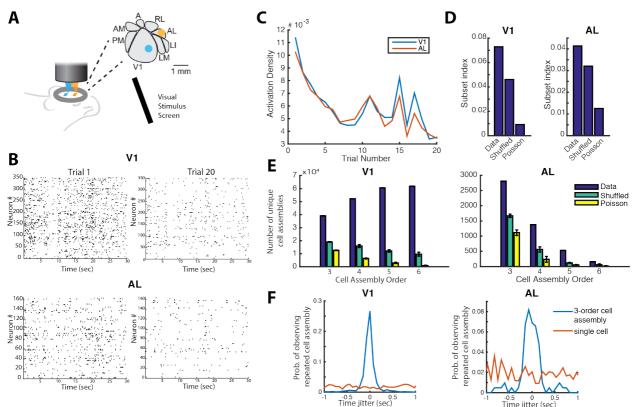
## Presence of high order cell assemblies in mouse visual cortices during natural movie stimulation

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Neocortical neurons work together to process sensory information. It has been hypothesized by Hebb 70 years ago that an assembly of neurons activate repeatedly during certain mental processes. A recently proposed theory of sequence learning, Hierarchical Temporal Memory (HTM), makes a number of specific predictions regarding the formation and function of high order cell assemblies<sup>1</sup>. In this study we tested these predictions of HTM in awake mouse visual cortices during presentation of a natural movie using wide field two-photon calcium imaging techniques<sup>2</sup>. We analyzed the statistics of spikes and their high order correlations, and compared them against surrogate models that shuffle spikes across trials or generate spikes according to a Poisson process. We found that (1) overall cell activity becomes sparser during repeated presentations of a natural movie, (2) cells active during a later trial tend to be a subset of the cells active during the first trial, (3) the chance of observing a high order cell assembly is much higher than predicted by the shuffled or Poisson models, and (4) high order cell assemblies are repeatable and occur at the same time relative to the stimulus, despite significant trial-to-trial variability. These observations are consistent with predictions of HTM theory and suggest that high order cell assemblies and high order correlations encode meaningful information regarding the visual stimulus.



A Simultaneous wide-field calcium imaging from area V1 and AL in awake mouse<sup>2</sup>. **B.** Population responses to natural movie stimuli in V1 (top, n=352) and AL (bottom, n=163) on the 1st and 20th trial. Spikes were inferred from calcium traces using a de-convolution technique. **C.** Population activity density decreases across trials for both areas. **D.** Subset index is defined as the fraction of cells that are active on both the first and the last trial at each frame, divided by the number of active cells on the first trial. The real data has a higher subset index than the trial-shuffled and Poisson models. **E.** The total number of unique cell assemblies across all trials. Cell assemblies are significantly more likely to occur in the real data than predicted by a trial-shuffled model or a Poisson model (p<0.001). **F.** Distribution cell assembly occurrence times relative to the median occurrence time. Cell assemblies tend to occur at the same time relative to the stimulus. This tight distribution is not observed for single cell activations.

## References

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- 2. Stirman, J. N., Smith, I. T., Kudenov, M. W. & Smith, S. L. Wide field-of-view, multi-region, two-photon imaging of neuronal activity in the mammalian brain. *Nat. Biotechnol.* **34**, 857–862 (2016).