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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:

We performed a bootstrapping-based power analysis described in the Methods section ("Analysis of behavioral data" subheader).

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:

We included detailed biological sample size information in Figure 1-table supplement 1. Additionally, technical replication information (bootstrapping) is present in all Figure legends, and detailed bootstrapping methods are described in the Methods section ("Analysis of behavioral data" subheader). Data exclusion criteria are clearly stated in the Methods section ("Behavioral data selection" subheader).



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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:

Detailed statistical methods are described in the Methods section ("Analysis of behavioral data", "Quantification of timescales from the GLM", "False discovery rate correction" subheaders). N and measures of center and dispersion are clearly specified in figure legends and figure 1-table supplement 1. Test, statistics and p-values are included in the main text. Exact p-values are given where possible, although note that for bootstrapping tests we report with p < x, since the exact p-value depends strongly on the choice of number of iterations. We have a resolution of 0.0001 for most reported tests.

(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:

Not applicable. All of our experiments have a within-subject design, i.e. every subject received optogenetic inactivations, and controls are laser-off trials. Separate controls validating the approach by performing laser experiments in mice not expressing ChR2 were performed in the context of a previous manuscript (Pinto et al 2019). We state this in the Results 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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A git repository with fully commented Python code to analyze the data and generate all figures is under final stages of preparation and will be made available upon publication of the manuscript (https://github.com/BrainCOGS/PintoEtAl2020 subtrial inact).

Some of the functions used to analyze Ca2+ imaging data in Figure 3 (GLM fitting in Matlab) are already publicly available at https://github.com/BrainCOGS/widefieldImaging. We will also deposit the full dataset

upon acceptance of the manuscript and include the data DOI in its final version.