

Date: August 6, 2021

Nam Nguyen, Manager of Review, JoVE

Dear Nam Nguyen, and reviewers,

Please accept our apologies as we missed the second vet review and did not include answers to these comments on our last resubmission. We have now included answers to these comments, as well as the new editorial comments sent to us on August 5<sup>th</sup> 2021.

Please find in the following pages, our answers to the editorial comments, and the vet review with our answers in blue. We also provide a revised manuscript to be considered for publication.

We remain available may you, the vets or the reviewers have further questions or comments.

Sincerely,

Thomas D. Prevot  
Project Scientist



### Editorial and production comments

Changes to be made by the Author(s) regarding the written manuscript:

1. Some minor changes are needed. Please see the comments in the attached manuscript.

*>> We have addressed all comments in the attached manuscript, removing the references to commercial brands, changing the format of the reference on page 2 to use numbered reference and changing the title of the manuscript to match the video.*

2. Please send us the rebuttal letter for the vet review comments.

*>> Sorry we missed answering this on our previous rebuttal. We addressed each point in the following pages.*

#	Time in the video	comment	Change in video required Yes/No	Change in text is sufficient Yes/No	Suggested Changes
1	3:29-4:14	Sound level changes significantly and is distracting	YES		Can the voice over be fixed? >> We addressed this issue by re-recording all sequences in the previous version.
2	3:29-4:14	The animal seems very habituated to the rolling and shelter test despite it being day 1. This will be misleading to individuals trying to emulate this handling style	YES		Can the video be reshot with animals who it truly is day 1 for? >> We changed the video of this sequence. In the new video, the mouse appears more alert, trying to flee, as expected on day 1.
3	9:22	There is a claim that this method will increase the ease of animal husbandry however, the technique takes at least 3-5 minutes per mouse. Considering even a small vivarium has 5000 cages of 5 animal per cage, the time commitment is substantial, potentially limiting the acceptance and use of this technique.	YES		Remove the last reference to animal husbandry >> We re-recorded this part as well and removed the mention of facilitating animal husbandry. The sentence now says that "implementing this technique has the potential to facilitate routing handling"(no reference to

					husbandry anymore)

### Vet Report

There are details not addressed in the video or text or contradictory between the two:

- Are these animals housed in ventilated or static caging systems?

>> *The mice of the present study were housed on static caging system. Other animals in our facility are housed in ventilated caging systems.*

- What enrichment was provided to these animals? The text states Yesterday's news was used for bedding but the video also shows a Nesting puck. Can this be more fully described in the text?

>> *Mice were provided with nesting puck to build nest. As this is now mandatory in all facilities, we did not include this in the methods, but added a mention at the very beginning of the result section (Line 626). It now says "Their home cage was filled with recycled newspaper as bedding material, as well as nesting material."*

- Is this handling technique done in an animal transfer station (ATS), biosafety cabinet (BSC), or in the open room? The video gives the appearance that the animals are in an open room, but most animals are usually handled in an ATS or BSC. Can this be better described in the text.

>> *This is an excellent comment that other reviewers also made. We do it in BSC, or in open room, depending on the study. For the present study, we did it in open room, and recorded it in open room. In the manuscript, we include option to work in the BSC (line 126-134). For the present studies, we now mention that the mice were handled in the open room (Line 629).*

- 438-440: the video shows animals housed 2 per cage but the text states that animals, at least males, were single housed. Figure 3 also shows 2 animals per cage with the heat map data. Can this be explained further or rectified?

>> *In the video, we showed animals group-housed as this is the most commonly used housing condition, and to avoid providing confusing messages on the video that would suggest that the handling technique needs to be done exclusively with single-housed mice. This is not the case, and we decided to show on the video that we can do it on group-housed mice. We were afraid that showing data from single-housed mice, and showed handling done in single housed mice in the video would make viewers think that mice need to be single housed for the handling to be efficient, which would decrease its adaptability and use in other facilities.*

*In the manuscript, we also describe that animals can be single-housed or group-housed (line 122). In this study, because we provide details on behavioral and neuroendocrine effects, we decided to single house the animals, but the core technique to be demonstrated in the video is the handling and not the behavioral and neuroendocrine results. These results are only here to support the efficacy of the technique at reducing anxiety like behavior and improving interaction with experimenters. Therefore, we made the video more generic than the approach that we used in the paper.*

*Regarding the heatmaps, they are representative heatmaps from animals being single-housed. Mice were not group housed in this experiment.*

- 455-456: Do you mean handling by the wrong method rather than bias of the facility staff?

>> Yes. Our facility staff is still transitioning from tail handling to tunnel or cup handling when doing the cage change. In order to limit tail handling of our tunnel or 3D handled mice during cage change, or more generically to limit the use of a different handling technique during the experiment, the experimenter changed all cages, using the appropriate handling technique relevant to each group. We changed the sentence to avoid any confusion. It now says: "During the time of the study, cage change was performed by the experimenter to ensure the use of the appropriate handling technique used for each group."