

Corresponding Author: Philip X. Joris

Manuscript Number: NN-A48694A

Manuscript Type: Article

Main Figures: 7

Supplementary Figures: 5

Supplementary Tables: 0

Supplementary Videos: 0

Reporting Checklist for Nature Neuroscience

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. For more information, please read [Reporting Life Sciences Research](#).

Please note that in the event of publication, it is mandatory that authors include all relevant methodological and statistical information in the manuscript.

► Statistics reporting, by figure

- Please specify the following information for each panel reporting quantitative data, and where each item is reported (section, e.g. Results, & paragraph number).
- Each figure legend should ideally contain an exact sample size (n) for each experimental group/condition, where n is an exact number and not a range, a clear definition of how n is defined (for example x cells from x slices from x animals from x litters, collected over x days), a description of the statistical test used, the results of the tests, any descriptive statistics and clearly defined error bars if applicable.
- For any experiments using custom statistics, please indicate the test used and stats obtained for each experiment.
- Each figure legend should include a statement of how many times the experiment shown was replicated in the lab; the details of sample collection should be sufficiently clear so that the replicability of the experiment is obvious to the reader.
- For experiments reported in the text but not in the figures, please use the paragraph number instead of the figure number.

Note: Mean and standard deviation are not appropriate on small samples, and plotting independent data points is usually more informative. When technical replicates are reported, error and significance measures reflect the experimental variability and not the variability of the biological process; it is misleading not to state this clearly.

| | | TEST USED | | n | | | DESCRIPTIVE STATS (AVERAGE, VARIANCE) | | P VALUE | | DEGREES OF FREEDOM & F/t/z/R/ETC VALUE | |
|----------------------------|--------------------|-----------------------|--------------|------------------------------------|-----------------------|-------------------------------------|---------------------------------------|-------------|-----------------------|-----------------|--|--|
| FIGURE NUMBER | WHICH TEST? | SECTION & PARAGRAPH # | EXACT VALUE | DEFINED? | SECTION & PARAGRAPH # | REPORTED? | SECTION & PARAGRAPH # | EXACT VALUE | SECTION & PARAGRAPH # | VALUE | SECTION & PARAGRAPH # | |
| example 1a | one-way ANOVA | Fig. legend | 9, 9, 10, 15 | mice from at least 3 litters/group | Methods para 8 | error bars are mean +/- SEM | Fig. legend | p = 0.044 | Fig. legend | F(3, 36) = 2.97 | Fig. legend | |
| example results, para 6 | unpaired t-test | Results para 6 | 15 | slices from 10 mice | Results para 6 | error bars are mean +/- SEM | Results para 6 | p = 0.0006 | Results para 6 | t(28) = 2.808 | Results para 6 | |
| + 2c | linear correlation | Methods | 16 | neurons from 16 gerbils | Fig. legend | all individual datapoints are shown | Figure 2c | p = 0.00001 | Figure | t(14) = -6.47 | Figure legend | |

| | | TEST USED | | n | | | DESCRIPTIVE STATS (AVERAGE, VARIANCE) | | P VALUE | | DEGREES OF FREEDOM & F/t/z/R/ETC VALUE | |
|---------------|----------------------|---|----------------------|--|--|----------------------|---------------------------------------|----------------------|---|----------------------|---|--------------------------|
| FIGURE NUMBER | WHICH TEST? | SECTION & PARAGRAPH # | EXACT VALUE | DEFINED? | SECTION & PARAGRAPH # | REPORTED? | SECTION & PARAGRAPH # | EXACT VALUE | SECTION & PARAGRAPH # | VALUE | SECTION & PARAGRAPH # | |
| + - | 2d | linear correlation | Methods | 16 | neurons from 16 gerbils | Fig. legend | all individual datapoints are shown | Figure 2d | p = 0.4 | Figure | t(14) = 0.918 | Figure legend |
| + - | 3b | linear correlation | Methods | 67 | datasets from 25 neurons from 24 gerbils | Fig. legend | all individual datapoints are shown | Figure 3b | p = 0.00000003 | Figure | t(65) = -6.26; r = -0.61 | Figure legend; Figure 3b |
| + - | 3d | linear correlation | Methods | 72 | datasets from 28 neurons from 26 gerbils | Fig. legend | all individual datapoints are shown | Figure 3d | p = 0.00005 | Figure | t(70) = 4.31; r = 0.46 | Figure legend; Figure 3d |
| + - | Results, paragraph 8 | two-sample one-tailed t-test | Results, paragraph 8 | 7/5 | datasets from resp. 5/3 neurons from resp. 5/3 gerbils (resp. 2 uM stry applied by pressure and 10 mM stry applied by iontophoresis) | Results, paragraph 8 | mean +/- SEM | Results, paragraph 8 | p = 0.5 | Results, paragraph 8 | t(10) = 0.092 | Results, paragraph 8 |
| + - | Results, paragraph 8 | two-sample one-tailed t-test | Results, paragraph 8 | 7/5 | datasets from resp. 5/3 neurons from resp. 5/3 gerbils (resp. 2 uM stry applied by pressure and 10 mM stry applied by iontophoresis) | Results, paragraph 8 | mean +/- SEM | Results, paragraph 8 | p = 0.04 | Results, paragraph 8 | t(10) = 1.95 | Results, paragraph 8 |
| + - | Results, paragraph 8 | paired sample one-tailed t-test | Results, paragraph 8 | 7 | datasets from 5 neurons from 5 gerbils | Results, paragraph 8 | mean +/- SEM | Results, paragraph 8 | p = 0.47 | Results, paragraph 8 | t(6) = -0.07 | Results, paragraph 8 |
| + - | 4d | one-way two-tailed ANOVA with Tukey's posthoc | Figure legend | n = 7, 7, 5, 4 (1, 10, 30, 100 microM) | neurons from 6 gerbils | Fig. Legend | mean +/- SEM | Methods | Rpk: p = n.a., 0.221, 0.002, 0.012; Rss, p = n.a., 0.049, 0.001, 0.0001; (1, 10, 30, 100 microM) | Figure legend | Rpk, F(3,19)=7.459; Rss, F(3,19)=12.731 | Figure legend |
| + - | 4e | one-way two-tailed ANOVA with Tukey's posthoc | Figure legend | n = 6, 6, 5, 4 (1, 10, 30, 100 microM) | neurons from 6 gerbils | Fig. legend | mean +/- SEM | Methods | -70 mV: p = n.a., 0.013, 0.026, 0.004. -90 mV: p = n.a., 0.535, 0.822, 0.021. -110 mV: p = n.a., 0.681, 0.096, 0.922. (1, 10, 30, 100 microM) | Figure legend | sag ratio at: -70 mV, F(3,17)=7.144; -90 mV, F(3,17)=3.704; -110 mV, F(3,17)=3.340; | Figure legend |
| + - | Results, paragraph 9 | paired sample two-tailed t test | Results, paragraph 9 | n = 100 | EPSPs per cell (5 cells), with and without strychnine | Results, paragraph 9 | not reported | not reported | p < 0.001 | Results, paragraph 9 | t(99) < -8.33 | Results, paragraph 9 |

| | | | | | | | | | | | | |
|--------|----------------------|---|----------------------|----|---|----------------------|---|--------------|---|----------------------|---------------------------|--------------------------|
| + - | Results, paragraph 9 | paired sample one-tailed t test | Results, paragraph 9 | 5 | datasets from 3 neurons | Results, paragraph 9 | not reported | not reported | p = 0.04 | Results, paragraph 9 | t(3) = 2.67 | Results, paragraph 9 |
| + - | Results, paragraph 9 | paired sample one-tailed t test | Results, paragraph 9 | 5 | datasets from 3 neurons | Results, paragraph 9 | not reported | not reported | p = 0.02 | Results, paragraph 9 | t(3) = 3.83 | Results, paragraph 9 |
| + - | Results, paragraph 9 | paired sample one-tailed t test | Results, paragraph 9 | 6 | datasets from 4 neurons | Results, paragraph 9 | not reported | not reported | p = 0.6 | Results, paragraph 9 | t(5) = -0.26 | Results, paragraph 9 |
| + - | Results, paragraph 9 | paired sample one-tailed t test | Results, paragraph 9 | 6 | datasets from 4 neurons | Results, paragraph 9 | not reported | not reported | p = 0.8 | Results, paragraph 9 | t(5) = -0.82 | Results, paragraph 9 |
| + - | 5h | linear correlation | Fig. legend | 18 | datasets from 18 neurons from 16 gerbils | Fig. legend | all datapoints are shown | Figure 5h | p = 0.77 | Figure legend | t(16) = -0.30; r = -0.075 | Figure legend |
| + - | 6f | linear correlation | Methods | 64 | datasets from 26 neurons from 24 gerbils | Fig. legend | all datapoints are shown | Figure 6f | p = 0.003 | Figure | t(56) = 3.07; r = 0.38 | Figure legend; Figure 6f |
| + - | 7c | linear correlation | Figure legend | 1 | neuron (data for 5 neurons from 5 gerbils reported in Results paragraph 16) | Fig legend | all data points are shown | Figure 7c | p = 0.0005 | Figure legend | t(4) = 10.552; r = 0.98 | Figure legend |
| + - | 7f | repeated measures two-tailed ANOVA with Tukey's posthoc | Figure legend | 5 | neurons from 3 gerbils | Fig. legend | mean +- SEM, individual data points also shown | Fig. legend | p = 0.006, 0.023, 0.332 (1.00, 0.75, 0.50 ms) | Figure legend | F(3,12)=29.702 | Figure legend |
| + - | 7h | repeated measures two-tailed ANOVA with Tukey's posthoc | Figure legend | 5 | neurons from 4 gerbils | Fig. legend | mean +- SEM | Fig. legend | p = 3.7E-5, 0.0003, 0.004 (1.00, 0.75, 0.50 ms) | Figure legend | F(3,12)=100.394 | Figure Legend |
| + - | 7i | repeated measures two-tailed ANOVA with Tukey's posthoc | Figure legend | 5 | neurons from 4 gerbils | Fig. legend | mean +- SEM | Fig. legend | p = 0.013, 0.0003, 8.1E-5 (1.00, 0.75, 0.50 ms) | Figure legend | F(3,12)=171.414 | Figure Legend |
| + - | Supp lem. Fig. 1f | linear correlation | Methods | 58 | datasets from 24 neurons from 22 gerbils | Fig. legend | individual datapoints are shown | Figure | p = 0.00000007 | Figure | t(56) = 6.23 | Figure legend |
| + - | Supp lem. Fig. 3i | one-tailed paired sample t-test | Fig. Legend | 8 | neurons from 8 gerbils | Fig. legend | individual datapoints are shown as well as the mean | Figure | p = 0.00007 | Figure | t(7) = 7.46 | Figure legend |
| + - | Supp l. Fig. 4 | linear correlation | Fig. Legend | 71 | datasets from 28 neurons from 26 gerbils | Fig. legend | all datapoints are shown | Figure | p = 0.3 | Figure | t(69) = 1.16 | Figure legend |

► Representative figures

1. Are any representative images shown (including Western blots and immunohistochemistry/staining) in the paper?

If so, what figure(s)?

Figure 1a includes a representative image of a biocytin labeled MSO neuron.

2. For each representative image, is there a clear statement of how many times this experiment was successfully repeated and a discussion of any limitations in repeatability?

If so, where is this reported (section, paragraph #)?

In the first paragraph in Results, we state that labeling was successful in half of cases, and that we are confident to identify unrecovered neurons as MSO neurons by means of the physiological properties.

► Statistics and general methods

1. Is there a justification of the sample size?

If so, how was it justified?

Where (section, paragraph #)?

Even if no sample size calculation was performed, authors should report why the sample size is adequate to measure their effect size.

No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications. This is mentioned in Online Methods, section Analysis, first paragraph.

2. Are statistical tests justified as appropriate for every figure?

Where (section, paragraph #)?

Statistical tests have been performed as indicated in the main text, Figure legends and Online Methods (section Statistics). Normal distribution of the data was assumed as described in Online Methods (section Statistics).

- a. If there is a section summarizing the statistical methods in the methods, is the statistical test for each experiment clearly defined?

Yes.

- b. Do the data meet the assumptions of the specific statistical test you chose (e.g. normality for a parametric test)?

Where is this described (section, paragraph #)?

Normal distribution of the data was assumed. This is described in Online Methods (section Statistics)

- c. Is there any estimate of variance within each group of data? Is the variance similar between groups that are being statistically compared?

Where is this described (section, paragraph #)?

Either all datapoints or error bars indicating SEM are shown for the figure panels where statistics has been performed, so that the variance can be judged. For data reported in the text, mean +/- SEM is reported unless indicated otherwise. For Suppl. Fig. 2 panel i, it can be seen that the variance in both groups is similar. For spike rate and ITD tuning with and without strychnine, variance was again of the same order of magnitude (Results, paragraph 8).

- d. Are tests specified as one- or two-sided?

Yes.

- e. Are there adjustments for multiple comparisons?

No, because the number of tests performed is low.

3. Are criteria for excluding data points reported?
Was this criterion established prior to data collection?
Where is this described (section, paragraph #)?
- For the main analysis (Fig. 3b,d), datapoints have been excluded where the ITD tuning was not meeting the criterion of significance. This was established prior to analysis. This criterion is Rayleigh test $\alpha < 0.001$. This is described in the Figure legend, and in Online methods (section Statistics).
4. Define the method of randomization used to assign subjects (or samples) to the experimental groups and to collect and process data.
If no randomization was used, state so.
Where does this appear (section, paragraph #)?
- For most analyses, cells were their own control (binaural response versus monaural response, with versus without strychnine). The decision to apply CNQX or strychnine, whether using iontophoresis or pressure, was made before contact with the neuron was established and therefore randomized to its properties. This is mentioned in Online Methods, section Pharmacology.
5. Is a statement of the extent to which investigator knew the group allocation during the experiment and in assessing outcome included?
If no blinding was done, state so.
Where (section, paragraph #)?
- No blinding was performed during experiments or analysis. This is mentioned in Online Methods, section Analysis, first paragraph.
6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included?
Where (section, paragraph #)?
- Yes, this is mentioned in the first paragraph of Online Methods.
7. Is the species of the animals used reported?
Where (section, paragraph #)?
- Yes, in the abstract, the first paragraph of Online Methods (in vivo experiments), and in Online Methods section In vitro slice experiments (in vitro experiments).
8. Is the strain of the animals (including background strains of KO/transgenic animals used) reported?
Where (section, paragraph #)?
- Yes, in the first paragraph of Online Methods (in vivo experiments), and in Online Methods section In vitro slice experiments (in vitro experiments).
9. Is the sex of the animals/subjects used reported?
Where (section, paragraph #)?
- Yes, in the first paragraph of Online Methods, and the first sentence of Online Methods, section In vitro slice experiments.
10. Is the age of the animals/subjects reported?
Where (section, paragraph #)?
- Yes, in the first paragraph of Online Methods (in vivo experiments), and in Online Methods section In vitro slice experiments (in vitro experiments).
11. For animals housed in a vivarium, is the light/dark cycle reported?
Where (section, paragraph #)?
- The gerbils used in the in vivo experiments were housed with a 10 hour light/dark cycle: lights turn on at 7 AM, and off at 9 PM. This is mentioned in Online Methods, first paragraph.
The gerbils used in the in vitro experiments were housed with a 12 hour light/dark cycle: lights turn on at 7 AM, and off at 7 PM. This is mentioned in Online Methods, section In vitro slice experiments.
12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?
Where (section, paragraph #)?
- The gerbils used in the in vivo experiments were housed with 6 or fewer per cage. This is mentioned in Online Methods, first paragraph.
The gerbils used in the in vitro experiments were housed with 10 or fewer per cage before weaning and 4 or fewer per cage after weaning. This is mentioned in Online Methods, section In vitro slice experiments.

13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)?
Where (section, paragraph #)?
- Not applicable.
14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported?
Where (section, paragraph #)?
- Animals did not have previous experimental history. This is reported in the first paragraph of Online Methods (in vivo experiments), and in Online Methods section In vitro slice experiments (in vitro experiments).
- a. If multiple behavioral tests were conducted in the same group of animals, is this reported?
Where (section, paragraph #)?
- Not applicable.
15. If any animals/subjects were excluded from analysis, is this reported?
Where (section, paragraph #)?
- The identification of MSO neurons was done according to criteria mentioned in Online Methods (section Analysis, paragraph 2).
- a. How were the criteria for exclusion defined?
Where is this described (section, paragraph #)?
- Based on the labeled cases, MSO neurons were defined as having mainly excitatory responses to sound played to either ear, ITD modulation in their sub- or suprathreshold responses and narrow EPSP halfwidths (<1.5 ms). This is described in Online Methods (section Analysis, paragraph 2).
- b. Specify reasons for any discrepancy between the number of animals at the beginning and end of the study.
Where is this described (section, paragraph #)?
- Not applicable.

▶ Reagents

1. Have antibodies been validated for use in the system under study (assay and species)?
- Not applicable.
- a. Is antibody catalog number given?
Where does this appear (section, paragraph #)?
- Not applicable.
- b. Where were the validation data reported (citation, supplementary information, Antibodypedia)?
Where does this appear (section, paragraph #)?
- Not applicable.
2. If cell lines were used to reflect the properties of a particular tissue or disease state, is their source identified?
Where (section, paragraph #)?
- Not applicable.
- a. Were they recently authenticated?
Where is this information reported (section, paragraph #)?
- Not applicable.

▶ Data deposition

Data deposition in a public repository is mandatory for:

- Protein, DNA and RNA sequences
- Macromolecular structures
- Crystallographic data for small molecules
- Microarray data

Deposition is strongly recommended for many other datasets for which structured public repositories exist; more details on our data policy are available [here](#). We encourage the provision of other source data in supplementary information or in unstructured repositories such as [Figshare](#) and [Dryad](#).

We encourage publication of Data Descriptors (see [Scientific Data](#)) to maximize data reuse.

- Are accession codes for deposit dates provided?

Where (section, paragraph #)?

Not applicable.

▶ Computer code/software

Any custom algorithm/software that is central to the methods must be supplied by the authors in a usable and readable form for readers at the time of publication. However, referees may ask for this information at any time during the review process.

- Identify all custom software or scripts that were required to conduct the study and where in the procedures each was used.

All analyses have been performed as described in Online Methods, using custom written scripts in MATLAB and IgorPro.

- If computer code was used to generate results that are central to the paper's conclusions, include a statement in the Methods section under "**Code availability**" to indicate whether and how the code can be accessed. Include version information as necessary and any restrictions on availability.

All scripts employ standard algorithms, described in Online Methods, that are widely known and commonly used in the field.

▶ Human subjects

- Which IRB approved the protocol?

Where is this stated (section, paragraph #)?

Not applicable.

- Is demographic information on all subjects provided?

Where (section, paragraph #)?

Not applicable.

- Is the number of human subjects, their age and sex clearly defined?

Where (section, paragraph #)?

Not applicable.

- Are the inclusion and exclusion criteria (if any) clearly specified?

Where (section, paragraph #)?

Not applicable.

5. How well were the groups matched?
Where is this information described (section, paragraph #)?
- Not applicable.
6. Is a statement included confirming that informed consent was obtained from all subjects?
Where (section, paragraph #)?
- Not applicable.
7. For publication of patient photos, is a statement included confirming that consent to publish was obtained?
Where (section, paragraph #)?
- Not applicable.

► fMRI studies

For papers reporting functional imaging (fMRI) results please ensure that these minimal reporting guidelines are met and that all this information is clearly provided in the methods:

1. Were any subjects scanned but then rejected for the analysis after the data was collected?
- Not applicable.
- a. If yes, is the number rejected and reasons for rejection described?
Where (section, paragraph #)?
- Not applicable.
2. Is the number of blocks, trials or experimental units per session and/or subjects specified?
Where (section, paragraph #)?
- Not applicable.
3. Is the length of each trial and interval between trials specified?
- Not applicable.
4. Is a blocked, event-related, or mixed design being used? If applicable, please specify the block length or how the event-related or mixed design was optimized.
- Not applicable.
5. Is the task design clearly described?
Where (section, paragraph #)?
- Not applicable.
6. How was behavioral performance measured?
- Not applicable.
7. Is an ANOVA or factorial design being used?
- Not applicable.
8. For data acquisition, is a whole brain scan used?
If not, state area of acquisition.
- Not applicable.
- a. How was this region determined?
- Not applicable.

9. Is the field strength (in Tesla) of the MRI system stated?
- a. Is the pulse sequence type (gradient/spin echo, EPI/spiral) stated?
- b. Are the field-of-view, matrix size, slice thickness, and TE/TR/flip angle clearly stated?
10. Are the software and specific parameters (model/functions, smoothing kernel size if applicable, etc.) used for data processing and pre-processing clearly stated?
11. Is the coordinate space for the anatomical/functional imaging data clearly defined as subject/native space or standardized stereotaxic space, e.g., original Talairach, MNI305, ICBM152, etc? Where (section, paragraph #)?
12. If there was data normalization/standardization to a specific space template, are the type of transformation (linear vs. nonlinear) used and image types being transformed clearly described? Where (section, paragraph #)?
13. How were anatomical locations determined, e.g., via an automated labeling algorithm (AAL), standardized coordinate database (Talairach daemon), probabilistic atlases, etc.?
14. Were any additional regressors (behavioral covariates, motion etc) used?
15. Is the contrast construction clearly defined?
16. Is a mixed/random effects or fixed inference used?
- a. If fixed effects inference used, is this justified?
17. Were repeated measures used (multiple measurements per subject)?
- a. If so, are the method to account for within subject correlation and the assumptions made about variance clearly stated?
18. If the threshold used for inference and visualization in figures varies, is this clearly stated?
19. Are statistical inferences corrected for multiple comparisons?
- a. If not, is this labeled as uncorrected?

20. Are the results based on an ROI (region of interest) analysis?

Not applicable.

a. If so, is the rationale clearly described?

Not applicable.

b. How were the ROI's defined (functional vs anatomical localization)?

Not applicable.

21. Is there correction for multiple comparisons within each voxel?

Not applicable.

22. For cluster-wise significance, is the cluster-defining threshold and the corrected significance level defined?

Not applicable.

► Additional comments

Additional Comments

No additional comments