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☐ 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.

For imaging experiments, between 3 and 7 mice were imaged per experimental paradigm; the number of mice was dependent on the yield of usable mice and the number of animals initially implanted and injected. All together, 122 animals were implanted for these experiments and 25 yielded usable mice, defined as those in which a field of well-expressing neurons was obtained. This was determined by screening before experimentation (Methods; paragraph 2). Selection was not based on any pre-specified effect.
The sample size for the number of cells was 175-300 cells per mouse, and was affected by the volume of viral GCaMP6s injected, the efficiency of Cre-mediated recombination and level of expression. While post-processing the imaging data (after PCA-ICA identification of individual cells) 1-5% of the identified components were discarded as artifacts and rest 95-99% were treated as cells and used for further analysis.

Sample sizes for behavioral experiments were determined by the current standard used for mice in behavioral neuroscience experiments, based on the minimal amount of mice required to detect significance with an alpha rate set at .05 in a standardly powered experiment.

2. Data exclusions

Describe any data exclusions.

Imaged mice were excluded from across-day learning analysis if the mice did not exhibit the social behaviors (mounting and aggression) associated with learning. However a comparison between examples of excluded vs included mice is presented in the manuscript.
Mice were excluded from analysis of unit (cellular) behavioral tuning (decoder performance & choice probability) if they did not show at least 2 seconds of the behavior of interest on a given day; this was done to avoid inaccurate measures of unit tuning.
Mice were excluded from the prolonged social interaction assay if they 1) did not mount the female encountered on day 1, or 2) showed separation of male and female representations prior to the day 1 exposure to conspecifics.

3. Replication

Describe whether the experimental findings were reliably reproduced.

Each experiment presented in the paper was repeated in multiple animals (between 3 and 7 per experiment). While a few animals were excluded from analysis due to atypical behavior (see above , Data exclusions and below), all results in the paper are drawn from the analysis of multiple animals. Importantly, the plasticity effects were consistent across experimental paradigms, whether they involved multiple brief exposures or a single prolonged exposure to conspecifics. We observed several cases of atypical mice that did not exhibit the appropriate behaviors even after multiple trials of social experience, and whose neural representations also did not segregate. These mice are included and discussed in the paper as evidence of individual differences within the population studied. They represented ~15-20% of animals and were observed at a similar frequency among unoperated controls. All of this information is presented in the text.

4. Randomization

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

Animals were assigned randomly to experimental and control groups, and within-animal controls were performed wherever possible.

5. Blinding

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

The investigators were not blinded during data collection. Blinding was used during analysis where the social behaviors were scored blind to the sex of the intruder mouse. Computational analysis was not performed blinded.

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 <u>exact sample size</u> (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 <u>central tendency</u> (e.g. median, mean) and <u>variation</u> (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.

A description of the software and code has been included in the Methods. Initial post-processing of raw data was performed using the Mosaic software package from Inscopix. Subsequent computational analysis was performed using commercial/custom code written in Matlab R2015a and is publicly available at: https://github.com/DJALab/VMHvl_imaging

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 restrictions will be placed on materials involved in this study. Mouse strains, viral vectors, implants and other hardware are already commercially available and sources have been mentioned in the Methods section of the manuscript.

9. Antibodies

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

No antibodies were used.

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.

> 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.

> Details on the mice used in this study have been included in the Methods section of this manuscript.
> Esr1-Cre knock-in mice, back-crossed into the C57BL6/N background (more than 10 generations), were bred and raised at Caltech. They are available from the Jackson Laboratory (JAX) as strain B6N.129S6(Cg)-Esr1tm1.1(Cre)And/J (stock number 017911). Only males, 12-14 weeks of age, were used in this study.
>
> Additional mice used for social interactions were males and ovariectomized females purchased directly from Charles-River labs at 12-14 weeks of age.

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.

> Study did not involve human research participants.