Response to Reviewers

Manuscript ID: JoVE50004 **Fiber-Optic Implantation for Chronic Optogenetic Stimulation of Brain Tissue**

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We thank the reviewers for their helpful comments and suggestions to improve the manuscript. To address concerns raised by the reviewers, we have modified our manuscript as per the reviewers’ input.

These changes and our response to the reviewers is itemized here and described in further detail in a point-by-point response below.

* We include more details about the surgical procedure.
* We add further details on stereotaxic implantation pertaining to the mouse and fiber optic implant.
* We describe a bad implant and the determining factor for a good implant, stated as the light output of the implant.
* We add in the discussion the possibility of light leak providing an unintended cue in behavioral studies and suggest a solution.
* We address some common problems and troubleshooting in the ‘discussion’ section.
* We clarify that the purpose of this article is to provide a visual complement to the method described in (Sparta et al., 2012).

**Reviewer #1**

**Q1: Reviewer 1 requested more details about the surgical procedure, including protocols involving anesthesia and other typical IACUC standards, stating: “Some of the details of the surgical procedure are a little thin. For instance, ketamine anesthesia is typically combined with xylazine or a similar agent; no dosage is given for isoflurane, etc. This will be familiar to rodent surgery labs but a published protocol should meet typical IACUC standards.”**

We included dosages for ketamine, xylazine and volatized isoflurane, as well as standard surgical practices such as disinfection of the surgical site and postoperative treatment with analgesics and antibiotics as per IACUC standards.

**Q2: Reviewer 1 requested more detail on the stereotaxic implantation.**

**“Some more detail on stereotaxic implantation would be useful – the skull should be leveled before inserting the fiber, approximate speeds for inserting to minimize damage, etc. The fiber is placed ‘directly above the region of interest’ – how close does it need to be for effective activation?”**

We add details about positioning the mouse in the stereotaxic rig and the rate of advance for fiber optic implant placement to minimize damage. We also added information about measuring the light output of the fiber optic parts in the protocol, and information about the proximity of the implant to the region of interest in the “Discussion” section.

**Q3: “How is a good working implant distinguished from a bad one, what is a rough range for acceptable power or throughput of the fiber for a working device?”**

We describe a bad implant. We also include details of testing the power output of the fiber optic implant, ensuring it can maintain 10 mW of light output as measured by a spectrophotometer.

**Q4: “When using an implant fiber during behavior, are there any issues with external light leak that the animal could use as an unintended cue, any strategies for reducing this (shielding etc)?**

We added a section in the “Discussion” section to address the issue of an unintended visual cue to the mouse in behavioral tests and suggest a possible solution, including the possibility of ‘blacking out’ of light using paint.

**Q5: “Some more information on typical problems and troubleshooting would also increase the usefulness of the protocol.**

More details about typical problems have been included in the ‘discussion’ section.

**Reviewer #2**

**Q1:** **“The one major concern with this submission is that it does not provide any obvious advance in approach or in detail as compared to the methodology described in (Sparta et al., 2012). While the authors acknowledge that the Sparta paper serves as the basis for the current manuscript, they do not make clear in what way their approach is different.”**

Although the existing text describes in detail a very elegant method, a visual demonstration can provide a very powerful complement to the text. We believe that a visual demonstration will enlighten some intricacies in the protocol that may not be obvious for labs just starting out with these procedures. We clarify in the ‘long abstract’ section that the purpose of the submission is to provide a video complement to the previously described method in (Sparta et al., 2012). Our main purpose is in fact to highlight this previously published method, and provide a visual demonstration to those less familiar with the parts and/or techniques.