

Supplementary Figure 1. Response reliability in stimulus, decision and other neurons.

a) Coefficient of variation of stimulus, decision, and other neurons. b) Response reliability measured as fraction of trials with a response in stimulus, decision, and other neurons. c) Validation accuracy of a classifier trained to predict trial type using single neuron activity for stimulus, decision, and other neurons. Other neurons are neurons that are neither stimulus, decision or trial-coding neurons. All subfigures: Mean \pm s.d., n = 4568 stimulus neurons, n = 2249 decision neurons, n = 34245 other neurons, two-sided Wilcoxon rank sum test, all comparisons are significant with p < 0.0001.





Supplementary Figure 2. Functional and spatial specificity of photostimulation.

(a) Mean trial selectivity of all neurons aligned so Target S (left) or Target R (right) is centered. (b) Mean photostimulation response of all neurons aligned so the targeted neuron is centered. Scale bars, 50 µm. Spatial footprints of neurons were simplified to circular masks 20 pixels (16 µm) in diameter. (c) Photostimulation response versus lateral distance to the nearest photostimulation site. n = 16096 neurons, 40 sessions, 7 mice. (d) Fraction of positive and negative followers versus lateral distance to the nearest photostimulation site. N = 80 target ensembles, 40 sessions, 7 mice. c-d, data are presented as mean ± s.e.m; errorbars are s.e.m.





(a) Calcium time courses of background neurons during different trial types (mean ± s.d., same experiments as in Figure 7b and Figure 6e). (b) Average calcium activity of all neurons (n = 191) in the example FOV in response to Photostimulation R during PhotoBoost (top), Photo-Disrupt (Middle) and Catch (bottom) trials. Red (grey) vertical bars mark the average frame range affected by (sham) stimulation artefact.

2 s



Supplementary Figure 4. Behavioiral performance and neuron identification after removal of lickport biased trials and depth distribution of stimulus and decision neurons.

a) Behavioral discrimination (Correct trials / (Correct + Incorrect trials)) across mice using only trials with or without lickport bias. Mean \pm s.d., n = 13 mice, grey Xs denote individual mice, two-sided Wilcoxon rank sum test. b) Percentage of stimulus and decision neurons per FOV using either all Correct and Incorrect trials ('All trials') or only Correct and Incorrect trials without a lickport bias ('Without lickport bias trials'). Mean \pm s.e.m., n = 14 FOVs (13 mice), two-sided Wilcoxon signed-rank test. c) Percentage of stimulus and decision neurons per FOV against recording depth (µm below the surface). Mean \pm s.e.m., n = 78 FOVs, two-sided Wilcoxon rank sum test. d) Neurons registered per FOV against recording depth (µm below the surface). Mean \pm s.e.m., n = 78 FOVs, two-sided Wilcoxon rank sum test.