Supplementary Information

Activity Recall in Visual Cortical Ensemble

Shengjin Xu, Wanchen Jiang, Mu-ming Poo, and Yang Dan

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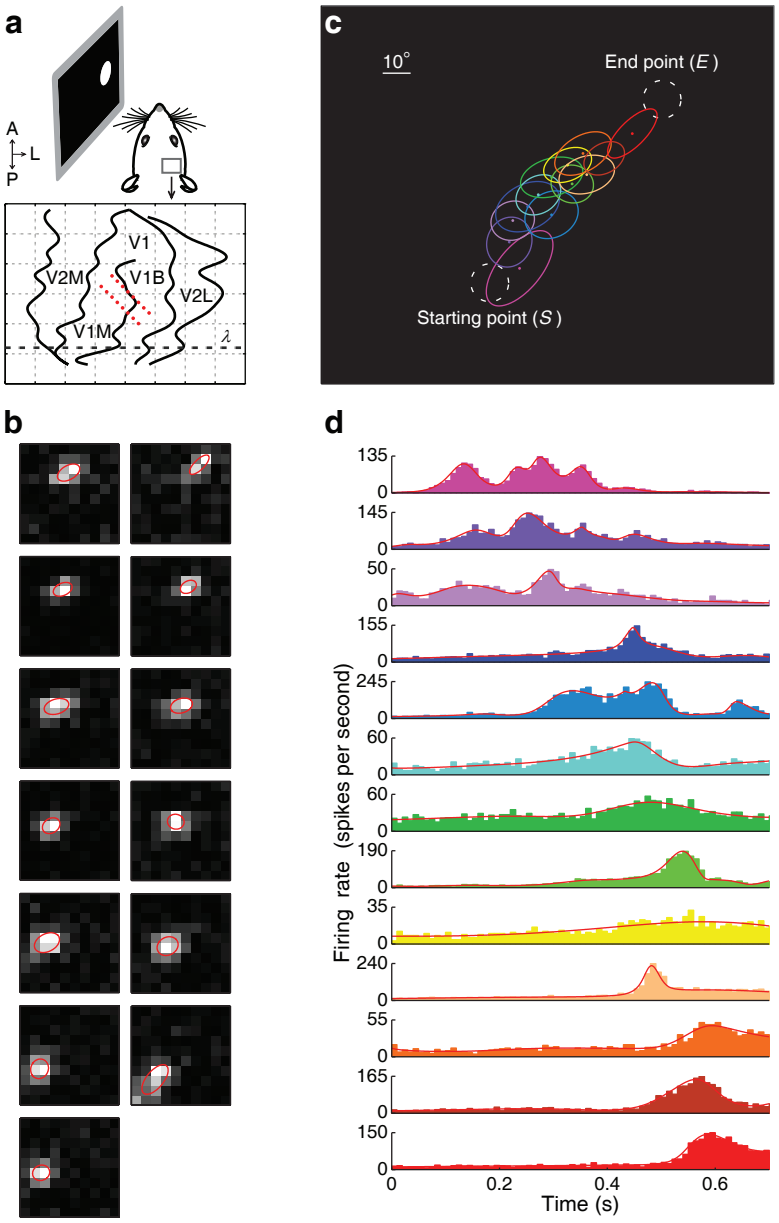

Supplementary Figure 1 RFs and conditioning-evoked sequential spiking of neurons in anesthetized rat V1. (**a**) Schematic of experimental setup. Visual stimuli were presented to left eye, multielectrode array (red dots) was inserted into right V1. Diagram of rat cortex was adopted from Paxinos and Watson[48], shown on 1 × 1 mm grid. (**b**) Multiunit RFs recorded simultaneously in a urethane-anesthetized rat. Red ellipse, contour of Gaussian fit at one standard deviation. (**c**) Superposition of RFs and visual stimuli. Colored ellipses, Gaussian fits of RFs. White dashed circles, "Starting point" (*S*) and "End point" (*E*) of conditioning moving spot. (**d**) PSTHs of the units during conditioning stimulation by a moving spot, ordered by distance between RF center and *S*. Red curves, PSTHs smoothed with Bayesian adaptive regression splines.

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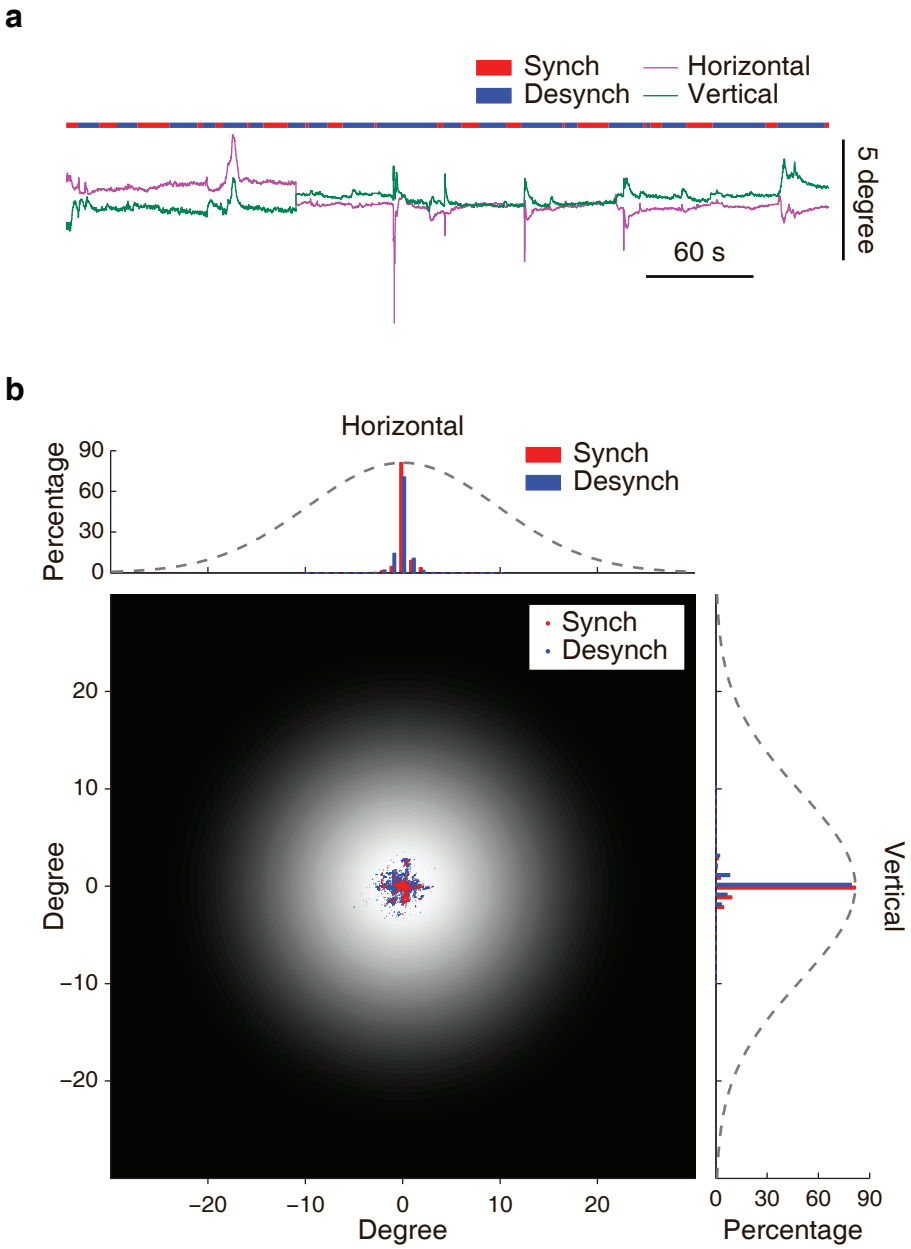

Supplementary Figure 2 Eye movement in awake head-fixed rats. (**a**) Example eye movement traces from a trained rat. Red and blue lines, periods of synchronized and desynchronized brain states (same as in **Fig. 6**). (**b**) Distribution of eye positions recorded in 5 experiments during test periods from 2 trained rats, in synchronized (red) and desynchronized (blue) brain states. Superimposed is a 2-D Gaussian function (luminance profile and gray dashed lines) whose size is matched to the average RF size of rat V1 neurons measured in our experiments.

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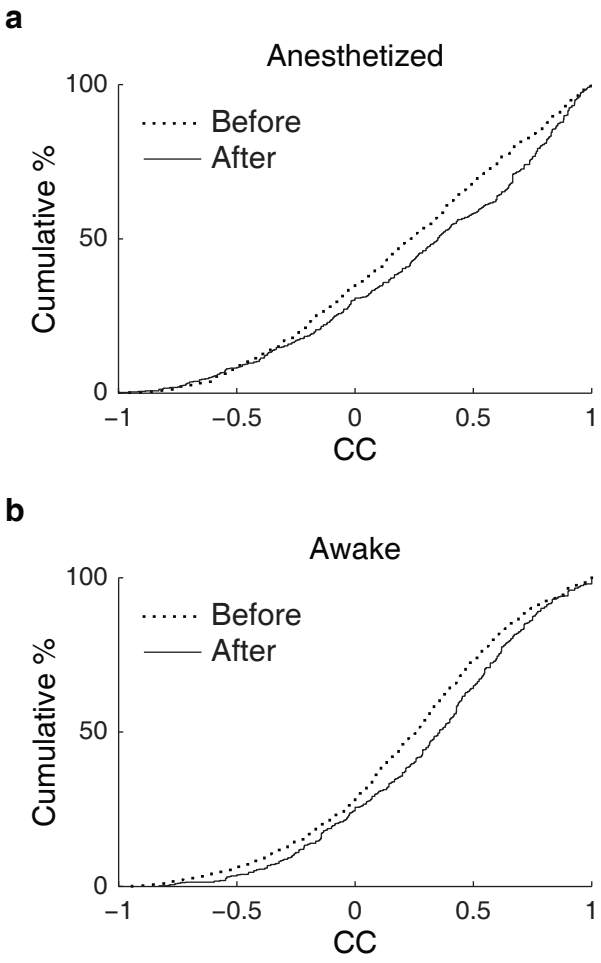

Supplementary Figure 3 Alternative analysis of spike sequence evoked by test cue at *S* in anesthetized and awake rats. Shown are the cumulative histograms of Spearman CCs between test stimulus *S*-evoked spiking sequence and conditioning stimulus-evoked spiking sequence, before (dotted line) and after (solid line) 100 trials of conditioning at 180° s^{-1} for anesthetized (**a**, n = 19 experiments) and awake (**b**, n = 18) rats. Conditioning caused significant rightward shift of the CC distribution (**a**, $P = 6.8 \times 10^{-5}$; **b**, $P = 1.1 \times 10^{-4}$, Kolmogorov-Smirnov test).

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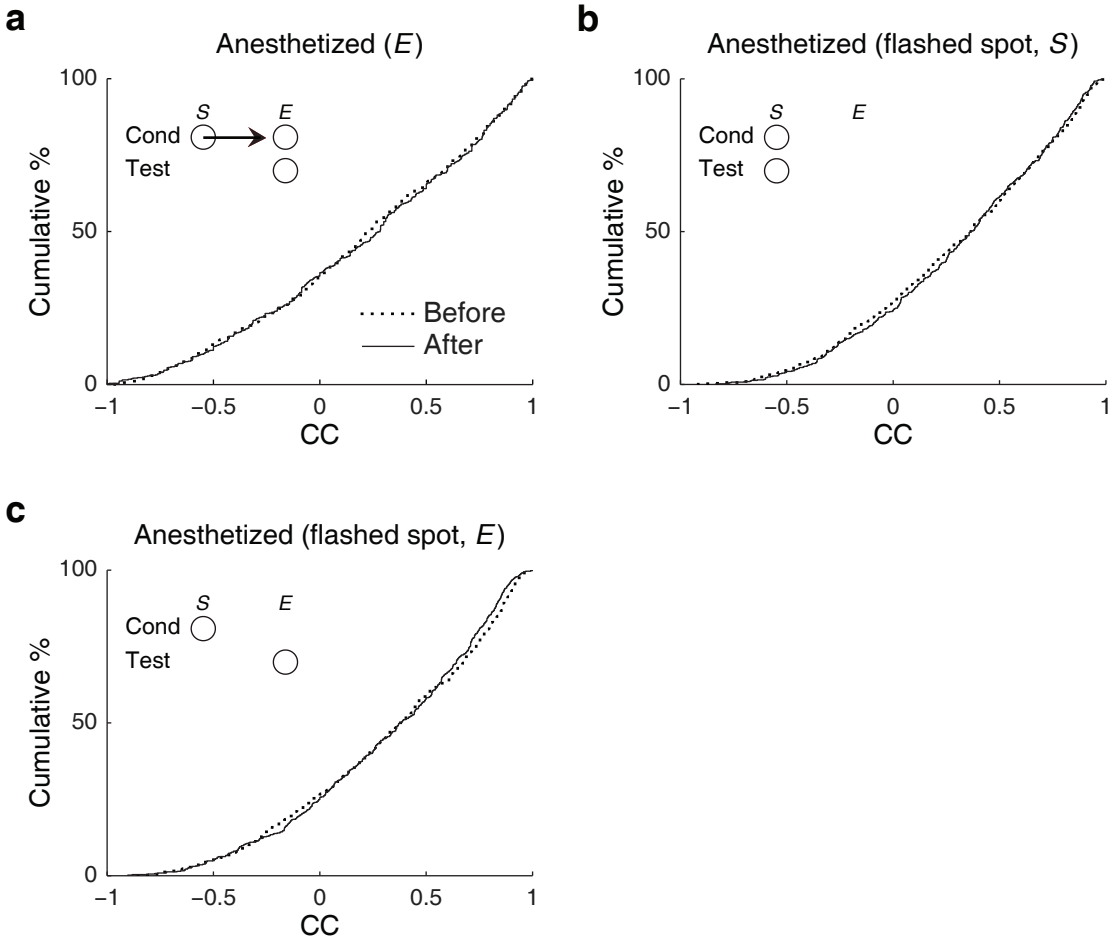

Supplementary Figure 4 Cumulative histograms of Spearman CCs for control experiments in anesthetized rats. (**a**) Cumulative histograms of CCs for test stimuli at *E* following 100 trials of *S* → *E* conditioning. CC was computed between test stimulus-evoked spiking sequence and RF position along the *E* → *S* axis, before (dotted line) and after (solid line) 100 trials of *S* → *E* conditioning at 180° s^{-1} for anesthetized rats (n = 19 experiments). There was no significant difference before and after conditioning ($P = 0.77$, Kolmogorov-Smirnov test). (**b**) Cumulative histograms of CCs for test stimuli at *S* following 100 trials of flashed-spot conditioning at *S* (n = 17 experiments). There was no significant difference before and after conditioning ($P = 0.47$). (**c**) Cumulative histograms of CCs for test stimuli at *E* following 100 trials of flashed-spot conditioning at *S*. There was no significant difference before and after conditioning ($P = 0.44$). Diagram in upper left region of each plot illustrates conditioning and test stimuli used in that experiment.

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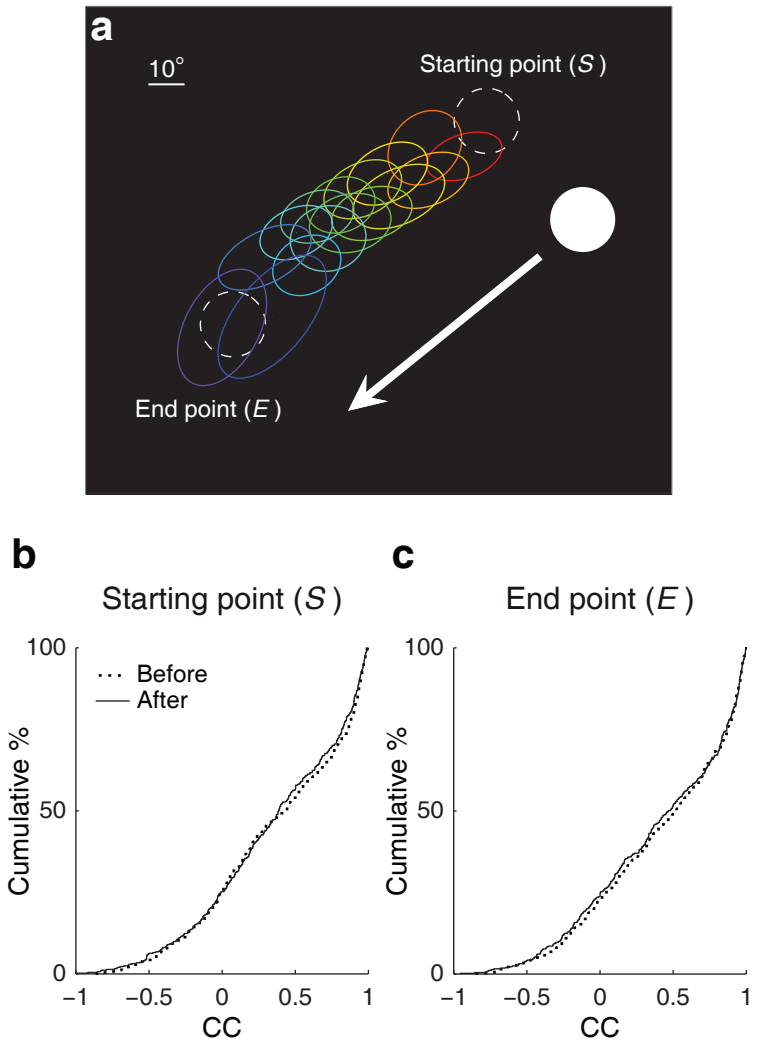

Supplementary Figure 5 Control experiment with the conditioning spot moving along a motion path parallel to, but not overlapping with, the long axis of the recorded RF distribution. (**a**) Superposition of RFs and visual stimuli. Colored ellipses, Gaussian fits of RFs. White filled circle and arrow indicate conditioning motion path. White dashed circles, "Starting point" (*S*) and "End point" (*E*) of test spot. (**b**) Cumulative histograms of Spearman CCs between *S*-evoked spike sequence and RF position, before (dotted line) and the first 2 min after (solid line) conditioning (n = 14 experiments). There was no significant difference (P = 0.80; Kolmogorov-Smirnov test). (**c**) Cumulative histograms of Spearman CCs for *E*-evoked spike sequence. The difference was not significant (P = 0.93).

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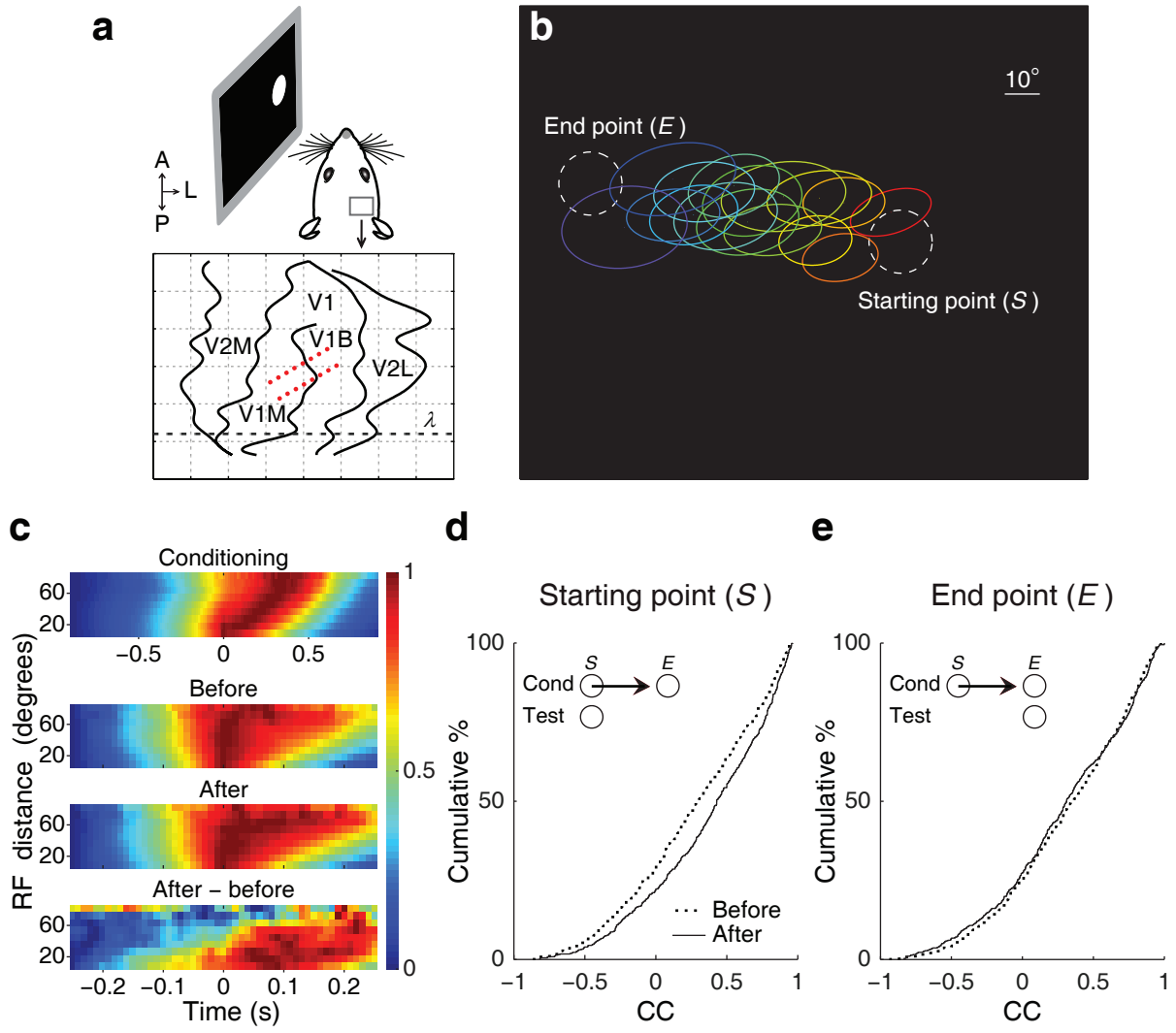

Supplementary Figure 6 Conditioning-induced increase of sequential spiking in anesthetized rats, with electrode array implanted at a different angle. (**a**) Schematic of experimental setup. Multielectrode array (red dots) was inserted into right V1. Diagram of rat cortex was adopted from Paxinos and Watson[48], shown on 1 × 1 mm grid. (**b**) Superposition of RFs and visual stimuli. Colored ellipses, Gaussian fits of RFs. White dashed circles, "Starting point" (*S*) and "End point" (*E*) of conditioning spot. (**c**) Top three rows, pairwise cross-correlation averaged from all experiments with this electrode array orientation (n = 18 experiments, including 8 experiments with 200 trials and 10 experiments with 50 trials of conditioning). Bottom row, difference between cross-correlation functions before and after conditioning. (**d**) Cumulative histograms of Spearman CCs between *S*-evoked spike sequence and RF position, before (dotted line) and the first 2 min after (solid line) conditioning for all 18 experiments. The difference was significant ($P = 4.3 \times 10^{-5}$; Kolmogorov-Smirnov test). (**e**) Cumulative histograms of Spearman CCs for *E*-evoked spike sequence. The difference was not significant ($P = 0.53$).

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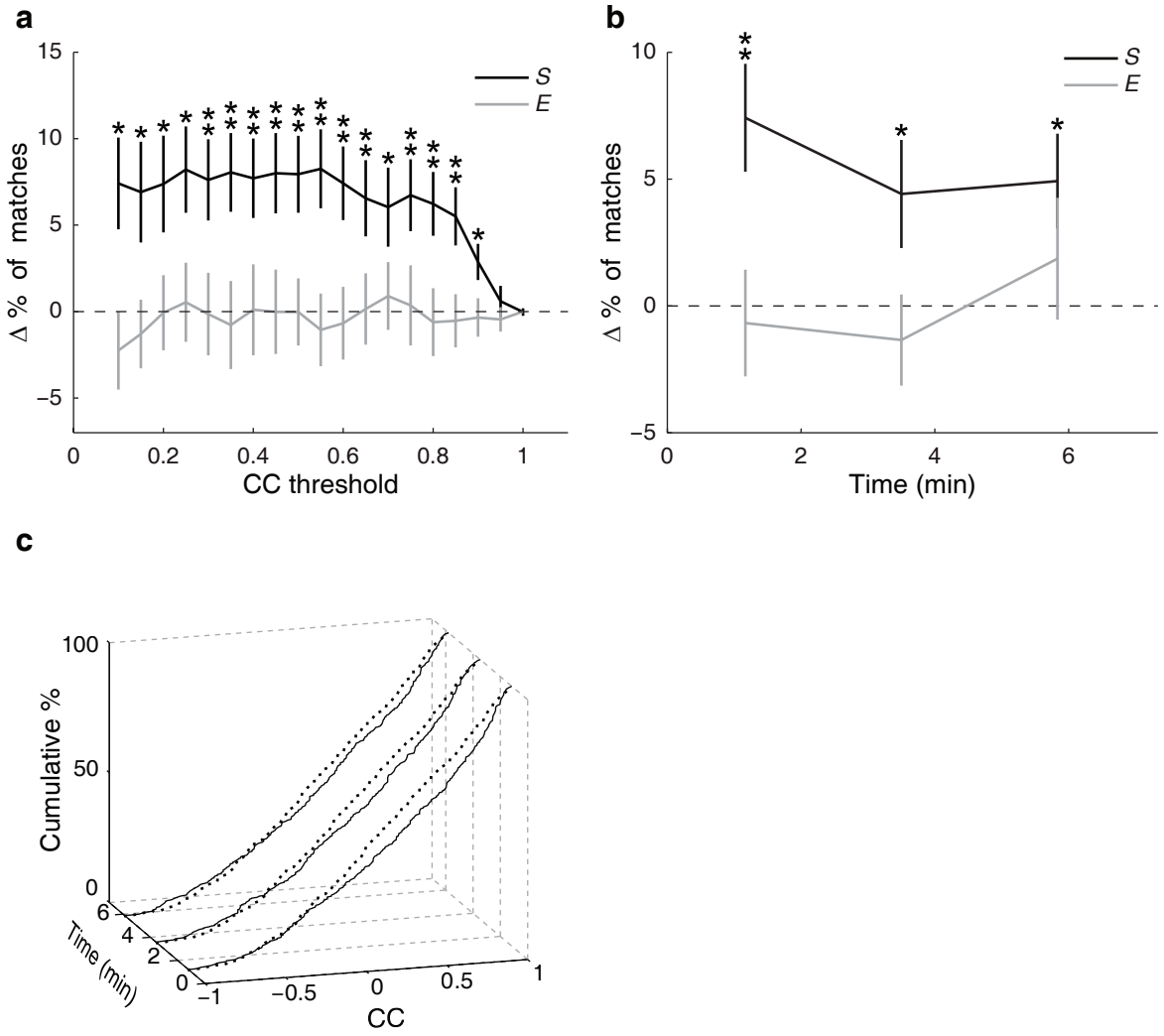

Supplementary Figure 7 Persistence of conditioning-induced increase in sequential spiking in anesthetized rats after 100 trials of conditioning at 180° s^{-1}. (**a**) Difference in the percentage of sequence matches before and after conditioning vs. CC threshold for test stimuli at *S* (black) and *E* (gray). The difference was significant at all CC thresholds below 0.9 for *S* ("*", $P < 0.05$; "**", $P < 0.01$; "***", $P < 0.001$; Wilcoxon signed rank test), but not at any threshold for *E*. Error bar, s.e.m. (**b**) Time course for decay of conditioning-induced increase in the percentage of matches (at CC threshold of 0.6). For *S*, the increase was significant at all time points (2 min, $P = 3.3 \times 10^{-3}$; 4 min, $P = 0.044$; 6 min, $P = 0.027$; Wilcoxon signed rank test) after conditioning. For *E*, the effect was not significant at any time. Error bar, s.e.m. (**c**) Cumulative histograms of Spearman CCs at different periods after conditioning (solid lines). Dotted line, histogram before conditioning. The difference is significant at 2 min ($P = 1.5 \times 10^{-3}$; Kolmogorov-Smirnov test) and 4 min ($P = 0.013$), but not at 6 min ($P = 0.079$).

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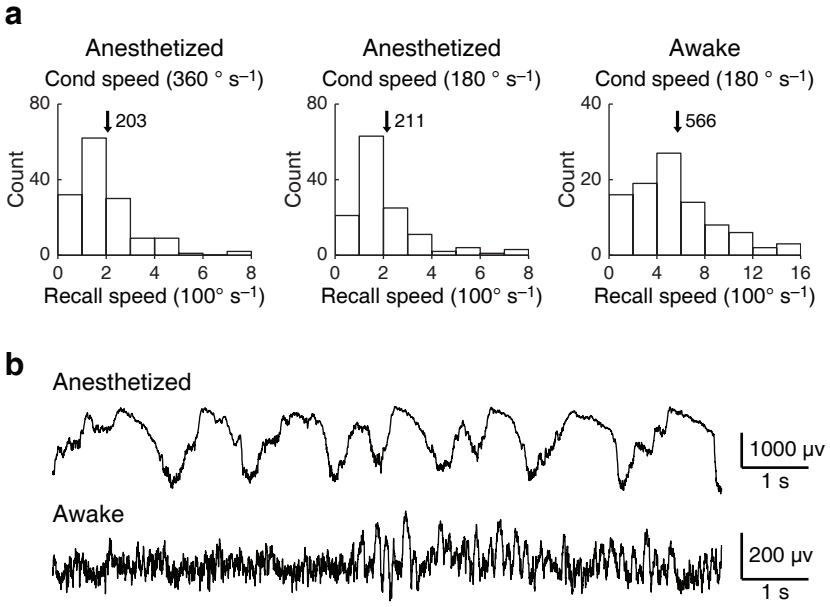

Supplementary Figure 8 Speed of cue-triggered recall of spike sequence in anesthetized and awake rats. (**a**) Left, histograms of speed for *S*-triggered recall of spike sequences in all anesthetized experiments (n = 18) with conditioning speed at 360° s^{-1}. Only matched trials with Spearman CC > 0.9 were included in this analysis. Arrow, mean. Middle, histograms of recall speed in all anesthetized experiments with 100 trials of conditioning at a speed of 180° s^{-1} (n = 19). Right, histograms of recall speed in all awake experiments (n = 18) with conditioning speed at 180° s^{-1}. (**b**) Example LFP traces recorded from a urethane-anesthetized and an awake rat.

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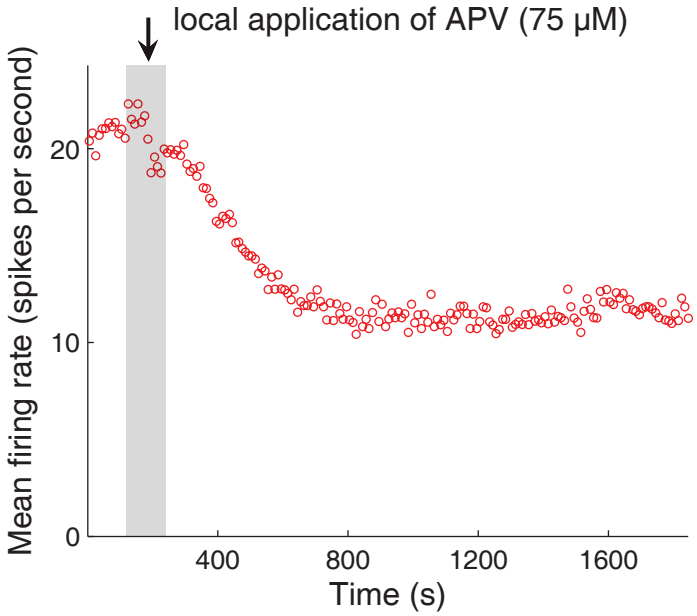

Supplementary Figure 9 Decrease in cortical firing rate after local application of APV. Each red circle represents mean spontaneous firing rate over a period of 10 s, averaged across all units recorded in the 18 APV experiments. The mean firing rate decreased to 57 ± 7% (s.e.m.) of the baseline and became stable within 10 min after the APV application. Experiments on sequence recall were performed ~ 30 min after APV application, when the firing rate was stabilized. Shadow area, period of APV application.

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Supplementary Movie. 1 Awake head-fixed rat during visual stimulation. Red circle indicates pupil size and position. Arrows point to reflections of the test stimuli (*S* and *E*, presented with an LCD monitor in front of the left eye) off the eye ball. The stationary bright spot on the left of the eye was the reflection of the infra-red light source that provides illumination for the camera. Horizontal and vertical eye positions are shown in the traces below. Also shown are simultaneously recorded LFP, together with red/blue lines indicating synchronized/desynchronized brain states, and the short bars below indicating periods of visual stimulation. Note that the desynchronized brain state is associated with some facial and whisker movement.

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