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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

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	LabVIEW (National Instruments). Custom software was not central to the paper.
Data analysis	Prism 7 (Graphpad), ImageJ (Fiji, Wayne Rasband, NIH), Igor Pro 6.3 (Wavemetrics, with TaroTools), Python 2.7 with packages noted in the methods. Custom software was not central to the paper.

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

Correspondence and requests for materials or data should be addressed to T.B. (t.branco@ucl.ac.uk).

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

For a reference copy of the document with all sections, see 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	Minimum sample sizes were predetermined from power estimates based on pilot experiments.
Data exclusions	For any data was excluded in the analysis, the criteria are clearly stated in the methods. This includes the pre-established exclusion of in vivo experiments with off-target optic fibre placement and viral infection, and in our behavioural analysis, the exclusion of a small number (n=3) of sensory stimulation trials where no stimulus-detection response was observed. For electrophysiological recordings, only cells with a stable series resistance of <30MOhm were analysed (a standard pre-established exclusion criterion for recording quality).
Replication	All experiments or analysis were reliably reproduced by at least two experimenters, independently. All datasets, except the calcium imaging, chemogenetic and optogenetic inhibition experiments, were acquired at two different institutions (MRC LMB and UCL SWC).
Randomization	Animals in test and control groups were litter mates and randomly selected.
Blinding	Behavioural experiments were not performed blind as the experimental setup is closed-loop and automatically delivers stimuli. Behavioural data was annotated blind and by five different experimenters.

Materials & experimental systems

Policy information about availability of materials

n/a	Involved in the study
☒	☐ Unique materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☐	☒ Research animals
☒	☐ Human research participants

Antibodies

Antibodies used	Standard, commercially available antibodies were used. Primary antibodies used are anti-GFP (1:1000, chicken; A10262, or rabbit; A11122, Life Technologies), anti-RFP (1:1000, rabbit; 600-401-379, Rockland) and anti-NeuN (1:1000, mouse; MAB-377, Millipore) and the secondary antibodies were Alexa-488 Donkey anti-rabbit and Goat anti-chicken, Alexa-568 Donkey anti-rabbit and Donkey anti-mouse, and Alexa-647 Donkey anti-mouse (1:1000, Life Technologies).
Validation	Anti-GFP A10262, Life Technologies: 110 citations, validated for IHC in mouse neural tissue. Anti-GFP A11122, Life Technologies: 1406 citations, validated for IHC in mouse neural tissue. Anti-RFP 600-401-379, Rockland: 106 citations, validated for IHC in mouse neural tissue. No reaction observed by manufacturer against Human, Mouse or Rat serum proteins. Anti-NeuN MAB-377, Millipore: >200 citations, validated for IHC in mouse neural tissue.

Research animals

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Animals/animal-derived materials	Adult male and female C57BL/6J wild-type (from Charles River and MRC LMB stock animals), VGluT2-ires-Cre (Jackson Laboratory, stock #016963) and VGluT2::EYFP (R26 EYFP, Jackson Laboratory #006148) mice were housed with ad libitum access to chow and water on a 12h light cycle and tested during the light phase.

Method-specific reporting

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ Magnetic resonance imaging