March 8th , 2019
Alisha Dsouza
Senior Review Editor
JoVE

Dear Dr. Dsouza,

Enclosed is a revised version of our manuscript (JoVE59072R1) “Microelectrode impalement method to record membrane potential from cannulated middle cerebral artery.” We thank the reviewers for the valuable critiques. We have addressed all the major and minor concerns raised by the reviewers. Please find the responses for the respective queries below.

Editorial comments:
Changes to be made by the author(s) regarding the manuscript:
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. **Response:** Reviewed and ensured there are no spelling or grammar issues.
2. Please provide an email address for each author. **Response: Provided on title page, line 11.**
3. Please expand your Introduction to include the following: advantages of the method over alternative techniques with applicable references to previous studies and Information that can help readers to determine if the method is appropriate for their application. **Response: Expanded introduction on page 2 from lines 129 to 138.**
4. Please include an ethics statement before your numbered protocol steps, indicating that the protocol follows the animal care guidelines of your institution. **Response: Included on page 3 from lines 149 to 153.**
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. You may use the generic term followed by “(see table of materials)” to draw the readers’ attention to specific commercial names. Examples of commercial sounding language in your manuscript are: Warner Instruments LLC, Sutter Instrument, MicroFil (WPI), Clampex 10, molecular devices, Living Systems Instrumentation, Milli-Q, Nikon Instruments Inc., Harvard Apparatus, etc. **Response: All commercial language is deleted.**
6. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets, dashes, or indentations. **Response: Adjusted the numbering.**
7. Please revise the protocol to contain only action items that direct the reader to do something (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.” Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible. Please move the discussion about the protocol to the Discussion. **Response: Revised the protocol accordingly.**
8. A schematic of the equipment and the setup as Figure 1 would greatly aid in the protocol.

Response: We revised the Figure 1 accordingly. We have included a schematic representation of the equipment and also explained in the figure legend and in results section.

9. Lines 191-193: Please specify what animals are used here. Are animals anesthetized before the procedure? Also, please describe how the surgical procedure is done and specify all surgical tools used. **Response: Specified the animal, anesthesia and surgical procedure on page 4 from lines 211 to 216. All surgical tools are provided in the table of materials.**

10. Please include single-line spaces between all paragraphs, headings, steps, etc. **Response: Included.**

11. After you have made all the recommended changes to your protocol (listed above), 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. **Response: Highlighted.**

12. 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. Notes cannot usually be filmed and should be excluded from the highlighting. Please do not highlight any steps describing anesthetization and euthanasia. **Response: Highlighted only imperative sentences.**

13. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted. **Response: Highlighted the details also.**

14. Figure 1: Please change "Agcl" to "AgCl". **Response: Changed.**

15. Figure 2: Please include an x-axis and explain what the dotted line represents. **Response: included and explained on page 7 from line 311 to 313.**

16. Figure 3: Please include a space between numbers and their corresponding units (100 nM). Please change the time unit "sec" to "s". Please define the error bars and the asterisk symbol in the figure legend. **Response: This figure is deleted in the revised version of the article.**

17. Table 1: Please include a space between numbers and their corresponding units (200 mV, 1 mV/MΩ). Please remove the period after "mV/MΩ" in the last row. **Response: Included.**

18. Table 2: Please apply subscript formatting to the number "2" in CaCl2. **Response: Applied.**

19. Please revise the Table of Materials to include the name, company, and catalog number of all relevant supplies, reagents, equipment and software in separate columns in an xls/xlsx file. Please sort the items in alphabetical order according to the name of material/equipment. **Response: Revised the Table of Materials accordingly.**

20. References: Please do not abbreviate journal titles. **Response: Complete Journal title is provided on page 9-10.**

Reviewer #1:

Manuscript Summary:

This is a well-thought out protocol paper investigating membrane potentials in whole middle cerebral arteries. This is very beneficial for the vascular community taking this one step further from cell culture to essentially an organ culture patch. Utilizing electrophysiological derived techniques, the authors are able to record membrane potentials in whole arteries. Only minor concerns exist.

Major Concerns: NA

Minor Concerns:
Line 108/109, Can this technique be done in other isolated artery preparations? **Response: Yes, previous studies have shown and used this method in isolated mesenteric arterial strips, lymphatic vessels (Harder and Sperelakis 1979 and Von der Weid et al., 2014).**
Line 154, catalog number of MicroFil **Response: Catalog number is provided in the table of materials in table 2.**
Line 251 and others in the representative results, please correlate to the fig. **Response: Revised results to correlate with the figures on page 6-7 from line 297 to 306.**
Line 254 did you show how to isolate MCA. That would be helpful utilizing an illustration. **Response: An illustration showing the dissection of MCA is incorporated into the figure 1 and shown as figure 1A. Description is provided in the legend on page 6 from line 298 to 300 as well as in the results on page 6 from line 263 to 269.**

Reviewer #2:
Manuscript Summary:
This manuscript should provide a useful adjunct for the JOVE video. However, the authors should address the following requests for clarification.

Major Concerns:

1. Line 137 - The electrometer manufacturer should be mentioned here. **Response: Mentioned in table of materials table 2.**

2. Line 150 - Insert "(when filled with 3M KCl)" after "resistances". **Response: Inserted on page 3 from line 170 to 171.**
3. Line 195 - A comment regarding setting of the axial length of the isolated artery would be useful as this will likely contribute to impalement success rate. **Response: Commented on page 5 from line 224 to 225.**
4. Line 206 - change "soaked" to "immersed". **Response: changed on page 5 from line 230.**
5. Line 212 - The brand/type of manipulator should be mentioned here. **Response: Mentioned in table of materials table 2**
6. Line 227 - Insert "depending on the level of intravascular pressure or other excitatory or inhibitory stimuli" after "-75mV". **Response: Inserted on page 5 from line 251 to 253.**
7. Line 227 - Delete "resting potential" and insert "transmembrane potential difference" . **Response: Inserted on page 5 from line 251 to 253**
8. Line 250 - Insert "the" after "to". **Response: Inserted on page 6 line 275.**
9. Lines 283-289, lines 339-350, and Figure 3 - The topic of this methods article is measurement of vascular smooth muscle membrane potential in intact arteries. The data in figure 3 are patch clamp data using isolated cells. These results are out of context, and the relevance and importance of the discussion (lines 339-350) are vague and speculative. These data and this discussion should be omitted. **Response: Deleted this figure. Both results and discussion are**

revised accordingly.
10. Lines 362 - 364 - This sentence is somewhat vague. Suggest changing to "Thus, this method can be used reliably to understand normal and altered Vm associated with disease, and may be useful in the development and application of pharmacological agents designed to modulate Vm, vascular tone and blood flow. **Response: Changed on page 8 from line 393 to 395.**

Reviewer #3:
Manuscript Summary:
Joey T et al. used microelectrode impalement method to record membrane potential from cannulated middle cerebral artery and it is really important in electrophysiology study. The method is useful for researchers to start the experiment. However, some problems should be addressed and additional data should be shown to clarify this completely method.

Major Concerns:
1: For equipment, I strongly suggest author provide a picture to show the equipment and point out which one is what? **Response: Modified Figure1 accordingly. Equipment is shown in the figure 1.**
2: It is better to provide parameter of P-97 puller for pull 80-120 MΩ resistances microelectrode. **Response: Parameters are provided on page 3 line 174 to 175.**
3: In representative results, authors should show the resting membrane potential when the microelectrode come into the vascular smooth muscle cell, but should show some positive response like high K+ solution-induced depolarization and K+ channel activation-induced hyperpolarization. **Response: 20mM KCl is used to induce depolarization and 20µM NS1619 a BK channel opener is used to elicit hyperpolarization. Both representative traces and data is shown in figure 3. Figure legends, and result section are also modified accordingly.**

4: In representative results, it is strange to show a result of perforated whole-cell patch clamp. The result is completely not related to the microelectrode method. **Response: This data is deleted in the revised version of the article.**

Minor Concerns:
Some redundant blanks are in the text: line 284, 256…. **Response: Deleted.**

Between unit and number should have a blank: line 260, 269, 270, 286…… **Response: Included space between unit and number across the manuscript.**