

# Integrating Neuronal Ensembles Constitute and Replay Hippocampal Engram

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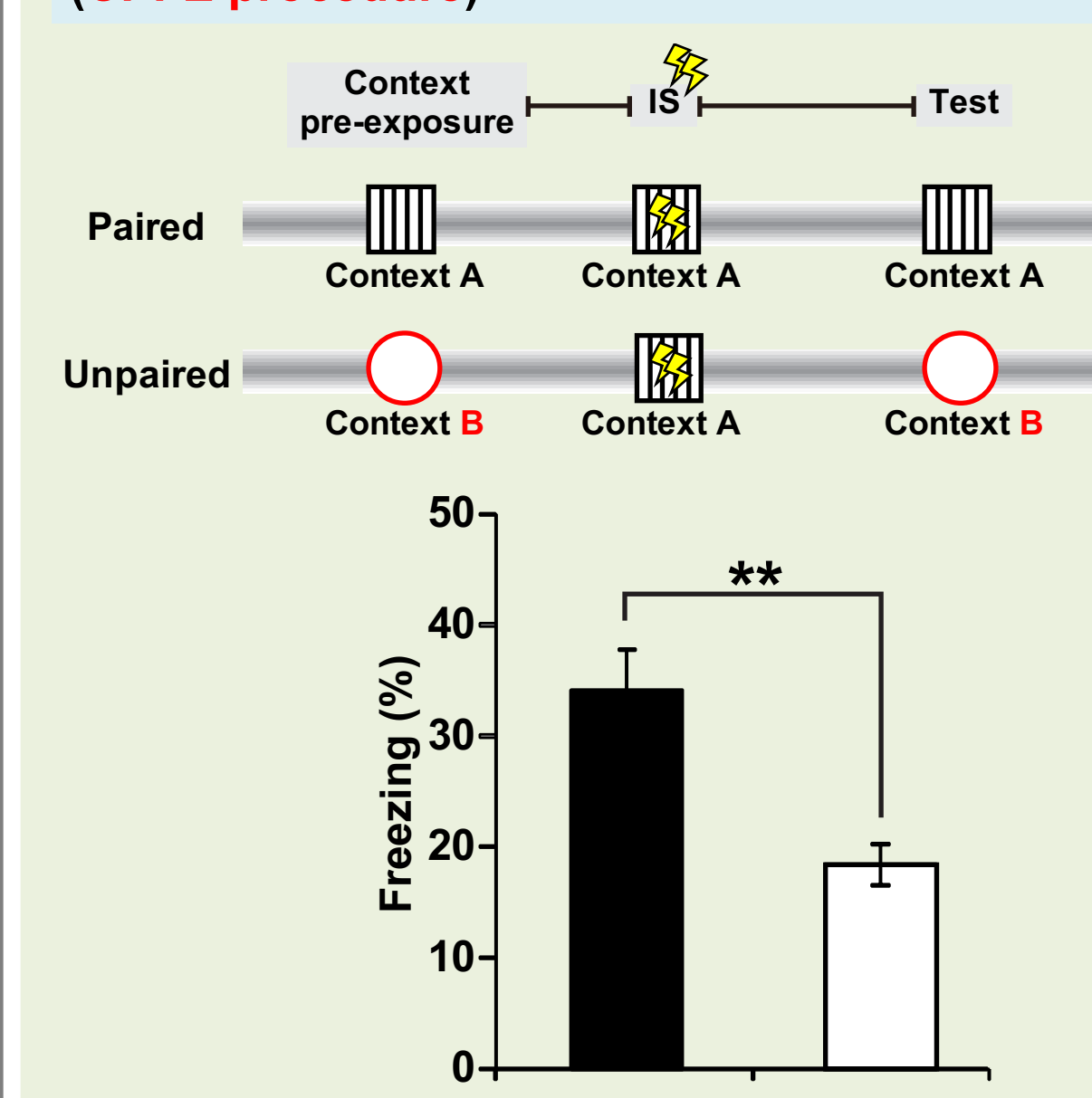
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## Summary

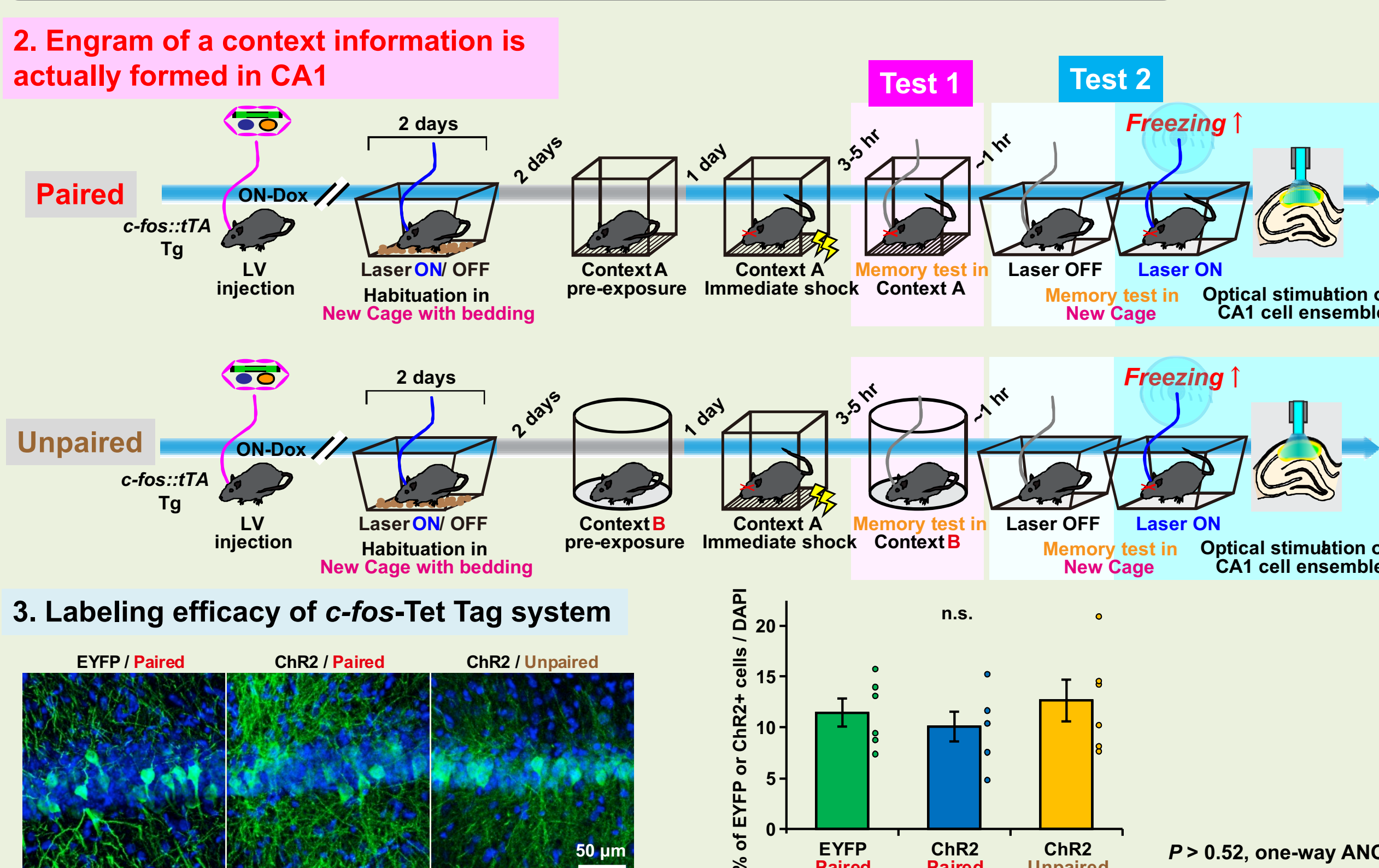
It is hypothesized that a memory is encoded in a subset of neurons, which is activated by physiological input derived from a corresponding event, called engram. The c-fos-TetTag system has been used to prove the engram theory, by manipulation of cells that showed activity-dependent gene expression driven by learning. However, the physical and basic activities that occur during learning and post learning in that group of cells, is still poorly understood. Here we show that the engram cells exhibit a remarkable synchronous activity representing the contextual memory in the form of several ensembles in engram cells. These ensembles carry on their activity not only during learning but also during post-learning sleep and retrieval sessions, in contrast to non-ensemble cells. A compatible imaging system was established to observe the neuronal activity of ~1000 CA1 neurons and the labeled engram cells; through a photoconvertible fluorescent protein KikGR (KikGR). The neuronal activity of hippocampal CA1 neurons was observed, through Ca<sup>2+</sup> influx with GCaMP7 in freely-moving animals by miniature head-mount microscopy. Our advanced imaging system of engram cells and non-ensemble cells provides deeper insights into the dynamics of the neural activity during contextual memory processing. Engram cells exhibit highly repetitive activity corresponding to remarkable synchrony during novel context exposure. Population vector distance (PVD) analysis indicates that total activity patterns of engram cells are stable and consistent across other sessions; sleep (NREM and REM) and retrieval, not only during learning. Furthermore, Non-negative Matrix Factorization (NMF) analysis extracted characteristic ensembles activity that were constructed by subgroup of engram cells, representing the persistent synchronous activity even during post-learning sleep (NREM and REM) sessions and retrieval session, but not in a distinct context. In contrast, these features were not seen in non-ensemble cells. These results suggest that there are several fundamental characteristics of the engram cells that give them superiority in encoding the ongoing event and consolidating the past ones.

## Results

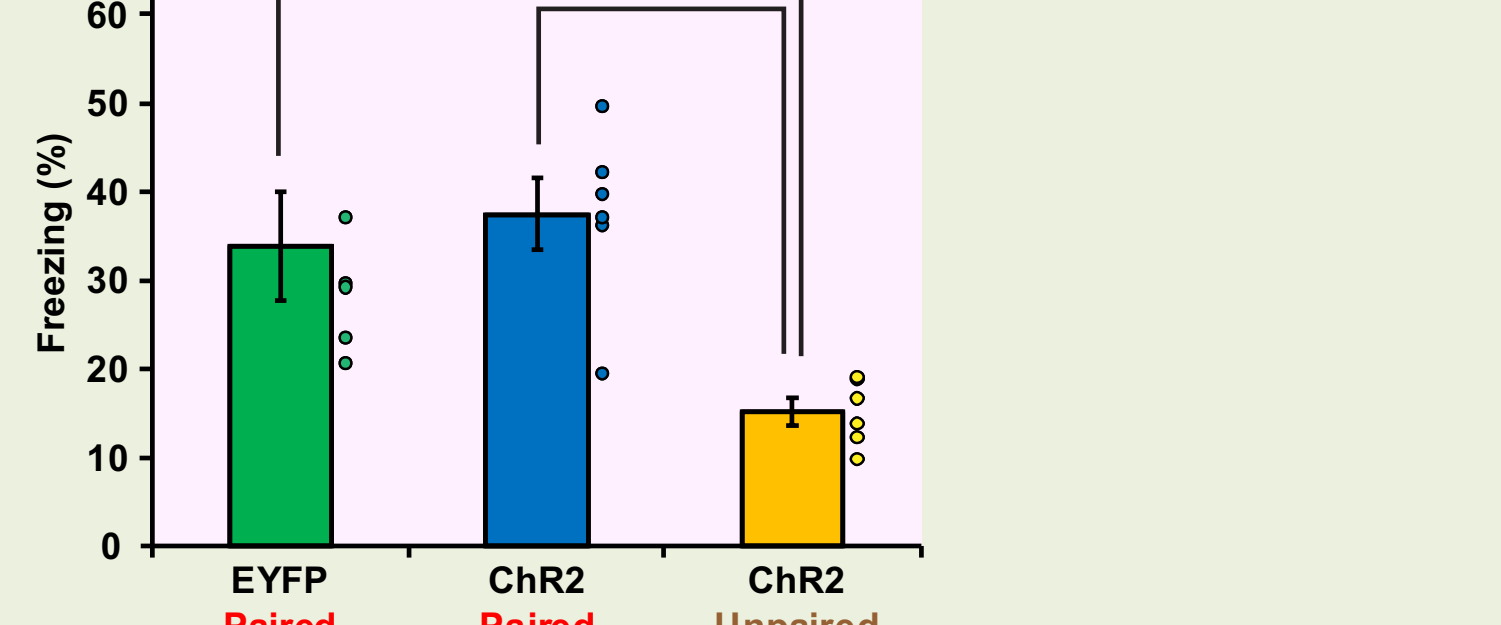
### 1. Context pre-exposure facilitation effect on immediate shock deficit procedure (CPFE procedure)



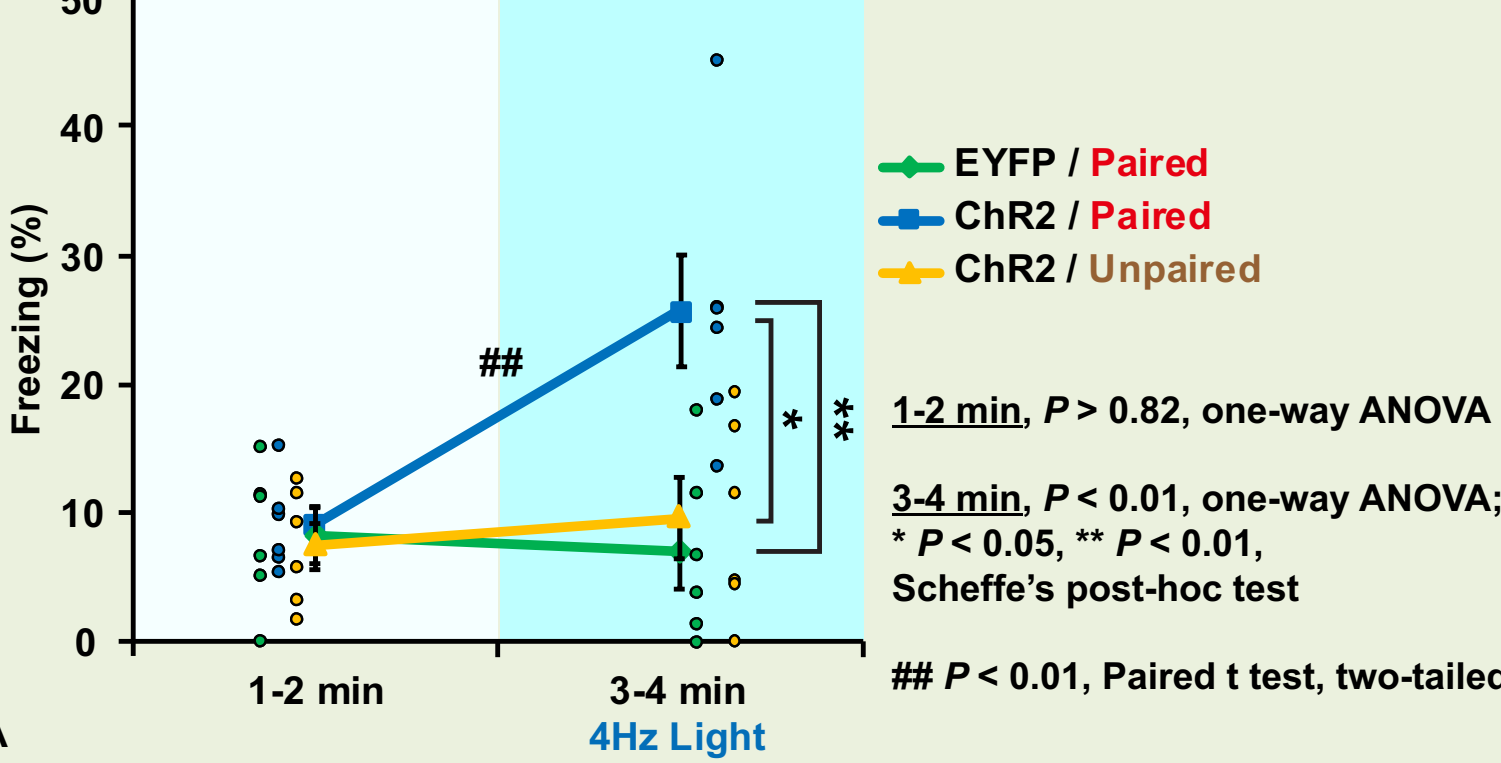
### 2. Engram of a context information is actually formed in CA1



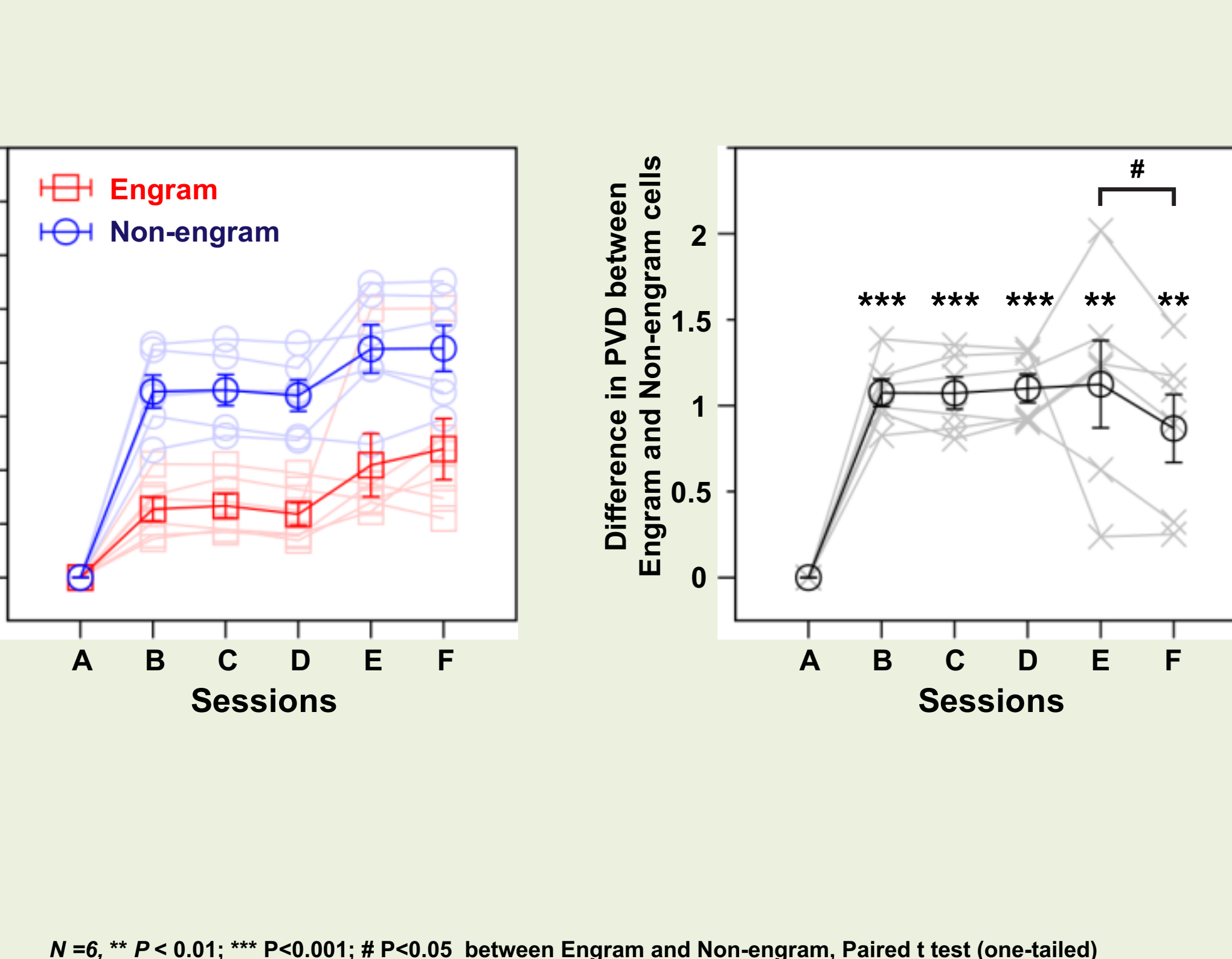
### Test 1



### Test 2

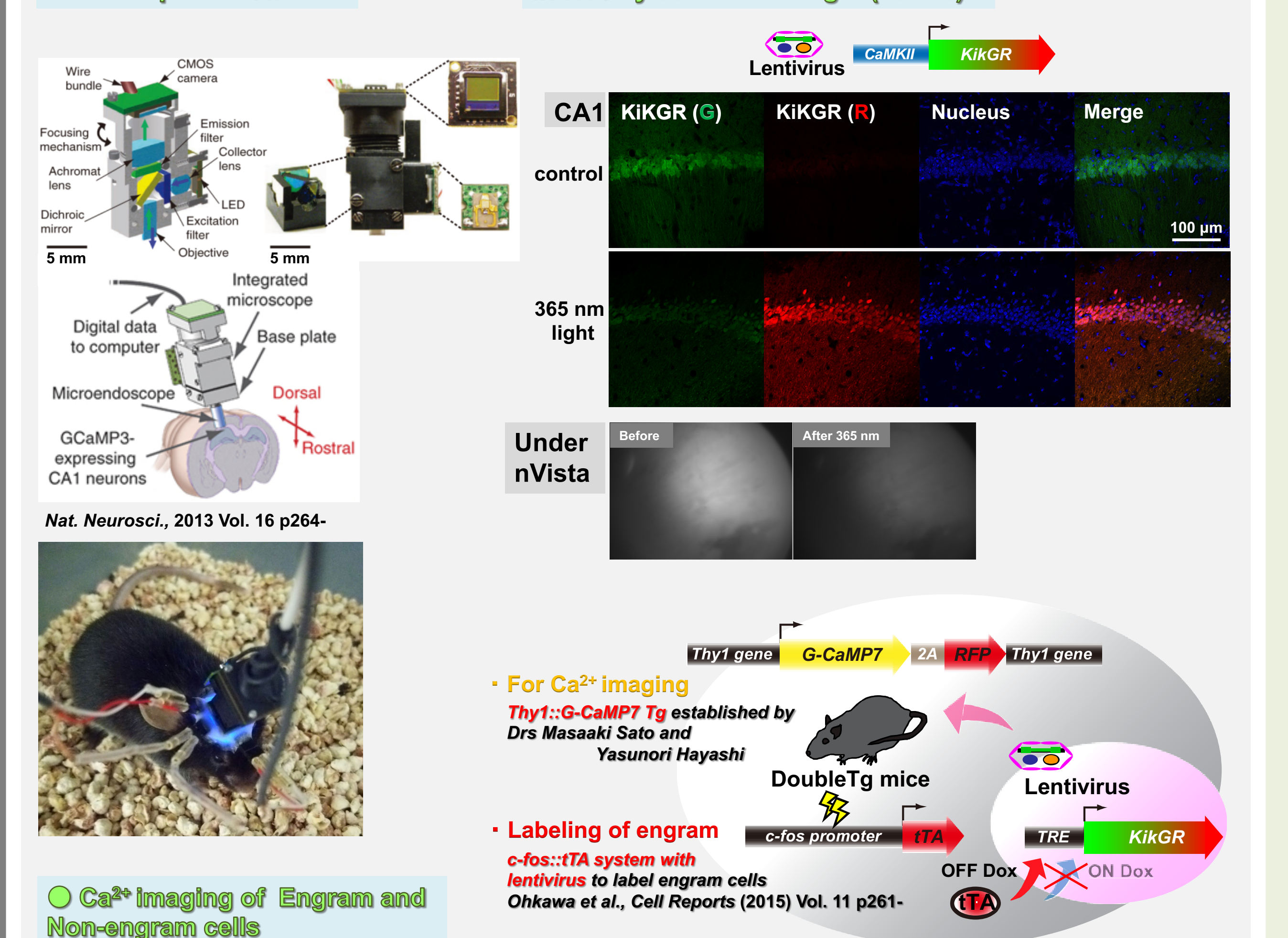


### 6. PVD is more consistent in Engram cells than Non-ensemble cells across sessions

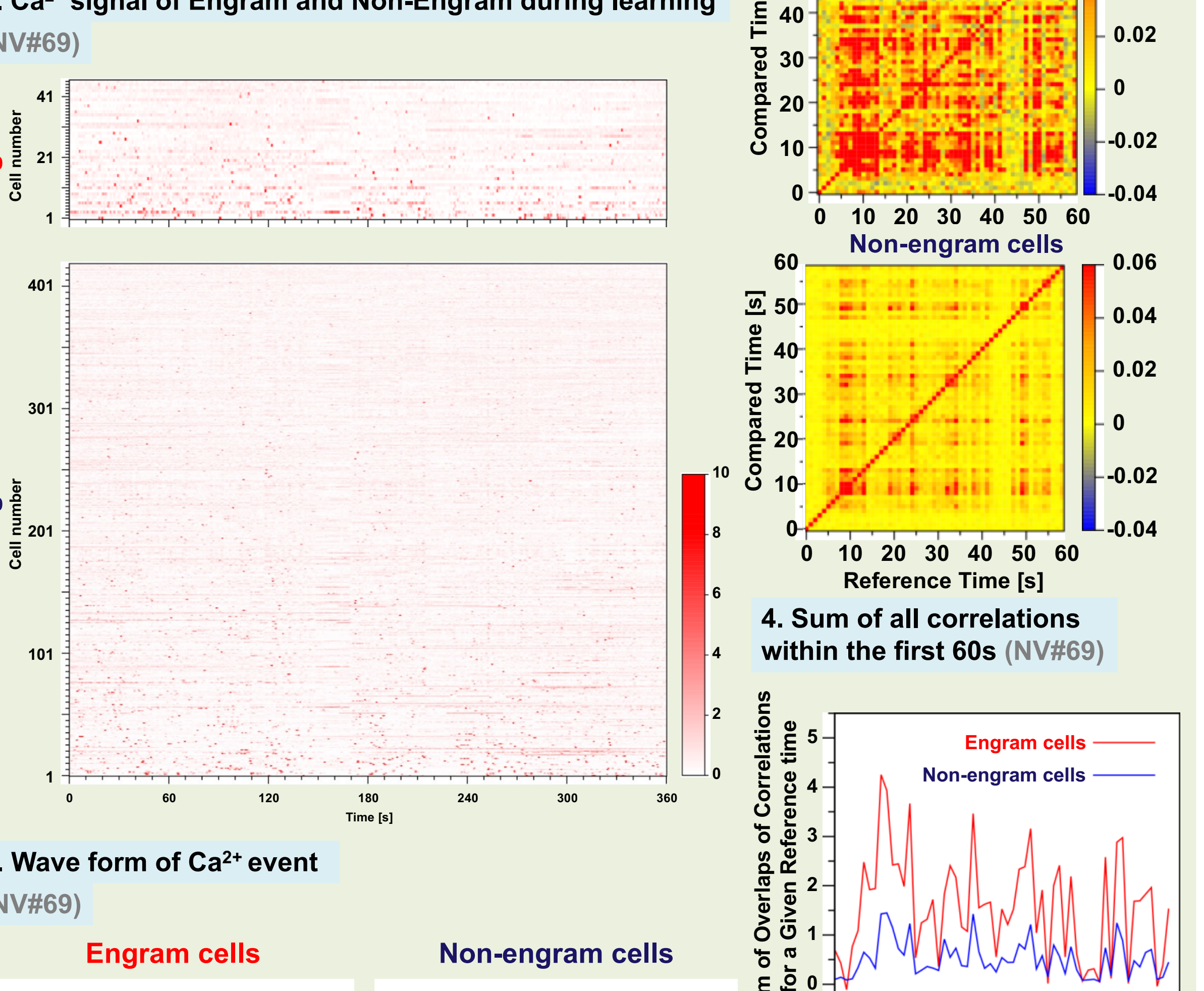


## Methods

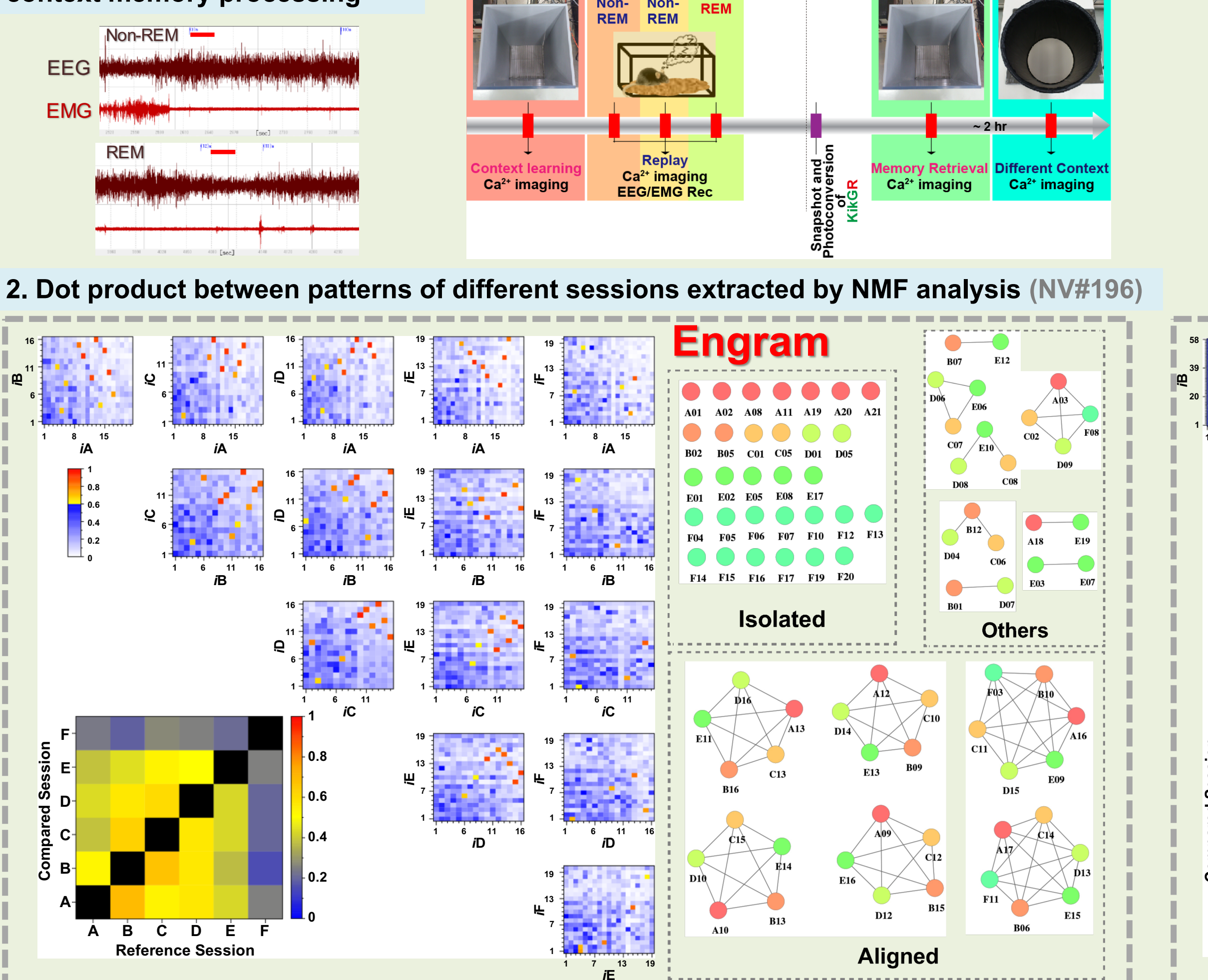
### 1. Engram and Non-ensemble cells (in case of learning)



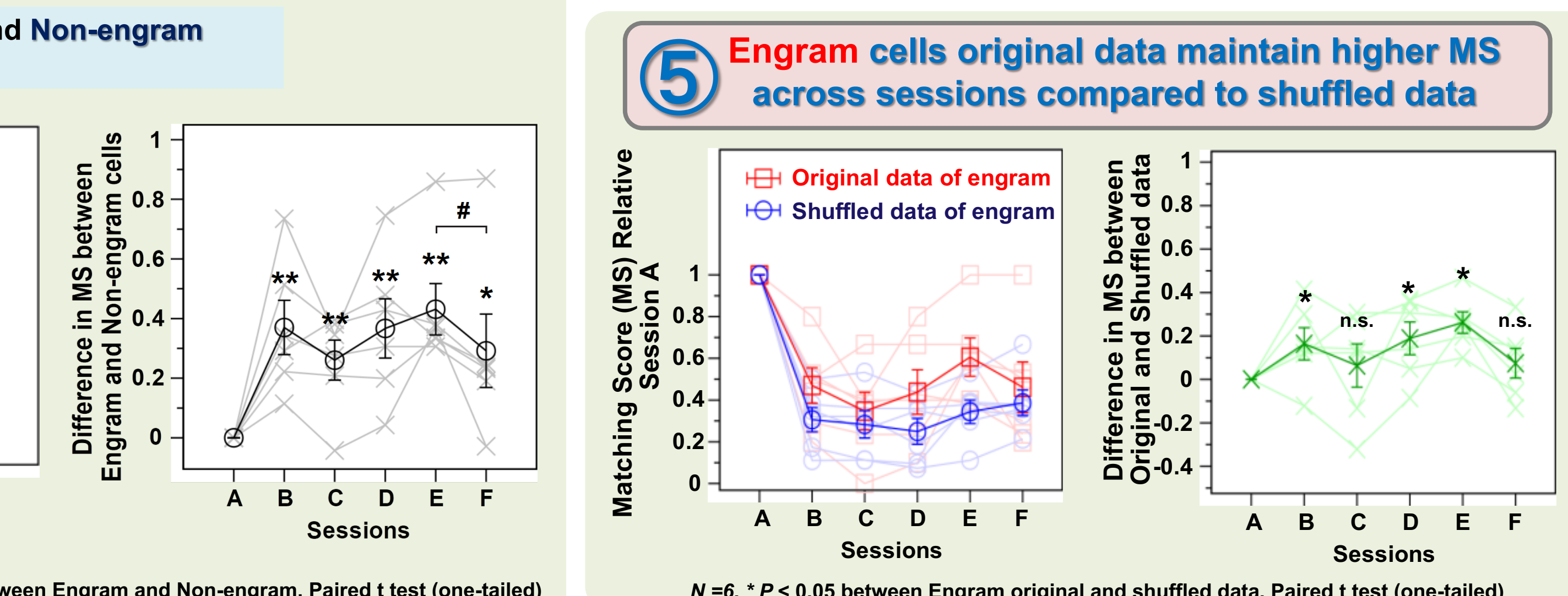
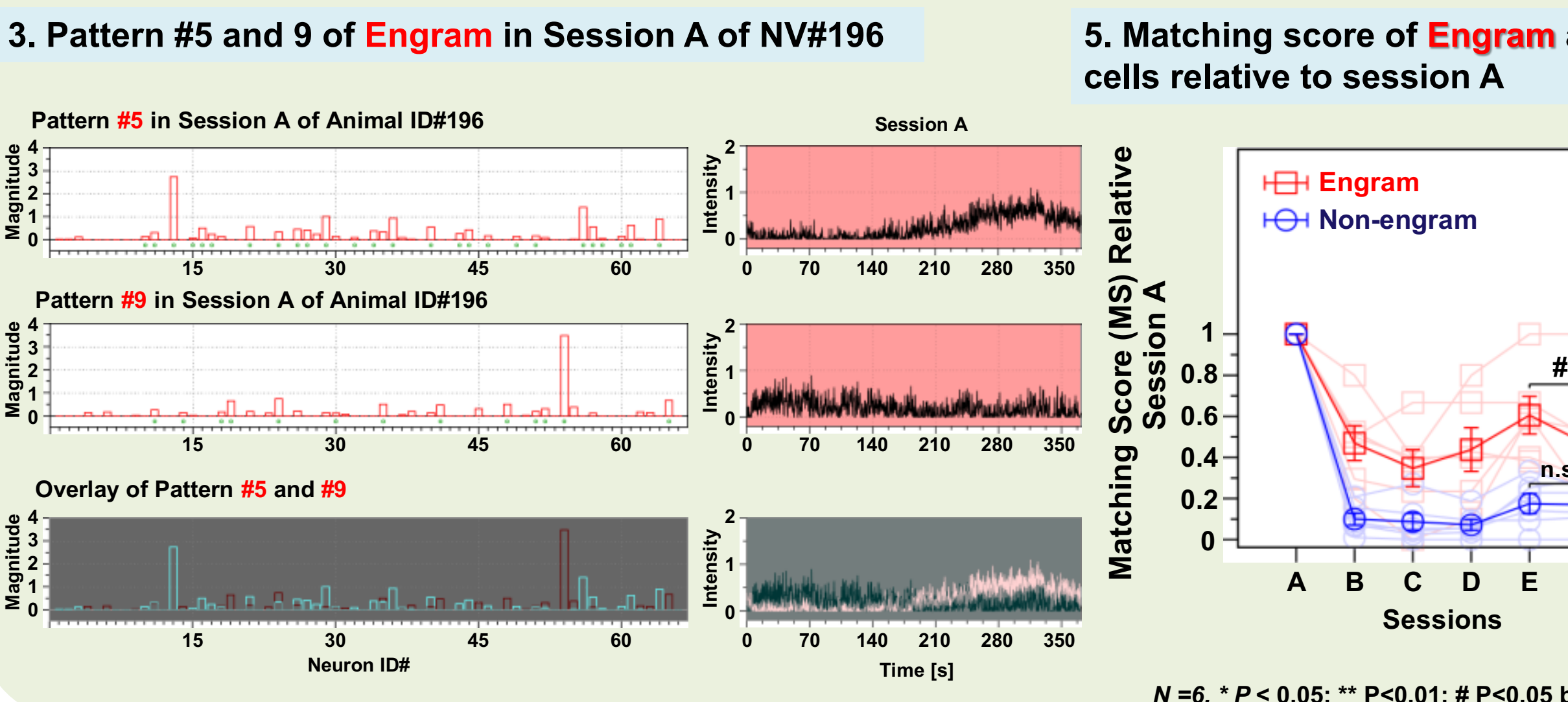
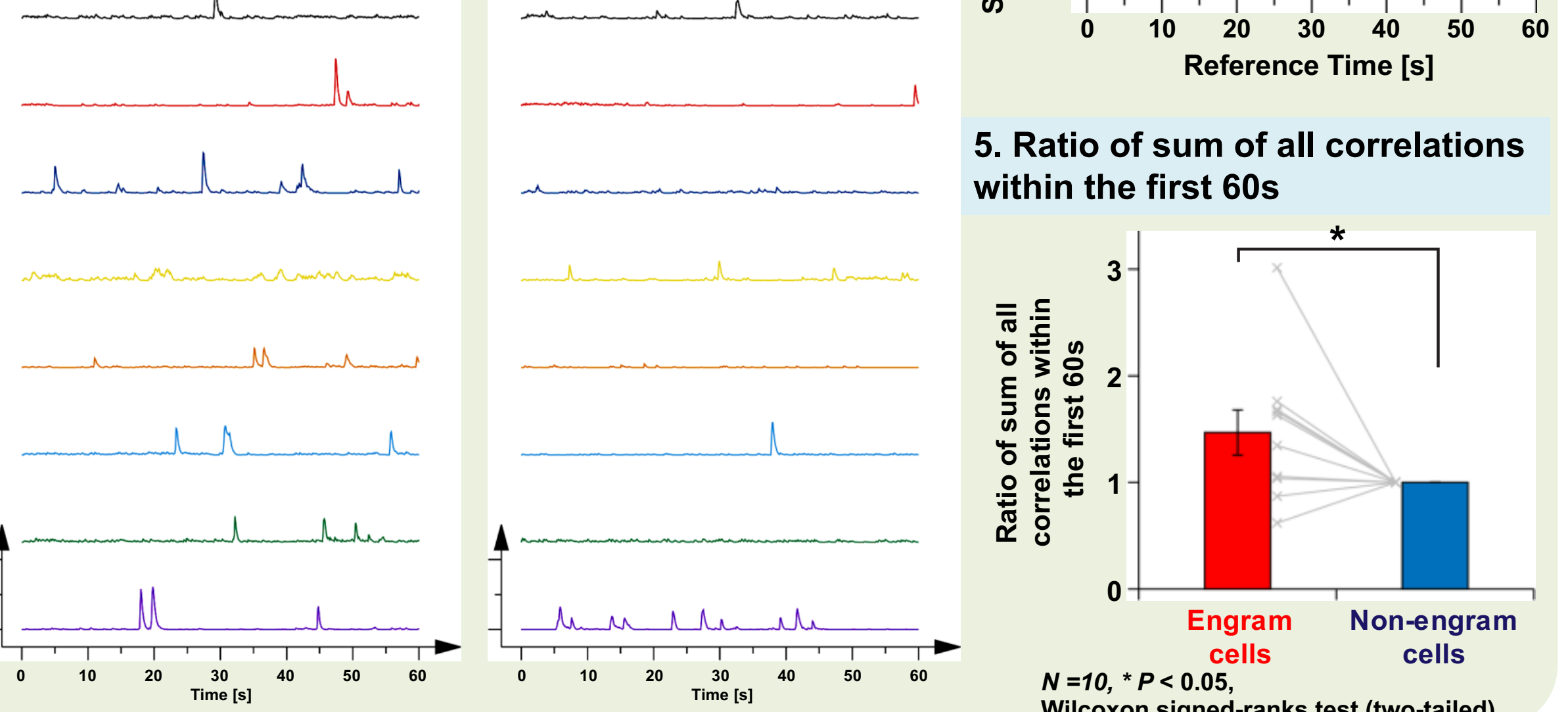
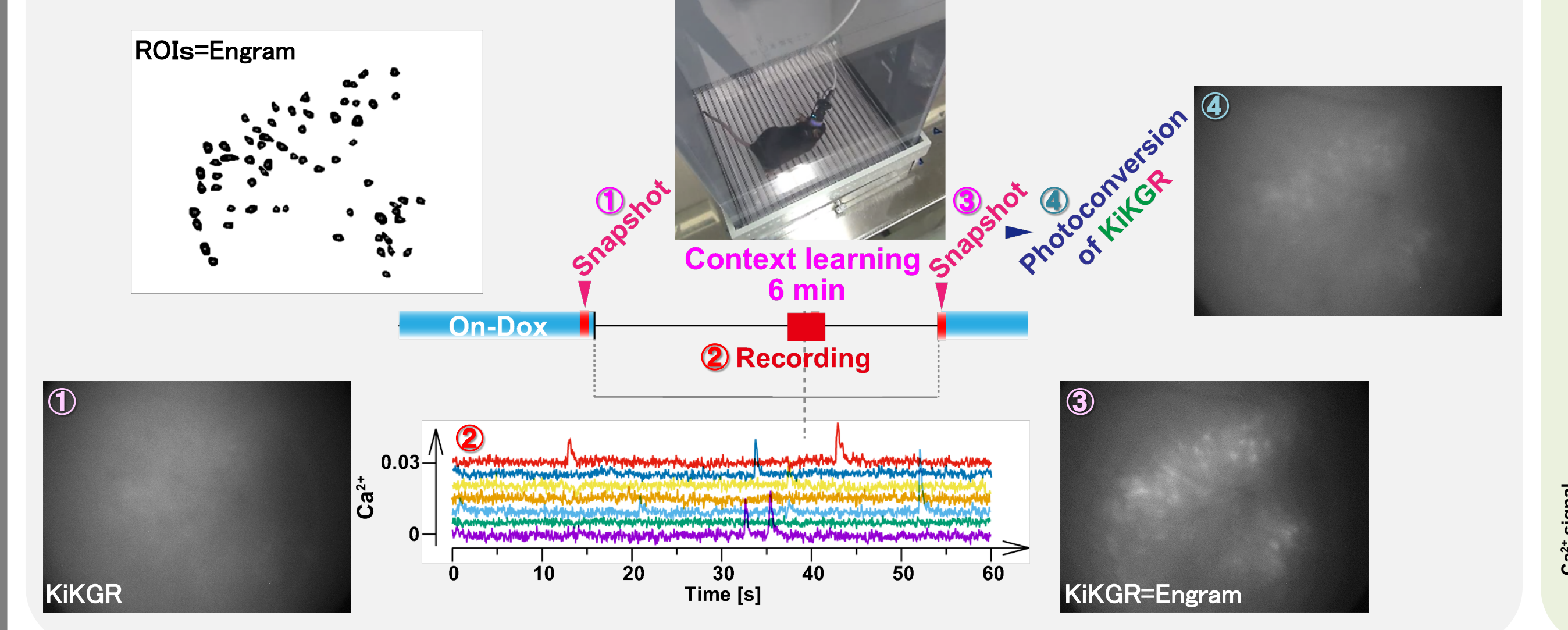
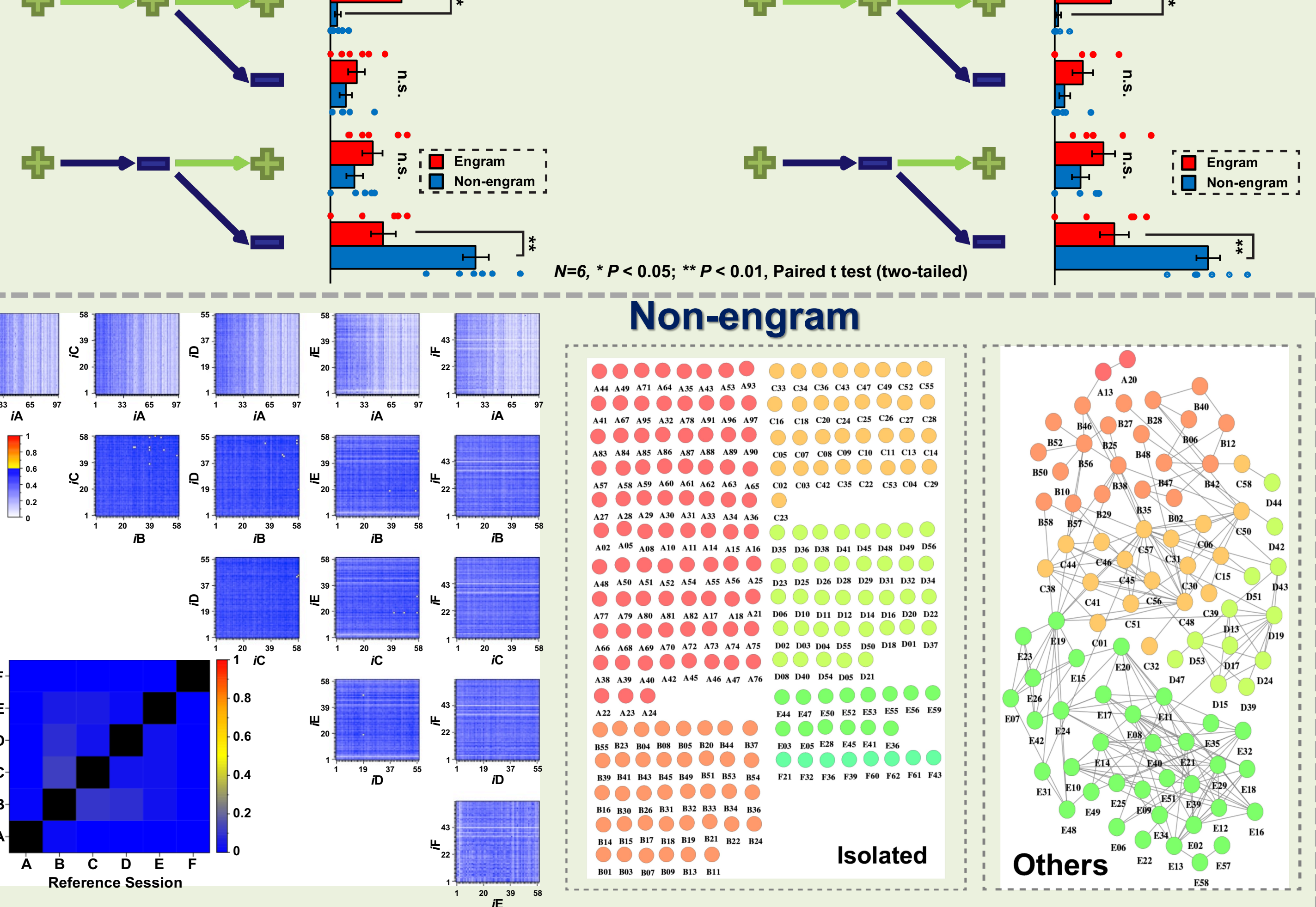
### 3. Repetitive activity is a characteristic feature of Engram cells during contextual learning



### 4. Ensemble activity patterns are preferentially reactivated in Engram cells during both resting and retrieval sessions



### 4. Engram patterns appeared in learning session are significantly highly reactive in both of either NREM or REM sleep and retrieval session compared with Non-ensemble patterns



## Question

How memory is represented and consolidated in a specific subpopulation of neurons?  
= "Engram"

The engram cells exhibit especial characteristics for encoding ongoing event and consolidation of past event during memory processing??

Contextual learning → Resting after learning → Memory retrieval

Cells that will be "Engram" → Identified by KikGR expression after learning

Photoconversion → Green fluorescence changes to Red (out of detection by nVista)

## Mathematical analysis

Activity pattern extraction (Non-negative Matrix Factorization)

$D \approx BC$

$E = \sum_{ij} (D_{ij} - \sum_k B_{ik} C_{kj})^2$

Dot product between patterns (Pattern matching)

To compare the patterns extracted across different sessions, cosine similarity between 2 vectors is used to quantify the similarity of 2 vectors. Pattern pair with > 0.6 is defined as a significant pair in this work.

Matching score (MS)

To compare the activity of engram cells and non-ensemble cells across different sessions, we calculated a matching score across sessions, which implies to what extent a pattern in the first session to be overlapped with any of the patterns extracted in the second session and vice versa.

Population vector distance (PVD)

The population vector distance is defined by the Mahalanobis distance, which implies the distance between 2 groups of neuronal activity vector normalized by their variance.

$C_{XY} = \frac{1}{\sqrt{N}} \sum_{i=1}^N \Theta((x_i^2 y_i^2) - 0, \sigma) - 0, \sigma$

## Conclusions

Contextual memory in the hippocampus is represented as distinct subsets of synchronous activity (defined by Ca<sup>2+</sup> transients) that comprise several ensembles of engram cells.

In contrast to non-ensemble cells, these ensembles maintain their activity not only during learning but also during post-learning sleep and retrieval sessions.

• We declare no Conflict of Interest (COI).

