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# Life Sciences Reporting Summary

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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.

Sample sizes were determined by the smallest sizes required for significant effects to reduce unnecessary animal use. Insignificant results were tested with a sample power analysis to ensure that tests were powerful enough to detect reasonable effects. Supplementary Methods, Section "Data analysis", paragraph 1

2. Data exclusions

Describe any data exclusions.

In experiments involving stereotaxic injections ("Viral vectors" section of Supplementary Methods), brains and animals with misses or partial hits of target areas were excluded from the study.

3. Replication

Describe whether the experimental findings were reliably reproduced.

yes, they were replicated in an appropriate number of cells and animals, as specifically described in figure legends and text

4. Randomization

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

1) In slice recordings, cells were randomly sampled throughout the full anatomical extent of NAc shell, by choosing
cells for recording using an objective that blinded the investigator to exact intra-NAc-shell location of the cell due to its small field of view
(high-magnification objective (x40) objective). After recording, the location of recorded cell was confirmed using a low-magnification large-field objective.
2) In whole-animal analyses, animals of the same genotype were grouped according to vectors injected into their brains,
and all groups had similar compositions based on sex (males), age, and bodyweight. Trials were interleaved in a Latin square to avoid order effects.

The above is explained in "Statistical analysis, blinding, randomization" Section of Supplementary Methods

5. Blinding

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

In slice recordings, cells were selected using an objective that blinded the investigator to exact intra-NAc-shell location of the cell due to its small field of view (x40 objective). In behavioral experiments, data collection and analysis were performed blind to the conditions of the experiments.

The above is explained in "Statistical analysis, blinding, randomization" Section of Supplementary Methods

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. | Data were analysed using commercial software: Origin Pro 8.6 (OriginLab), Prism 6.0 (GraphPad), ImageJ, and Ethovision XT (Noldus). No custom codes were used. |

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. | all materials are commercially available |

9. Antibodies

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

Commercial antibodies were used, which were validated in our system (mouse brain) both in previous publications, and within this study, as follows:

Anti orexin-A (sc-8070, 1:2000, Santa Cruz Biotechnology): the specificity of this antibody in our system (mouse brain) was previously validated by Western blotting and by observing a pattern of staining comparable to that of another antibody, as described in Brischoux et al, The Journal of Comparative Neurology 2008, 510: 607-630. Furthermore, the specificity of this antibody was also previously validated by our lab, in mouse brain slices, by demonstrating loss of staining in orexin cell knockout mice (Gonzalez et al, Current Biology 2016, 26(18): 2486-91).

Anti MCH (1:1000, Phoenix Pharmaceuticals, Burlingame, CA): the specificity of this antibody in our system (mouse brain) was previously validated by demonstrating loss of antibody staining in brain slices of MCH cell knockout mice (Whiddon and Palmiter, J Neurosci 2013, 33(5): 2009-2016).

In addition, we performed further checks of antibody specificity based on the following logic. The antigens we stained (orexin and MCH) are biologically found exclusively in the LH cells, and thus no staining in extra-hypothalamus regions served as a reasonable control for antibody specificity. We also tested the relevant antibodies in the same brains, and found no colocalisation between them, as expected from the biological reality (orexin and MCH peptides are biologically found in separate cells).

The specificity of secondary labels was confirmed by observing lack of cell body staining in mouse brain slice preparations where the primary antibody was omitted.

10. Eukaryotic cell lines

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

no eukaryotic cell lines were used

b. Describe the method of cell line authentication used.

no eukaryotic cell lines were used

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

no eukaryotic cell lines were used

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 commonly misidentified cell lines were used

## 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.

mice, different genetic strains (as described in detail in Supplementary Methods), male and/or female (as specified for different experiments in Supplementary Methods), age 8-12 week old (adult).

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.

the study did not involve human research participants