

## 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

Collection of fluorescence video:  $\mu$ Manager software(  $\mu$ Manager, version 1.4),  
Control of behavior task: Arduino IDE (Arduino, version 1.8 )  
Recording of pupil diameter: Spinnaker SDK software (FLIR Systems, version 1.2 )  
Coordination/triggering of all hardware: Spike2 (Cambridge Electronic Design Limited, version 5.21 ),

Data analysis

All data was analyzed with custom MATLAB (Mathworks) code, which are made available in GitHub repositories at [github.com/west0883/](https://github.com/west0883/).  
Individual repositories and their purpose are listed in Table 3 within the manuscript

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 generated and analyzed in this study are available from the authors upon request.

## 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](https://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

No sample-size calculation was performed, but the determination of the number of animals needed was based on past wide-field calcium imaging studies using similar imaging and analytic techniques.

Data exclusions

One mouse (mouse #4) only rarely walked spontaneously (6% of the number of walk periods as the next-lowest walking mouse) and was not included in the spontaneous locomotion analysis. Exclusion criteria were not pre-established.

Replication

The experiments were performed in 7 animals across a large number of trials (~1500 total instances per behavior period across animals).

Randomization

The order of motorized treadmill periods and parameters were randomized at the start of each trial.

Blinding

Blinding was not performed during data collection or analysis. Blinding was not necessary as all analyzed measurements were performed with automated processes applied the same to all data.

## 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
<input checked="" type="checkbox"/>	Antibodies
<input checked="" type="checkbox"/>	Eukaryotic cell lines
<input checked="" type="checkbox"/>	Palaeontology and archaeology
<input type="checkbox"/>	Animals and other organisms
<input checked="" type="checkbox"/>	Clinical data
<input checked="" type="checkbox"/>	Dual use research of concern

**Methods**

n/a	Involved in the study
<input checked="" type="checkbox"/>	ChIP-seq
<input checked="" type="checkbox"/>	Flow cytometry
<input checked="" type="checkbox"/>	MRI-based neuroimaging

**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

Seven C57BL/Thyl-GCaMP6f mice 3-12 months in age were used in these experiments (The Jackson Laboratory).

## Wild animals

N/A

## Reporting on sex

Sex was not considered in the study design. Seven animals were used (3 female, 4 male). The number of animals of each sex included in this study is not sufficient for meaningful evaluation of potential differences between sexes.

## Field-collected samples

N/A

## Ethics oversight

All animal studies were approved by and conducted in conformity with the Institutional Animal Care and Use Committee of the University of Minnesota.

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