

Life Sciences Reporting Summary

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For further information on the points included in this form, see [Reporting Life Sciences Research](#). For further information on Nature Research policies, including our [data availability policy](#), see [Authors & Referees](#) and the [Editorial Policy Checklist](#).

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study. For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

► Experimental design

1. Sample size

Describe how sample size was determined.

No statistics were used to determine sample size, but sample sizes were chosen based on common practice in animal behavior experiments.

2. Data exclusions

Describe any data exclusions.

Based on predetermined criteria, cells with a peak firing rate less than 1 Hz were excluded. Animals in which the virus injection missed the target brain region (vMT) were excluded.

3. Replication

Describe the measures taken to verify the reproducibility of the experimental findings.

In the paper, we show data reproducing the main results (vMT activation and vMT-to-mPFC activation) using two different methods: chemogenetic and optogenetic techniques. All attempts to reproduce our results were successful.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Half of the mice in each cage were randomly assigned to either control or treatment groups. All control mice were treated with the same experimental procedures, except a control virus was injected instead.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

The experimenter was blind to group allocation during data collection and analyses.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a Confirmed

- ☐ ☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- ☐ ☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ A statement indicating how many times each experiment was replicated
- ☐ ☒ The statistical test(s) used and whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☐ ☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- ☐ ☒ Test values indicating whether an effect is present
*Provide confidence intervals or give results of significance tests (e.g. *P* values) as exact values whenever appropriate and with effect sizes noted.*
- ☐ ☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- ☐ ☒ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on [statistics for biologists](#) for further resources and guidance.

► Software

Policy information about [availability of computer code](#)

7. Software

Describe the software used to analyze the data in this study.

Biobserve Software: Behavior tracking data
TINT Software, Axona and MATLAB: Analyzing spike data
Image J Cell Counter: Image analysis and cell counts
GraphPad Prism and R: Statistics

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* [guidance for providing algorithms and software for publication](#) provides further information on this topic.

► Materials and reagents

Policy information about [availability of materials](#)

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

No restrictions are placed on unique materials.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

All antibodies are widely used commercially available antibodies. Antibodies were validated in WB and IHC by the manufacturer.
rabbit-anti-c-Fos: EMD Millipore, ABE457, LOT:2878126 (1:1000)
rabbit-anti-DsRed: Living Colors, 632496, LOT:1509043 (1:1000)
Alexa Flour 594 donkey anti-rabbit: invitrogen, A21207, LOT:1668652
Alexa Flour 488 donkey anti-rabbit: invitrogen, A2206, LOT:1874771

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

No eukaryotic cell lines were used.

b. Describe the method of cell line authentication used.

No eukaryotic cell lines were used.

c. Report whether the cell lines were tested for mycoplasma contamination.

No eukaryotic cell lines were used.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by [ICLAC](#), provide a scientific rationale for their use.

No eukaryotic cell lines were used.

► Animals and human research participants

Policy information about [studies involving animals](#); when reporting animal research, follow the [ARRIVE guidelines](#)

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

Male and female C57BL/6 mice 8-12 weeks old at the time of viral injection were used.

Policy information about [studies involving human research participants](#)

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

This study did not involve human research participants.