

CC: "Leonardo A. Molina" leonardo.molina@ucalgary.ca, "Victorita E. Ivan" victorita.ivan@uleth.ca, "Aaron Gruber" aaron.gruber@uleth.ca

Dear Dr. Luczak,

Your manuscript, JoVE59812R2 "Using neuron spiking activity to trigger closed-loop stimuli in neurophysiological experiments.," 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: (.psd, ai, .eps., .svg). Please ensure that the image is 1920 x 1080 pixels or 300 dpi. Additionally, please upload tables as .xlsx files.

Your revision is due by **Jul 16, 2019**.

To submit a revision, go to the [JoVE submission site](#) and log in as an author. You will find your submission under the heading "Submission Needing Revision". Please note that the corresponding author in Editorial Manager refers to the point of contact during the review and production of the video article.

Best,

Nam Nguyen, Ph.D.
Manager of Review

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Editorial comments:

Changes to be made by the Author(s):

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

We did additional careful proofreading to eliminate any grammatical errors.

2. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.

We inserted references in multiple steps to address this.

3. 1.6: What are the hole diameters? How many holes?

Now we clarify that typically, 4-8 screws with diameter ~0.5mm are used to anchor the implant.

4. 1.7: What are the coordinates?

We now clarify that the presented protocol for closed-loop stimulation will work properly, regardless if coordinates of implanted electrodes are set to motor cortex, sensory cortex or any other brain area.

5. 1.8: How much acrylic is applied?

Now we clarify that the amount of dental acrylic should be enough to sturdily attached implant but it should not extend beyond implant to contact soft tissue.

6. Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

We now eliminated personal pronouns.

7. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.

We would appreciate your advice on this topic. Our software is designed to work only on one type of recording system out of multiple types available on the market. Thus not mentioning explicitly in the main text for which system our protocol is designed may cause significant confusion. It is like reading instruction how to fix something, only to discover on the last page that it does not apply to my case. With other publishers, we never experienced restriction on specifying company names in the main text to disambiguate products. Therefore we kindly ask to allow us to mention right in the front, that our protocol is specifically designed only for Neuralynx system.

Reviewers' comments:

Reviewer #1:

The authors addressed all of my concerns and recommendations.
I am happy to support it being accepted.

Thank you.

Reviewer #3:

Manuscript Summary:

This article presents an easy to implement matlab software for stimulation upon detection of single or multiple neuronal activity. It is nice to present this as a method to be implemented in research. I think that both Introduction and Discussion do not discuss many articles and the methods of closed-loop systems that they use. That could be interesting to enable comparison between different methods and recommendations when other methods are good to use and when this method outstands all the others.

Major Concerns:

- I think optogenetics is just one of the possible stimulation options. In human patients, there is a lot of interest in electrical stimulation. Is this also possible to apply using your software? If that is the case, you should mention one of the Neuropace studies as well, since they have applied electrical closed-loop stimulation to suppress epilepsy in a large amount of patients (Heck et al. 2014 Epilepsia, for example) in introduction. Another system for detection and delivering of therapy is Peters et al. J Clin Neurophysiol 2001.

Thank you for the suggestion. Those studies are now added in the Introduction.

- I am not familiar with Neuralynx. Is this system especially used in animal studies? This should be added in abstract line 35.

Yes, this system is mostly used in animal studies, especially in rodents. This is now specified in the Abstract.

- Page 3 line 129: I do not understand what kind of spike information is loaded. Is this an electrocorticography with epileptic spikes, or is this neuronal spiking data. I would like to see an example of the data that can be loaded.

This is neuronal spiking data, not epileptic spikes. Example of single neuronal spike is shown in Figure 2.

- Page 3 line 131. I do not understand what you mean with neuronal ensemble. Is this the kind of event you would like to detect to trigger stimulation? I would like to see an example of such a neuronal ensemble.

By neuronal ensemble we mean group of neurons. We now clarify that users select one or multiple neurons to trigger stimulation.

- Page 4 line 152, I only see a bold dashed line in figure 2, but not the displayed spike waveforms in real time, which you mention in line 152. Could you update the figure with such a plot as well?

Spike waveforms is plotted in the left top corner of Figure 2 but it was not clearly visible, as it was a dark blue trace on a black background. We have now changed the color of the spike waveform to yellow to better illustrate it.

- Page 4 line 147: what kind of clusters were observed when sorting the spikes? What were the properties of these clusters, and what were the criteria for a spike belonging to a cluster, and did all clusters trigger a stimulation?

This is a good question. Each cluster corresponds to spikes from a different neuron. For example, if a neuron is close to an electrode, then spikes from that neuron will have larger amplitude than spikes from neurons located farther away from the recording electrode. Thus, spike waveform features, like amplitude, will form separate clusters corresponding to different neurons. Differentiating spike feature clusters is often difficult, and there are multiple methods developed to facilitate this process. We now clarify this point in the text and we provide reference for more details on this topic.

- Discussion: I do not see that many references to other articles using a closed-loop system and what they're protocols are. What makes this protocol different/better than others?

We added 6 new references (including suggested above by the Reviewer) in the Introduction where we provide an overview of closed-loop literature. In the Discussion, we specify that the advantage of our method is that it only requires software installation, and it does not require any new hardware for users who already own a Cheetah recording system. Thus, for many labs studying animal electrophysiology, our protocol offers a low cost solution to implement closed-loop stimulation.

Minor Concerns:

- Page 2, line 102, Software installation: on separate computers that are. This is just one of the weird grammar examples (see also Abstract line 29-30, page 4 line 134). This should be checked!

Thank you for pointing it out. We corrected those sentences and we carefully checked grammar in the rest of manuscript.

Reviewer #4:

Manuscript Summary:

Paper describe the protocol for using closed-loop systems with optogenetics, specifically for triggering stimuli based on the activity of single neurons. Paper is well written and clear.

Protocol and scripts are step-by step explained in a clear way. Authors well addressed all potential critical points. No further significant concerns has raised.

Major Concerns:

non major concerns are present

Thank you.