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	CLAX (columbus instruments) for metabolic parameters monitoring; Doric Neuroscience Studio (Doric lenses) for fiber photometry recording; EthoVision XT and MediaRecorder for Phenotyper recordings (Noldus) ClampFit 11.2
Data analysis	Microsoft Excel (2024), GraphPad Prism (v10.4.0), Adobe Illustrator CC (v28.3) for preparation of figures; Python (v3.11.5) within the JupyterLab environment (v3.6.3), MATLAB R2023a and RStudio (v4.3.0) for data analysis; Doric Neuroscience Studio (v6.5.0.0) for fiber photometry data analysis; Image J (v1.54m), CellProfiler for image processing; Oxymax (columbus instruments) for metabolic parameters data analysis; Additional code is available at https://github.com/UVACircMetNeuLab/glp1r-reward-circuit

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

Data and code available on LabArchives at https://mynotebook.labarchives.com/share/Guler%2520Lab%2520Notebook/MTQuM3wxMTY0NDYxLzExL1RyZWVOb2RlLzI1NDM4MDU2N3wzNi4z or on Github at https://github.com/UVACircMetNeuLab/glp1r-reward-circuit.

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	No humans participants in this study
Reporting on race, ethnicity, or other socially relevant groupings	No humans participants in this study
Population characteristics	No humans participants in this study
Recruitment	No humans participants in this study
Ethics oversight	No humans participants in this study

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	Sample sizes were based on prior experience with similar experiments. For behavioral and metabolic studies, we aimed for a minimum of 10 animals per group when possible. While we did not perform a formal power analysis for each experiment in this study, previous analyses in our lab (using Cohen's d) have shown that, with $\alpha = 0.05$ and power = 0.8, a sample size of ~6 animals per group is sufficient to detect a ~40% difference in mean with ~25% standard deviation, and ~12 animals are sufficient to detect a ~20% difference in mean with ~20% standard deviation. Sample sizes in this study therefore ranged from 6 to 12 animals per group.
Data exclusions	Animals were excluded if histological validation revealed mistargeted viral injections and/or fiber optic implant or lack of expression of the intended payload in the targeted brain region. This was determined post hoc through brain section analysis. For electrophysiology experiments, cells with unstable resting membrane potentials were excluded from the analysis and no cells were considered statistical outliers. For behavior in Phenotyper chambers, mice that failed to meet a baseline criterion of ≥50 food hopper head entries between ZT 12–14 were excluded to ensure sufficient engagement with the feeding setup. Of 59 mice tested, 12 did not meet this threshold and were excluded.
Replication	All experiments were independently replicated at least twice, and reproducibility was confirmed. Data from biological replicates were combined for final analysis and presentation.
Randomization	Animals were randomly assigned to experimental or control groups. For behavioral experiments, littermates were randomly distributed across conditions. When feasible, both control and treatment groups were tested simultaneously. The positions of animals in behavioral testing chambers were also randomized to prevent spatial bias.
Blinding	Blinding was not performed due to the limited number of researchers with the required expertise to carry out the experiments and interpret the data. However, we took care to ensure consistent experimental outcomes across replicates and relied on objective quantification methods (e.g., electrophysiology traces, automated behavioral readouts) to reduce subjectivity in data analysis.

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

All relevant information regarding the antibodies used is provided in the Methods section. The following primary antibodies were used for fluorescent labeling: anti-c-Fos (rabbit, 1:1000; Synaptic Systems, #226003, RRID:AB_2231974), anti-DsRed (rabbit, 1:1000; Takara Bio, catalog no. 632496, RRID:AB_10013483), anti-TdTomato (goat, 1:1000; Arigobio, catalog no. ARG55724), anti-hGLP1R (rabbit, 1:200; Invitrogen, catalog no. PA5-97789, RRID: AB_2812404), anti-HA (rabbit, 1:1000, Cell Signaling, catalog no. 3724), anti-Th (rabbit, 1:500; Chemicon, catalog no. AB152), and anti-GFP (goat, 1:500; Rockland, catalog no. 600-101-215). The secondary antibodies (Jackson ImmunoResearch) used were Cy2-conjugated donkey anti-rabbit (1:250; catalog no. 711-225-152, RRID:AB_2340612), Cy3-conjugated donkey anti-rabbit (1:250; catalog no. 711-165-152, RRID:AB_2307443), Cy5-conjugated donkey anti-rabbit (1:250; catalog no. 711-175-152, RRID:AB_2340607), Cy3-conjugated donkey anti-goat (1:250; catalog no. 705-165-147, RRID:AB_2307351), and Alexa-Fluor® 488 donkey anti-goat (1:250; catalog no. 705-545-003, RRID:AB_2340428).

Validation

All primary antibodies used contain RRID and have been validated for immunohistochemistry by the specific vendor and confirmed in our lab. Also, most of them have been validated in our previous publications. For anti-hGLP1R (Invitrogen, catalog no. PA5-97789, RRID: AB_2812404) we validate the antibody by comparing to tissue that does not express hGLP1R.
1) Anti-DsRed (rabbit, 1:1000; Takara Bio, catalog no. 632496, RRID:AB_10013483)
https://www.takarabio.com/products/antibodies-and-elisa/fluorescent-protein-antibodies/red-fluorescent-protein-antibodies?srsltid=AfmBOoqD91H3-b532bZmFEbmZhP2af8p6iNTVibmkLsKFiGD-gF8fPyY
2) anti-TdTomato (goat, 1:1000; Arigobio, catalog no. ARG55724)
https://www.arigobio.com/anti-tdTomato-antibody-ARG55724.html
3) anti-hGLP1R (rabbit, 1:200; Invitrogen, catalog no. PA5-97789, RRID: AB_2812404)
https://www.thermofisher.com/antibody/product/GLP1R-Antibody-Polyclonal/PA5-97789
4) anti-HA (rabbit, 1:1000, Cell Signaling, catalog no. 3724)
https://www.cellsignal.com/products/primary-antibodies/ha-tag-c29f4-rabbit-monoclonal-antibody/3724?srsltid=AfmBOop53B9GSxUDmSteNbLtMokdrpydLCLIf9CswdIAp86HB7bTsoRe
5) anti-Th (rabbit, 1:500; Chemicon, catalog no. AB152)
https://www.sigmaaldrich.com/US/en/product/mm/ab152?srsltid=AfmBOop81-1Y0UfCXvLd2x5JIrryQe4syIGPogRy_T4r62ZyFngwtjgv
6) anti-GFP (goat, 1:500; Rockland, catalog no. 600-101-215)
https://www.thermofisher.com/antibody/product/GFP-Antibody-Polyclonal/600-101-215M
Note: anti-c-Fos (rabbit, 1:1000; Synaptic Systems, #226003, RRID:AB_2231974) has been discontinued but well validated before. Example: Zan et al., 2025, Neuron, PMID: 41043420

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

We used 8-week or older male and female mice. Strains information:
1) C57BL/6J (Strain #:000664, RRID:IMSR_JAX:000664)
2) Glp1r-ires-Cre (STOCK Glp1rtm1.1(cre)Lbrl/RcngJ, Strain #:029283, RRID:IMSR_JAX:029283)
3) Glp1r S33W mice have been generated in this study and available on JAX (STOCK Glp1rem1Aglu/J, Strain #:040551 RRID:IMSR_JAX:040551
4) Dat-ires-Cre (B6.SJL-Slc6a3tm1.1(cre)Bkmn/J, Strain #:006660, RRID:IMSR_JAX:006660)
5) Gcg-Cre mice (C57BL/6J-Tg(Gcg-cre)-1Mmsc/Mmmh, stock #051056-MU, RRID:MMRRC_051056-MU)
6) Ai14 tdTomato reporter line (B6.Cg-Gt(ROSA)26Sortm14(CAG-tdTomato)Hze/J, strain #007914, RRID:IMSR_JAX:007914)
7) Glp1r flox/flox mice (B6(SJL)-Glp1rtm1.1Stof/J, strain #035238, RRID:IMSR_JAX:035238)

Wild animals

No wild animals were used in this study.

Reporting on sex	We used both male and female mice for most experiments. In cases where a specific sex was excluded, a scientific rationale is provided. Full details are available in the main text and Methods section.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	All experiments were conducted in compliance with the Association for Assessment of Laboratory Animal Care policies and approved by the Institutional Animal Care and Use Committees of the University of Virginia, University of Washington, and University of California, Irvine.

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

Plants

Seed stocks	No plants were used in this study
Novel plant genotypes	No plants were used in this study
Authentication	No plants were used in this study