nature neuroscience

Corresponding Author:	Sherman	# Main Figures:	6
Manuscript Number:	NN-A48900B	# Supplementary Figures:	
Manuscript Type:	Article	# Supplementary Tables:	
		# Supplementary Videos:	

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

		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 #
+ -	2b	one-way ANOVA + Tukey		5	virus injected mice	Results P4	error bars are mean +/- SEM	Results P4; Fig. legend	150-180°: p=0.007 180-210°: p=2x10^-5 210-240°: p=4x10^-6 240-270°: p=4x10^-7 270-300°: p=1x10^-9 300-330°: p=5x10^-10 330-360°: p=4x10^-10	Results P4; only 150-180° and 330-360°	F(23,96)=25.48	Not specified
+ -	2e	one-way ANOVA + Tukey		5	virus injected mice	Results P4	error bars are mean +/- SEM	Results P4; Fig. legend	0-30°: p=4x10^-10 30-60°: p=0.02	Results P4; only 0-30°	F(23,96)=11.41	Not specified
+ -	2f	one-way ANOVA + Tukey		5	virus injected mice	Results P4	error bars are mean +/- SEM	Results P4; Fig. legend	0-30°: p=4x10^-10	Results P4	F(23,96)=9.52	Not specified
+ -	4c	one-way ANOVA + Tukey		7	virus injected mice	Results P6	error bars are mean +/- SEM	Results P6; Fig. legend	0-30°: p=2x10^-7 300-330°: p=0.01 330-360°: p=5x10^-7	Results P6	F(23,120)=7.16	Not specified
+ -	4d	one-way ANOVA + Tukey		7	virus injected mice	Results P6	error bars are mean +/- SEM	Results P6; Fig. legend	All p > 0.05	Results P6	F(23,120)=1.47	Not specified
+ -	4e	one-way ANOVA + Tukey		7	virus injected mice	Results P6	error bars are mean +/- SEM	Results P6; Fig. legend	150-180°: p=0.002 180-210°: p=2x10^-5 210-240°: p=8x10^-6 240-270°: p=0.01	Results para 5; only 210-240°	F(23,120)=9.16	Not specified
+ -	5b	unpaired t-test		3	virus injected mice	Results P7	error bars are mean +/- SEM	Results P7; Fig. legend	p=3x10^-6	Results P7	t(2.49)=3.99	Not specified

Representative figures

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

   If so, what figure(s)?

Yes, it is necessary to show representative images, particularly of airflow traces depicting characteristic responses to light.

Figures:
1a-b,e-f
2c-d
3a-g
4b, f-h
5a
6b,c,d

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 #)?

Yes, this is clearly stated in the manuscript and the figure legends.
Figures:
1a-b,e,f (Results, P1)
2c-d: the n reported (Results, P3-4)
3a-g: the n reported (Results, P5)
4b, f-h: the n reported (Results, P6)
5a: the n reported (Results, P7)
6b,c,d: the number of units and mice reported (Results, P8-9)

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.

A justification for the sample sizes was performed and included in our last grant submission, but was not explicitly included in the manuscript methods. We used a power analysis based on the formula:
$n > 2*((Z2\alpha+Z2\beta)S/D)^2$ (Armitage and Berry, 1987)
As this power analysis was not performed a priori, we have included a statement in the methods stating: "No statistical methods were used a priori to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications (Tan et al., 2008; McKay et al., 2005)."

In summary, we use a significance level (α) of 0.05, and we assume a power (β) of 0.8; therefore using a table for standardized deviates for two tail areas of normal distribution (Armitage and Berry, 1987), $Z2\alpha=1.96$ and $Z2\beta=0.842$. S is the standard deviation of frequency, based on our previous experience (McKay et al, 2005, Pagliardini et al, 2011). For example, in calculating phase shifts, conservative estimates of S and D based on our initial experiments would give a values of S=~20 degrees and D=~80 degrees. Our effects, at their peak, were in fact much stronger than this, but we wanted to make conservative estimates with limited data in front of us. Based on these numbers, the power analysis formula produced a needed n > 1. Given the large and consistent effect that was observed, our data was statistically significant after n = 3 in every grouping so we were comfortable with the numbers that we obtained.

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

   Where (section, paragraph #)?

Yes, we used t-tests when comparing only two groups and one-way ANOVAs for comparing multiple groups. This is described in the manuscript (Methods, #12). A Tukey HSD test was done post hoc for multiple comparisons.

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

Yes, this is clearly defined in the methods section in paragraph 12. Statistical tests are either t-tests for comparing two groups or ANOVA + Tukey HSD post hoc for multiple groups.

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 #)?

Data distribution was assumed to be normal but this was not formally tested.

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 #)?

Yes, all data values are reported as mean +- SEM and all plotted data contains errors bars representing the SEM as well. This is mentioned in the manuscript throughout the Results section and the Methods section (Methods, #12).

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

All tests completed are two-sided.

e. Are there adjustments for multiple comparisons?

Yes, the Tukey HSD test adjusts for multiple comparisons.

3. Are criteria for excluding data points reported?

Was this criterion established prior to data collection?

Where is this described (section, paragraph #)?

Data points were not excluded.

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 #)?

Microinjections of cre-dependent virus were performed with the investigator blind to whether the animal was Cre+ or Cre-.

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 #)?

While no explicit action was taken to unblind the investigator during the subsequent photostimulation or photoinhibition experiments, the investigator was effectively unblinded after the first few light pulses, since the light effect in positive animals was very significant and entirely absent in negative animals. This was not made explicit in the Methods section.

6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included?

Where (section, paragraph #)?

Yes, this is reported in the manuscript (Methods, #1). Animal use was in accordance with the guidelines approved by the UCLA Institutional Animal Care and Use Committee.

7. Is the species of the animals used reported?

Where (section, paragraph #)?

Yes, this is reported in the manuscript (Methods, #1).

8. Is the strain of the animals (including background strains of KO/ transgenic animals used) reported?

Where (section, paragraph #)?

Yes, the strain is reported in the manuscript. The mouse strain used is GlyT2-Cre and the background strain is C57Bl6 (Methods, #1).

9. Is the sex of the animals/subjects used reported?

Where (section, paragraph #)?

Yes, this is reported in the manuscript (Methods, #1).

10. Is the age of the animals/subjects reported?

    Where (section, paragraph #)?

Yes, this is reported in the manuscript (Methods, #1).

11. For animals housed in a vivarium, is the light/dark cycle reported?

    Where (section, paragraph #)?

Yes, this is reported in the manuscript (Methods, #1). It was 12:12 with the light cycle running from 9 am to 9 pm.

12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?

    Where (section, paragraph #)?

Yes, this is reported in the manuscript (Methods, #1).

13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)?

    Where (section, paragraph #)?

No, this was not reported. The only applicable experiments are the experiments involving photostimulation of awake, behaving ChR2-transfected mice. All of these experiments were done during the light cycle.

14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported?

    Where (section, paragraph #)?

N/A

    a. If multiple behavioral tests were conducted in the same group of animals, is this reported?

       Where (section, paragraph #)?

Yes, mice underwent a series of experiments that are described in the Methods from paragraphs #2-6. Transfected mice used for single-unit recording underwent virus injections (Methods, #2) and then a distinct terminal experiment involving concurrent photostimulation or photoinhibition while recording from neurons (Methods, #7).

15. If any animals/subjects were excluded from analysis, is this reported?

    Where (section, paragraph #)?

N/A

    a. How were the criteria for exclusion defined?

       Where is this described (section, paragraph #)?

N/A

    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 #)?

N/A

Reagents

1. Have antibodies been validated for use in the system under study (assay and species)?

Yes, all antibodies used in this manuscript have been validated and regularly used in the mouse brain for immunohistochemistry.

a. Is antibody catalog number given?

Where does this appear (section, paragraph #)?

The antibodies used are described in the manuscript (Methods, #9), including host species, concentration, company name, and catalog number. Just to repeat:
rabbit anti-SST-14 (T4103; Peninsula Labs)
mouse anti-NeuN (MAB377; Millipore)
chicken anti-GFP (GFP-1020; Aves Labs)
rabbit anti-glycine (AB5020; Millipore)

b. Where were the validation data reported (citation, supplementary information, Antibodypedia)?

Where does this appear (section, paragraph #)?

These four antibodies are frequently used in mice and have been well validated. The validity of the SST-14 antibody has been shown in both rat (Tan et al, 2008; 2010; Pagliardini et al, 2011) and mice (Bouvier et al, 2010; Kam et al, 2013). The GFP antibody has used successfully in mice as well (Bouvier et al, 2010). In addition, the glycine antibody has been extensively used and validated as a marker of glycinergic neurons in mice and rats (Poyatos et al, 1997; Zeilhofer et al, 2005). The NeuN antibody has been extensively used in mice throughout the field of neuroscience reearch.

2. If cell lines were used to reflect the properties of a particular tissue or disease state, is their source identified?

Where (section, paragraph #)?

N/A

a. Were they recently authenticated?

Where is this information reported (section, paragraph #)?

N/A

Data deposition

Data deposition in a public repository is mandatory for:

a. Protein, DNA and RNA sequences
b. Macromolecular structures
c. Crystallographic data for small molecules
d. 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.

1. Are accession codes for deposit dates provided?

Where (section, paragraph #)?

N/A

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.

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

I have written three custom scripts within the Igor software platform to analyze our data once collected in and exported from LabChart. The three scripts are computing values for: Phase Response (Results, #2-3, 5), Laser Delay (Results, #4), Inflation Reflex (Results, #6)

2. 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.

No, the scripts have not been deposited anywhere, but they are available upon request. It is worth noting that these scripts simply parse the data and perform the necessary calculations for graph preparation. There is nothing particularly novel about them. Analyzing the airflow traces in relation to laser pulses simply went beyond what LabChart could do out of the box.

Human subjects

1. Which IRB approved the protocol?

   Where is this stated (section, paragraph #)?

   N/A

2. Is demographic information on all subjects provided?

   Where (section, paragraph #)?

   N/A

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

   Where (section, paragraph #)?

   N/A

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

   Where (section, paragraph #)?

   N/A

5. How well were the groups matched?

   Where is this information described (section, paragraph #)?

   N/A

6. Is a statement included confirming that informed consent was obtained from all subjects?

   Where (section, paragraph #)?

   N/A

7. For publication of patient photos, is a statement included confirming that consent to publish was obtained?

   Where (section, paragraph #)?

   N/A

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?

   N/A

   a. If yes, is the number rejected and reasons for rejection described?

      Where (section, paragraph #)?

      N/A

2. Is the number of blocks, trials or experimental units per session and/or subjects specified? Where (section, paragraph #)?	N/A
3. Is the length of each trial and interval between trials specified?	N/A
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.	N/A
5. Is the task design clearly described? Where (section, paragraph #)?	N/A
6. How was behavioral performance measured?	N/A
7. Is an ANOVA or factorial design being used?	N/A
8. For data acquisition, is a whole brain scan used? If not, state area of acquisition.	N/A
a. How was this region determined?	N/A
9. Is the field strength (in Tesla) of the MRI system stated?	N/A
a. Is the pulse sequence type (gradient/spin echo, EPI/spiral) stated?	N/A
b. Are the field-of-view, matrix size, slice thickness, and TE/TR/flip angle clearly stated?	N/A
10. Are the software and specific parameters (model/functions, smoothing kernel size if applicable, etc.) used for data processing and pre-processing clearly stated?	N/A
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 #)?	N/A
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 #)?	N/A
13. How were anatomical locations determined, e.g., via an automated labeling algorithm (AAL), standardized coordinate database (Talairach daemon), probabilistic atlases, etc.?	N/A

14. Were any additional regressors (behavioral covariates, motion etc) used?	N/A
15. Is the contrast construction clearly defined?	N/A
16. Is a mixed/random effects or fixed inference used?	N/A
a. If fixed effects inference used, is this justified?	N/A
17. Were repeated measures used (multiple measurements per subject)?	N/A
a. If so, are the method to account for within subject correlation and the assumptions made about variance clearly stated?	N/A
18. If the threshold used for inference and visualization in figures varies, is this clearly stated?	N/A
19. Are statistical inferences corrected for multiple comparisons?	N/A
a. If not, is this labeled as uncorrected?	N/A
20. Are the results based on an ROI (region of interest) analysis?	N/A
a. If so, is the rationale clearly described?	N/A
b. How were the ROI's defined (functional vs anatomical localization)?	N/A
21. Is there correction for multiple comparisons within each voxel?	N/A
22. For cluster-wise significance, is the cluster-defining threshold and the corrected significance level defined?	N/A

Additional comments

Additional Comments	N/A