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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 <u>EQUATOR Network</u>), life science research (see the <u>BioSharing Information</u> <u>Resource</u>), or the <u>ARRIVE guidelines</u> 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: <u>editorial@elifesciences.org</u>.

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 sizes were determined using power analysis based on preliminary data or through literature searches. Assuming a 30% change in behavior or physiology is meaningful, with a power level of β =0.80 and a significance level of α =0.05, the sample size used for experiments is at least n=8/group. For these reasons, most of our behavioral data have n=10/group and physiology recordings have at least n=8 cells/group.

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:

Biological replicates are repeated measurements of biologically distinct samples that capture random biological variation. Technical replicates are repeated measurements of the same sample that represent independent measures of the random noise associated with protocols. For behavioral tests, biological replicates (two cohorts of mice) were used to account for availability of transgenic animals, EtOH drinking variability, and adequately powered sample size. For immunohistochemistry, while multiple cohorts of tissue were collected, all biological replicates were run in the same immunostain with the same imaging and analysis parameters. Further, we took four slices per animal and measured bilateral c-Fos and Pdyn expression. For electrophysiology, again, multiple cohorts of transgenetic mice were used as biological replicates. Outliers were tested with Grubb's test. One cell was excluded as it fell outside the inclusion criteria. This information is outlined in the Statistics section.



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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:

The statistical tests performed for each experiment are described in the Statistics section; however, each statistical comparison is stated in the Figure Legend. Raw data points are presented in the Figures in addition to the Mean +/- SEM. For slice recordings, both the number of cells and number of mice are reported in the Figure Legends. Exact p-values are reported for all analyses.

(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:

C57BL/6J mice from Jackson laboratory were randomly assigned to treatment groups. Transgenic mice were bred in-house and litter mates were divided up into different treatment groups. Behavioral data were recorded and quantified by Ethovision XT13, and burying behavior was hand-scored using BORIS (Behavioral Observation Research Interactive Software) by a blind observer. This information is found in the Methods section.

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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Representative behavioral heatmaps of time and space in the homecage (Fig 1, 3, 5, and 7), c-Fos and in situ hybridization immunohistochemistry (Fig 2), spontaneous synaptic transmission traces (Fig 4) and optically-evoked electrophysiological traces (Fig 6) are shown in the Figures. We do not provide extra data tables, but are willing to do so.