

## Life Sciences Reporting Summary

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Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study. For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

### ► Experimental design

#### 1. Sample size

Describe how sample size was determined.

Due to the absolute novelty of PA-Nic, sample sizes were not determined prior to experimentation. We were guided by prior studies, cited in the Online Methods, for sample size expectations.

#### 2. Data exclusions

Describe any data exclusions.

Outlier data points were removed (rarely) via the ROUT method as described in the Online Methods.

#### 3. Replication

Describe the measures taken to verify the reproducibility of the experimental findings.

All experiments were independently replicated. Where samples were taken from animals, every reasonable effort was made to replicate findings in more than one animal.

#### 4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Where applicable, animals were randomly assigned to experimental groups via informal randomization.

#### 5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

For chronic nicotine treatments, investigators performing recordings were blind to the treatment during the first ~50% of data collection to assure unbiased results.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

#### 6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a Confirmed

- The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- A statement indicating how many times each experiment was replicated
- 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 any assumptions or corrections, such as an adjustment for multiple comparisons
- Test values indicating whether an effect is present  
*Provide confidence intervals or give results of significance tests (e.g.  $P$  values) as exact values whenever appropriate and with effect sizes noted.*
- A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on [statistics for biologists](#) for further resources and guidance.

## ► Software

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

### 7. Software

Describe the software used to analyze the data in this study.

Standard commercial software: MS Excel, GraphPad Prism, OriginLab, Clampfit, pClamp 10, MATLAB, PrairieView 5

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* [guidance for providing algorithms and software for publication](#) provides further information on this topic.

## ► Materials and reagents

Policy information about [availability of materials](#)

### 8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

All reagents and materials are available from the authors upon reasonable request.

### 9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

goat anti-ChAT (Millipore; cat# AB144P; clone n/a; lot 2843074)  
 rabbit anti-DsRed (Clontech; cat# 632496; clone n/a; lot not available)  
 anti-goat Alexa 488 (Invitrogen; cat# A11055; clone n/a; lot 1827671)  
 anti-rabbit Alexa 647 (Invitrogen; cat# A31573; clone n/a; lot 1826679)  
 Validation:  
 Supplementary Figure 2d: anti-ChAT and anti-DsRed staining cross-validate each other since their signals should overlap in medial habenula of ChAT-Cre::Ai14 mice.  
 Supplementary Figure 6c: anti-DsRed staining simply increased the signal from the endogenous tdTomato expressed specifically in GAD2 neurons in IPN.

### 10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

no cell lines were used in this study

b. Describe the method of cell line authentication used.

no cell lines were used in this study

c. Report whether the cell lines were tested for mycoplasma contamination.

no cell lines were used in this study

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by [ICLAC](#), provide a scientific rationale for their use.

no cell lines were used in this study

## ► Animals and human research participants

Policy information about [studies involving animals](#); when reporting animal research, follow the [ARRIVE guidelines](#)

### 11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

species: mus musculus  
 strain: C57BL/6 (Jackson Labs strain numbers 000664, 006410, 007914, 010802)  
 sex: male and female  
 age: 2 to 24 weeks old

Policy information about [studies involving human research participants](#)

### 12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

this study did not involve human research participants