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	LabJack LJStreamM from Doric photodetector (photometry signals) Customed Python program for Arduino (Licking and various event timestamps)
Data analysis	R v4.1: GLMM statistical analysis MATLAB R2021a, R2022a: Data preprocess and construction; Standard statistical analysis; custom in-house code deposited with Code Ocean as described in Code Availability section.

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

Source data are provided in a Source Data file. Examples of raw data are provided as Supplementary Data files.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	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 sample size for mouse studies was established by ensuring adequate statistical power, drawing insights from comparable previous research. Nevertheless, we identified increased variabilities in learning capabilities, dopamine signal reactions to cues and rewards, probe placements, and instances of unexpected learning difficulties among certain mice in the early stage of the study. Consequently, we augmented the number of subjects for the experimental conditions.
Data exclusions	Data were collected on a daily basis; however, for the purposes of this longitudinal study, we specifically focused on the relevant sessions. It's worth noting that there were instances where the implant became detached, rendering the data unrecoverable. Additionally, a few mice did not meet the required learning index criteria and exhibited a consistently low discrimination index over an extended period. In such cases, mice that failed to meet the learning criteria were excluded from the subsequent phases of the study.
Replication	Every mouse participating in the study experienced an identical learning paradigm, which encompassed daily exposure to both the cue and reward within the apparatus. Our study was composed of multiple cohort studies, as opposed to a single cohort study, with a small-scale replication integrated into the current research. Although there were nuanced distinctions in how individual subjects developed lick response patterns, these differences eventually converged into similar profiles. This convergence underscores the internal validity of the study's design and the replication of its outcomes.
Randomization	This study involved the randomization of several factors. Firstly, we utilized an F1 hybrid line (FVB x B6) bred in-house. To ensure diversity within each cohort, we intentionally separated siblings into distinct experimental groups and selected study mice from various parentage as much as possible. Secondly, surgical preparations were conducted solely based on the age of the subjects. Thirdly, the assignment of the experimental condition for the initial conditioning cue side was randomized. Lastly, we readied a set of nine identical recording apparatus. During daily training sessions, each mouse was assigned to a randomly chosen chamber to prevent potential adaptation to a specific environmental setup.
Blinding	The surgical preparation was carried out without knowledge of the experimental groups. Additionally, the experimenters remained unaware of the learning performance level exhibited by each individual subject.

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

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used: Chicken polyclonal anti-GFP antibody (abcam, ab13970) at 1:2000 dilution. Rabbit monoclonal anti-mu opioid receptor antibody (abcam, ab134054) at 1:500 dilution. Goat anti-chicken Alexa Fluor 488 (Thermofisher, A-11039) and Goat anti-rabbit Alexa Fluor 647 (Thermofisher, A-21245) at 1:300 dilution.

Validation: Anti-GFP antibody (abcam, ab13970) is widely used and has been referenced in more than 3000 publications. Specification of anti-mu opioid receptor antibody (abcam, ab134054) was validated in several publications such as PMID 20851148, 21957251 and 26290245.

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: Strain information is stated in method in the manuscript as 'F1 hybrids on C57BL/6J (Jackson Laboratory, strain ID #: 000664) and FVB (Taconic, model #FVB) background with the approval of the Committee on Animal Care at the Massachusetts Institute of Technology (MIT). F1 hybrids were produced from FVB mice in which Pde6brd1 and Disc1 were bred out ('corrected FVB').' The experiment took place when the mice were between 3 and 6 months old, encompassing the surgical preparation as well.

Wild animals: N/A

Reporting on sex: Both male (n = 41) and female (n = 26) mice were used. Total 67 mice included.

Field-collected samples: No field-collected samples were used in this study.

Ethics oversight: All mouse husbandry and experimental procedures were conducted with the approval of the Committee on Animal Care at the Massachusetts Institute of Technology.

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