

## Peer Review

# Review of: "A Contextual Fear Conditioning Paradigm in Head-Fixed Mice Exploring Virtual Reality"

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This paper illustrates the development of CFC behavior paradigms in the head-fixed mouse and verifies the place cell remapping during CFC in this setting. This new method expands the possibilities for performing dendritic calcium imaging or voltage imaging in association with behavior, as shown recently. Such insights will add significant value to the field.

However, in general, water restriction, which accompanies body weight loss immediately after surgery, is harsh, and the high dropout rate suggests that the system could be revised (e.g., by extending the recovery period or using reward-based motivation) to reduce intervention. Below are some minor points for consideration.

**For further clarification of the experimental procedures:** The authors could mention the total time the animals were exposed to each of the control and CFC VRs on Day 0 in all paradigms (especially Paradigms 2 and 3). When the ISI was changed, I presumed that the CFC VR was shortened from 10 + 6 min to 10 + 2 min (a bit more than 10 + 2 min) or that it was done in such a way that the total time exposed to the VRs remained the same. It would be fair if the total time spent experiencing CFC VR were kept the same as for the control VRs. Additionally, one technical improvement related to this timing: In the free-moving version of CFC, the mice are typically kept in the chamber for a few tens of seconds after the last shock to avoid associating the shock with unwanted contexts, such as the opening of the apparatus door, handling, odor, or the home cage.

**For the sake of clear presentation:** In Paradigms 2 and 3, the authors mention using two “novel” VRs, but at first glance, when readers compare Figure 1 with Figures 3 and 5, it may seem contradictory to call the VRs “new” since they are pre-trained (on Day -1) for both VRs. To avoid confusion, the authors could add a small panel in all the figures showing the training VRs (in Paradigm 1, it’s the

same as the familiar VR, but in Paradigms 2 and 3, it's different from both the control and CFC VRs?) where the mice are initially trained, and possibly mention whether the training involves water rewards.

Another thing the authors could check is that in Sup Figure 5B, the statistics above the "Recall Day 2" graph show  $p = 0.05$ . This does not intuitively match the data shown in the graph.

**Psychological interpretation:** In the main text, the authors often discuss the extinction of contextual fear memory. This could be mentioned when comparing it to another paradigm of memory "decay". Therefore, the term "extinction" should be used with caution or in a more limited manner.

## **Declarations**

**Potential competing interests:** No potential competing interests to declare.