

Dear Dr Hangya,

Thank you for reviewing our preprint. We have now submitted a revision based on the peer-review comments to enhance clarity, and figures 1 and 2 were revised. The supplemental file repository was updated and a DOI created.

Our responses to reviewers are below in **green**. A revised manuscript is submitted on bioRxiv and a copy in track-changes mode is attached as a separate file.

Best wishes,

Mahesh Karnani

Review by Ede Attila Rancz, 24 Feb 2024 14:50

Hartmann et al. present a new, open-source device to measure mouse foraging behaviour in a close-to-natural environment.

The presentation of the design choices and the build instructions are exemplary, allowing pretty much any lab to build its device. The use of food deprivation, swapping of feeding areas and pharmacogenetic interference with specific neuronal pathways elegantly demonstrates the device's utility in investigating motivated behaviours, particularly motivational switching.

I congratulate the authors on their approach and the work done and look forward to exciting experimental work using the device. I only have a few minor suggestions.

Thank you.

In the current form of the supplementary material, the Bill of Materials points to tinkercad files. I suggest referencing the OSF repo files, which are likely more future-proof. In addition, a "version of record" depository should be produced upon publication. Further versions can then be used for future modifications and improvements to the design.

We have now added links to the OSF repository files in table 1 of the supplement, and created DOI [10.17605/OSF.IO/YB67Q](https://doi.org/10.17605/OSF.IO/YB67Q) for the current version.

I want to draw the authors' attention to two similar publications which use a similar approach to designing novel research equipment. These papers and repositories could guide readers willing to design their tools, and the authors may consider referencing them.

<https://doi.org/10.1371/journal.pone.0211571>

<https://doi.org/10.1016/j.jneumeth.2023.110002> (conflict of interest: this paper is from my lab)

Indeed, there are many open-source tools using similar approaches (e.g., the 256 projects listed at <https://edspace.american.edu/openbehavior/>), and we have now cited several of them in the discussion, p13 2nd paragraph.

"sleep mix" is a colloquial term and should be removed.

Now removed.

In the methods section, I struggled to follow what viral constructs were injected for what purpose in which animals. While it is deducible, it requires unnecessary effort, and I suggest restructuring these few sentences.

Now re-written, p3.

Relatedly, the histology figures referenced in the manuscript are missing from the supplementary material. They are present on the OSF data repository without legends, making it difficult to square with the methods section.

Apologies for this oversight. The histology figures are now added to the Supplement with legends, p30-37.

I struggled to understand what epoch in Figure 2 could correspond to the "appetitive" epoch mentioned in the second line of the results.

Added in Figure 2C.

In Figure 1C, please mark the position of the FED3, SWD, HSD and SEM devices.

Added.

Please provide the weight of the individual mice during the whole experiment in Figure 2.

Added as Figure 2B.

A further minor suggestion to ensure animal wellbeing is automatic monitoring of mouse weight and foraging with a warning system (i.e. automatic warning email if a mouse drops below 85% of initial weight or doesn't visit the maze for 12 hours).

We added the warning for BW drop below 85% in separate script files in the repository, called `Switch_maze_functions_email.py` and `Switch_maze_main_email.py`. We set this up as a separate copy of the standard maze scripts, as the email functionality may require trouble shooting from new users who wish to change email settings, or if our default mail address' rules change in the future. We added a note of this in the Supplement, p29 paragraph 3.

A sentence about the device's compatibility with tethered optical and electrophysiological measurement systems is warranted.

Added at top of p6 and top of p13.

Review by Ewelina Knapska, 08 Mar 2024 08:41

The Switchmaze is a new device for measuring motivation and drive switching in mice, enabling the observation of behavioral features not accessible in classical motivation tests. These features include the ability to monitor the switching between different rewards over extended periods. The Switchmaze employs a clever idea to spatially separate appetitive, consumatory, and termination phases of feeding. Importantly, it does not require food or water restriction and mirrors the feeding and drinking patterns observed in mice living in their natural environment. The device has the potential to unveil new patterns of behavior in mice and their corresponding neuronal correlates. Moreover, the system is open source, ensuring easy accessibility to the community. While the manuscript is generally well-written, I have some comments that could enhance its readability.

Thank you.

1. Abstract: The phrase "measured as the ratio of single probe entries to continuous exploitation runs" may not be clear to readers unfamiliar with the paper's context.

We have now explained this more clearly.

2. Introduction: It would be beneficial to provide a clear definition of the drive being referenced. Now it is stated that it is a contemporary one that is distinct from earlier drive reduction theory.

We have now defined this more clearly.

3. Two viral constructs were used in H-hM4Di animals: 'For 4 out of 9 H-hM4Di animals, the injections contained 30-150 nl of 1:5 mixture of AAV9-CMV-Cre-tdTomato (1012 GC/ml) and AAV8-hSyn-DIO-HA-hM4Di-mCitrine (1013 GC/ml), and the other 5 out of 9 animals received 30 nl of AAV8-hSyn-hM4Di-mCherry (2*1013 GC/ml.'. Did the behavioral results for these subgroups differ?

There was no difference in the behavioral result between these subgroups. The MSR change due to C21 in the Cre:DIO-hM4Di mixture group was $-9.4 \pm 49.5 \%$, and in the hSyn-hM4Di group it was $-5.6 \pm 42.5 \%$ ($P=0.9$). We have now added this to the text, p3 third paragraph.

4. In the methods section, it is stated that 'In PFC-hM4Di specimens, the hypothalamic injection centroid was always within the lateral hypothalamic area (LH) with a minority of cases with expression elsewhere (1/11) or difficult to detect expression (1/11). In all cases the mPFC layer 5 (L5) neurons expressed hM4Di-mCitrine, with difficult to detect expression in 1 case out of 11 (same individual as the above difficult to detect hypothalamic expression) and abundant expression in all others.'. It is unclear whether the case with difficult-to-detect expression was included in the analysis.

We have now added a note that we included all cases on p4, first paragraph. This was done because it is not clear what the relationship between effective expression level of hM4Di and detectable expression level of mCitrine was, and the difficult-to-detect expression was in some cases due to poor preservation of the tissue.

5. The description of upward closing doors is not entirely clear. Including additional explanation or a short video illustrating their operation would enhance understanding.

We added a diagram into Figure 1E and a clearer description in the text on p6, first paragraph.

6. In the description of behavioral experiments, the total number of tested animals should be clearly stated (Figures 2 and 3), and any potential differences between groups should be discussed. Is it possible that dominant mice in some groups might attempt to block access to food or water for others?

We added a statement of the total number of animals in each experiment on p4, second paragraph. As for dominance effects, none were identified, nor were the experiments designed to detect this. It is unclear how such a dominance effect would be visible in our data, other than if some animals would not enter the foraging enclosure. This did not happen, as seen, e.g., in Figures 2A,D and 3D. However, as there is a possibility that social effects such as hierarchy affect the results in some way, we have noted this in the discussion, p13 third paragraph.

7. The data in Figure 4 are difficult to follow. The time series data (left and middle panels) have a 4-hour bin width, while the bar graphs (right panels) are measured from the last 6 hours before lights-on. It would be helpful to explain the rationale behind choosing 4-hour and 6-hour bins for data quantification. Do shadowed areas mark dark phases of the light-dark cycle? Also, why is N=20 for habituation and N=22 for challenges? Are these the same animals?

Thank you for pointing out this missing information. We have now explained the choices clearer in the text (p3, last paragraph and p4, second paragraph) and figure legend. We estimated the 4-hour bin width to be the lowest time resolution that provides reasonably precise values of MSR. As a ratio between singles and runs, in blocks that occur somewhat stochastically across hours (see Figure 2A), this parameter becomes highly variable at low time bins as the absolute values in numerator and denominator may approach 1. The 6-hour bin was selected based on the duration of DREADD activation from Alexander et al, 2009 *Neuron* **63**, 27–39.

The shadowed areas indeed denote the dark phase – now noted in the Figure legend.

The N=20 for habituation is because the work was done during the covid-19 pandemic, and unfortunately there were unexpected long pauses in some animals' habituation time-courses. The challenges had a higher N because they were simpler to collect consistently across the cohort as they occur within fewer consecutive days. 15 animals were in both cohorts, and this is now noted on p4, second paragraph.

8. Figure 6 presents data for the entire 6-hour period, but actual chemogenetic modulation might have lasted for a shorter duration (not controlled). It would be beneficial to show the data in shorter time bins, such as 3-hour intervals.

We have replotted the data in 3-hour bins below (Figure R1). The choice of a 6-hour period for the manuscript is based on two factors: 1) published pharmacokinetics data indicating that the effect of chemogenetic modulation lasts for 6 hours (Alexander et al, 2009 *Neuron* **63**, 27–39; Jendryka et al 2019 *Sci Rep* **9**, 4522), and 2) MSR can be highly variable in short time windows which may contain a low amount of trials (e.g., animal 3 in Figure 2A might yield an imprecise MSR value from the latter 3 h bin). We have added a note of this on p3, last paragraph.

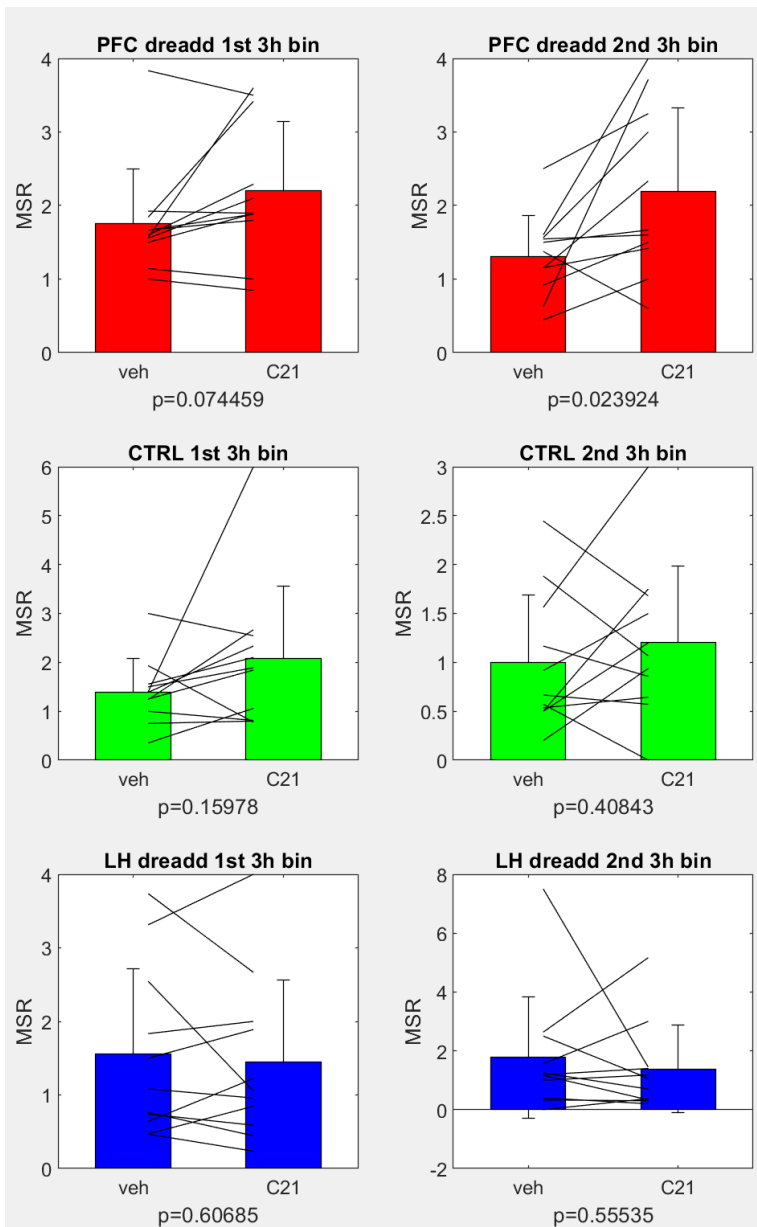


Figure R1, Data from Figure 6A replotted in 3-h bins. One might interpret this as the increased MSR developing in the later 3-h bin in the PFC dreadd cohort, however, with a caveat that MSR may not be ideally precise from short time bins for a relatively small cohort of animals.

- **Title and abstract**
 - Does the title clearly reflect the content of the article? Yes, No (please explain), I don't know
 - Does the abstract present the main findings of the study? Yes, No (please explain), I don't know
- **Introduction**
 - Are the research questions/hypotheses/predictions clearly presented? Yes, No (please explain), I don't know
 - Does the introduction build on relevant research in the field? Yes, No (please explain), I don't know
- **Materials and methods**
 - Are the methods and analyses sufficiently detailed to allow replication by other researchers? Yes, No (please explain), I don't know
 - Are the methods and statistical analyses appropriate and well described? Yes, No (please explain), I don't know
- **Results**
 - In the case of negative results, is there a statistical power analysis (or an adequate Bayesian analysis or equivalence testing)? Yes, No (please explain), I don't know n/a
 - Are the results described and interpreted correctly? Yes, No (please explain), I don't know
- **Discussion**
 - Have the authors appropriately emphasized the strengths and limitations of their study/theory/methods/argument? Yes, No (please explain), I don't know

The limitations of the method are not discussed. For instance, the role of social hierarchy as a potential confound of the results should be mentioned.

- Are the conclusions adequately supported by the results (without overstating the implications of the findings)? Yes, No (please explain), I don't know

We have now added a discussion about potential social effects such as hierarchy on p13 third paragraph.