

Journal of Visualized Experiments (JoVE)
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Mainz, den 25.10.16

Dear Jaydev Uppon,

Please find attached a revised version of our manuscript JoVE55415 entitled "Introducing Clicker Training as a Cognitive Enrichment for Laboratory Mice" for publication as an video-article in JoVE. We greatly appreciate the careful and constructive revision. All comments have been addressed, with corresponding changes made directly to the manuscript where appropriate. Detailed responses to the editorial comments are included on the next pages.

We are looking forward to your response.

Sincerely,



Nadine Baumgart

Editorial comments:

•**NOTE: Please download this version of the Microsoft word document (File name: 55415_R1_091416) for any subsequent changes. Please keep in mind that some editorial changes have been made prior to peer review.**

•Please keep the editorial comments from your previous revisions in mind as you revise your manuscript to address peer review comments. For instance, if formatting or other changes were made, commercial language was removed, etc., please maintain these overall manuscript changes.

•Scattered grammar and formatting issues should be corrected:

-Title – Is there some confusion as to the effect of clicker training? Please remove the question mark and also consider rephrasing the title as an investigation into the effect of clicker training.

-New Title: **Introducing Clicker Training as a Cognitive Enrichment for Laboratory Mice**

-Line 102: Also routinely performed laboratory procedures like general handling, restrain, blood or tissue sampling can cause stress responses."

➔ "Furthermore, routinely performed laboratory procedures like general handling, restraining, blood or tissue sampling can cause stress responses, which can be addressed by measuring different parameters such as stress hormones or behaviour"

-Line 153: "The reward must not be a risk for animal health." This is a bit awkward.

➔ Use food rewards, which are vacuum-packed food or animal feed that meets the requirements of food safety standards. This sentence makes "The reward must not be a risk for animal health." Unnecessary. The remarked sentence got deleted.

-Step 2.2.1: Second sentence should be "alternate" rather than "alter."

➔ Word is changed

-Step 2.3.2, 2.4.7, 2.4.10: Is "the access of the tunnel" the right term here? Perhaps "entrance"?

➔ The term is changed to "end of the tunnel" throughout the entire document

-Step 2.3.6 has an extraneous close parentheses and is missing a period at the end of the sentence.

➔ Step 2.3.5 is deleted and 2.3.6 modified to make the step more clear

-Step 2.4.8: "Allows" should be "allow."

➔ Is modified to allow

-Figure legend for Fig. 7 has capitalized "Mouse" when it is not necessary to do so.

➔ Is now "mouse"

•Formatting

-Step 1.1 would make more sense presented at the end of the section rather than the beginning.

➔ Step 1.1 now is step 1.6 at the of the section

-Step 2.2.3: The punctuation/formatting here is a little confusing.

➔ The formation is changed by adding more sections

•Additional detail or clarification is needed:

-Step 2.4.10 says to present the reward as soon as the mouse shows "this behavior" appropriately, but as written it is not clear specifically to what "this behavior" refers.

➔ "This behaviour" is changed into "mouse starts continuously to reenter the tunnel"

-Step 2.6 needs to be rewritten so it is clearer what "the hand" refers to. The experimenter's other hand (the one not holding the target stick) one assumes?

➔ One explaining sentence is inserted as Step 2.6.1

➔ "the hand" is modified to "the experimenter's hand"

-Step 3.1.1: What kind of "grid"?

➔ As step 3.1.1 is no longer part of the protocol it is no further addressed

•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.

•JoVE reference format requires that the DOIs are included, when available, for all references listed in the article. This is helpful for readers to locate the included references and obtain more information. Please note that often DOIs are not listed with PubMed abstracts and as such, may not be properly included when citing directly from PubMed. In these cases, please manually include DOIs in reference information.

➔ The bibliography style is changed from Nature to JOVE

•IMPORTANT: Please copy-edit the entire manuscript for any grammatical errors you may find. The text should be in American-English only. This editing should be performed by a native English speaker (or professional copyediting services) and is essential for clarity of the protocol and the manuscript. Please thoroughly review the language and grammar prior to resubmission. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version

➔ The manuscript has been edited by Elsevier professional copyediting service

•NOTE: Please include a line-by-line response letter to the editorial and reviewer comments along with the resubmission.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

The authors present an interesting, novel and valuable approach towards the stress free handling and cognitive enrichment of mice. The presented method and data will be of interest for the audience, I have

just a few comments that should be addressed by the authors before the manuscript can be recommended for publication:

Major Concerns:

1) Page 4, line 100: Are there any hints that mice are capable to develop "trusting relationships" with humans? Or is the goal of this approach just to decrease anxiety when being handled (a process of learning that a situation is not harmful)?

- ➔ The goal of this approach is to decrease anxiety when being handled and the hope would be that the mice experience this as a relationship of trust.

2) I see that it is an important proof of the training success to test for "welfare" parameters. I agree it is important to mention the tests you use (restraint, floating) and to show the resulting data. Nevertheless, I disagree that there is a need to describe the tests in this manuscript in detail. I assume the prospective reader of this article aims to refine his or her mouse handling/housing when applying the described training. Describing two stressful tests (restraint, floating) here may imply that there is a need to regularly and routinely test the welfare effects of the training.

- ➔ The detailed description of the stress test is not anymore part of the protocol, as the concerns have a point.

In contrast, for establishing and describing such a training, as you do here, I would additionally expect to add more data that prove that the training is reducing anxiety and increases welfare. Did you assess any physiological data (endocrine, heart beat etc.)? Or other behavioural signs of good wellbeing (e.g. certain home cage behaviours)? I do not think you have to include this data now, but please highlight the need to further evaluate the effects of training in the future.

- ➔ No such data were obtained
- ➔ "In further studies supplementary information about the mice wellbeing, such as a whole behavioral battery, physiological data as corticosterone levels or the impact of training on brain development should be gained"

Minor Concerns:

1) The style of the manuscript should be improved by a native speaker.

- ➔ The manuscript has been edited by Elsevier professional copyediting service.

2) Please, change gender to sex in the whole manuscript. Gender is a socio-cultural definition, sex a biological.

➔ Gender is changed to sex in the whole manuscript

3) When talking about "cupping" in the description of the training, please cite Hurst et al.

➔ An additional sentence regarding cupping is inserted and Hurst et al. are cited

4) 2.2.2): remove the food? How?

➔ An additional sentence is inserted

5) Figure 10, 11: mean +/- SEM?

➔ The columns are expressed in % of all tested mice.

Additional Comments to Authors:

N/A

Reviewer #2:

Manuscript Summary:

This manuscript concisely describes a method for clicker training mice that results in ease of handling and reduced stress to the animals. The goals of the study were described adequately and the authors documented the technique through the figures. It is admirable that a study like this was undertaken since mice are typically not given any positive reinforcement training (PRT). While I am not certain that this technique will be used widely simply because of the sheer numbers of mice used in research, I believe it provides a good example of how PRT can and should be used for mice to decrease stress and theoretically improve scientific data.

Major Concerns:

I have no major concerns about the paper.

Minor Concerns:

I have a few minor concerns.

While the authors acknowledge that the study has limitations because they only used inbred mice, I think they should also address the issue that there are many strains of mice with different behavioral characteristics that may or may not lend themselves to successful PRT.

➔ A sentence referring to this issue is inserted in line 444-446.

There are also some minor grammatical corrections that need to be made for example:

Line 65 should be 3Rs Principles (and throughout)

- ➔ 3R Principle is not changed into 3Rs Principles
- ➔ „Bailoo, J. D., Reichlin, T. S., & Würbel, H. (2014). Refinement of experimental design and conduct in laboratory animal research. ILAR Journal / National Research Council, Institute of Laboratory Animal Resources, 55(3), 383–91. <http://doi.org/10.1093/ilar/ilu037>” use as well “3R Principle”

Line 103 - restraint, not restrain

- ➔ changed

Line 341 - Figure 10 not Figures

- ➔ changed

Line 342 - Figures 11 and 12 not Figure

- ➔ changed

Additional Comments to Authors:

No additional comments.