



## eLife's transparent reporting form

We encourage authors to provide detailed information *within their submission* to facilitate the interpretation and replication of experiments. Authors can upload supporting documentation to indicate the use of appropriate reporting guidelines for health-related research (see [EQUATOR Network](#)), life science research (see the [BioSharing Information Resource](#)), or the [ARRIVE guidelines](#) for reporting work involving animal research. Where applicable, authors should refer to any relevant reporting standards documents in this form.

If you have any questions, please consult our Journal Policies and/or contact us: [editorial@elifesciences.org](mailto:editorial@elifesciences.org).

### Sample-size estimation

- You should state whether an appropriate sample size was computed when the study was being designed
- You should state the statistical method of sample size computation and any required assumptions
- If no explicit power analysis was used, you should describe how you decided what sample (replicate) size (number) to use

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

No explicit power analysis was used. Detailed information about sample size can be found in the figure legends. At least 5 flies and tens to hundreds of cells were used for each condition and genotype. The number of flies in behavior and flies (cells) in calcium imaging was similar or bigger than in previous articles using the same methods. All statistics were done between flies, to avoid underestimation of the error. Details can be found in the "Behavioral analysis" and "Two photon data analysis" sections of the material and methods.

### Replicates

- You should report how often each experiment was performed
- You should include a definition of biological versus technical replication
- The data obtained should be provided and sufficient information should be provided to indicate the number of independent biological and/or technical replicates
- If you encountered any outliers, you should describe how these were handled
- Criteria for exclusion/inclusion of data should be clearly stated
- High-throughput sequence data should be uploaded before submission, with a private link for reviewers provided (these are available from both GEO and ArrayExpress)

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:



For behavioral experiments, each fly was considered a biological replicate. Flies with forward walking speed below a threshold (2mm/s) were discarded from every experimental condition. Further details can be found in the Material & Methods section of the manuscript.

For 2 photon imaging, each fly (but not cells within one fly) was considered a biological replicate. Further details can be found in the Material & Methods section of the manuscript.

No outliers were encountered.



### Statistical reporting

- Statistical analysis methods should be described and justified
- Raw data should be presented in figures whenever informative to do so (typically when N per group is less than 10)
- For each experiment, you should identify the statistical tests used, exact values of N, definitions of center, methods of multiple test correction, and dispersion and precision measures (e.g., mean, median, SD, SEM, confidence intervals; and, for the major substantive results, a measure of effect size (e.g., Pearson's r, Cohen's d)
- Report exact p-values wherever possible alongside the summary statistics and 95% confidence intervals. These should be reported for all key questions and not only when the p-value is less than 0.05.

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

Details on statistical methods can be found in the Material & Methods section of the manuscript. Statistical tests, methods of multiple test correction and exact value of N (for both #flies and #cells) are also given in the figure legends. The code provided along with the data files (see the 'Additional data files' section below) also includes code for statistical testing.

(For large datasets, or papers with a very large number of statistical tests, you may upload a single table file with tests, Ns, etc., with reference to sections in the manuscript.)

### Group allocation

- Indicate how samples were allocated into experimental groups (in the case of clinical studies, please specify allocation to treatment method); if randomization was used, please also state if restricted randomization was applied
- Indicate if masking was used during group allocation, data collection and/or data analysis

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

Each genotype constituted an experimental group. Therefore, randomization and masking were not applicable to our study.

### Additional data files ("source data")

- We encourage you to upload relevant additional data files, such as numerical data that are represented as a graph in a figure, or as a summary table
- Where provided, these should be in the most useful format, and they can be uploaded as "Source data" files linked to a main figure or table
- Include model definition files including the full list of parameters used
- Include code used for data analysis (e.g., R, MatLab)
- Avoid stating that data files are "available upon request"

Please indicate the figures or tables for which source data files have been provided:



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All data used to generate plots (individual fly/cells responses), i.e. response traces over time, peak velocities, slopes etc, are provided in source data files as either “.mat” (MatLab) or “.pickle” (Python) files. Mean and SEM values can be extracted using the provided analysis code.

Analysis code in Matlab and Python is available at Github:

<https://github.com/silieslab/Ketkar-Gur-MolinaObando-etal2022>.

Data are available on Zenodo: <https://doi.org/10.5281/zenodo.6335347>.