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 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 (*n*) 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 *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 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. *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

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	Behavior data was collected using Matlab 2017b (MathWorks) and RPvdsEx (Tucker Davis Technologies). Auditory stimuli for imaging experiments were produced using RPvdsEx (Tucker Davis Technologies). Two photon data was collected using ScanImage (Vidrio; version 3.1 and version 2021)
Data analysis	Two photon excitatory and axon imaging data was preprocessed using the Suite2p algorithm v0.12-0.13 (Patchiariu, et al., 2016). All behavior data and preprocessed two photon imaging was analyzed using custom code written in Matlab 2017b (MathWorks). Fiji and QuickNII (Puchades, et al., 2019) was used to process immunohistochemistry images. The code that support the findings of this study are further available on Github (https://github.com/kam953/Martin_et_al_2024) or from the corresponding author upon reasonable request.

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 data that support the findings of this study are further available on Github (https://github.com/kam953/Martin_et_al_2024) and figshare (https://figshare.com/projects/Vagus_nerve_stimulation_recruits_the_central_cholinergic_system_to_enhance_perceptual_learning_KA_Martin_et_al_/131516) or from the corresponding author upon reasonable request. Source data are provided with this paper. Allen Mouse Brain Atlas was used.

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	*n/a*
Reporting on race, ethnicity, or other socially relevant groupings	n/a
Population characteristics	n/a
Recruitment	*n/a*
Ethics oversight	*n/a*

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	The number of animals used for behavior experiments was determined by performing a power analysis with power of 0.8. For animals for the optogenetic inhibition experiments (Figure 7), we determined the number of animals necessary to detect a 11.7% increase in performance (seen in Figure 2e) with a power of 0.8. For other experiments, sample size was based on group size from published papers using similar methods (calcium imaging: Kuchibhotla et al. 2017, Betzel et al. 2019, Schiavo, et al., 2020)
Data exclusions	For calcium imaging analysis, neurons with GCaMP in the nucleus ('filled cells') with abnormal calcium traces were excluded from further analysis. This criteria was established based on previously published work showing that filled neurons exhibit abnormal electrophysiological properties (Tian et al. 2009; Yang et al. 2018). Experimental and sham VNS implanted animals that were not stimulated for at least 19 days were excluded so we could compare stimulation across days for consistent time periods.
Replication	All experiments were successfully reproduced across animals and neuronal populations. Our main behavioral results (Figure 2 and 3) were successfully replicated across two cohorts of animals and across several types of analyses. Behavioral results in Figure 6 and 7 were consistent

	across two cohorts of animals. Axon imaging results were consistent across two cohorts of animals. Neuronal responses over time were successfully reproduced across animals (Figure 4h-l).
Randomization	Animals could not be randomly assigned to experimental groups, as VNS cuff impedance determined whether cuffs were viable or not viable. Animals used during optogenetic experiments were randomly assigned to experimental group prior to onset of behavior when we injected either channelrhodopsin, Arch, or a fluorophore. Tones during both behavior and for two-photon imaging experiments were presented pseudorandomly such that the order was randomized, but there was a set number of trials for each frequency presented.
Blinding	Blinding was not performed since experimental group was determined by VNS cuff impedance (see above). For the optogenetic activation experiments with control, we added control animals and additional experimental animals to facilitate blinding. The experimenter was blind to the virus type (either Channelrhodopsin or tdTomato) injected prior to behavior onset.

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 antibodies (1:500): Chicken Anti GFP IgY (Abcam catalog # ab1397) and Rabbit Anti mCherry IgG (Abcam catalog # ab167453) Secondary antibodies (1:500): Goat anti-chicken IgY Alexa Fluor 488 (Thermo Fisher Scientific cat. # A11039) and Goat anti-Rabbit IgG (H+L) Cross-Absorbed Secondary Antibody, Alexa Fluor 555 (Thermo Fisher Scientific Part number # A21428)
Validation	On the manufacturer websites (Abcam or Thermo Fisher Scientific), a data sheet is provided for each antibody describing the antibody's specificity measured using Western blots and/or other protocols; antibodies were not otherwise validated.

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	70 mice were used for experiments. Mice were 6-20 weeks old with a C57BL/6J background. Throughout the experiments, we used: 31 wild type animals (15 males and 14 females; The Jackson Laboratory; Stock No: 000664), 37 ChAT-Cre animals (21 males and 16 females; The Jackson Laboratory; Stock No: 028861), and 4 TH-Cre animals (3 males and 1 female; The Jackson Laboratory; Stock No: 008601). Animals were housed at room temperature (~22 C) with relative humidity of ~45%.
Wild animals	This study did not involve wild animals.
Reporting on sex	This study considered sex in its design and used approximately the same number of animals of each sex for experiments (39 males, 31 females, additional information provided above). There were no significant sex based differences in learning rates (Figure 1e).
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	All procedures were approved under NYU Langone Institutional Animal Care and Use committee protocols.

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

Plants

Seed stocks	There were no seed stocks used in this study
Novel plant genotypes	There were no novel plant genotypes used in this study
Authentication	No novel seed stocks or genotypes were generated for this study.