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:

Sample size was determined according to the accepted practice for animal research and but no statistical methods were used to predetermine sample size. We used no less than 10 animals per experiment (excluding Fig. 6I and mosaic analysis of UAS:EGFP and UAS:Cdc42-(T17N)-EGFP in figure 7C,D). This is due to technical challenges in labeling of OXT neurons specifically projecting to neurohypophysis (Wircer et al eLife 2017) and further compounded by the fact that injections were performed in eggs from incross of robo2 +/- fish.

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:

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The number of replicates and repeats were determined according to the customary in the field. Each experiment was performed at least twice. In the STORM imaging (Fig. 3A-D; Fig. 6D-E), ISH expression (Fig. 4A) and mosaic overexpression assays (Fig. 3I-N, 7C,D, Fig. 7 – fig supplement 1), we used biological replicates only, as multiple synapses from each animal were imaged and/or quantified.

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:

Data is presented as mean ± standard error of the mean (SEM) and analyzed using PRISM software. All data sets were tested for departures from normality with Shapiro-Wilks test. Students t-test or Mann-Whitney was used for all comparisons between two groups. ANOVA or Kruskal-Wallis H test (when samples departed from normal distribution) were used for comparing multiple groups. Two-way ANOVA was used when necessary. All data sets were corrected for multiple comparisons. Dunn's pairwise comparisons, student t-test and Bonferroni comparisons were used as post-hocs. Cohen's d test was performed to measure effect size. Detailed statistical description and sample size are found in the figure legends and methodology.

(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:

Larvae were randomly selected and assigned for DMSO vs Cytochalasin D treatments (Fig. 3E-H). For experiments with *robo2* mutants, larvae from in cross of heterozygous adults were genotyped post-imaging.

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:

Source data as summary tables is provided for all graphs and plots shown (Fig. 1E; 2B-E and G-J, 3E-H and J,K,M,N, 4D-F, 5O,R, 6I, 7C,D). R codes used for data import and analysis is provided.