol can be modified for different tissues or species by adding a brief discussion of these points to the last paragraph of the discussion (noted with Track Changes).

Overall, I highly recommend this m/s to be published and preferably have an open access, so that it helps a wider community, esp. in the developing world. A few minor comments

Line 80: External to what?

-Edited description to be more specific: “Because these sensilla protrude from the surface of the labellum”

L 126: Not critical, but do you know the approximate flow rate?

-No, we do not know the flow rate.

L 129-30: Why is it important to have the reflected light? White paper is not visible in the figures.

**-Added clarifying note to Protocol step 1.1.6.
- Edited Figure 1 to show more of the white paper disc**

L 177: What is W&W 1989.

-Removed erroneous non-EndNote format citation

L 200: Did you use borosilicate or quartz glass? Did it have filament? Were electrodes backfilled? Please provide glass details in the table.

**-Added “borosilicate” and “with filament” details to Protocol step 1.2.2
-Changed “Fill” to “Backfill” in Protocol step 2.3**

L 274: It has been shown that washing the sensillum tip with water-filled electrodes between stimuli helps. Do authors like to clarify, if such procedure were performed here.

-The authors were previously unaware of this procedure. We do not perform this procedure and as such, we do not mention it in the protocol.

References need some editing, eg. References 23 and 31 are not complete.

-We have replaced the incomplete EndNote reference files with complete ones.

Reviewer #4:

Manuscript Summary:

The authors describe how to record electrical activities from external taste receptors in *Drosophila*, using a procedure they run in their own laboratory. This is a nice paper. I have only minor recommendations listed below. In addition, it might be useful to add a comment concerning the way hairs are stimulated. In my laboratory, new users are often worried about the responses of the mechanoreceptor. Obtaining successful recordings without activating the mechanoreceptor is greatly improved if the recording electrode is aligned with the main axis of the sensillum.

(in response to Reviewer #1's similar suggestions)

-We have added a description of mechanosensory firing and how to avoid it in the third paragraph of the discussion section. To accommodate this description, we made minor changes to the introductory sentence to that paragraph, as well as the beginning of the following sentence (changing "Third," to "Fourth,").

-We have also edited Figure 6 (and the Figure 6 legend) to include two representative traces of mechanosensory firing (alone and with bitter GRN firing) as panels D and E.

Major Concerns:

N/A

Minor Concerns:

Page 3:

Line 71 "GRNs. 8,10, 14": "14" = inappropriate citation: Fujishiro et al do not mention bitter chemicals in their paper.

-Removed 14 citation

Line 83: "which uses a tungsten electrode inserted into the sensillum to record neuronal activity": The side-wall recording uses a "glass electrode". This approach was introduced independently by Morita and Dethier. People also used W electrodes inserted at the base of the sensillum as it was used in olfactory recordings but this technique is not "side-wall"

-Changed "tungsten" to "glass"

-Distinguished between tungsten/socket base method and side-wall method by adding description of tungsten method

-Included relevant references

Page 4

Line 91: a word is missing here (TTC does not suppress the neuron but the response of that neuron): "suppresses responses from the osmolarity-sensitive GRN". However, I am not sure that describing this cell (the W cell) as "osmolarity-sensitive" is right. For example, Wieczorek and others showed that some W cells have a genuine sensitivity to sugars.

-Clarified sentence as suggested

Page 7:

Line 216 "Always be careful to avoid touching the labellum with the forceps at all times during the preparation process.": why? Because of potential mechanical damages?

-Clarified note for Protocol Step 2.7 as suggested

Line 242: we use up to x570

-Added qualifying language to magnification power in protocol step 3.2

Page 11:

Line 396 "sidewall": NO, the recordings are made from the socket of the hair.

Line 398: you are mixing references of papers using 2 different techniques: 30 is with tungsten from the socket base (which is not too injurious) and 31 is with sidewall recording (which is potentially injurious as you need to crack the side of the wall of the hair).

-Distinguished between tungsten and side-wall recordings in discussion paragraph, altering placement of relevant references accordingly