Optogenetic SWR-silencing destabilizes newly formed hippocampal cell assemblies

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Introduction

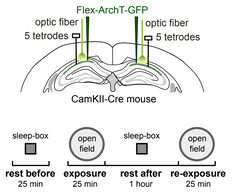
New memories are initially labile, and consolidation-processes during sleep are believed to contribute to their future retrieval. According to the functional cell assembly hypothesis, memories are represented at the network level by the temporally coordinated activity of subsets of neurons. This suggests that memory retrieval should be mediated by reinstatement of the same "memory-representing" cell assemblies that were active during encoding. To promote such reinstatement, newly-formed cell assemblies of the hippocampus are thought to be strengthened by their reactivation in sleep, which predominantly occurs during hippocampal sharp-wave/ripples events (SWRs). However, a direct relation between the off-line reactivation of cell assemblies and their subsequent reinstatement during memory retrieval has not been demonstrated.

Methods

[a] Hippocampal multi-unit recordings in freely-moving mice

Mice (n = 5) were implanted with a custom-made headstage to enable multi-channel recordings during exploratory behavior and sleep. Local field potential and extracellular action potentials were recorded with ten independently movable tetrodes bilaterally targeted to the dorsal CA1 region of the hippocampus. Action potentials discharged by single neurons were identified based on waveform-similarity and spike train dynamics (see McNamara et al., 2014). Firing rate and autocorrellogram-shape were used to classify isolated units as putative principal cells. The location of the animal was tracked with small light-emitting diodes attached to the headstage.

An hour after exploring either a novel or familiar open-field environment, animals were put back in the same environment. In the intervening period, animals rested in a "sleep-box". Reactivation during this sleep/rest period was investigated by comparing the expressed neural patterns with those expressed during a sleep/rest-session recorded before the first exploration.



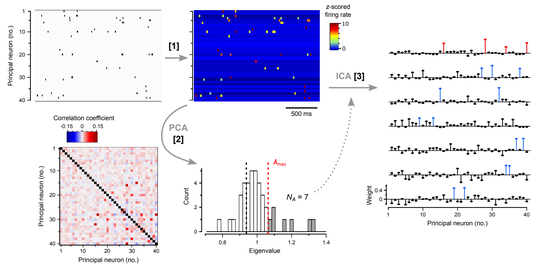
[b] Identification & tracking of hippocampal principal cell assembly-patterns

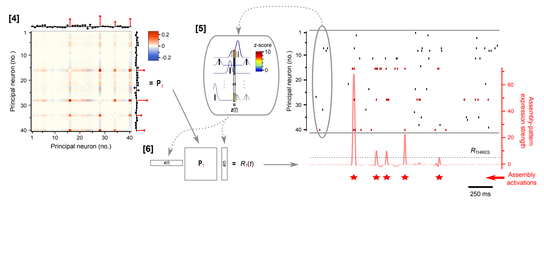
Assembly-pattern identification

After binning (25 ms) and normalizing (z-score) each neuron's spike-counts **(1)**, principal component analysis (PCA) was applied to the resulting matrix to find the number of statistically significant patterns **(2)**, as estimated by the number of eigenvalues N_A exceeding the Marcenko-Pastur threshold λ_{max}. Independent component analysis (ICA) was then used to identify N_A assembly-patterns, given by weight-vectors indicating the contribution of each neuron to that pattern **(3)** (Lopes-dos-Santos et al., 2013).

Tracking of assembly-pattern expression

For each assembly-pattern, a "projector matrix" was constructed by taking the outer product matrix of its weight-vector and setting the diagonal to zero **(4)**. The spike-trains were convolved with a Gaussian kernel **(5)**. The assembly-pattern expression strength was then taken as the quadratic form of the projector matrix with the convolved and z-scored spike-trains **(6)**. Assembly activations were defined as peaks in the expression strength exceeding R_{THRES} = 5.

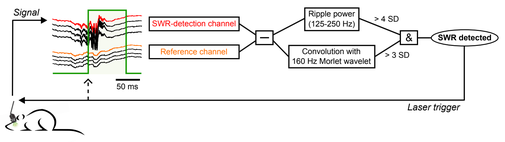




[c] Optogenetic silencing of hippocampal principal cells during on-line detected SWRs

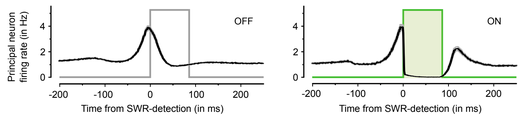
On-line detection of SWRs

Based on the power of the recorded signal in the ripple frequency band, more than 80% of the off-line identified SWRs were detected in real time with an average latency of 7.7 ms before the SWR peak power.



Silencing of principal cells using ArchT

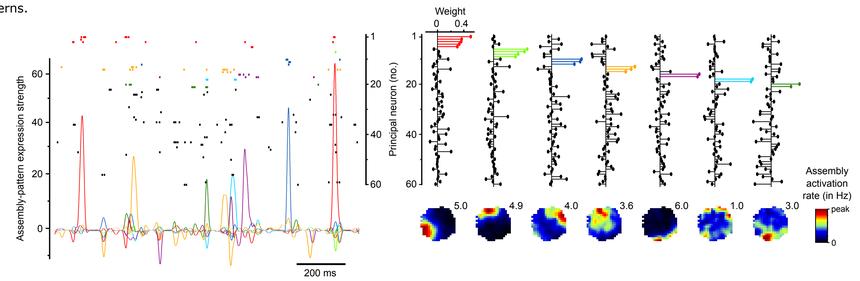
CamKII-Cre$^{+/+}$ mice (n = 4) were injected with a Cre-recombinase-dependent AAV vector carrying a gene for ArchT. Upon detection of each SWR, a laser-pulse (561 nm) was delivered to the hippocampus through two optic fibers bilaterally implanted targeting the dorsal CA1 pyramidal layer.



Results

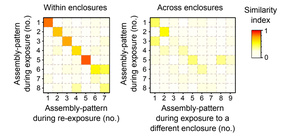
(1) Short-timescale coordination of hippocampal neurons forms spatially-selective assembly-patterns

This panel shows assembly-patterns identified from the 25 ms co-fluctuation of neuronal discharges during an exposure-session, together with a 2 sec example-trace depicting the spike-trains of the 60 simultaneously recorded hippocampal principal cells along with the expression strength time-course of each detected pattern. The "assembly maps" at the bottom-right illustrate the strong spatial tuning characteristic for most of these patterns.



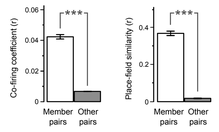
Detected assembly-patterns are environment-specific

Patterns expressed during exposure to an enclosure are more similar to those expressed during re-exposure to that same enclosure (left) than to those expressed in a different enclosure (right).



Assembly-patterns correspond to cell assemblies?

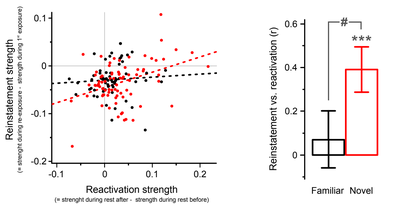
For each assembly-pattern, a corresponding "cell assembly" is defined as those neurons with weight exceeding 2 SD above the mean. Pairs of neurons assigned in this way to the same cell assembly indeed have strong correlated firing activity (left) and large place field overlap (right).



(2) Reinstatement of assembly-patterns representing novel environments depends on their off-line reactivation

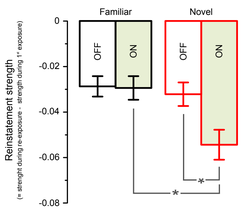
Reactivation predicts subsequent reinstatement

After exposure to a novel, but not a familiar, enclosure.



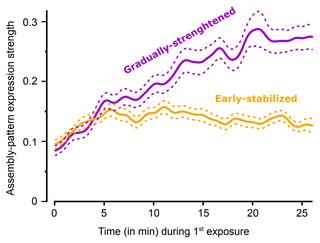
Silencing of SWR reactivation impairs subsequent reinstatement

After exposure to a novel, but not a familiar, enclosure.

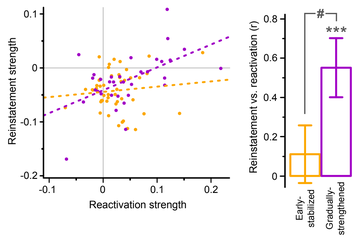


(3) Gradually-strengthened, but not early-stabilized, assembly-patterns require reactivation

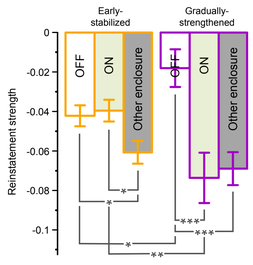
If repeated co-activation of member-neurons strengthens a newly-formed assembly, then the strength of that assembly is expected to increase throughout its formation. We divide assembly-patterns representing novel environments into two groups based on the dynamics of their expression strength during the initial exposure session: "gradually-strengthened" patterns have a significant increasing linear trend while "early-stabilized" patterns do not.



Reactivation predicts reinstatement only for gradually-strengthened patterns



SWR-silencing impairs reinstatement only of gradually-strengthened patterns



Discussion

We found that the reinstatement of assemblies representing a novel, but not a familiar, environment was both predicted by their off-line reactivation and impaired by closed-loop optogenetic silencing timed to SWRs. However, we discovered that reactivation was only required for the reinstatement of assemblies whose expression was gradually strengthened during encoding of a novel place. The context-dependent reinstatement of assemblies whose expression did not gain in strength beyond the first few minutes of encoding was not dependent on reactivation. Yet, both sets of assemblies were spatially selective and equally represented the environment. This demonstrates that co-existing hippocampal representations of space can markedly differ in their consolidation mechanisms.

References

Lopes-dos-Santos et al. (2013) Detecting cell assemblies in large neuronal populations. *J Neurosci Methods* **220**: 149-166.
McNamara et al.(2014) Dopaminergic neurons promote hippocampal reactivation and spatial memory persistence. *Nat Neurosci* **17**: 1658-1660.

Acknowledgements

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