

## 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

In vitro data was collected using the open source software ephus (vidrio technologies). In vivo data was collected using exper (written by Tomas Hromadka while at Cold Spring Harbor Lab).

Data analysis

In vitro data was analyzed using the open source companion software to ephus (vidrio technologies). In vivo data was analyzed using exper analysis scripts (written by Tomas Hromadka while at Cold Spring Harbor Lab). iDisco data was analyzed with the open source suite ClearMap (<http://www.idisco.info>).

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 stored in CCNY servers and 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 predetermine sample sizes, but our sample sizes are similar to those reported in previous publications (13, 21, 39, 42)

Data exclusions      No data were excluded.

Replication      Multiple investigators acquired and analyzed the data and multiple recording methodologies were employed. In all cases replication was successful.

Randomization      In vivo spiking data were analyzed automatically without consideration of hemisphere identity.

Blinding      iDisco immuno staining was performed blind to the experimental conditions (vocalizations vs sweeps).

## 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      cFos: rabbit anti cFos, sc-52, (1:200, Santa Cruz)  
donkey anti-rabbit Alexa 647 (1:500, Life Technologies)

Validation      All antibodies are validated for species by manufacturer

## Animals and other organisms

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

Laboratory animals      Species: mice. Strains: male CBA/J and male Ntsr1-cre

Wild animals

no wild animals were used in this study

Field-collected samples

no samples were collected from the field