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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.

Sample size was chosen based on preliminary experiments and literature in the field.

2. Data exclusions

Describe any data exclusions.

No data was excluded.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All attempts at replication were successful under the experimental conditions defined.

4. Randomization

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

After eclosion, virgin female flies with the same genotype were sorted into groups of 10-15 flies per vial at random. All flies in each vial were administered with the same treatment regime. For each experiment, the experimental and control flies were collected, treated, and tested at the same time.

5. Blinding

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

Blinding was not performed as almost all data acquisition and analysis was automated.

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 exact sample size (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 central tendency (e.g. median, mean) and variation (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.

Daily activity measurements were taken using Drosophila activity monitors (DAMS, Trikinetics) and actograms were generated using the ActogramJ software. For video-assisted tracking experiments, Ethovision or the Caltech FlyTracker (http://www.vision.caltech.edu/Tools/FlyTracker/) software were used to process videos. Bout analysis was subsequently performed using simple custom python scripts, which are available upon request. Gait analysis was performed using the FlyWalker software package (http://biooptics.markalab.org/FlyWalker/). All statistical analysis was performed using Prism Software (GraphPad, version 7).

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 unique materials were used.

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.

No eukaryotic cell lines were used.

c. Report whether the cell lines were tested for mycoplasma contamination.

No eukaryotic cell lines were used.

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.

No commonly misidentified cell lines were used.

▶ 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.

For our studies, we used the model organism, Drosophila melanogaster. Majority of the experiments used 7-day-old adult virgin female flies, except for Extended Data Figure 1b in which 7-day-old male flies were also used. The fly lines used include: Canton-S (#64349), Imd-/- (#55711), UAS-dTrpA1 (#26264), Tdc2-GAL4 (#52243), TβH-GAL4 (#48332), Th-GAL4 (#8488), Ddc-GAL4 (#7009), Gad1-GAL4 (#51630), ChAT-GAL4 (#60317), Elav-GAL4 (#46655), UAS-TβhRNAi (#27667), UAS-DTI (#25039), and pBDPG4U-GAL4 (#68384) lines from Bloomington Drosophila Stock Center at Indiana University. Other fly stocks used were OregonR (kindly provided by A. A. Aravin and K. Fejes Tóth), TβHM18 (kindly provided by M. H. Dickinson) Tdc2R054, and tsh-GAL80 (kindly provided by D. J. Anderson). To minimize the effect of genetic background on behaviors, mutant fly lines were outcrossed for at least three generations onto a wild-type background.

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.

This study did not involve human research participants.