



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.

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:

This information can be found in both method section, lines 882-889 and in the figure legends 8 and 9.

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:



I would like to state that due to the nature of this study, which is mainly neuroanatomical and molecular signature analysis of PACAP signaling, within the known sensory-motor hubs and circuits, the above points do not apply entirely to this study.

However, for the final behavioral part we wanted to make an example of our analysis and conclusions, we stated the number of animals used and the reasons for sample size determination, both in the method sections and in the figure legends 8 and 9.

In particular, for DISH histochemistry procedure, lines 816 "Mice (n=5/group)..."; for behavior experiment: lines 843-844: "Subjects: C57BL/6N wild type and PACAP deficient male mice, age 8-10 weeks, n=9 (N=18) were used". This latter sample size was determined based on the availability of animals, pilot data and published literature.

For "criteria for exclusion of data...": we did exclude 4 of the PACAP deficient subjects and their wild type pairs and we declared (lines 870 - 872): "The jumping behavior (movement in the Z-dimension) is only symbolized with *zig-zags* (in motion traces). Four mice exhibited this behavior and due to the aim of this study - the place preference assessment within X, Y dimensions - these subjects were discarded for the motion trace analysis (reflected in pixels in 2D heat maps). PowerPoint traces were skeletonized with ImageJ and movement traces from n = (9-5=) 5 animals were used".



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:

This information can be found in the legends for figures 8 and 9.

(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:

For behavioral assessment the off-line analyst was blind to the experimental condition of the animal (line 858-859). Other points do not apply here.

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:

The script for constructing the heatmap of figure 8 is included in this submission.