

5/10/2020

Journal of Visualized Experiments Editorial Office
1 Alewife Center, Suite 200
Cambridge, MA 02140 USA

Re: Resubmission of revised manuscript JoVE61504, entitled "*Systems analysis of the neuroinflammatory and hemodynamic response to traumatic brain injury.*"

Dear Dr. DSouza

We have revised our manuscript entitled "*Systems analysis of the neuroinflammatory and hemodynamic response to traumatic brain injury,*" and we are now resubmitting it for publication in *Journal of Visualized Experiments*. We thank you and the reviewers for the careful examination of our manuscript. We greatly appreciate the feedback and were glad to see that our work was appreciated by both reviewers, with reviewer #1 recognizing that "the description of the methods and analysis pipeline (including critical controls) is excellent and it provides a step-by-step approach for the reader."

Below we address questions and comments raised by both reviewers. In each response, we copy the original comment (black), provide a response (red), and then indicate text changes (if any) made to accommodate the comment. In regards to reviewer #2's comment regarding the scope of the manuscript, we have discussed this issue with Claudia Espinosa-Garcia, Guest Editor, and she determined that the current scope is well-aligned with the theme of this special issue and should remain as is. Please feel free to contact us with any further questions. We hope you find that the manuscript in its revised form is now suitable for publication.

Sincerely,



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Reviewer 1

“This is an excellent JoVE paper. I congratulate the authors for providing clear protocols that detail the use of system analysis approaches to analyze neuroinflammatory and hemodynamic responses after mild TBI. The introduction and methods are beautifully written and are very clear. The protocols are detailed, yet concise, and this is very helpful for the reader[...]Again, the description of the methods and analysis pipeline (including critical controls) is excellent and it provides a step-by-step approach for the reader. The authors also provide a nice discussion section that includes strengths and limitations of the techniques/analysis platforms.”

Comment 1: Line 144. The authors should cite their study using the repeated mTBI model that results in long-term impairments in cognitive function.

Response: This reference is “Increasing Recovery Time Between Injuries Improves Cognitive Outcome After Repetitive Mild Concussive Brain Injuries in Mice”, Meehan 2012. We include this reference on Line 107 after we stated that “Key features of this model include[...]persistent (up to 1 year) cognitive deficits that emerge only after multiple hits[Meehan 2012].”

Comment 2: Fig 2. The authors should consider adding representative righting reflex data for mice following Sham and mTBI surgery as a reference data set for the TBI model.

Response: We added a supplemental table containing a representative data set.

Reviewer 2

Major issues:

Comment 1: I hope the authors will considering dividing this paper into multiple papers.

Response: We appreciate the Reviewer’s comment and have extensively discussed this very point amongst ourselves and with Claudia Espinosa-Garcia, Guest Editor of special issue “..”. Ultimately we concluded the unique aspect of our work is the integration of multiple experimental techniques, this integration gives us the capacity to investigate to disease mechanisms and as such we felt it necessary to maintain the current scope of the manuscript.

Editorial Comments

Comment 1: The JoVE protocol should be almost entirely composed of numbered short steps (2-3 related actions each) written in the imperative voice/tense (as if you are telling someone how to do the technique, i.e. "Do this", "Measure that" etc.). Any text that cannot be written in the imperative tense may be added as a brief “Note” at the end of the step (please limit notes).

1) Long descriptive sections of the protocol such as on lines 147-164, 248-262 should be made into steps/notes (limit 1 note per step) or can be moved to Representative Results or Discussion. The JoVE protocol should be a set of instructions rather a report of a study. Any reporting should be moved into the representative results.

Response: This protocol effectively covers the unique integration of four experimental procedures. We have therefore included a necessary introductory paragraph for each procedure section to aid in reader

comprehension. This text would not be appropriate for Representative Results. We would be happy to work with you to determine a better placement for this text, but as of now we feel this is the most appropriate location to aid in reader comprehension.

Comment 2: 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 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.

Response: Thank you for the suggestion. We now add specific details for button clicks and software actions in RStudio to execute the PLSR analysis. The accompanying code is sectioned according to steps in the protocol. Steps were added to download all relevant programs and R packages, enabling easy replication of the protocol.

Text change: Sections 4.1 to 4.3

Comment 3: Please adjust the numbering of your protocol section to follow JoVE's instructions for authors, 1. should be followed by 1.1. and then 1.1.1. if necessary and all steps should be lined up at the left margin with no indentations. There must also be a one-line space between each protocol step.

Response: Thank you for clarifying that this type of numbering is necessary, we adjusted the numbering of our protocol accordingly.

Comment 4: After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. There is a 10-page limit for the protocol text, and a 3- page limit for filmable content. If your protocol is longer than 3 pages, please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps.

- 1) The highlighting must include all relevant details that are required to perform the step. 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 included in the highlighting.
- 2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.
- 3) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.
- 4) Notes cannot be filmed and should be excluded from highlighting.

Response: We confirmed that the protocol is less than 10 pages and we have highlighted ~2.5 pages for filming a cohesive narrative.

Comment 5: JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

Response: We have ensured that we thoroughly address all of these concerns in our discussion.

Comment 6: Fig 6 is called out before 5. Please re-order figures.

Response: Thank you for bringing this to our attention, we re-ordered figures 5 and 6.

Comment 7: JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Examples of commercial sounding language in your manuscript are McMaster-Carr, Kimwipe, Kimberly-Clark, SPCM-AQ4C-IO, Perkin-Elmer, (Flex05-8ch, www.correlator.com, cOmplete™, etc.

1) Please use MS Word's find function (Ctrl+F), to locate and replace all commercial sounding language in your manuscript with generic names that are not company-specific. All commercial products should be sufficiently referenced in the table of materials/reagents. You may use the generic term followed by "(see table of materials)" to draw the readers' attention to specific commercial names.

Response: Thank you, we removed all of the commercial language from our manuscript except Luminex, which is a distinct type of immunoassay.

Comment 8: Please sort Table of Materials in alphabetical order.

Response: Thank you for the comment. We have made this correction.