Sleep reactivation predicts awake reinstatement of hippocampal cell assemblies

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Introduction

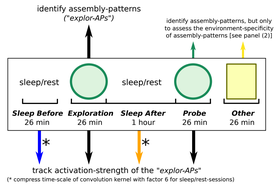
New memories are initially labile, and consolidation-processes during sleep are believed to contribute to their future retrieval. According to the “functional cell assembly hypothesis” (Hebb, 1949), memories are represented at the network level by the temporally coordinated activity of subsets of neurons. One implication of this hypothesis is 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 consolidated by their reactivation in sleep, which predominantly occurs during hippocampal sharp-wave/ripples events (SWRs). However, a direct relation between the (sleep) reactivation of cell assemblies and their subsequent (awake) 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 were allowed to sleep. Reactivation during this sleep/rest was investigated by comparing the expressed neural patterns with those expressed during a sleep/rest-session recorded before the first exploration. After putting the animals back in the same environment, animals were exposed to another environment to assess the environment-specificity of the expressed assemblies.



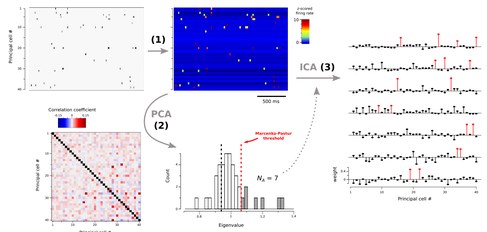
[b] Identification & tracking of principal cell assembly-patterns

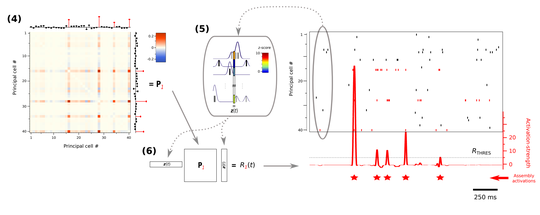
Assembly-pattern identification

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)**, which is given by the number of eigenvalues exceeding the Marcenko-Pastur threshold (= N_A). 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 activity

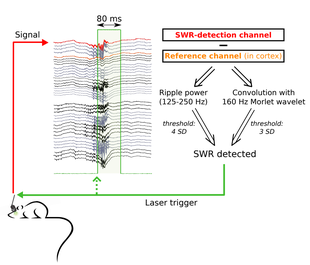
For each assembly-pattern, a “projector matrix” was constructed by taking the outer product matrix of its weight-vector and setting the diagonal to zero **(4)**. The spike-trains were convolved with a Gaussian kernel **(5)**. The instantaneous assembly-pattern activation-strength was then taken as the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



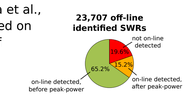


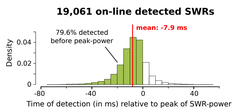
[c] Optogenetic silencing of hippocampal principal cells during on-line detected SWRs

On-line detection of SWRs



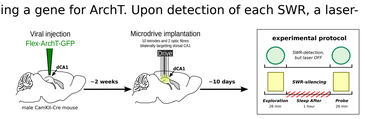
The performance of the real-time SWR-detection algorithm was evaluated by comparing the on-line detected SWRs with off-line identified SWRs (McNamara et al., 2014), based on 20 hours of sleep/rest-recording:



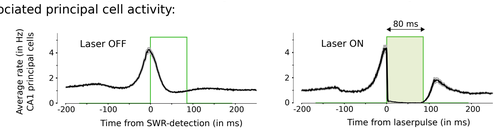


Silencing of principal cells using ArchT

Male adult CamKII-Cre$^{+/+}$ mice (n = 2) 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.



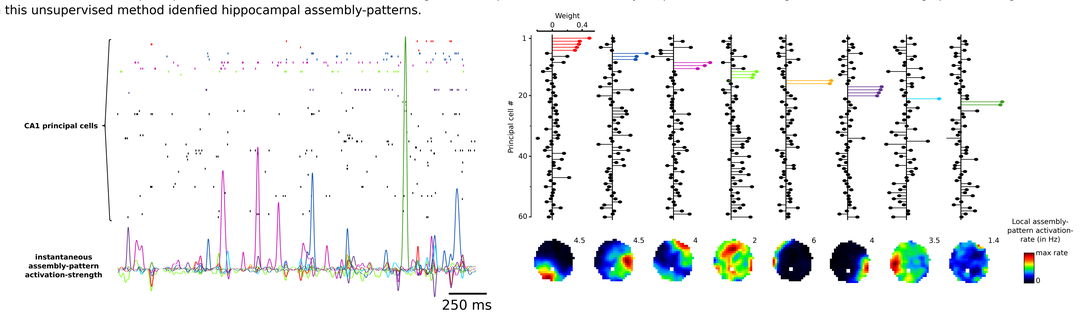
This closed-loop optogenetic feedback system succeeded to transiently silence SWR-associated principal cell activity:



Results

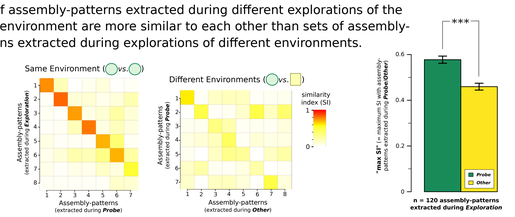
(1) Identified hippocampal assembly-patterns exhibit strong spatial tuning

This panel shows the weight-vectors of the 8 assembly-patterns extracted during a representative 26-min exploration-session, together with a 3-sec example-trace depicting the recorded spike-trains of the 60 simultaneous recorded principal cells and the tracked activation-strength of each pattern. The "assembly maps" at the bottom-right illustrate the strong spatial tuning characteristic for most of the with this unsupervised method idenfied hippocampal assembly-patterns.



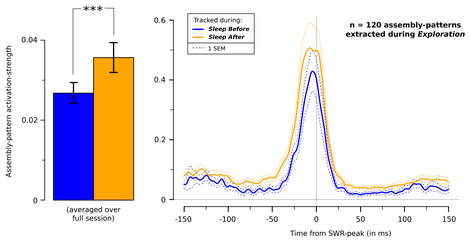
(2) Assembly-patterns are environment-specific

Sets of assembly-patterns extracted during different explorations of the same environment are more similar to each other than sets of assembly-patterns extracted during explorations of different environments.



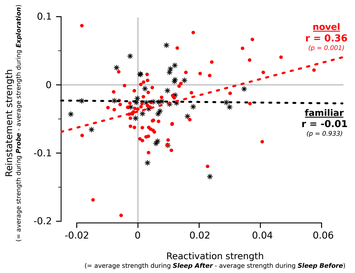
The similarity index (SI) between two patterns is given by the inner product of their weight-vectors.

(3) Assembly-pattern reactivation locked to SWRs



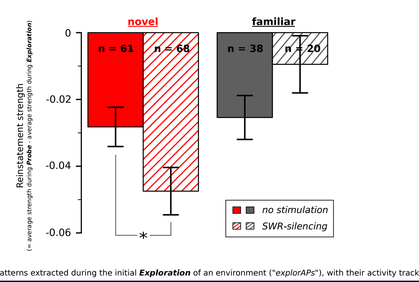
(4) Reactivation of assembly-patterns predicts their subsequent reinstatement

After exploration of a novel - but not a familiar - environment



(5) Silencing of SWR-associated reactivation impairs subsequent assembly-pattern reinstatement

After exploration of a novel - but not a familiar - environment



In panels (4) and (5), the data-points are assembly-patterns extracted during the initial ***Exploration*** of an environment (“*explorAPs*”), with their activity tracked during the indicated session

Discussion

Based on short-time scale (25 ms) interactions, groups of hippocampal neurons are identified whose combined discharges are internal representations of space. The environment-specificity of these assembly-patterns suggests that new patterns are formed in the hippocampus during exploration of a novel environment. We found that the strength at which those assembly-patterns are reactivated during the sleep after predicts their subsequent reinstatement strength during re-exposure to the same environment. Moreover, optogenetic silencing of hippocampal principal cells during on-line detected SWRs impairs that reinstatement. Importantly, after exploration of a familiar environment, when the cell asssemblies underlying the spatial map are presumably more stable, neither result holds. We thus provide direct evidence for a causal role of SWR-associated (sleep) reactivation on the (awake) reinstatement of newly-formed hippocampal cell assemblies.

References

Hebb (1949) The Organization of Behavior. New York: Wiley & Sons.
Lopes-dos-Santos et al. (2013) Detecting cell assemblies in large neuronal populations. *J Neurosci Methods* **220**: 149-166.
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Acknowledgements

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