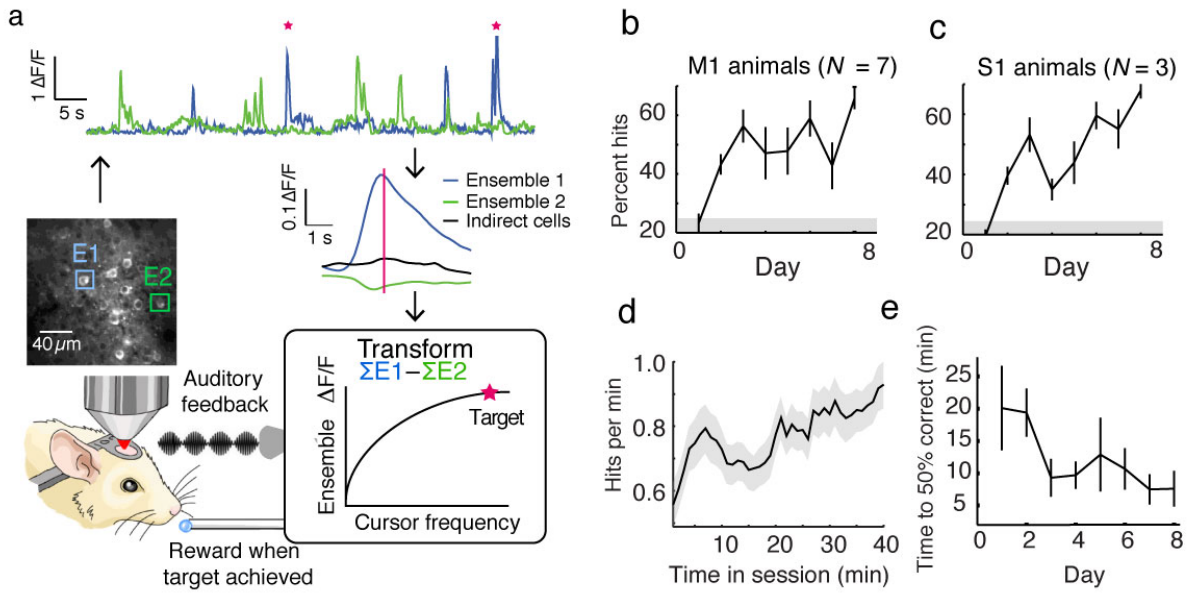
**SUPPLEMENTAL FIGURES**




**Supplemental Figure 1. Mice rapidly learn to intentionally modulate calcium dynamics. a.** Task schematic. **b & c.** Behavioral performance over 8 days for animals trained to use M1 (**b**) and S1 (**c**). **d.** Hit rate increases over the course of individual sessions. Shaded region denotes s.e.m., 10 mice. **e.** Animals achieve a criterion performance of 50% hits faster over the course of training. N=8 days, 10 mice



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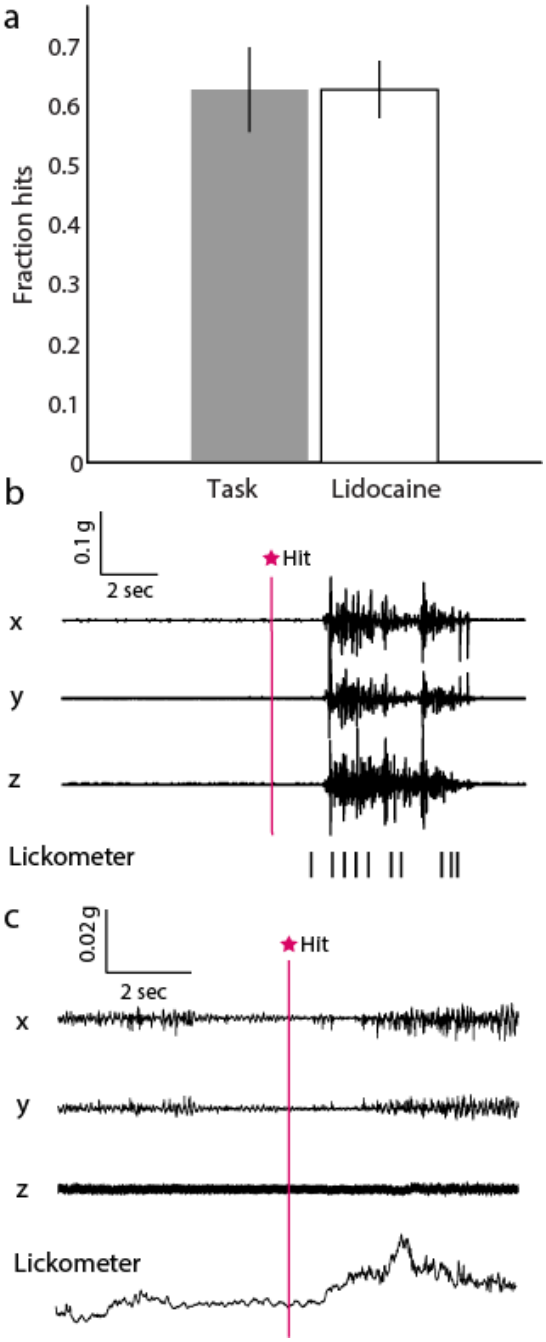


**Supplemental Figure 2. Task performance does not rely on natural movements. a.** Performance on the task was not impaired when we injected lidocaine into the mystacial pad to block whisker movements and somatosensory input. Error bars denote s.e.m. **b.** Accelerometer traces for a single trial with no motion or licking behavior before target achievement, despite clear activity afterwards as mice consume the reward. **c.** Hit-locked accelerometer traces averaged over one entire training session for one animal shows animal does not rely on systematic motion for task.



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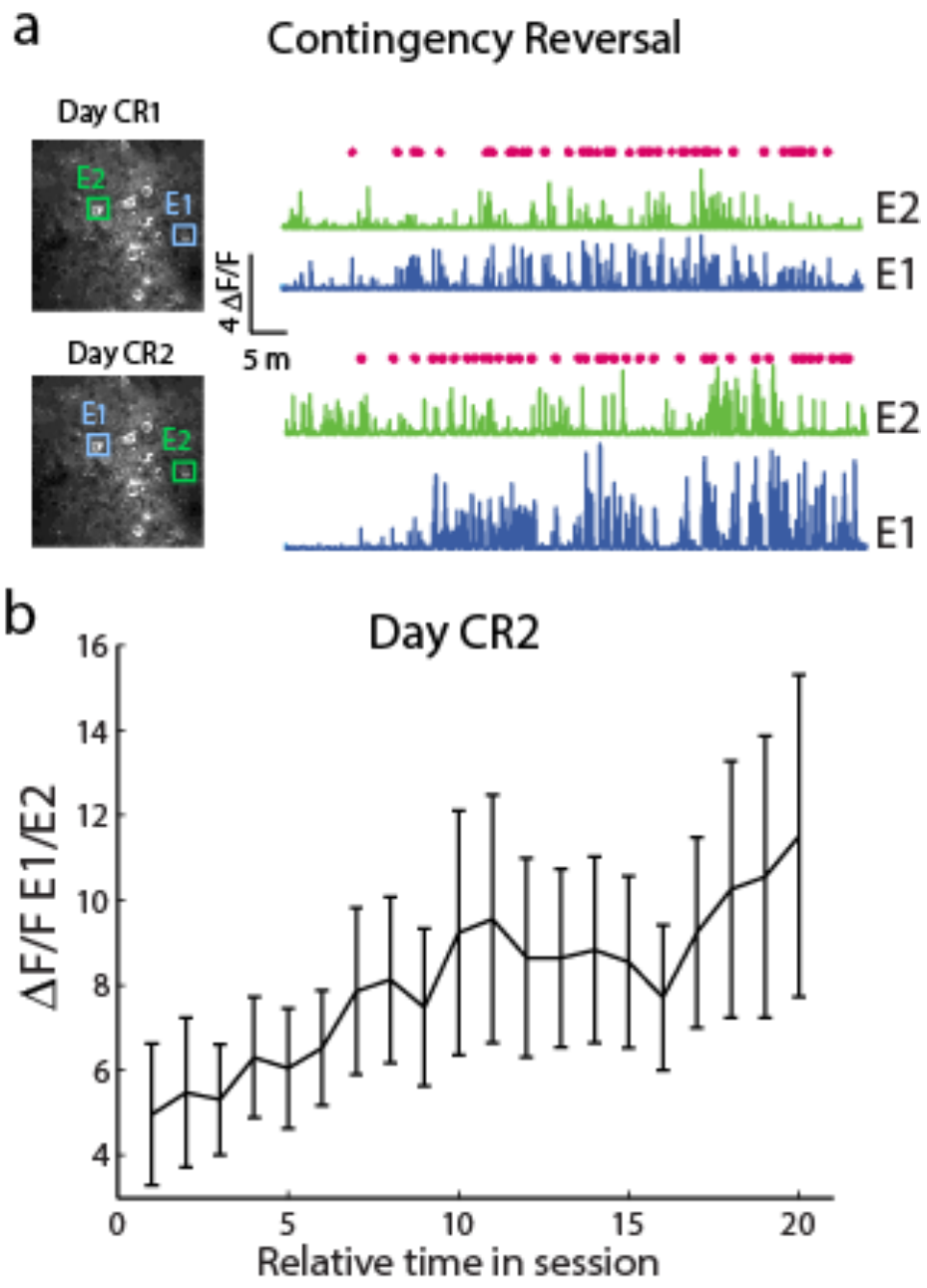


**Supplemental Figure 3. Fluorescence modulations during contingency reversal experiments. a.** When the identity of output cells is changed from day 1 to day 2, animals learn to reverse the activity patterns of their output neurons. **b.** ΔF/F E1/E2 ratio increases over the course of training on day CR2 (N=3 mice, 20 time points, R2=0.832, p=0.02, t(18)=13.32). Error bars s.e.m.



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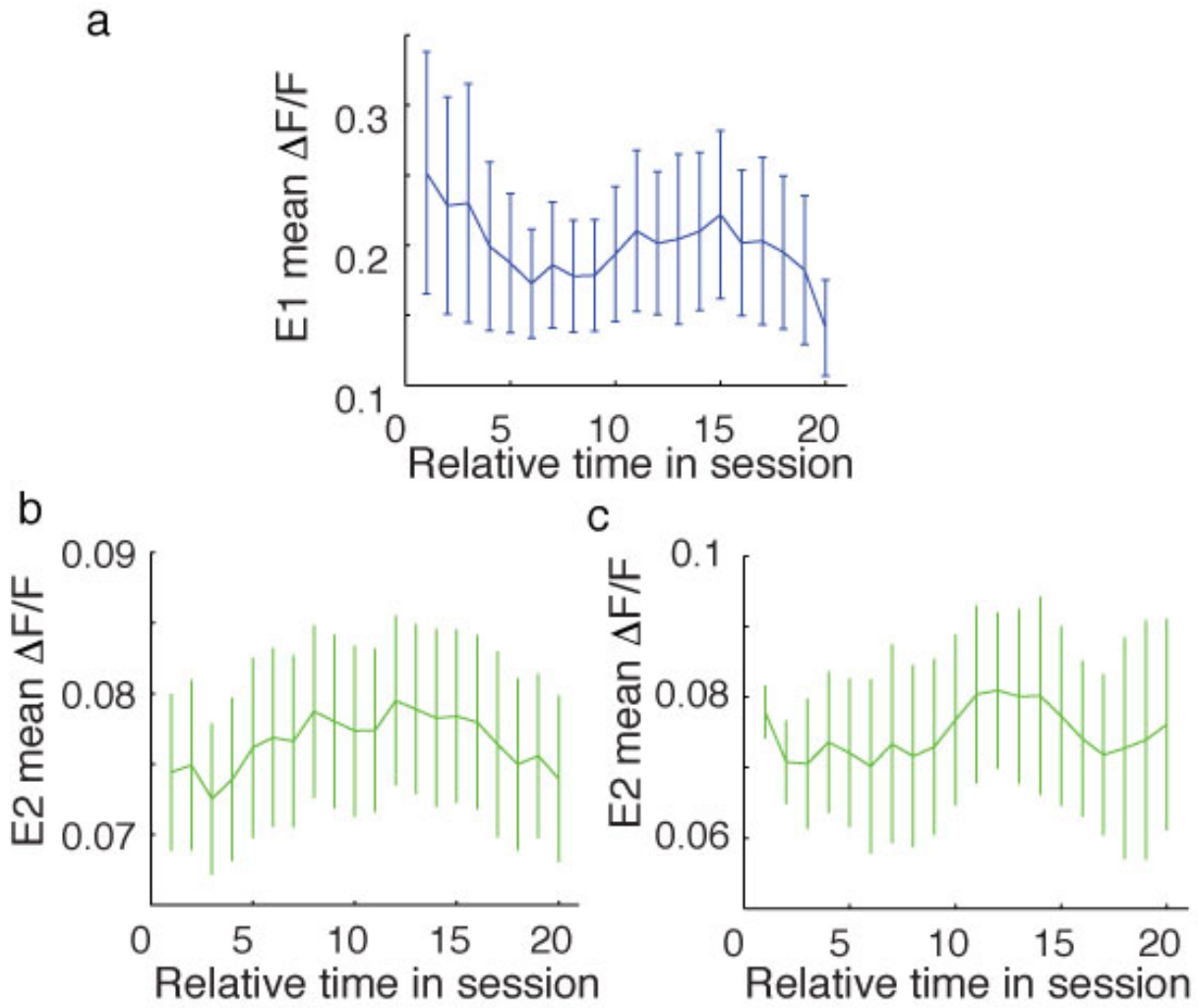


**Supplemental Figure 4. Fluorescence modulations during contingency degradation experiments a.** Mean fluorescence decreases in E1 cells during the contingency degradation manipulation. **b.** Mean ΔF/F is unchanged in E2 cells over the course of a normal training session (N=5 mice, 20 time points, p=0.234,t(18)=1.23). **c**. Mean ΔF/F is unchanged in E2 cells during the contingency degradation manipulation (N=5 mice, 20 time points, p=0.13,t(18)=-1.58). Together, these results suggest that animals primarily perform the task by modulating mean activity in E1 cells and do not coarsely modulate the mean activity of E2 cells. Error bars in all panels represent mean +/- SEM.



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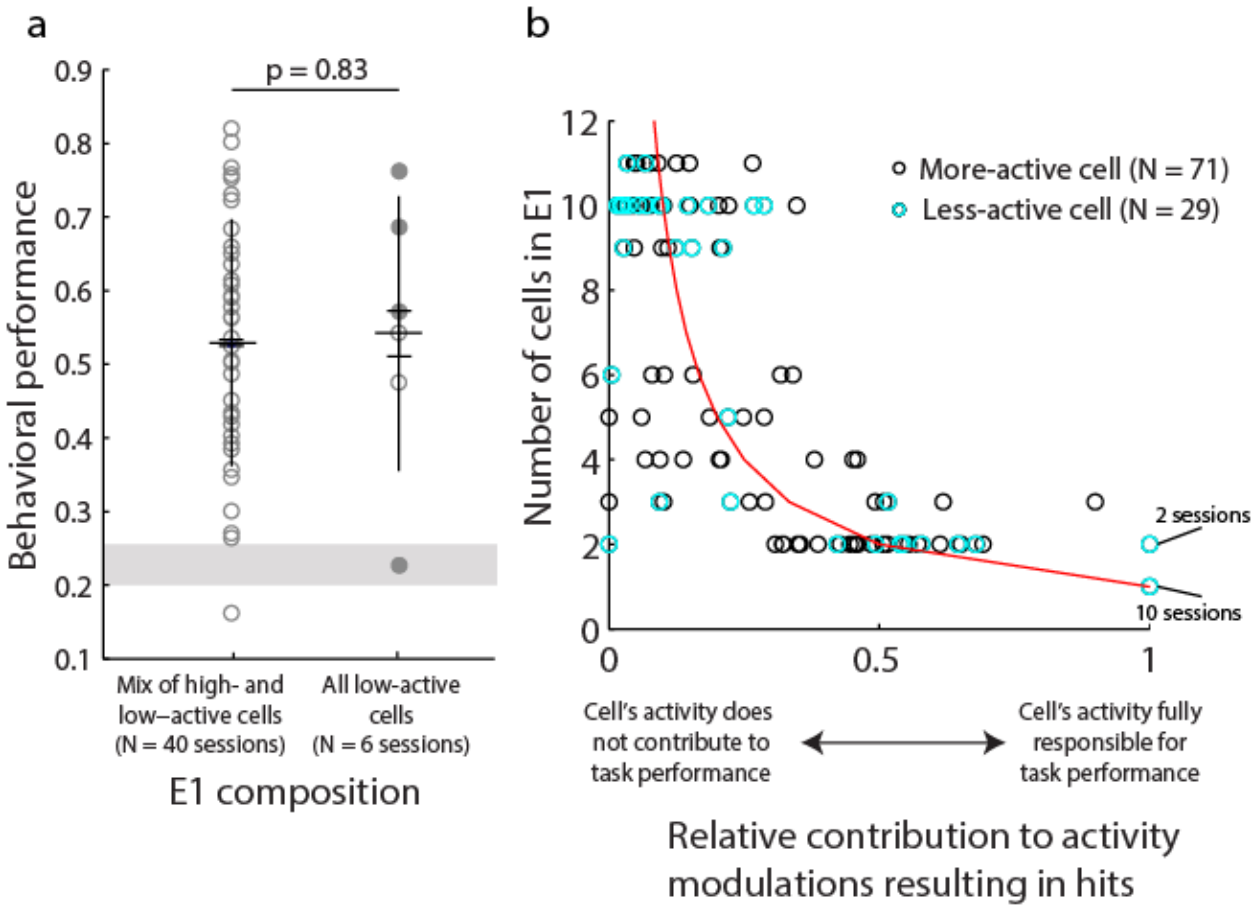


**Supplemental Figure 5. Performance does not depend on baseline activity in E1 cells.**

**a.** Behavioral performance plotted against the makeup of E1. In 40 sessions, E1 was composed of a mix of more- and less-active cells (defined as cells in the lowest quintile of spontaneous activity rates). In 6 training sessions, E1 was solely comprised of low-active cells, and in 4 cases all cells in E1 had zero baseline activity (filled circles); in three cases the animal still performed the task above chance. Performance in both conditions was significantly better than chance (t-test, high- and low-active ensembles, p=2.2e-15, t(38)=11.69; low-active ensembles, p=0.02, t(5)=3.7). **b**. Analysis of the contribution of individual E1 cells to learned modulation of E1 ensemble activity. Fluorescence modulations around hits were calculated for each E1 cell, and normalized to the sum of modulations in the entire ensemble. Each cell's relative contribution to these modulations was then averaged over the entire training session, and plotted against the number of cells in the E1 ensemble. A bimodal distribution of contributions around 0 and 1 would suggest the task were carried solely by one cell in an ensemble. If all cells contributed equally, the distribution would peak around 1/N (red curve), where N = number of cells in the ensemble. The animals appear to use a combination of both strategies. Less-active neurons (in blue) contributed to hits in a manner indistinguishable from more-active neurons.



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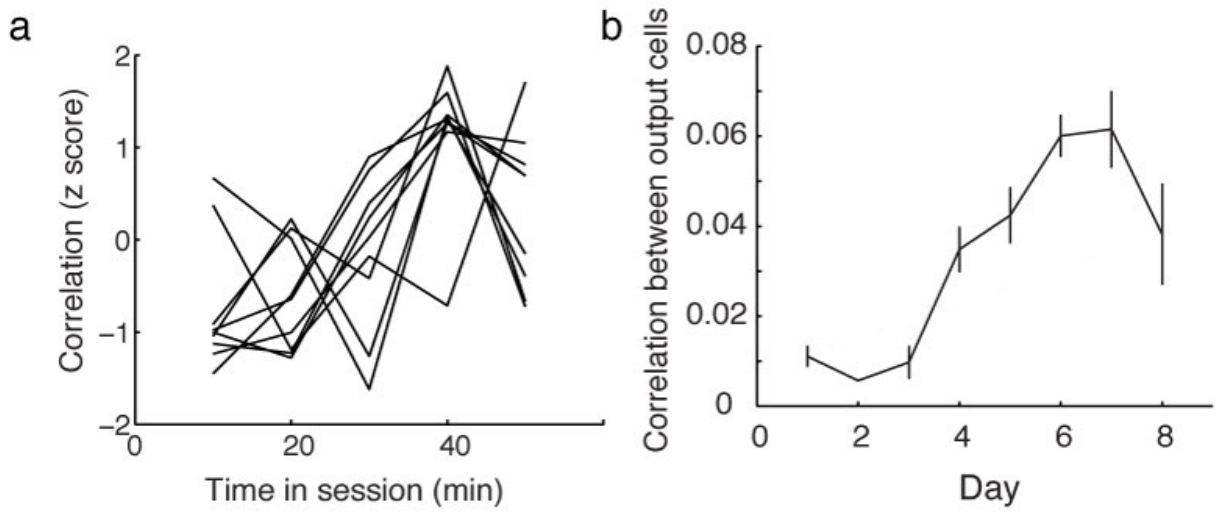


**Supplemental Figure 6. Correlations between output cells increase over the course of a session and the course of training. a.** Z-scored correlations between output cells over the course of a behavioral session shown separately for each animal tested. All animals exhibit the same increasing trend that was seen in the mean. **b.** Correlations between output cells increase across days of training on the task (N=8 days, 10 mice, p=0.011, t(6)=3.59). The composition of ensembles varied across days, so this may reflect meta-learning rather than an increase in correlations between individual cells.



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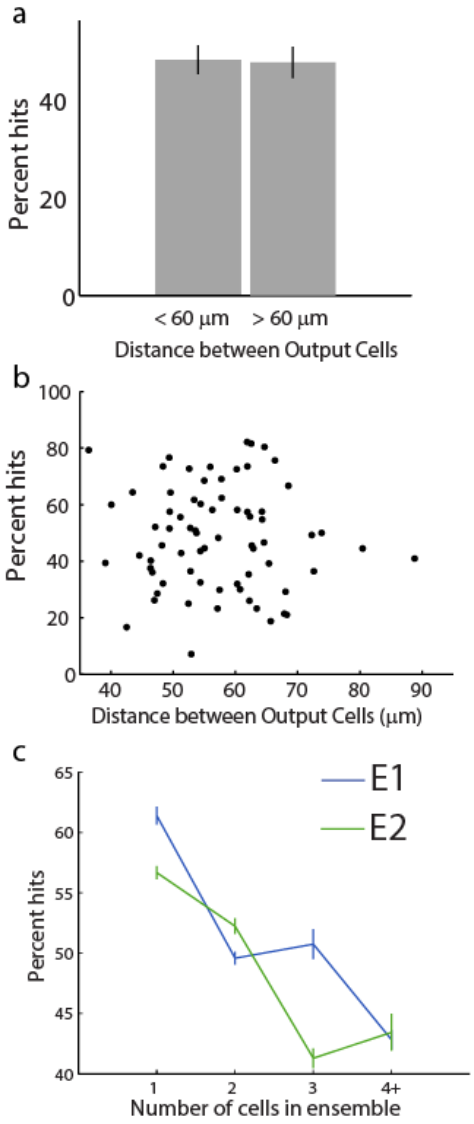


**Supplemental Figure 7. Performance is not affected by the distance between output cells, but does depend on the number of cells in an ensemble. a.** There is no significant difference in performance during sessions in which the output cell ensembles were separated by less than 60 µm and those in which the ensembles were separated by more than 60 µm. Error bars denote s.e.m. **b.** Scatter of performance vs. distance between E1 and E2. **c.** Average performance vs. number of cells in E1 (blue) and E2 (green). Error bars denote s.e.m.



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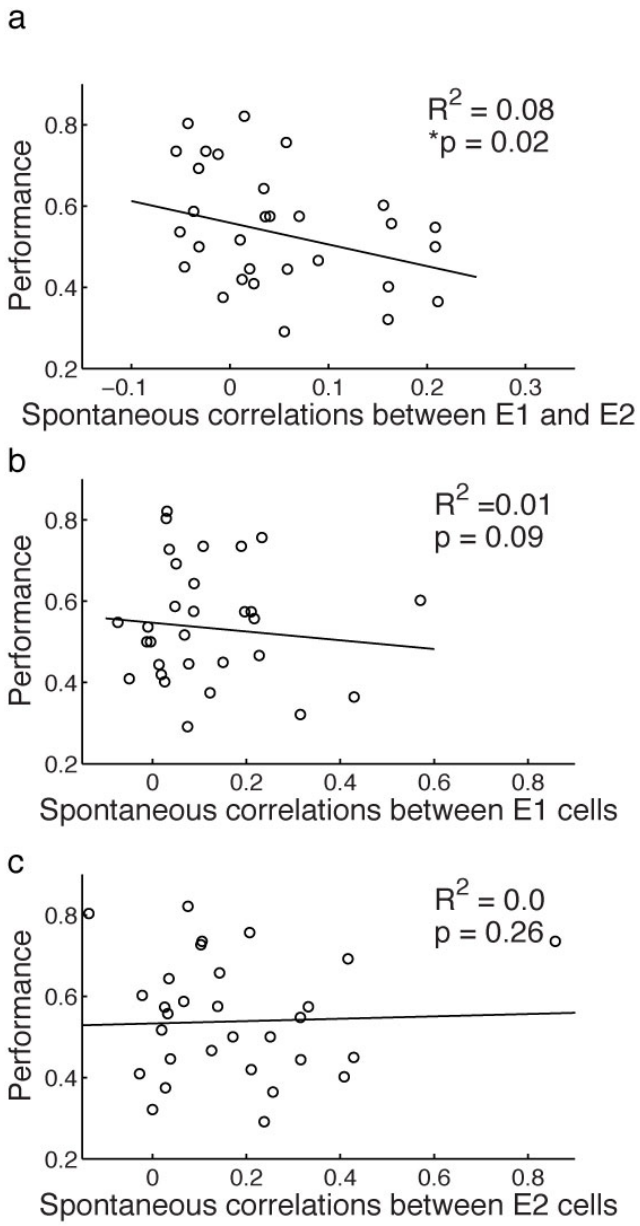


**Supplemental Figure 8. Performance is affected by baseline correlations between ensembles. a.** Animals performed worse when E1 and E2 cells were more spontaneously correlated than when they were less correlated. (N=31 sessions, 10 mice). This effect was not evident **b.** within E1 or **c.** within E2.



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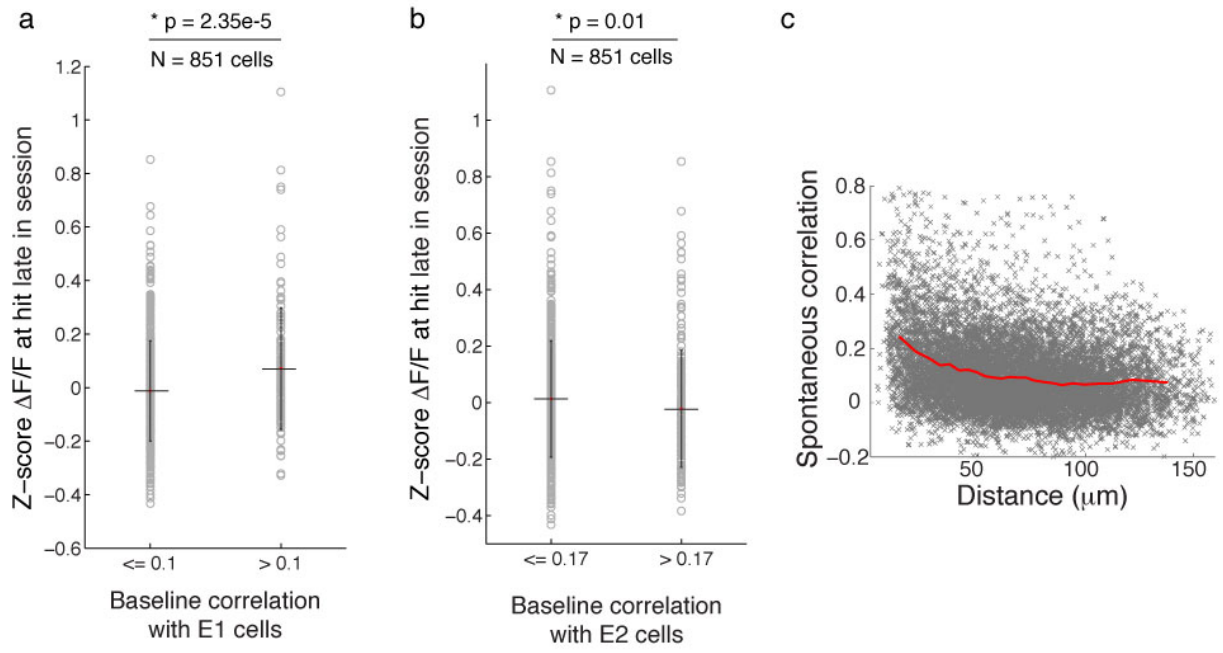


**Supplemental Figure 9. Indirect cells initially more correlated with output cells maintain output cell-like activity late in session a.** Indirect cells that were more spontaneously correlated with E1 cells (highest quintile of spontaneous correlations, >0.1) increase activity during task performance more than cells that were initially weakly, or anti-, correlated (t-test, p = 2.35e-5, t(849)=4.26). Horizontal bar indicates mean, vertical bar indicates standard deviation. **b.** Indirect cells that were more spontaneously correlated with E2 cells (highest quintile of spontaneous correlations, >0.17) show reduced activity during task perfomance compared with less-correlated cells (t-test, p = 0.01, t(849)=-2.6). Horizontal bar indicates mean, vertical bar indicates standard deviation. **c.** Spontaneous correlations between L2/3 neurons in mice fall off rapidly with short distances (binned mean shown in red, S1 and M1 data pooled).



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