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Research Report

Hippocampal replay sequence governed by spontaneous brain-wide dynamics

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Abstract

Neurons in the hippocampus exhibit spontaneous spiking activity during rest that appears to recapitulate previously experienced events. While this replay activity is frequently linked to memory consolidation and learning, the underlying mechanisms are not well understood. Recent large-scale neural recordings in mice have demonstrated that resting-state spontaneous activity is expressed as quasi-periodic cascades of spiking activity that pervade the forebrain, with each cascade engaging a high proportion of recorded neurons. Hippocampal ripples are known to be coordinated with cortical dynamics; however, less is known about the occurrence of replay activity relative to other brain-wide spontaneous events. Here we analyzed responses across the mouse brain to multiple viewings of natural movies, as well as subsequent patterns of neural activity during rest. We found that hippocampal neurons showed time-selectivity, with individual neurons responding consistently during particular moments of the movie. During rest, the population of time-selective hippocampal neurons showed both forward and time-reversed replay activity that matched the sequence observed in the movie. Importantly, these replay events were strongly time-locked to brain-wide spiking cascades, with forward and time-reversed replay activity associated with distinct cascade types. Thus, intrinsic hippocampal replay activity is temporally structured according to large-scale spontaneous physiology affecting areas throughout the forebrain. These findings shed light on the coordination between hippocampal and cortical circuits thought to be critical for memory consolidation.

Significance Statement

During periods of quiescence, hippocampal neurons replay spiking activity sequences from previous behavioral events, believed to be vital for learning and memory. The mechanisms behind these replays, however, remain largely elusive. Our research demonstrates that the replay of a movie-induced sequence in the hippocampus is intricately linked with a broader spontaneous dynamic across the brain, characterized by sequential activations across neurons from various brain areas. These results imply that hippocampal replays emerge and are structured based on the intrinsic resting-state circuit dynamics and also offer insights into the interplay between hippocampal and cortical circuits pivotal for memory consolidation.

Introduction

Learning and memory are the cornerstones of intelligence. The hippocampus is a key brain structure involved in these functions. A remarkable finding about the rodent hippocampus is the fact that its place-selective neurons ("place cells") can replay sequences of activity previously induced by active exploration of a spatial environment. Often these replay episodes take place during rest and sleep and are typically manifest in a temporally compressed form (1–4). Such replay sequences are associated with prototypical electrical events originating in the hippocampus called sharp wave-ripple complexes (SPW-R) (5–7). These events, which are evident as high-amplitude bursts in hippocampal local field recordings, have been proposed to play an important role in the consolidation of episodic memory (8–11).

The nature of hippocampal firing sequences during quiescent periods is a matter of active research. Increasing evidence suggests that these sequences, rather than being induced by the external experience itself, are fundamentally a product of internal circuit dynamics (12–16). A puzzling finding is that in addition to replaying previous sequences generated during active behaviors,

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hippocampal place cells also appear to "preplay" a firing sequence that is only encountered later during exposure to a novel environment (17–19), suggesting that the hippocampal sequences exist before experience. Similarly, in a related subclass of hippocampal neurons, firing sequences appear to be generated not by the registration of external events, but instead by the passage of time (20). The sequential firing of these "time neurons" can occur in the absence of changing environmental or body-derived inputs (21–24). These findings of preplays and time neurons have propelled a new theory that episode-specific activity sequences of hippocampal neuronal assembly roll forward as a result of the self-organization of the brain and this temporal flow of activity is determined by intrinsic neuronal architecture (12–14, 17, 25).

A distinct type of sequential activations has recently been shown to shape neural firing across the forebrain, extending beyond the hippocampus to include multiple cortical and thalamic structures (26). Like the replay, preplay, and time sequences observed in the hippocampus, these widespread patterns operate autonomously in the absence of external perturbations. They are expressed as stereotypic spiking cascades that affect a large proportion (~70%) of the neural population in all tested forebrain areas. They are synchronized and quasi-periodic, with individual sequences lasting between 5 and 15 seconds. Moreover, each individual neuron bears a consistent temporal signature in its peak firing, leading or lagging the population peak by a fixed temporal interval. Importantly, these single-cell spiking sequences, which are expressed at many locations across the forebrain, were found to be synchronized with the slow modulation of hippocampal SPW-R occurrence (26). This synchronization with hippocampal ripples raises the question of whether these widespread sequential spiking cascades might stem from the same self-generated brain dynamics as the hippocampal replays, which also concur with the hippocampal ripples as sequential activations.

In this study, we investigated this possibility by analyzing population neuronal recordings from the visual cortex and hippocampus of the mouse under conditions conducive to replay activity. Using data available through the Allen Visual Coding project (27, 28), we first evaluated the activity of individual neurons in the visual cortex and hippocampus recorded during the viewing of a movie. Neurons in both areas yielded responses associated with particular moments or events in the movie, forming temporal sequences of neuronal spiking. The activity of these apparently time-selective neurons during subsequent periods of rest recapitulated the movie-induced sequence in a temporally compressed manner in the hippocampus, but not the visual cortex. We then investigated the relationship between these movie-related replay events and previously reported spontaneous firing cascades that engulf the brain during rest (26). Importantly, the hippocampal replay events were temporally aligned to the spiking cascades, indicating that the replay activity in the hippocampus is one facet of a larger-scale pattern of sequential neural dynamics expressed spontaneously across the brain. A fine-scale analysis further revealed that forward and reverse hippocampal replays appeared, respectively, during two fundamental types of spiking cascade events of shorter duration. Together, these findings indicate that the hippocampal replay events are generated and structured according to resting-state circuit dynamics manifest as the spiking cascade across a large portion of the brain.

Results

We analyzed large-scale neuronal recordings in mice from the Visual Coding project of the Allen Institute (27, 28). The dataset includes the spiking activity of a large group of neurons simultaneously recorded from various brain cortical and subcortical regions. We focused on the spiking data of ~10,000 neurons recorded from 14 mice in 44 brain regions (730 ± 178 neurons per mouse, mean ± SD) during two movie sessions and one spontaneous session (Fig. 1A and B). In each of the two movie sessions (i.e. prerest and postrest ones), the same 30-s movie clip was repeatedly presented to mice 30 times. The spontaneous session was free of visual stimulation, and the 14 mice remained stationary for extended periods (>20 min) (see Materials and methods for stationary quantification).

Hippocampal and visual neurons showed time-selective response during movie watching

We first examined time-selective responses of neurons during movie watching (i.e. the prerest movie session, which was used to obtain all main results, whereas the postrest session was used to check consistency with the relevant data presented in the supplementary material; the same hereafter). To do this, a time course of time-specificity score was computed for each neuron to quantify its firing rate increase at a specific moment (i.e. a 0.5-s time bin) compared with other periods of the movie. The peak score quantifies the amplitude of the time-selective response, whereas the time to achieve this peak is regarded as the time field of the neuron (Fig. 1C; see Materials and methods for details). The neurons with a significant ($P < 0.05$) peak time-specificity score were regarded as time-selective neurons. After sorting the neurons by the time field, sequential activations of the neurons are clearly visible as a diagonal band in the averaged spiking activity during movie watching. The number of identified CA1 time-selective neurons showed a significant disparity between the real dataset and the null distribution generated by shuffling neuronal spikes within each movie trial (Fig. S1A). Similar results were obtained for the postrest movie session (Fig. S2A) and an extended group of mice (Fig. S3). Such time-specific responses were even stronger for the visual neurons (Figs. S1B and S2B). Both the CA1 and visual time-selective neurons demonstrated reproducibility across presentations of the same movie, with the CA1 time-selective neurons showing relatively more variability (Fig. S4). It is worth noting that the definition of time-selective neurons in our study differs from the conventionally defined time neurons (20, 22). In this case, their activity (particularly visual neurons) may have been responses to time-dependent visual features rather than only reflecting the passage of time.

The movie-induced sequence of the CA1 time-selective neurons replays at rest

We then studied whether the firing sequence observed during the movie watching would replay at rest, similar to the place-cell firing sequence during maze running (6, 7). We adapted a template-matching method to detect the replay events. Briefly, the resting-state spiking data were divided into time segments according to troughs of the global mean spiking rate (vertical dotted lines in Fig. 2B) similar to the previous study (26), but the global mean signal was first low-pass (<5 Hz) filtered to generate fine-scale segments whose duration (506 ± 186 ms) roughly matched up with the known timescale of hippocampal replays. A delay profile was computed to describe the order of sequential activations of time-selective neurons within each segment and then correlated (Spearman's rank correlation) with their firing sequence during the movie watching (Fig. 2A and B; see Materials and methods for details). The replay

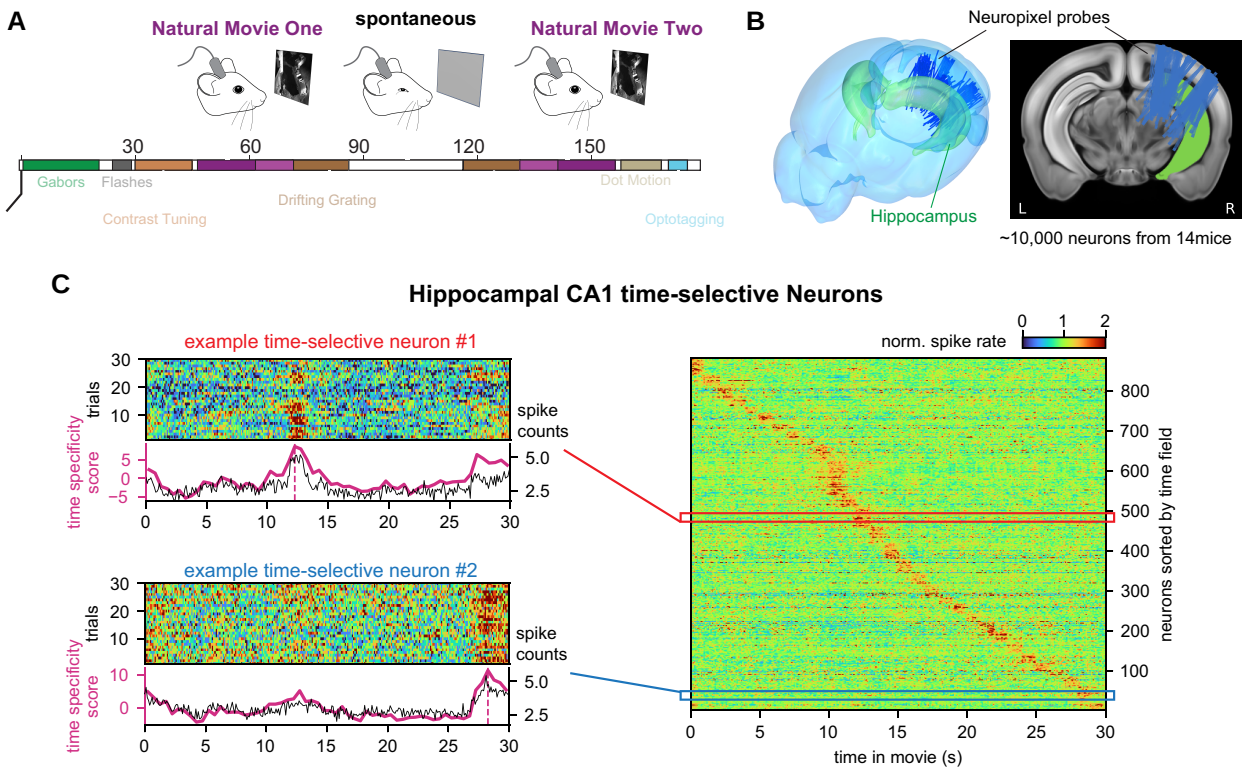

Fig. 1. Time-specific responses of hippocampal neurons during movie watching. A) Illustration of the "functional connectivity" stimulus set of the "Visual coding—neuropixels" project, which includes a 30-minute spontaneous resting session and two natural movie sessions used in this study. B) 3D locations of 6,171 channels on 79 probes from 14 mice (left) and their projection onto the 2D middle slice of the brain template (right) in Allen Mouse Common Coordinate Framework. C) Left: two example CA1 neurons showing strong time-selective responses that are consistent across different trials of movie watching. The time courses of the mean spiking rate and the time-specificity score, both peak within the neuron's time field. The spiking rate was normalized as a percentage change relative to its temporal mean. Right: The averaged ($N = 30$ trials) spiking activity of the CA1 time-selective neurons during the movie watching from all mice. The neurons were sorted according to their time field. The red and blue lines identify the two example neurons.

events were then detected as time segments showing significant ($P < 0.01$) positive (forward) and negative (reverse) correlations (red and blue segments in Fig. 2B). The same procedure was repeated for randomized movie sequences ($N = 200$) to create a null distribution for the replay counts. In 11 out of 14 mice, the number of replay events of the CA1 time-selective neurons was significantly higher than what would be expected from the randomized controls. The above analysis was repeated for three control cases: the equal number of VIS time-selective neurons showing the strongest time-selective responses to the movie, the CA1 nontime-selective neurons that did not show significant time-selective responses, and the CA1 time-selective neurons derived from the shuffled data described above. Significant replay events were seen in none of these cases, including the visual time-selective neurons that had a stronger time-specific response in the movie sessions than the CA1 time-selective neurons (Figs. 2D and S6C). Consistent with the previous findings (6, 7, 29), independently detected SPW-R events using local field potential (LFP) from CA1 regions (see Material and methods and Fig. S5) peaked around the center of the replay events of the CA1 time-selective neurons (Figs. 2E and S6D).

Hippocampal replays co-occur with brain-wide spiking cascades

We further investigated the potential link between the replay events and previously reported brain-wide cascades of neuronal firing (26). The slow spiking cascades can be clearly seen in the resting-state recordings after sorting all recorded neurons from various brain regions according to their principal delay profile (Fig. 3A), i.e. the first principal component of delay profiles of coarse-scale time segments (see Ref. (26) for more details). This coarse-scale principal delay profile represents the direction of sequential activations of the spiking cascade (Fig. S7A and B). The cascade started with slow and sequential entrainments of the negative-delay neurons (top group of neurons in Fig. 3B) at the early phase and then reached a tipping point featuring the rapid transitioning to the activation of the positive-delay neurons (bottom group of neurons in Fig. 3B), which were then slowly and sequentially disengaged in ~1–3 s (Fig. 3A and B). The cascade involved ~70% of all recorded neurons from various brain regions (26), and the region-specific mean spiking activity showed significant modulations at the cascade in every recorded brain region (Fig. 3C). Tracking the occurrence of the CA1 replays along with the spiking cascade revealed an interesting pattern: the reverse replays of a movie sequence in the CA1 time-selective neurons are much more likely to occur around the fast-transitioning (black arrows, defined as the sharp activation of positive-delay neuron, see Materials and methods for details) of the spiking cascades (Fig. 3D). This observation is consistent with the distribution of the reverse replays over the cascade cycle (Fig. 3E and F). The forward replays displayed an opposite modulation and were less likely to appear around this transitioning point (time zero in Fig. 3E and F). In comparison, the reverse replays detected for the three control groups of neurons, including the visual time-selective neurons, did not show significant modulations across the spiking cascade cycle, particularly at the transitioning point (Fig. S7C).

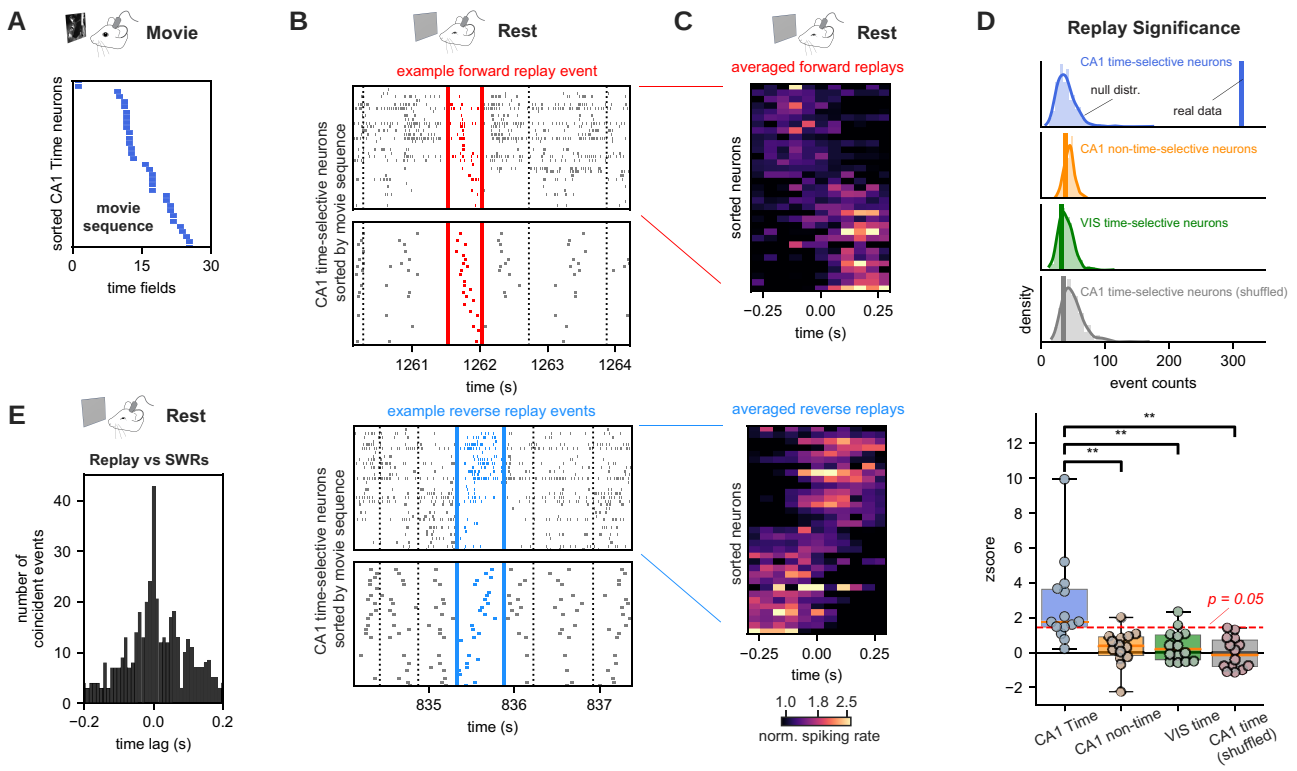

Fig. 2. The movie-induced sequence of hippocampal time-selective neurons replayed at rest. A) The movie-induced hippocampal sequence constructed based on the time fields of the CA1 time-selective neurons in a representative recording session. B) Examples of forward and reversed replay sequences from the same session as in (A). For each case, the upper panel shows the raw spiking data and the lower panel shows the computed delay profiles for each neuron. The segment boundaries, determined by fluctuations in the global mean signal, are indicated by dotted lines. Detected forward and backward replay events are delineated by bold lines (see Materials and methods for more details). C) The averaged pattern of the forward (top) and reverse (bottom) replays in the neural population, aligned to the segment center occurring throughout the rest period. D) Top: The number of detected replay events was compared against a null distribution built by repeating the same analysis on randomized movie sequences for the representative mouse. The same result was derived for the CA1 time-selective neurons, CA1 nontime-selective neurons, visual (VIS) time-selective neurons, and CA1 time-selective neurons identified from the shuffled data (from top to bottom). Bottom: The box plot of z-scores summarizing the difference between the real counts of replay events and the null distributions from all 14 mice. E) The distribution of hippocampal ripples relative to the detected replay events of the CA1 time-selective neurons from all 14 mice.

Distinct microcascades mark forward and reverse replay events

Our investigation of the fine-scale structure of resting-state spiking activities revealed the existence of shorter subsecond timescale global events that exhibited a temporal profile similar to the spiking cascade (Fig. 4A and B). We term these subsecond global events "microcascades." Briefly, these finer-scale events featured similar sequential transitions between the negative-delay neurons and the positive-delay neurons as the coarse-scale cascade, but the positive-delay neurons were only briefly activated for <100 ms. Importantly, they were often associated with single SPW-R events (Fig. 4A, black arrows). To better understand the fine-scale dynamics, we correlated the delay profiles of the fine-scale time segments with the coarse-scale principal delay profile. The resulting sequential scores (i.e. normalized correlations) were significantly (Kolmogorov-Smirnov test; $P = 0$) stronger than randomized controls (Fig. 4C). Unlike the sequential scores of the coarse-scale segments that mostly showed large positive values (Fig. S7A), the fine-scale segments have both large sequential scores of negative and positive values, corresponding to sequential transitions along two opposite directions. In addition, the fine-scale principal delay profile, i.e. the first principal component of all delay profiles of fine-scale segments, is highly similar to the coarse-scale principal delay profile (Fig. S9B), suggesting that both slow (seconds) and fast (hundreds of milliseconds) cascade dynamics feature sequential activations along a similar direction.

We then extracted the fine-scale segments with significant ($P < 0.001$) negative and positive sequential scores and called them the P-N (positive-delay neurons to negative-delay neurons) and N-P microcascades, respectively. Their averaged patterns clearly showed sequential activations along and opposite to the principal delay profile direction (Fig. 4E and F, left panels). The brief positive-delay neuron activation at these microcascades was tightly coupled by a sharp increase in the SPW-R probability (Fig. 4D). Most importantly, the reverse and forward replays of movie sequence in the CA1 time-selective neurons co-occurred with the N-P and P-N microcascades, respectively (Fig. 4E and F, right panels). At the same time, the sequential scores of the reverse and forward replay segments are biasedly distributed toward the negative and positive values, respectively (Fig. 4G). These results remained similar with removing the microcascades, mostly the N-P type, at the fast-transitioning point of the slow spiking cascades (Fig. S10). Forward and reverse replays were also detected with Bayesian decoding methods for the CA1 time-selective neurons (5, 30), and all the major results remain similar (Figs. S11 and S12). In comparison, there is no significant modulation found for the three control groups of neurons across the microcascade cycles (Fig. S8).

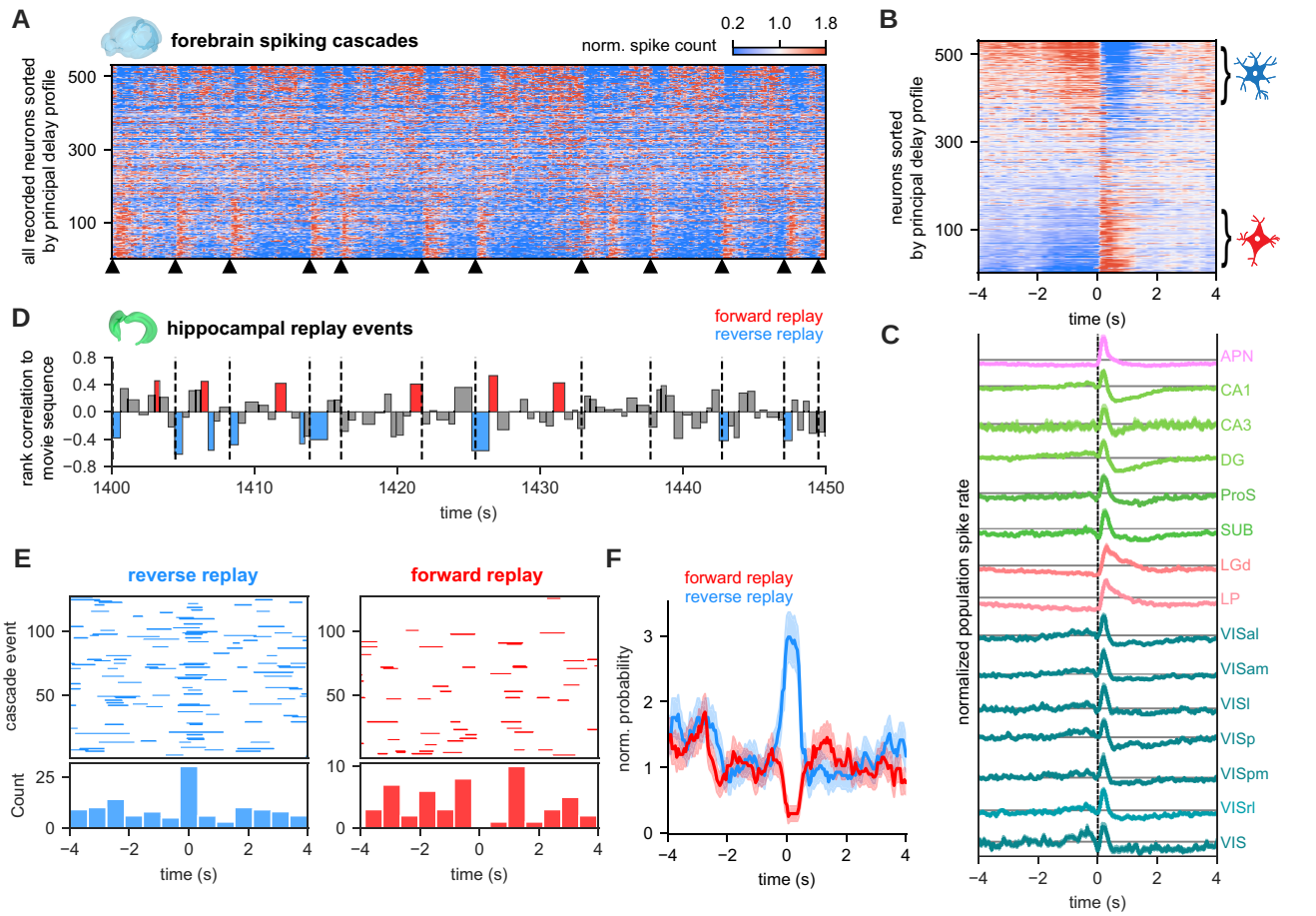

Fig. 3. Hippocampal replay events temporally locked to spiking cascades across the forebrain. A) A 50-s example of spiking data during resting state in a representative mouse, with all recorded forebrain neurons sorted by the principal delay profile. Black arrows indicate fast-transitioning points from negative-delay neurons to positive-delay neurons ("cascade midpoint"). B) The averaged pattern of the spiking cascade from the representative mouse. C) The averaged population spiking rate of 15 brain regions, each containing more than 100 neurons, during the slow spiking cascade. D) Bar plot showing rank correlations between the movie sequence and the delay profile of CA1 time-selective neurons during the 50-s time period in (A). Colored bars indicate sequences with sufficient correlation to identify them as replay sequences. E) Distribution of detected reverse (top left) and forward (top right) replays across the cycle of spiking cascades from the representative mouse. Each row corresponds to a spiking cascade, and short horizontal lines denote detected replays. The length of each line represents the duration of the corresponding replay event. Their distributions are summarized in the histograms (bottom). F) Normalized probability of forward and time-reversed replays across the cascade cycle, summarized for all 14 mice. The shaded region denotes the area within 1 SEM ($N = 1,787$).

Discussion

Here we examined the activity of a large population of neurons from throughout the brain during hippocampal replay following passive movie viewing in rodents. We found that the hippocampal replay of the movie sequence coincided and correlated with brain-wide cascades of sequential neuronal activation that involve many forebrain structures. The forward and reverse replays were aligned with two types of microcascade of opposite temporal orders, with the latter type also featuring the fast-transitioning point of the coarse-scale cascade structure. Consequently, the movie-related hippocampal sequences appear to be predetermined according to the sequence of these spontaneous cascade events.

The embedding of hippocampal replays in the highly structured, resting-state global dynamics supports recent theory about the self-organized nature of the hippocampal neural sequences (12, 15, 20, 25). The replays of movie-related hippocampal sequences observed here are similar to what has been repeatedly reported for maze-running-related place-cell sequences (21). Interestingly, the place-cell sequences were also found to "preplay" before the maze running (17, 31). While such preplays had once been explained as the internal dynamics for action planning (21), this planning interpretation may not explain the preplays occurring even before animals see the maze track (17, 19). The preplay finding is however consistent with another line of research into hippocampal time cells (14, 20, 32) since both suggested the self-generated nature of hippocampal sequences. It was found that hippocampal neuronal sequences can be robustly formed with animals running on a wheel without apparent changing of environmental or body-derived inputs, suggesting that they actually represent self-generated dynamics for time encoding (20).

The existence of apparent time cells has led to the idea that the sequential firing in the hippocampus during a temporally structured event may be internally generated rather than driven by a sequence of external stimuli (12, 20). The new theory would reconcile the "preplay" and "replay" findings if self-generated sequential dynamics generally follow a pre-existing temporal order. Here we showed that the movie evoked the time-selective responses, and thus the temporal activation sequences, of both the hippocampal and visual neurons. The sequence of the hippocampal time-selective

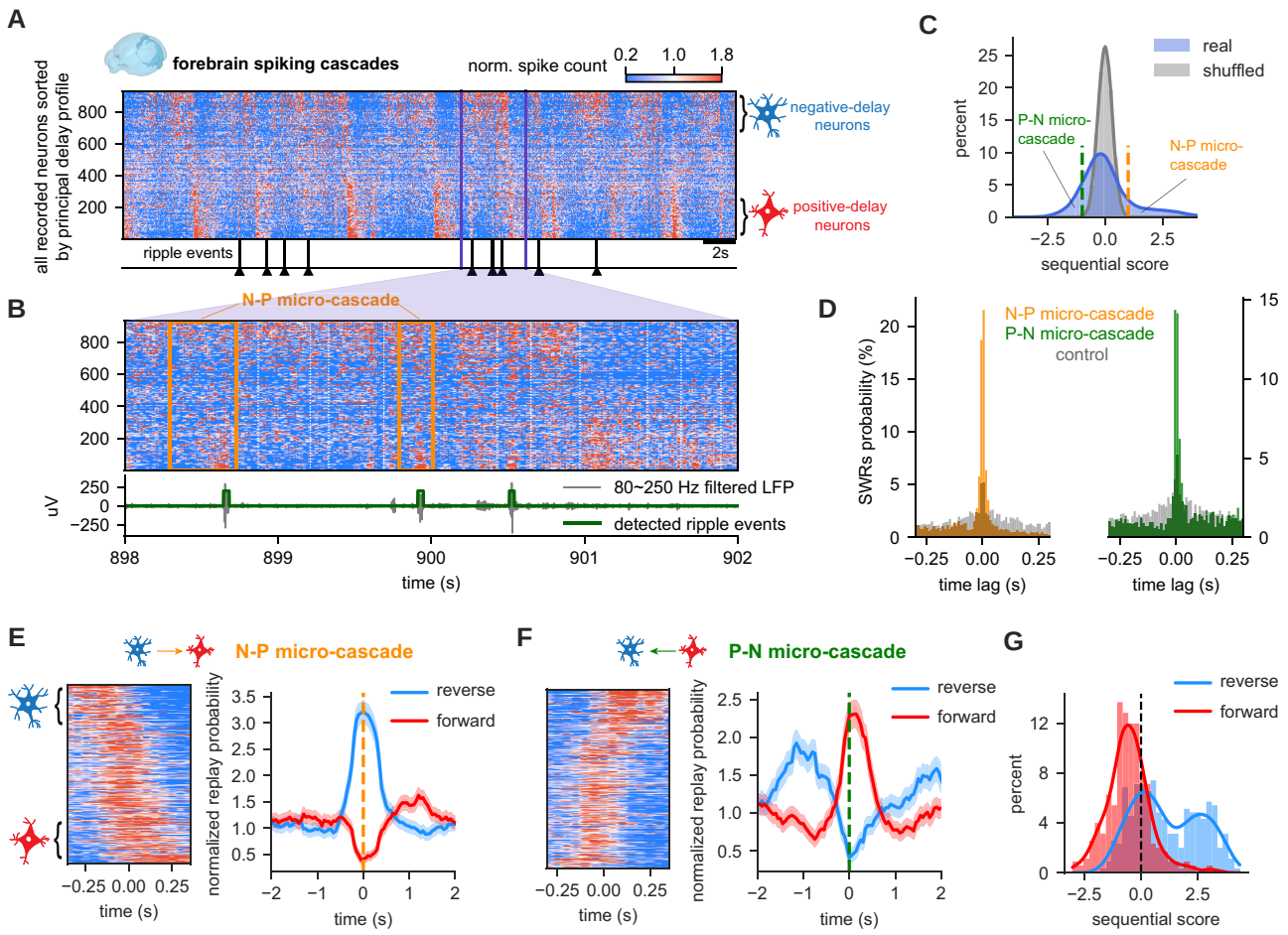

Fig. 4. Forward and reverse hippocampal replay events associated with distinct microcascades. A) Example resting-state spiking data with finer (20 ms) temporal resolution. The bottom trace displays the identified SPW-R events. B) A 4-s horizontally zoomed-in segment from (A). The spiking data was divided into fine-scale segments based on the troughs of the finely filtered (<5 Hz) global mean signal (dotted lines). Analysis of fine structure revealed microcascades, characterized by a temporal sequence across neurons expressed over a duration of less than 0.5 s. These microcascades were frequently associated with SPW-Rs. C) The distribution of sequential scores of all the fine-scale segments. The sequential score is the normalized correlation between the delay profile of the fine-scale segments and the coarse-scale principal delay profile. The fine-scale segments with significant ($P < 0.001$) positive (right side of the distribution) and negative (left side of the distribution) sequential scores were defined as the negative-to-positive (N-P) and positive-to-negative (P-N) microcascades, respectively. D) The probability of SPW-R across the cycle of the N-P and P-N microcascades for all 14 mice. They were aligned according to the brief peaks of positive-delay neuron activations in the microcascades. E) N-P microcascades were predominantly observed in conjunction with reverse replay events. Left: Representative mouse's averaged patterns of N-P microcascades. Right: Normalized probabilities for forward and reverse replays throughout the cycle of N-P microcascades, aggregated for all 14 mice. F) P-N microcascades were primarily observed alongside forward replay events. Similar presentation to (E). G) Sequential score distributions for the fine-scale segments containing forward and reverse hippocampal replays.

neurons, but not the visual neurons with stronger time-selective responses, was found to replay during the rest period after the movie watching. The difference might be due to the fact that the time sequences in the hippocampus result from the firing order imposed by its neural substrate, while the order observed in the visual cortex is imposed by time-specific movie features.

Hippocampal ripples/replays have been found to be coordinated with cortical activities in the visual cortex (33), the motor cortex (34), and the prefrontal area (35) during sleep and awake rest, playing a critical role in memory consolidation. Consistent with such cortical-hippocampal coupling, recent studies using large-scale recordings and imaging found that the hippocampal ripples are associated with brain-wide neural dynamics of specific spatiotemporal patterns (26, 36–39). This study extended these previous findings by showing that the hippocampal replays are actually embedded in pre-existing, self-organized global brain dynamics, consisting of coarse- and fine-scale spiking cascades of neuronal populations (26). Most importantly, these resting-state activity cascades featured sequential activations of the whole-brain neuronal populations along a specific direction. This temporal direction may represent a general direction that governs the sequential brain activations of different timescales and across different populations, including the hippocampal sequence during the movie watching (30 s), the coarse spiking cascades (5–15 s), the microcascades and the replays of the movie sequence (~hundreds of milliseconds). Different cascades along this general time frame explain replay types of different directions and timescales. The microcascades of opposite directions correspond to the forward and reverse replays of fast timescale and might also be related to their preferential occurrence at the DOWN-to-UP state transitions (40, 41), whereas the coarse-scale cascades may explain the replays seen on seconds timescale (42). The embedding of the hippocampal replays in the global cascade dynamics could have certain advantages at least theoretically.

First, the global dynamics may open a critical time window for the hippocampal-cortical interactions that are essential for memory consolidation. The spiking cascades involved ~70% of brain neurons in various cortical and subcortical areas. Particularly around the rapid transitioning point, most of the recorded neurons, including the hippocampal and cortical neurons, fired within a very brief (hundreds of milliseconds) time window, and created an opportunity for information transfer between the hippocampus and the cortex. The hippocampal-cortical interplay has been observed previously as slow (~10 s) comodulations of the cortical delta-band power and the hippocampal ripples (43, 44). The ripples were also found to trigger widespread cortical fMRI responses of the seconds timescale (36). These hippocampo-cortical interactions may represent the same brain process as the spiking cascade, which was coupled to slow modulations of both the cortical delta power and hippocampal ripples (26).

Second, the embedding of the hippocampal replays in the global dynamics could be an efficient way of consolidating learning and memory. Different daily life experiences can be encoded in neuronal sequences of different subgroups of hippocampal neurons (15, 21, 24). The spiking cascades that entrain most brain neurons would then be able to replay them all at once through a global sequential activation following the pre-existing principal direction.

Finally, the global spiking cascades may provide the driving forces for the hippocampal replays. The importance of the hippocampal replays makes their occurrence unlikely to rely completely on random fluctuations of spontaneous brain activity. In the absence of external perturbations during rest and sleep, the self-generated dynamics could be critical for driving these events in a controllable way. The highly organized spiking cascades would serve this purpose by driving the replay events and warranting their re-occurrences. Nevertheless, it remains unclear what in turn drives the spiking cascades. Modulatory influences from the various neurotransmitter systems, including the cholinergic system (45–47), are among the possibilities. The resting-state global brain activity measured by fMRI and electrocorticography has been linked to subcortical arousal-regulating areas (48), particularly the major locations of the cholinergic neuron (49, 50). In fact, the deactivation of the basal forebrain cholinergic regions effectively suppressed the resting-state global activity. The spiking cascades, which are shown as the global brain activity of single neuron level, were phase coupled to slow pupil dilations (26), which have previously been shown to be linked to the activation of cholinergic neurons (51). This explanation would be consistent with the known role of the cholinergic projections in the generation of the hippocampal ripples (52–54).

Materials and methods

This study utilized the Allen Visual Coding Neuropixels dataset (28), which includes neural spike data and behavior measurements from 14 mice. The neural spikes were sorted using the Kilosort2 pipeline (55). The 30-s movie was evenly segmented into 60 time fields. For each time field, the time-specificity score was defined as the t-score that compares the neuron's firing rate at that specific time field to the averaged firing rates at the remaining time fields.

For replay event detection, we binned spikes with a time interval of 50 ms, and candidate events were segmented based on the troughs of the global mean spiking signal. We then assessed the similarity between the firing sequence of each event and the neuron firing sequence during the movie using Spearman's rank correlation. Forward replay and reverse replays were defined as events showing significant positive and negative correlations ($P < 0.01$), respectively. To identify ripple events, we employed an offline ripple detection method, utilizing the LFP signal recorded from the hippocampal CA1 region (56).

We binned spikes with the time interval of 200 ms for coarse-scale cascade detection conformed with a previous study, where a delay profile decomposition method was used to derive the principal direction (i.e. the principal delay profile) of the sequential cascade (26). The negative- and positive-delay neurons were defined as those neurons with significant positive or negative principal delay values in the cascade, respectively ($P < 0.001$, one-sample t-test). For detecting microcascades, we used time bins of 50 ms to derive the spike rate. The N-P and P-N cascades were defined as sequential events that significantly (negatively for N-P and positively for P-N) correlated with the principal direction of the coarse-scale cascade.

More details are available in SI Materials and methods.

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Supplementary Material

Supplementary material is available at *PNAS Nexus* online.

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Author Contributions

Y.Y. and X.L. contributed to the conception, design of the work, and data analysis; X.L. also devoted efforts to the supervision, project administration, and funding acquisition; Y.Y., D.A.L., J.H.D., and X.L. contributed to data visualization, and writing the paper.

Preprints

This manuscript was posted on a preprint: https://www.biorxiv.org/content/10.1101/2022.09.05.506667v1.full.

Data Availability

We used the Neuropixels Visual Coding dataset from the Allen Institute (27, 28). All the multimodal data are available at https://portal.brain-map.org/explore/circuits/visual-coding-neuropixels. The Python code that produced the major results of this paper will be available at https://github.com/psu-mcnl/Neural-Seq.

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