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 sensillun. 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 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 so 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 contains 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**