

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 [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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 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)
☐	☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

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

Data collection	Imaging : ScanBox (Neurolabware, version: 2.5) Stimuli design: Matlab (MathWorks) R2021b (Custom code) Stimuli presentation: RVPdsEx (Tucker-Davis Technologies, version 90), Matlab R2021b (Custom code)
Data analysis	Custom code for data analysis: Matlab R2021b

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 and reviewers. We strongly 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 preprocessed imaging data used for this manuscript is publicly available at 10.5281/zenodo.14743696. Additional data can be made available upon reasonable request to the corresponding author (K.V.K.).

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	NA
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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

Field-specific reporting

Please select the one below that is 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/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size	No sample size calculation was performed prior to the study. The number of bats (n = 5) is consistent with the bat electrophysiological literature (Sarel et al., 2022 n = 4, Salles et al. 2020 n = 5). Note that our approach yields a substantially larger number of recorded cells with a total of 9,443 neurons (Sarel et al., 2022, n= 430 single-units, Salles et al. 2020 n = 575 single-units).
Data exclusions	Only recorded imaging sites with visually identifiable cells were analyzed (27 imaging sites over 5 bats). Sites with fewer than 50% of cells with significant tone-evoked responses were excluded from the vocalization analysis (6 excluded sites, so 14 sites for the analysis in original 3 bats, 7 sites for analysis in the 2 new bats for the revision).
Replication	Findings remain similar when performing analyses using neurons, sites, or individual animals as replicates.
Randomization	Sound presentation was randomized within blocks of auditory stimuli.
Blinding	No, this study is an observational study of sound encoding at the cell level in healthy animals. This experimental procedures require hands-on adjustments based on animal condition (e.g., surgery, optical imaging in awake passively listening animal), full blinding is not be feasible.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed 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 & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used	Primary antibody: 1) Goat Anti-GFP (Novus Biologicals, Catalog #: NB100-1770) 2) Rabbit Anti-GAD67 (ThermoFisher, Catalog #: PA5-21397) Secondary antibody: 1) Donkey anti-goat Alexa Fluor 488 (Jackson ImmunoResearch, Catalog #: 705-545-003) 2) Donkey anti-Rabbit Cy3 (Jackson ImmunoResearch, Catalog #: 711-165-152)
Validation	Fluorescence microscopy was used for visual identification. These antibodies are routinely employed in rodent studies. Goat anti-GFP and donkey anti-goat Alexa Fluor 488 were used to enhance GFP fluorescence associated with CaMKII-GCaMP6f. Immunostaining was specific to the infection site (the IC) and corresponded with in vivo imaging results at the IC surface. Double staining with rabbit anti-GAD67 and donkey anti-rabbit Cy3 revealed that GFP-labeled cells (green) occasionally overlapped with GAD67-positive cells (red), as expected.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	No
Wild animals	Big brown bats, <i>Eptesicus fuscus</i> , were collected from exclusion sites in the State of Maryland under a permit issued by the Maryland Department of Natural Resources (no. 55440). Epiphyseal-diaphyseal bone fusion was used to determine that data from all bats included in this manuscript were adults. All procedures were approved by the Institutional Animal Care and Use Committees at Johns Hopkins University (protocol no. BA17A107), where this research was conducted. All animals were euthanized following the experiment in accordance with protocol no. BA17A107.
Reporting on sex	Findings apply to both male and females. Our data set contains n= 5 bats, 3 females and 2 males. We do not report sex-based analyses as we found no differences (median responsivity, KW test, $p = 1$; % of social selective cells, KW test, $p=0.564$; % of navigation selective cells, KW test, $p=0.248$).
Field-collected samples	The study did not involve samples collected in the field.
Ethics oversight	The Institutional Animal Care and Use Committees at Johns Hopkins University approved protocol: no. BA17A107

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

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA