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Corresponding author(s): Philip Ryan

☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

See Methods - Statistics and figure preparation section - paragraph 2.
A power analysis was performed for an effective sample size using http://powerandsamplesize.com. Based on a pilot study for fluid deprivation, we used a mean of 1.8 and standard deviation of 0.4. Assuming a significance level of 0.05 and power of 0.8, we calculated a sample size of 7 per group if means were 1.5-fold different with a two-tailed Student's t-test.

2. Data exclusions

Describe any data exclusions.

See Methods - Microscopy section.
Following imaging, any mouse whose targeted injection site was missed or demonstrated very sparse expression (≤6 fluorescent neurons/section), suggesting inadequate injection, was excluded from experimental analysis. In addition, any mouse that was unilaterally injected was included in experimental analyses for stimulatory (1 unilateral injection in each group of hM3Dq-injected OxtrPBN and OxtPVH mice) and control groups, but was excluded from inhibitory (TetTox- and hM4Di-injected) groups. For infusion experiments, mice whose targeted injection was missed were excluded (2 mice).

3. Replication

Describe whether the experimental findings were reliably reproduced.

See Methods - Mice section - paragraph 3.
All fluid and food experiments were performed with at least two cohorts of mice and data were combined, unless otherwise stated.

4. Randomization

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

See Methods - Mice section - paragraph 3.
Animals in each litter were randomly assigned to either experimental or control groups.

5. Blinding

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

See Methods - Fos experiments - paragraph 4; and Food and saline intake - paragraph 1.
1) The investigator who quantified Fos was blinded to the identity of the conditions.
2) The experimenter was not blinded to the identity of the experimental versus control groups.

Note: all studies involving animals and/or human research participants must disclose 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 (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals 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

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.	See Methods - Statistics and figure preparation - paragraph 1 Data analysis and generation of histograms were performed using GraphPad Prism Version 6.01 for Windows. Three-way mixed design ANOVA were performed using IBM SPSS Statistics for Windows v.20. Calcium fluorescence data was analyzed using OriginPro v. 2016.

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 for-profit company.	No unique materials were used.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	See Methods - immunohistochemistry section. The ordering information for the antibodies used are all referenced. The companies provide validation data for histological staining of fixed tissue.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	N/A
b. Describe the method of cell line authentication used.	N/A
c. Report whether the cell lines were tested for mycoplasma contamination.	N/A
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.	N/A

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 details on animals and/or animal-derived materials used in the study.	See Methods - Mice section - paragraphs 1 & 2 for details.

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.	N/A