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Corresponding author(s): Dr Dorela Shuboni-Mulligan

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Reporting Summary

Nature Research 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 Research policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

Statistics

| For | all st | atistical 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. | |
| X | | 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted Give <i>P</i> values as exact values whenever suitable. | |
| × | | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings | |
| X | | 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 <i>d</i> , Pearson's <i>r</i>), 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 | n about <u>availability of computer code</u> |
|--------------------|--|
| Data collection | Ethovision XT 14 with Mouse behavior Module and ParaVision 5 |
| Data analysis | IBM SPSS, FIJI, Cosinor Software (Roberto Refinetti, circadian.org), and 3D Slicer 4.8 |

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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

The data that support the findings of this study are available from the corresponding author upon request.

Field-specific reporting

Life sciences study design

| Sample size | Sample sizes were determined by the authors experience with circadian rhythms, needing a minimum of 8 mice per group. |
|-----------------|---|
| Data exclusions | No available data was excluded from the experiments. |
| Replication | We are currently using the video scoring system in subsequent experiments and find similar results in young mice. The results of the study find similar but more detailed findings as those reported in the literature. |
| Randomization | The experiment examined age and could therefore not be randomly assigned. |
| Blinding | Scorers of behavior and brain volume size were blinded to the groups during the process. |

All studies must disclose on these points even when the disclosure is negative.

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

Methods

| n/a | Involved in the study | n/a | Involved in the study |
|-----|-------------------------------|-----|------------------------|
| × | Antibodies | × | ChIP-seq |
| × | Eukaryotic cell lines | × | Flow cytometry |
| × | Palaeontology and archaeology | | MRI-based neuroimaging |
| | 🗴 Animals and other organisms | | • |
| × | Human research participants | | |
| × | Clinical data | | |
| × | Dual use research of concern | | |

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | Male C57BL/6 Mice at 6 weeks and 18 months old |
|-------------------------|--|
| Wild animals | n/a |
| Field-collected samples | n/a |
| Ethics oversight | IACUC at the NCI, NIH Bethesda MD |

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

Magnetic resonance imaging

Experimental design Design type Design specifications Mice were perfused w

| Design specifications | Mice were perfused with Gad doped Para-formaldehyde and placed in Flourinert and scanned with a 14.1T Bruker Microimaging. |
|---------------------------------|--|
| Behavioral performance measures | n/a |
| Acquisition | |
| Imaging type(s) | Structural |
| Field strength | 14.1 |
| Sequence & imaging parameters | FLASH sequence, TE=5ms, TR=50ms, FOV=1.6x0.8x0.8cm, Resolution=32x32x32um, Averages=10 |
| Area of acquisition | Whole brain |

🗶 Not used

Used

Preprocessing

| Preprocessing software | FIJI and 3D Slicer 4.8 |
|----------------------------|------------------------|
| | |
| Normalization | (n/a |
| | |
| Normalization template | (n/a |
| | |
| Noise and artifact removal | (n/a |
| | |
| Volume censoring | n/a |
| | |
| | |

Statistical modeling & inference

| Model type and settings | n/a | |
|--|---|--|
| Effect(s) tested | n/a | |
| Specify type of analysis: 🗌 Whole brain 🔲 ROI-based 🛛 🕱 Both | | |
| Anato | omical location(s) A neuro-anatomical expert identified structures and defined them manually. | |
| Statistic type for inference (See <u>Eklund et al. 2016</u>) | n/a | |
| Correction | n/a | |
| | | |

Models & analysis

n/a Involved in the study

| × | Functional and/or effective connectivity |
|---|--|
| × | Graphanalysis |

X Multivariate modeling or predictive analysis