March 19, 2015

Dear Editor,

We thanks for your careful assessment of our recent resubmission “Rat Model of Photochemically-Induced Posterior Ischemic Optic Neuropathy” (**JoVE52402**). We have now addressed all of the issues raised for our manuscript that were suggested by the editors. We feel that the manuscript is greatly improved through the suggestions from editors, and hope the editors find the revised manuscript appropriate for JoVE.

**Responses to comments:**

**Editorial comments:**  
1) Video comments:  
a) Frame size/proportions issues  
i) The thin green lines seen on the left and top of frame are an artifact generated in the conversion to our web player's size and are not present in the original video. The artifacts were likely created because we had to scale the video up to fit our web player's size. Future submissions should be at least 512x384.

RESPONSE: Thank you, we have exported the video size at 720x540 and hopefully this will solve the problem. Note that we had downsized it because the submission required an initial size of <50MB. We will certainly comply with that on all future submissions. The recent proliferation of higher pixel density cameras will facilitate this for our future work.

b) Audio issues  
i) The audio levels need to be normalized to between -6 and -12 dB below peaking. In particular, Yan Wang's narration is lower than most of the rest of the narration.

RESPONSE: Done as requested.

c) Text/formatting issues  
i) 6:25 – They numbered this chapter "II. I" and the next chapter (at 8:42) "II. II". Either this chapter should just be "II" and the next one "III", or this should be "II. A" and the next "II. B"

RESPONSE: Done as requested.

2) Vet Review Report:  
6:46-6:51 - Surgical scrub only once performed. Change in the appropriate sections in the text, line 137 and line 177 for both surgical sites, that “the skin needs to be scrubbed at least twice alternating skin disinfectant and alcohol”.  
In line 246, it states "clean the surgical area...". It should rather state "surgically scrub" the incision site.

RESPONSE: All edited as requested, see lines 114-115, 128, 169-170, and 239.

3) Please take this opportunity to thoroughly proofread your manuscript to ensure that there are no spelling or grammar issues. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version.

RESPONSE: We have carefully given the manuscript an additional proofreading.

4) If your figures and tables are original and not published previously, please ignore this comment. For figures and tables that have been published before, please include phrases such as “Re-print with permission from (reference#)” or “Modified from…” etc. And please send a copy of the re-print permission for JoVE’s record keeping purposes.

RESPONSE: All figures/tables are original.  
  
**Reviewer #1:**   
Major points: None  
Minor points:  
Line 75, Should disease be diseases?  
Line 83: of "an" animal model?  
LINE 171: How much saline approximately?

RESPONSE: All corrected/edited as suggested. Please see lines 67, 75 and 164.

On lines 145 and 180 of the text says to make a "small incision". The length of the incision can be estimated when watching the video, but perhaps replace "small" with an exact length, e.g. "a ~1cm incision". The same applies to line 179 where the text says to make an incision "along the skin behind the eye". Since the location of the ischemic injury is specific (3-4mm behind the ONH) it would be help to define the exact surgery location.

RESPONSE: All details added as suggested. Please see lines 142-143, 174-175 and 172.  
  
Erythrosin B is stated to be circulated for "a few seconds". Since irradiation is started right after erythrosin B injection, an accurate time estimate or a range would benefit to prevent any misinterpretation of a "few seconds". For instance, how long after injection is the dye still sensitive to laser irradiation? On line 209, the word "week" is used. Is this meant to be "weak"? In the video you mention anesthetizing the rat via intraperitoneal injection, however on line 116 it references an intraparietal injection.

RESPONSE: All details corrected as suggested. Please see lines 209, 203 and 107.  
  
Overall this is a nice study. Congratulations!

RESPONSE: We thank the reviewer for the supportive comments.  
  
**Reviewer #2:**   
*Manuscript Summary:* The authors of this video clearly describe their technique for producing posterior ischemic optic neuropathy in the rat.  
*Major Concerns:* None; the procedure is beautifully described and illustrated.  
  
*Minor Concerns:* It would have been nice for the authors to introduce themselves at the beginning of the video so that the viewer knows who is speaking.

RESPONSE: We have included visual name labels rather than adding verbal introductions.

There is a fair amount of background noise when Dr. Yang and Mr. Brown are speaking. In addition, the volume must be changed (increased) when Dr. Yang is speaking and decreased when Drs. Watson and Goldberg are speaking. This is a particular issue when Dr. Yang is speaking because of her asian accent.

RESPONSE: Corrected as noted in the editorial suggestion above.

*Additional Comments to Authors:* One would think that you could purchase (or fashion) blunt- or fine-tipped forceps that could lock in place so that you did not have to hold them at a precise length (or as precise as possible) during radiation of the nerve.

RESPONSE: True, and we even tried to fashion some ourselves! But the space was too tight to manage a locking, opening forceps, so we hold them after all.  
  
**Reviewer #3:**   
The video and its accompanying manuscript are extremely clear and provide detailed step-by-step procedures to implement this model in virtually any laboratory with an interest in ischemic optic neuropathies. This model provides a valuable in vivo platform to study the pathophysiology of posterior ischemic optic neuropathy and potentially other CNS ischemic diseases.  
  
RESPONSE: Thank you!