

Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a Confirmed

- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ 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 all covariates tested
- ☐ ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☐ ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated
- ☐ ☒ Clearly defined error bars
State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on [statistics for biologists](#) may be useful.

Software and code

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

Data collection QCapture Suite 2.98.2, ZEN 2012, IgorPro 6.10A

Data analysis Prism 6, RStudio Version 1.1.453, BitPlane AutoAligner, IMARIS x64 8.0.1, BitPlane AutoAligner x64, ImageJ

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/authors/policies/ReportingSummary-flat.pdf](https://www.nature.com/authors/policies/ReportingSummary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications (DeNardo et al., 2015).
Data exclusions	No animals or data points were excluded from analysis.
Replication	The major findings of this manuscript were reproduced in all samples observed for this study. In order to ensure reproducibility, we used both male and female animals and repeated experiments three and five times per condition.
Randomization	No experimental manipulations were used in this study. No preference was given towards particular embryos or mice for IUE or viral injections.
Blinding	Blinding to experimental condition was not possible in these studies due to the obvious anatomical or laminar differences in different groups.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	The following primary antibodies were used in this study: rabbit polyclonal anti-RFP (1:800, Rockland, #600-401-379), chicken polyclonal anti-GFP (1:1000, Abcam, ab13970), guinea pig polyclonal anti-PV, (1:2000, Swant, PVG-213), rat monoclonal anti-SOM (1:250, Millipore, MAB354), rabbit polyclonal anti-VIP (1:500, Immunostar, #20077), mouse monoclonal anti-NeuN (1:500, Millipore, MAB377), mouse monoclonal anti-GAD67 (1:800, Millipore, MAB5406), and rat monoclonal anti-HA (1:500, Roche, #11-867-423-001). The following secondary antibodies were used: donkey antibodies conjugated to Alexa 488, Cy3, or Cy5 (Jackson ImmunoResearch, 150 µg/mL).
Validation	All antibodies were validated by the companies we obtained them from prior to purchase. Additionally, all antibodies were verified many times by multiple lab members in house, and in all cases have been verified in peer-reviewed publications (Taniguchi et al., 2011; Ishino et al., 2017; Steinecke et al., 2017).

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	HEK293T - These cells were obtained from ThermoFisher. B19G2, EnvA2, and HEK293T-TVA - These cell lines used for rabies virus amplification, pseudotyping, and titring were obtained from the Salk Institute Viral Vector Core along with rabies seed virus that they produced for us.
Authentication	None of the cell lines have been authenticated (besides acquiring them from a reliable source).

Mycoplasma contamination

Cell lines were not tested for mycoplasma contamination.

Commonly misidentified lines
(See [ICLAC](#) register)

No commonly misidentified cell lines were used in this study.

Animals and other organisms

Policy information about [studies involving animals](#): [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals

Homozygous Cre/Flp dual responsive frt-STOP-frt (FSF)-loxP-STOP-loxP (LSL)-RFP mice (Ai65) were bred to homozygous PV-ires-Cre, SOM-ires-Cre, and VIP-ires-Cre knockin mice to produce PV-ires-Cre/+;Ai65/+, SOM-ires-Cre/+;Ai65/+, and VIP-ires-Cre/+;Ai65/+ mice. Homozygous FSF-LSL-Synaptophysin-YFP (SypYFP) mice were bred to homozygous PV-ires-Cre mice to produce PV-ires-Cre/+;FSF-LSL-SypYFP/+ mice. Homozygous LSL-RFP mice (Ai14) were bred to homozygous SOM-ires-Cre mice to produce SOM-ires-Cre/+;Ai14/+ mice. Homozygous PV-ires-Cre mice were bred to mice homozygous for Ai65 and heterozygous for Dlx5/6-Flp (Miyoshi et al., 2010) to produce PV-ires-Cre/+;Dlx5/6-Flp/+;Ai65/+ mice. Homozygous FSF-RFP mice were bred to Swiss Webster (SW) mice to produce FSF-RFP/+ mice. Wild-type SW mice were used for control experiments to test specificity of RV-mediated mono-transsynaptic tracing.

PV-ires-Cre, SOM-ires-Cre, VIP-ires-Cre, Ai65, Ai14, FSF-LSL-SypYFP, FSF-RFP, and Dlx5/6-Flp mice were first backcrossed with SW mice at least three times and then maintained as homozygotes. Female mice with SW background were used for breeding.

Both males and females were used for analyses. Experiments were performed on E12.5, E15.5, and E16.0 embryos, and all adult animals used were between P21 and P36.

Wild animals

No wild animals were used in this study.

Field-collected samples

No field-collected samples were used in this study.