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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

There was no prior information on the effect sizes and variability with this strategy. We therefore estimated the number of mice would be conservatively the same as in a previous set of experiments that used a manual approach (Browne et al 2017). For Figures 2D, 2E and Figure 3, we used 8 mice each with 6 trials. For Figure 2F, we used 6 mice with 10 trials each for the response probabilities to patterns of stimuli. The automated quality control described in Materials and methods slightly reduced these numbers as clearly stated in the figure legends. For Figure 4B we used 8 mice each with 3-6 trials, and for Figure 4E and 4F we used 10 mice each with the first 8 trials that passed quality control as described in Materials and methods. For Figure 5, we used 11-12 mice.

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 and technical replicates are stated in each figure legend and in Materials and methods. Experiments were conducted using six or more mice from at least two litters (biological replicates). On each experimental day, mice were tested with multiple trials (technical replicates) for multiple conditions. We did not perform any outlier tests. In Figure 2F, one of the seven TRPV1-Cre::ChR2 mice was removed from the dataset because it was unusually hypersensitive to stimuli - displaying saturating responses to stimuli that were subthreshold (Protocol 3) in all other mice tested - preventing comparison of values across a dynamic range.



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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:

Details of the statistical tests used are provided in the Statistics subsection of the Materials and Methods section. Statistical values are given in the figure legends and the main text.

(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:

All experimental designs were within-subject (all animals underwent the same stimulation protocols). As a result, no randomization or blinding of animal identity was carried out during development and characterization of the system. Stimuli were delivered in different pseudorandomised sequences where possible. Behavioral analysis was fully automated using custom code.

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:

All components necessary to assemble the optical system are listed in Figure 1 - table 1. A Solidworks assembly, the optical system control and acquisition software and behavioral analysis toolkit are available at https://github.com/ browne-lab/throwinglight. The data that support the findings of this study are associated with figures as source data.