**Responses to Editorial and Reviewers’ Comments**

**Response to the 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. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

Response: The final version of the manuscript has been edited by a medical editor at the authors’ Institution.

2. Please abbreviate all journal titles.

Response: Done.

3. Please define all abbreviations before use.

Reponses: Done.

4. Please use focused images of uniform size/resolution (at least 300 dpi).

Response: Done.

5. Please print and sign the attached Author License Agreement (ALA). Please then scan and upload the signed ALA with the manuscript files to your Editorial Manager account.

Response: Done.

6. 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.

Response: Done.

7. 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.

Response: Done.

8. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step.

Response: Simplified.

9. 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.

Response: Added.

10. What is the age/gender of the mouse?

Response: Added in 1.1.

11. Please specify all instruments used throughout.

Response: We have added Table 1 summarizing the instruments.

12. 2.1: What instrument is used for the incision?

Response: Scissors. We have added in the text.

13. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. The highlighted steps should form a cohesive narrative with a logical flow from one highlighted step to the next. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

Response: Protocol 1, 2, and 3.

14. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.  
  
Response: We have modified the steps to follow a logical flow.   
  
  
**Response to Reviewer #1:**

*Manuscript Summary:*  
This very informative manuscript provides a very detailed description of an experimental procedure that can be used to chronically assess mouse brain neurons activity through dual open skull windows and 2-photon microscopy.  
  
*Major Concerns:*  
N/A  
  
*Minor Concerns:*  
The following are comments for X Lin, T Zhao, W Xiong, S Wen, X Jin, and X-M Xu about their manuscript titled "Imaging neural activity in the primary somatosensory cortex using Thy1-GCaMP6 transgenic mice".  
  
PROTOCOL  
1. Preparing the animal for imaging  
1.1) Sentences 1 and 2 (about the ethanol cleaning and the heating pad) appear to be also stated in 1.3 and 1.4 where they are more applicable.

Response: Dropped sentence 1 from 1.1. Moved sentence 2 to the beginning of 1.3.

1.2) Add 'and has no corneal reflex' to sentence 2. Delete 'original' from …the original anesthetic…' in sentence 3. Delete '…and every 8-12 h postinjury for 2 d…' from sentence 4 as this is stated in 2.13 where it is more applicable.

Response: Thanks! Very helpful.

1.4) Is povidone-iodine also used?

Response: Yes and added.  
  
2. Chronic cranial window preparation

2.1) Refer to Figure 3 as well in sentence 1.

Response: Since Figure 2 is a schematic drawing describing window creation, we prefer not to refer to Figure 3 in sentence 1.

2.5) What size burr (drill bit) is recommended? (Response: Information provided) Is not ACSF applied to the skull not '…the S1 area…'as stated in sentence 3? (Response: Thanks! The sentence has been modified.) What temperature is the ACSF at? (Response: Room temperature, clarified) It is suggested to break up sentence 4 by ending it at '…overheating.'. (Response: Done, thanks!) The next sentence could be started at 'Replace…'. Should '…and…' in sentence 6 be '…to…'? (Response: Changed) Why is Fig. 2C being referred to after the last sentence? (Response: removed)

2.8) Is the right cortex kept moist while the left cortex is exposed?

Response: Yes, a change has been made.

2.9) Is ACSF left between the coverglass and the dura or is this space dried completely? (Response: Yes, added to the sentence.) A picture from the side showing the 2 coverglasses glued and cured together may be helpful. (Response: Difficult to show because the glue is transparent.)

2.10) In sentence 4, should it be (Fig. 2G-H) instead of (Fig. 2G-F)?

Response: Thanks, corrected.

2.11) Is this more applicable in 3.1? How much isoflurane would you recommend? Is this mixed with oxygen? If so, at what flow rate (L/min)?

Response: Isoflurane anesthesia has been moved to 3.1 as suggested.

Figure 2. What is the ddH2O used for?

Response: To increase the resolution of the microscopy with a water-immersion objectives for imaging.   
  
3. Two-photon imaging

3.2) What manufacturer of a 2P microscope and manipulator would you recommend?

Response: Company information added to the manuscript.

3.8) Delete '…side of the…' in sentence 4?

Response: Deleted.

Figure 4 Legend A2. Would '…structures…' be more appropriate than '…cells…'?

Response: Cells are more appropriate in this manuscript.  
  
4. Data processing

4.4) and Figure 5 Legend D. Do you want these equations to be the same?

Response: The equations are the same now. Thanks!  
  
REPRESENTATIVE RESULTS  
Since monitoring calcium dynamics is used as a premise for developing this experimental procedure, should not paragraph 2 be presented before paragraph 1?

Response: We prefer to keep the current order of the paragraphs.

Paragraph 2, Sentence 1: '…single cell…' or '…single neuron…'?

Response: Changed.

Paragraph 2: Is Figure 6 being discussed here?

Response: Fig. 6 has been referenced in the text.

DISCUSSION  
Have any differences in results been observed between 2 or 1 brain hemisphere being exposed and having a cranial window?

Response: We have not made a direct comparison in this study. The comparison will be performed in future hypothesis-driven studies.

Paragraph 2: What about studies of sensory dysfunctions and/or pain? Can various peripheral stimulation strategies (for example, optogenetic, electrical, chemical) be used?

Response: Good point; we have added this to the 2nd paragraph of the Discussion.

Paragraph 3, Sentence 4: Should Figure 3 be referred to here? Would this paragraph be more applicable in the PROTOCOL section?

Response: Yes, Figure 3 has been referenced in the text.

*Additional Comments to Authors:*  
N/A  
  
  
**Reviewer #2:**  
*Manuscript Summary:*  
N/A  
  
*Major Concerns:*  
The authors state in several sections (Long Abstract, Introduction and Discussion) that dynamics of neural activity can be viewed in symmetric brain regions, which suggests that 2 regions of the brain can be observed at the same time. It is not clear what the authors mean by this description and maybe it can be re-stated.

Response: In the Long Abstract, we stated “This method enables recordings and quantifications of neural activity in bilateral mouse brain regions one at a time in the same experiment for a prolonged period *in vivo*.”  
  
*Minor Concerns:*  
Line 87 indicates a 40 fold range of dosing for Buprenorphine. Can the authors be more specific for a preferred dose.

Response: In 1.3) we have stated: “Inject buprenorphine subcutaneously (s.c.; 0.05-0.10 mg/kg).”

Line 152 regarding chronic experiments it is not indicated if the skin incision is closed for the 7-10 days between placement of the viewing ports and 2 photon observations.

Response: No suture is needed to close the wound since the skin is replaced by the optical window. The wound margins are covered by dental cement (new 2.15).

Line 292 regarding Figure 1 and Figure 2 - There is a vertical line labeled as 5 um across the cortical layers. This is not correct as indicated in the figures.

Response: Thanks! We have corrected the mislabeling.

Line 326 regarding Figure 6 - What do the arrows in Panels B and C indicate

Response: We have indicated arrows in Figure 6. Thanks again.   
  
*Additional Comments to Authors:*  
N/A