

Dear JoVE Editorial members, December 14, 2014

We are submitting a revised manuscript (JoVE52859\_R1\_110514) entitled “A Method for Remotely Silencing Neural Activity in Rodents During Discrete Phases of Learning” for your consideration for publication in *The Journal of Visualized Experiments*. We appreciate the peer review comments and agree that they will improve the manuscript. We have addressed each of the peer review comments as described below:

***Changes made by the science editor:***

1. Changes have been made to the manuscript. Please accept or address all tracked changes.

**Response:** I did not notice editorial changes made to the document.

***Changes made by the authors:***

1. Proofread

**Response:** The document has been thoroughly copy edited. Several changes were made throughout and are indicated in red font.

2. Include DOIs when available

**Response:** DOIs have been added.

**REVIEWER 1**

**Major concerns:** None

**Minor concerns:**

1. Modify line 100 pertaining to “neural activation in RSC is attenuated…”

**Response:** Reviewer 1 is correct in that this manuscript does not show attenuation of neuronal activity. The attenuation effect has been omitted from **line 110** and a discussion of verification of attenuation of electrophysiological activity has been added to the section of the Discussion pertaining to Limitations of the techniques **(line 619).**

2. Modify Figure 1 so that the viral construct does not look like a plasmid.

**Response:** Figure one has been modified; the viral vector is now displayed as linear.

3. Modify the legend for Figure 3 by including the number of replicates per group and including the meaning of the error bars (STDEV or SEM).

**Response:** The Figure 3 legend has been adjusted as specified **(line 517).**

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**REVIEWER 2**

**Major concerns:**

1. Reviewer 2 commented on the selection of antibodies used to detect mcitrine, which is the fluorescent reporter that is contained in the viral construct used in this protocol. Reviewer 2 stated that mcitrine is a derivative of DsRed, not GFP. They continue by including that GFP antibodies should not be used to detect mcitrine protein expression.

**Response:** While we appreciate the efforts of Reviewer 2 to improve the immunohistochemical section of this manuscript, we respectfully disagree with the comments. In fact, mcitrine is a derivative of YFP, not DsRed. The bioluminescent protein, GFP was originally obtained from the Aequorea Victoria jellyfish. Because GFP, YFP and mcitrine are modifications of the same protein, GFP antibodies are well suited for their detection. In contrast, fluorescent proteins in the red family (i.e., DsRed) were derived from tropical coral reefs. Reviewer 2 is referred to the Abcam antibody website that verifies that GFP antibodies detect mcitrine: <http://www.abcam.com/gfp-antibody-ab5450.html#description_images_2>. Furthermore, in my personal correspondence with Bryan Roth’s lab, (Dr. Roth is one of the pioneers that established the DREADD technology) they recommend the GFP antibody for the detection of mcitrine expression. This information can also be verified by viewing the August 20 entry on the chemogenetic users blogspot at <http://chemogenetic.blogspot.com/> and on the official DREADD wiki site: <http://pdspit3.mml.unc.edu/projects/dreadd/wiki/WikiStart>. These sites are maintained by the group (Roth lab) that made the viral construct used in this protocol.

2. Reviewer 2 suggested that sections of the manuscript pertaining to experimental design and the consideration of controls be expanded. They suggested that a discussion of the types of data analysis that can be coupled with chemogenetic experiments be included.

**Response:** We enthusiastically agree with the Reviewer’s comments that experimental design and subsequent quantitative analyses of behavioral paradigms that involve pharmacological and/or other types of experimental manipulations is essential. Text pertaining to the importance of experimental design and analyses as well as references to publications that use DREADD in combination with a variety of behavioral designs and statistical approaches are now included in the introduction **(line 84).** Also included is reference to a comprehensive review of the current uses of DREADD, including a chart with 29 publications that use the many variations of the DREADD approach. In addition, a more detailed description of the controls used in the present study has been included in the Introduction **(line 112).** Lastly, a description of the statistical methods used to analyze the data in the present manuscript is included **(line 514, 516).** However, it is our strong conviction that due to the wide variety of manipulations, experimental groups and test phases that may or may not be included in any particular experiment, it would be inappropriate to include instructions as to how future experiments conducted by other investigators should be designed or quantified.

3.Reviewer 2 suggested including a description of the limitations of the chemogenetic approach.

**Response:** A paragraph describing some limitations of the chemogenetic approach was added to the discussion section **(line 619).**

**Minor concerns:**

4. Reviewer 2 requested clarification on which AAV serotypes require the use of Biosafety Level 2 precautions.

**Response:** Text describing the difference has been added to complement the existing text pertaining to safety precautions **(line 117 and 162).**

5. The reviewer suggested an expanded discussion of the types of DREADDs available. The reviewer requested information about why a construct with the hSyn promotor was used and where we obtained our viral vectors.

**Response:** A section has been added to the discussion describing the types of DREADDs that are available, a comprehensive review is identified and a reference to the DREADD wiki, which lists many available options for DREADDS was included **(line 84).** Text pertaining to the function of the hSyn promotor was included in the legend for Figure 1 **(line 492).** In keeping with the guidelines established by the JoVE editorial office, the source of the DREADDs used in this protocol has been listed in the reagents section of the manuscript.

6. Minor misc. comments were addressed

**Response:** The range of the titre of the virus has been changed from *4.2 X 1012* to *1012* **(line 582).**

**Response:** The word “isoflurane” is now spelled correctly and m-citrine was changed to mcitrine throughout the text.

**Response:** Additional advantages of the DREADD approach have been added to the introduction **(line 84).**

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**REVIEWER 3**

**Major concerns:** None

**Minor concerns:**

**Response:** The time course for CNO treatment has been changed from 2-4 hours to 2-5 hours **(line 45).**

Introduction

**Response:** Additional limitations of traditional cannulation methods have been added **(line 61).**

**Response:** the word “that” was deleted **(line 96).**

**Response:** Information about the subjects that comprise the control group was added **(line 112).**

Procedure

**Response:** Clarification was provided about the use of a fume hood **(line 199).**

**Response:** A sentence describing that isoflurane anesthesia is used throughout the surgery was included **(line 208).**

**Response:** The word “redact” was replaced with the word “retract” **(line 225).**

**Response:** Because spread of the virus can vary from surgery to surgery and it may differ depending on which brain regions are targeted, an exact amount of spread of virus was not provided in this revision. However, to address the reviewers important point, text instruction experimenters to conduct pilot studies to determine the spread of the viral construct in their preparation was included **(line 244).**

**Response:** The word “triple” was replaced with the word “topical” **(line 258).**

**Response:** The metal walls of the operant chamber were described **(line 275).**

**Response:** Clarification about the time that the brain should be soaked in paraformaldehyde was added **(line 420 & 587).**

**Response:** The published immunohistochemistry protocol that is supplied by the company that produces the DREADD was referenced **(line 432 & 585).**

**Response:** In keeping with the guidelines established by the JoVE editorial office, a specific brand name of mounting media was not specified in the text of the manuscript, but remains included in the reagents section of the manuscript**.**

**Response:** We politely disagree with the reviewers suggestion to include information about the filters used for the fluorescent imaging of DREADDS because it is our contention that this information is beyond the scope of the present protocol. Furthermore, investigators can choose from a number of fluorescent secondary antibodies, which would influence the filters that would be appropriate.

**REVIEWER 4**

**Major concerns:** None

**Minor concerns:**

1. The reviewer requested a rationale for the time parameter for CNO.

**Response:** A justification for the time course of CNO injection and bioactivity has been provided **(line 369).**

2.Similarly to Reviewer 2, Reviewer 4 suggested including a description of the limitations of the chemogenetic approach.

**Response:** A paragraph describing some limitations of the chemogenetic approach was added to the discussion section **(line 619).**

3. Similarly to Reviewer 2, Reviewer 4 suggested including an expanded discussion of the types of DREADDs available.

**Response:** A section has been added to the discussion describing the types of DREADDs that are available and a reference to a comprehensive DREADD review, which lists many available options, was added **(line 84).**

We look forward to hearing from you.

Respectfully,