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Corresponding author(s):   YUKI OKA

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

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 to determine sample size were used. Sample size is similar to recent papers in behavioural neuroscience.

2. **Data exclusions**

   Describe any data exclusions.

   > All data were included with verified virus expression

3. **Replication**

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

   > Key surgeries, behavioral and histological assays were replicated by more than two lab members.

4. **Randomization**

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

   > No statistical randomization methods were used. However, mice used for data collection were both males and females, at least 8 weeks of age

5. **Blinding**

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

   > The investigators were not blinded during data collection.

   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.

> Custom MATLAB(2016 a) code, GraphPad Prism 7.02, ImageJ 1.50b, Leica LAS X and Microsoft Excel 2016 were used to analyze data. Code is
> available from the corresponding author upon reasonable request.

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 the availability of materials used. Some transgenic strains may be obtained from the original authors

9. Antibodies

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

> The primary antibodies (1:500 dilution) used were - goat anti-c-Fos (Santa Cruz, SC-52G, Lot #I0216), rabbit anti-NOS1 (Santa Cruz, sc-648, Lot #K2415), rabbit anti-GAD65+GAD67 (Abcam, ab183999, Lot #GR260178-2), chicken anti-GFP (Abcam, ab13970, Lot #GR236651-18) and rat anti-mCherry (Thermo Fisher, M11217,Lot #SF251482).

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.

> N/A

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

> N/A

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.

> N/A

▶ 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.

> Details of mice used for this study are given in the Methods section of the manuscript.
> The following mice from the Jackson Laboratory were used (C57BL/6J; stock number 000664, Slc32a1 (Vgat)-Cre; stock number 016962, Ai9; stock number 007909, Ai3; stock number 007903).
> GLP1r-Cre and Ai110 were generously provided by Fiona Gribble (Cambridge) and David Anderson (Caltech), respectively.
> At least 8 weeks of age of both male and female mice were used for this study.

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.

> The study did not involve any human research participants.