**From:** [em.jove.f367.5ad79a.1417bba7@editorialmanager.com](mailto:em.jove.f367.5ad79a.1417bba7@editorialmanager.com) [<mailto:em.jove.f367.5ad79a.1417bba7@editorialmanager.com>] **On Behalf Of** Alisha DSouza  
**Sent:** 27 April 2018 22:24  
**To:** Gibson, Claire L. (Dr.) <[cg95@leicester.ac.uk](mailto:cg95@leicester.ac.uk)>  
**Subject:** Revisions required for your JoVE submission JoVE58191

CC: [mt307@le.ac.uk](mailto:mt307@le.ac.uk), [mk450@le.ac.uk](mailto:mk450@le.ac.uk), [jj167@le.ac.uk](mailto:jj167@le.ac.uk)  
  
Dear Dr. Gibson,  
  
Your manuscript, JoVE58191 Alternative Surgical Approach for Middle Cerebral Artery Occlusion allowing Reperfusion via Common Carotid Artery Repair in Mice., has been editorially and peer reviewed, and the following comments need to be addressed. Note that editorial comments address both requirements for video production and formatting of the article for publication. Please track the changes within the manuscript to identify all of the edits.  
  
After revising and uploading your submission, please also upload a separate rebuttal document that addresses each of the editorial and peer review comments individually. Please submit each figure as a vector image file to ensure high resolution throughout production: (.svg, .eps, .ai). If submitting as a .tif or .psd, please ensure that the image is 1920 pixels x 1080 pixels or 300 dpi.  
  
Your revision is due by **May 18, 2018**.  
  
To submit a revision, go to the [JoVE submission site](http://www.editorialmanager.com/jove) and log in as an author. You will find your submission under the heading "Submission Needing Revision".  
  
Best,  
  
Alisha DSouza, Ph.D.  
Senior Review Editor  
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**Editorial comments:**  
The manuscript has been modified to include line numbers and minor formatting changes. The updated manuscript **58191\_R0.docx** is located in your Editorial Manager account. In the revised PDF submission, there is a hyperlink to download the .docx file. **Please download the .docx file and use this updated version for future revisions.** The file is also attached.  
  
You will find Editorial comments and Peer-Review comments listed below. Please read this entire email before making edits to your manuscript.  
NOTE: Please include a line-by-line response to each of the editorial and reviewer comments in the form of a letter along with the resubmission.   
  
**Changes recommended by the JoVE Scientific Review Editor:**  
  
• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.  
  
• **Protocol Language:** Please ensure that ALL text in the protocol section is 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 “Note”, however, notes should be used sparingly and actions should be described in the imperative tense wherever possible.  
1) Examples not in imperative tense: 1.1.2, 2.11.1, 2.12, 2.13, 4.3

*Response:* These have been amended appropriately.  
  
• **Protocol Detail:** 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. 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:* We are satisfied with the amount of detail given.  
  
• **Protocol Highlight:** 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, anesthesia, and euthanasia cannot be filmed and should be excluded from highlighting.  
*Response:* Completed.

• Please define all abbreviations at first use.

*Response:* Completed.  
  
• Please use standard abbreviations and symbols for SI Units such as µL, mL, L, etc., and abbreviations for non-SI units such as h, min, s for time units. Please use a single space between the numerical value and unit.

*Response:* Completed.  
  
• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

*Response:* provided and completed.

**Comments from Peer-Reviewers:**   
  
  
**Reviewers' comments:**  
  
**Reviewer #1:**   
Manuscript Summary:  
This is a nicely written paper describing the alternative approach and modifications to animal model of stroke by intraluminal suture method. The new procedures are certainly refinement of existing transient MCAO model and will benefit the stroke research community working with animal model of stroke. Following the new procedures will reduce the mortality and variability encountered with existing transient stroke model. The methods are described in detail and easy to follow steps.  
  
Major Concerns:  
None  
  
Minor Concerns:  
I have following suggestions to improve the manuscript:  
1. Abstract only states using alternative surgical approach will decrease the variabilities in outcome measures. In the abstract it would be nice to mention briefly about the specific alternative approaches (use of tissue pads sealing, avoidance of ECA ligations).

*Response:* This detail has been added.  
2. Providing a better description and precautions (thawing, freezing, shelf life) of Baxter sealant either in section 3.5 or table will be helpful. More researchers will adopt the method if it is made clear that price and effort for adopting this technique is comparatively low.

*Response:* This detail has been added.  
3. It appears that LD-CBF measurements (figure 3) in pre-repair (CCA repaired) show less variability than post repair. The CBF increase in post repair group is more compared to pre repair showing increased perfusion of the MCA territory. It would be helpful to compare pre and post repair reperfusion CBF for the significance. Reperfusion CBF level will certainly have an impact on the outcome measures. This is very interesting finding and should be further highlighted and discussed.

*Response:* It does appear, in Figure 3, that the LD-CBF measurements show less variability in pre-repair than post repair. In terms of significance, as shown on the graph, the LD-CBF response is significantly (\*\*\* P < 0.001) increased at post-repair compared to pre-repair.

4. It would be helpful for the new investigator to include an additional panel in figure 2 showing the anatomy of the arteries (circle of Willis, ECA, ICA, incision site etc). This can be reproduced from other publications.

*Response:* We have not added this figure because, as the reviewer suggests, this kind of figure is available in a number of other publications. In addition, this paper is probably aimed more at those with some prior experience of MCAO rather than a complete novice.

5. It would be nice to include discussions on the need for comparing the new approach on long term general wellbeing, functional outcome measures and extended patency of the sealed CCA. Also, it would be nice to compare the effect of ECA ligation on behavioral measures such as tongue protrusion tests affecting the feeding behavior.  
*Response:* The authors agree that it would be good to study the effects of long term behavioural responses following this refined vessel repair. This is outwith the scope of the current publication which is focused on detailing the methods associated with the vessel repair procedure.

This is an important, timely and nicely done study. Congratulations.  
  
  
**Reviewer #2:**   
Manuscript Summary:  
The current MS describes the development of a method for the refinement of the operation technique of the middle cerebral artery occlusion (MCAO) by intraluminal filament insertion to mimic human ischemic stroke. There is some novelty to this method, in that it differs from well-established operation techniques not restoring blood-flow in the brain, and might work as a refinement to the MCAO surgery.  
  
The authors should be applauded for tackling the restoration of blood-flow after occlusion of the MCA. In general, the manuscript in general is well written. However, I suggest some minor changes addressing the following problems:  
  
Major Concerns:  
The authors describe short-term macroscopic effects only (MRI- Measurements) no improvement in functional outcome was assessed. This would improve the ms. and validate the CCA-repaired-method.  
*Response:* The authors agree that it would be good to study the effects of long term behavioural responses following this refined vessel repair. This is outwith the scope of the current publication which is focused on detailing the methods associated with the vessel repair procedure.

Minor Concerns:  
Procedure:  
The authors allow local deviations from their protocol (page 8, line 315-316. I suggest that the authors allow the use of local protocols prior the actual MCAO-surgery (lines 126-213).  
*Response:* There will inevitably be some local variation in acclimatisation protocols but where feasible these should be as consistent as possible.

Representative results:  
1. The authors state "Typically in MCAO procedures the occluding filament is inserted into the CCA and the ECA is ligated to prevent this filament passing into the ECA rather than the ICA. Avoidance of ECA ligation and addition of analgesia showed a trend towards improved weight loss at 48 hours post-MCAO, when compared to data from previous studies undertaken by the same surgeon for the same MCAO time using ECA ligation with no analgesia whereas lesion volume (LV) appeared unaffected, see Figure 1." Could the authors discriminate between the possible impact of analgesia and/or saving the ECA?

Response: It is not possible to distinguish this statistically as both variables (i.e. ECA ligation avoidance and analgesia addition) were implemented at the same time.

2. The phrase "a trend towards improved weight loss" might be misleading. As far as I understand, an improved weight loss means losing more weight, which would be contra productive.

*Response:* This has been changed to a trend towards reduced weight loss.

3. Page 10, para 6, lines 397-399 should be moved to the methods section.  
*Response:* Given the format of this paper it appears clearer to leave this in the results section as it shows diagrammatically what is achieved.

4. Regarding the figures no description of the statistical tests used are given.

*Response:* Some text has now been added to the start of the results section.

5. Did the authors use Levene Test for Equality of Variances?

*Response:* Yes, the Levene’s test for equality of variances was used (spelling mistake of Lavene has been corrected in manuscript).  
  
Discussion:  
2nd paragraph, lines 490-497. What is the significance of these results, regarding the "repaired CCA"-method? Could the authors discuss that or omit this paragraph, since no experimental data are given.

*Response:* We have omitted this paragraph and the references renumbered accordingly.

Figures:  
It might be better, if you would use the identical sorting of data sets on the x-axis, like CCA ligated on the left and CCA repaired on the right consistently to make reading the figures easier.

*Response:* This refers to figures 3 and 4 which are taken directly (as referenced) from our previous publication. Within each figure set the data is presented in consistent manner.  
  
Considering that, I believe that the manuscript needs a revision before it can be considered for publication. After this is done, I would be glad to review the paper again and discuss the procedure.