## nature portfolio

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## **Reporting Summary**

Nature Portfolio 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 Portfolio policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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For all stati	stical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.				
n/a Confir	med				
□ × Th	ne exact sample size $(n)$ for each experimental group/condition, given as a discrete number and unit of measurement				
	statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly				
	ne statistical test(s) used AND whether they are one- or two-sided nly common tests should be described solely by name; describe more complex techniques in the Methods section.				
	description of all covariates tested				
X	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons				
□ × A	A full description of the statistical parameters 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)				
☐ ⊠ Fo	or null hypothesis testing, the test statistic (e.g. $F$ , $t$ , $r$ ) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted we $P$ values as exact values whenever suitable.				
⊠ ☐ Fo	or Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings				
⊠ ∏ Fo	or hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes				
☐ X Es	timates of effect sizes (e.g. Cohen's $d$ , Pearson's $r$ ), indicating how they were calculated				
·	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.				
Softwa	re and code				
Policy infor	mation about <u>availability of computer code</u>				
Data colle	Ection LabVIEW 2013 ScanImage 2019				
Data anal	CalmAn (1.8.3) Suite2p (0.7.1)				
For monus:-	The analysis code is publicly available at: www.github.com/ivan-voitov/loops.				
	its 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 and estrongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

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

Field-spe	ecific reporting				
	ne below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.				
X Life sciences	Behavioural & social sciences Ecological, evolutionary & environmental sciences				
For a reference copy of	the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>				
Life scier	nces study design				
All studies must dis	sclose on these points even when the disclosure is negative.				
Sample size	bata was collected from 23 animals. When possible, given the limitations such as viral vector mediated gene expression, cranial window mplant condition, etc., animals were recorded from in multiple sessions. No sample size calculation was performed prior to the start of the xperiments. Sample sizes were progressively increased until the onset of the COVID pandemic, when all experiments were halted.				
Data exclusions	mals which entered behavioural training but did not achieve adequate behavioural performance, as measured by trial completion, were luded from all further study. Animals with cranial window implants which were in poor condition (e.g.exhibited dura regrowth) were luded from further study.				
Replication	Reproducibility was confirmed by having multiple animals in each experimental condition.				
	Multi-area optogenetic silencing experiments (Fig. 1) were replicated in 9 mice. Cell-body imaging experiments (Fig. 2 and Fig. 3) were replicated in 7 mice. Axonal imaging and silencing experiments (Fig. 4) were replicated in 7 mice.				
	All attempts at replication were successful, given the data exclusion criteria outlined above.				
	Preliminary data was collected at another institute in another country (Biozentrum, University of Basel).				
Randomization	e were no comparisons across sampled animal populations in this study. All animals used in this study were chosen as the first available the breeding populations at the local animal facility.				
Blinding	There were no explicit control groups in this study (all experimental controls were within-animal).				
	g for specific materials, systems and methods on from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material,				
'	ted is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.				
Materials & ex	perimental systems Methods				
n/a Involved in the study					
Antibodies					
Eukaryotic Palaeontol	cell lines				
	nd other organisms				
	search participants				
Clinical dat					
	esearch of concern				
Animals and	other organisms				
Policy information	about <u>studies involving animals</u> ; <u>ARRIVE guidelines</u> recommended for reporting animal research				
Laboratory anima					

3 animals were Ai-148 × Cux-creER mice (JAX 030328 and JAX 012243, Jackson Laboratory).

3 animals were Ai-148 mice (JAX 030328, Jackson Laboratory).

7 animals were PV-Cre mice (JAX 017320, Jackson Laboratory).

Wild animals

There were no wild animals used in this study.

Field-collected samples

No field-collected samples were used in this study.

Ethics oversight

All experiments were performed under the UK Animals (Scientific Procedures) Act of 1986, under project license PPL PD867676F, following local ethical approval by the Sainsbury Wellcome Centre Animal Welfare Ethical Review Body.

Note that full information on the approval of the study protocol must also be provided in the manuscript.