Dear Dr. Bajaj,

Thank you and the reviewers for your helpful comments to strengthen the manuscript. We now submit a revised manuscript to address those comments and suggestions. We now include an additional figure to address concerns about descriptions of surgical landmarks, and provide a point-by-point response to the specific comments below. Original comments are in black, acknowledgement and rebuttal are in blue.

Lindsey Macpherson
Assistant Professor of Biology
The University of Texas at San Antonio
lindsey.macpherson@utsa.edu

Bryan Fowler, MS
Graduate Research Assistant
Macpherson Lab, UTSA
Bryan.Fowler@utsa.edu
925.451.6576

Editorial comments:

Changes to be made by the Author(s):

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

2. Please revise the following lines to avoid overlap with previously published work: 243-247
Section has been revised.

3. Please provide an email address for each author.
An email address has been added for both authors.

4. Please revise the text, especially in the protocol, to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).
Use of "we" and "you" has been eliminated from the protocol.

5. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.
For example: BD Intramedic polyethylene tubing; vetbond glue;
Done, brand specific names have been changed to generic names such as "veterinary glue," "polyethylene tubing (specifications)", etc.

6. Being a video based journal, JoVE authors must be very specific when it comes to the humane treatment of animals. Regarding animal treatment in the protocol, please add the following information to the text:
a) Please move information about using transgenic Snap25-GCaMP6s animal to the beginning of the protocol along with information about age, sex etc
Snap25 animals have been addressed as a potential transgenic line to use for this experiment at the beginning of the protocol. Suggested ages and sex have been included with the previously included suggested weight.

b) What happens to the animal after this procedure? Please specify the euthanasia method without highlighting it.
The fact that this is a terminal procedure has been highlighted at the beginning of the protocol. Preferred method of euthanasia has been briefly addressed at the last step of the protocol.

c) Please specify the use of vet ointment on eyes to prevent dryness while under anesthesia.
This step has been added to the protocol.

d) For survival strategies, discuss post-surgical treatment of animal, including recovery conditions and treatment for post-surgical pain.
N/A. See point 6B.

e) Discuss maintenance of sterile conditions during survival surgery.
N/A. See point 6B.

f) Please specify that the animal is not left unattended until it has regained sufficient consciousness to maintain sternal recumbency.
N/A. See point 6B.

g) Please specify that the animal that has undergone surgery is not returned to the company of other animals until fully recovered.
N/A. See point 6B.

7. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.
Tense has been shifted to the imperative.

8. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. Please add more specific details (e.g., button clicks for software actions, numerical values for settings, etc) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.
Major issues have been addressed with the addition of pictures for the particularly difficult portions of the protocol.

9. Please format the manuscript as: paragraph Indentation: 0 for both left and right and special: none, Line spacings: single. Please include a single line space between each step, substep and note in the protocol section. Please use Calibri 12 points and one-inch margins on all the side. Please include a one line space between each protocol step and then highlight up to 3 pages of protocol text for inclusion in the protocol section of the video.
The specifications have been adopted and the desired section has been highlighted.

10. Please include a scale bar for all images taken with a microscope to provide context to the magnification used. Define the scale in the appropriate Figure Legend.
Scale bar is addressed in both figures for every panel.

11. As we are a methods journal, please add the following points to the Discussion:
a) Any limitations of the technique
Discussion now directly addresses limitations due to the invasive nature of this procedure.

b) The significance with respect to existing methods
This is now addressed within the discussion.

12. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage–LastPage (YEAR).] For more than 6 authors, list only the first author then et al. Please include volume and issue numbers for all references, and do not abbreviate journal names.
Endnote with the JoVE style was used. Aberrant references have been corrected independently.

_____

Reviewers' comments:
Reviewer #1:
Manuscript Summary:

Spatiotemporal activity of geniculate ganglion neurons offers a valuable information on how taste information is encoded and processed. Yet, microscopic access to the geniculate ganglion has been difficult due to the complex anatomy. This manuscript describes the detailed protocol for in vivo calcium imaging on geniculate ganglion neurons from surgical preparations to imaging. This reviewer believes this manuscript will provide a helpful guide for readers who wants to adopt this technique. I have only a few minor comments.

Minor Concerns:
1. I think the prior protocol worked on the same topic by An Wu & Gennady Dvoryanchikov (Protocol Exchange, 2015) needs to be cited. It would be also helpful if the authors discuss improvements over the prior protocol.
Done.

2. Although the video will be provided, I think the schematics or photographs on the anatomy can be highly helpful. For example, opening up of tympanic bulla and removal of temporal bone is not easy to follow without any prior knowledge on the anatomy.
Figures have been added for more complex parts of the protocol. Specifically: an initial image to orient readers to the surgical areas, where to blunt dissect musculature to expose the bulla, where to break into the bulla, a schematic of what the interior of the tympanic bulla should look like with critical features labelled (tensor tympani and cochlea), an image of post cochlea breaking showing the point used to pull up the temporal bone, and an image of the completed procedure showing an exposed geniculate.

Reviewer #2:
Fowler and Macpherson elegantly describe their techniques for in vivo imaging of mouse geniculate ganglion using GECIs. This manuscript provides a brief, yet informative introduction on the geniculate ganglion and the taste system. The authors describe other current methods for measuring peripheral nerve activity including whole nerve recording and single fiber recording and clearly explain the advantages of the herein described calcium imaging. Previous studies using this technique (utilizing 2-photon, confocal, or epifluorescence microscopy) are cited and clearly demonstrate how useful this technique is to the field of gustatory sciences. Lastly, the expression of GECIs is covered as the authors suggest the possibility of using Cre-mediated expression or viral delivery.
The protocol itself is logically prepared and easy-to-follow from equipment prep to data analysis. I have done similar mouse preps and had no trouble following the steps, even without pictures or video. I only have a few clarifications, listed below.
Overall, this is an excellent description of a delicate surgical prep. This technique is an invaluable addition to the quiver of methods to study gustatory peripheral nerve responses and has the potential to answer many yet outstanding questions in the field of gustatory science.

Questions/clarifications
1) Would the authors comment on potential feedback from the CNS? Typically in gustatory whole nerve recording, the nerve is transected distal to the ganglion body prior to applying stimulus.
This is a potentially interesting area to investigate. This method for calcium imaging keeps the entire ganglion and it's peripheral and central processes intact. Therefore, there is the potential for modulation by the CNS. We are not aware of any studies that have compared CT nerve responses to taste stimuli in transected vs intact preparations.

2) What does the future hold for this technology? The authors touch on this in their discussion of using photoconvertible fluorophores. But perhaps the authors could expand on this a bit more such as, could this be adapted for awake, behaving mice?
This is now addressed more extensively within the discussion, and the fact that this is best suited as a terminal procedure has been highlighted.

3) Line 72: change wording to imply that green fluorescence increases with increasing Ca
Changed to reflect that increased Ca concentration causes increased green fluorescence.

4) Line 138: what size/diameter PE tubing?
Tubing entry has been amended to declare the I.D. and O.D.

5) Line 192: would you provide more details on the objectives used? Specifically the minimum required working distance (or even an estimate, knowing there will be variation in preps and mice)
This information is now provided within the protocol.

Reviewer #3:
Manuscript Summary:
In the manuscript, titled "In vivo calcium imaging of mouse geniculate ganglion neuron responses to taste stimuli" Bryan Fowler et. al. have demonstrated imaging of individual geniculate ganglion in response to taste stimuli using GCaMP. The surgical procedure to expose the geniculate ganglion followed by fluorescence image acquisition by using a microscope and digital camera to record the taste-evoked response has been presented. The technique is presented very elaborately and the surgical procedure is easily understandable.

Major Concerns:
While the description for the experiment is in detail, it would be better to have some clear pictures of the surgical setup and a photo (or a cartoon) which shows the anatomy of the mouse geniculate ganglion which can help the reader understand overall procedures in addition to the video.

Figures have been added for more complex parts of the protocol. Specifically: an initial image to orient readers to the surgical areas, where to blunt dissect musculature to expose the bulla, where to break into the bulla, a schematic of what the interior of the tympanic bulla should look like with critical features labelled (tensor tympani and cochlea), an image of post cochlea breaking showing the point used to pull up the temporal bone, and an image of the completed procedure showing an exposed geniculate.

Minor Concerns:
1. Regarding figure 1, there is no explanation for the responses of umami and sour which responded differently than the others. Also, the figure contains many neurons and it would be better to indicate which cells responded to the specific five tastes.
The figure (now figure 2) has been updated to address the lack of significant umami response and the fact that sour and bitter co-responders are more common than other co-responders. The figure contains a picture of a snapshot pre stimulus and of the ganglion during a single tastant trial, AceK.

2. Line 230: "although bitter tastants such as quinine and cycloheximide can producemore prolonged responses" reviewer demands the representation or reference of this fact.
Claim has been removed as it references currently unpublished work.