Distinct preplay of multiple novel spatial experiences in the rat

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The activity of ensembles of hippocampal place cells represents a hallmark of an animal's spatial experience. The neuronal mechanisms that enable the rapid expression of novel place cell sequences are not entirely understood. Here we report that during sleep or rest, distinct sets of hippocampal temporal sequences in the rat preplay multiple corresponding novel spatial experiences with high specificity. These findings suggest that the place cell sequence of a novel spatial experience is determined, in part, by an online selection of a subset of cellular firing sequences from a larger repertoire of preexisting temporal firing sequences in the hippocampal cellular assembly network that become rapidly bound to the novel experience. We estimate that for the given context, the recorded hippocampal network activity has the capacity to preplay an extended repertoire of at least 15 future spatial experiences of similar distinctiveness and complexity.

It is a matter of debate whether specific sequences of place cells (1) encoding different novel spatial experiences in the rat are formed exclusively during the experiences (2–4) or are, in part, preconfigured in the form of correlated temporal sequences that are expressed before the experience (5–7). Previous work in mice has shown that in the naïve animal, temporal firing sequences expressed in association with sharp-wave ripples during sleep or rest can preplay future spatial sequences of place cells encoding novel environments (5). However, it remains unclear whether place cell sequences encoding a novel spatial experience are preconfigured as a dominant cluster of temporal sequences preferentially preplaying the subsequent experience or whether they are selected from an existing larger repertoire of temporally organized sequences. The latter would confer the hippocampal network with the capacity to rapidly encode multiple parallel spatial experiences, but the extent of this capacity has not been addressed experimentally. Here we investigate these two issues.

Results

Preplay of Future Place Cell Sequences and Spatial Trajectories.

Ensembles of place cells were recorded from the CA1 area of the hippocampus in three experimentally naïve rats during sleep/rest sessions and during subsequent first-time exploration of 1.5-m-long linear tracks (Fig. 1A). The sleep/rest sessions were conducted in a sleep/rest box surrounded by high opaque walls that blocked the rats' view of the room except for a limited portion of the ceiling. After the naïve rats went through an ~1-h sleep/rest period, a linear track (*Materials and Methods*) was introduced into the room for the first time. Place cell sequence templates were computed by ordering the place cells based on the location of their place field peak firing during exploration of the linear track (Fig. 1B, *Right*). Spiking events were detected during sleep/rest frames (8) as epochs of multiunit activity recorded from at least six different pyramidal cells with a <100-ms interspike interval in the multiunit activity, flanked by epochs of >100 ms of silence (4, 5, 9). For each spiking event, the order of cells firing during sleep/rest (temporal sequence) was correlated with the order in which they subsequently fired as place cells during exploration of the linear track (spatial sequence) (2, 4, 5, 9). During an event, if a cell fired more than one spike, only

the first spike was further considered to calculate the “temporal” sequence of neuronal firing (2, 8, 9). Similar results (Fig. S1) were obtained when individual cell firing rates were accounted for by considering all of the spikes emitted during the events (4, 10). An event was considered as preplay of the future place cell sequence (Fig. 1B, *Left*) when the absolute value of the corresponding temporal-spatial correlation exceeded the 97.5 percentile of a distribution of absolute correlation values calculated between the event and 500 surrogate spatial sequences created by shuffling the original spatial order of place cells (5).

We used a Bayesian reconstruction algorithm (5, 11, 12) to decode the rat's position from the neuronal activity recorded during the first run session on the novel track or during the preceding sleep/rest session in the box. The decoded (reconstructed) position during the run was compared with the actual trajectory of the animal (Fig. 1C). The cumulative error (Fig. 1D, red lines) and average error (Fig. 1E, red lines) of the reconstructed position were significantly smaller than the distribution of errors obtained by shuffling for 500 times the time bins (Fig. 1D and E, *Left*) or position bins (Fig. 1D and E) of the original reconstructed position of the animal during the run. Moreover, in a significant number of cases, the firing rate-based Bayesian decoding of the neuronal activity of “all” spikes during each spiking event detected as above during sleep/rest revealed patterns of activation of place cells subsequently coding spatial trajectories on the novel linear track that was only later explored for the first time (Fig. 1F). For each event, the decoded trajectory during the preceding sleep/rest was significant if the original score for the line fitting along the trajectory (5, 12) exceeded the 99th percentile of each of the two distributions of 500 scores ($P < 0.01$; Fig. 1F). These scores were calculated by fitting a line through the surrogate probability distributions of positions (12) (*Materials and Methods*) obtained by shuffling the time bins or position bins of the original probability distribution function decoded during the sleep/rest session (5, 12).

Statistical Significance of Preplay of Single Novel Spatial Experience.

Of all the detected spiking events during sleep/rest in the three rats, 9.8% were classified as significant preplay of the future spatial sequences because they were significantly correlated with place cell sequences expressed during the first-time exploration of the novel track. Preplay sequences spanned 82.6% of the track length on average ($n = 1,388$; $P < 10^{-200}$, binomial probability test; Fig. 1G and Fig. S2). They occurred in the forward or reverse order (forward:reverse ratio = 1.04) and generally were significantly correlated specifically with place cell sequences of one direction of movement (85.3% of preplay sequences were

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Fig. 1. Preplay of future place cell sequences and spatial trajectories during sleep/rest in the naïve rat. (A) Schematic of the sequence of experimental sessions (one-track experiment). Preplay events were detected during the first sleep/rest session of naïve rats (*Left*) and correlated with the place cell sequences on the linear track (*Right*). (B) Examples of preplay spiking events during the sleep/rest session in the sleep box preceding the first run on the novel linear track (first 10 boxes; *Left*) and the corresponding place cell sequence during the run (*Right*) in one naïve rat. Tick marks (first 10 boxes; *Left*) indicate individual spikes during preplay events. For each event and for each participating cell, the first spike emitted during the event is represented in red, whereas all of the remaining spikes are in gray. The numbers in the boxes are the correlation values between the temporal order of spiking and the spatial order of activation corresponding to each preplay event. Corresponding local field potential recordings are shown above the spiking events. The horizontal arrow (*Right*) indicates the order of the place cell activation during the run. (C) Bayesian decoding of the rat's position from the spiking activity of all cells during one lap run on the novel track (250-ms bins, animal velocity >10 cm/s). The heat map represents the decoded position of the animal, whereas the yellow line displays its actual position on the track. (D) Cumulative probability distribution of the error of the Bayesian decoding of the rat's position (red curves) compared with the distribution of errors of 500 shuffles (blue curves) of time bins (*Left*) and position bins (*Right*). (E) Average error of the Bayesian decoding of the rat's position (red lines; scaled up 10 times) compared with the distribution of average errors of 500 shuffles (blue curves) of time bins (*Left*) and position bins (*Right*). (F) Examples of significant decoding of a future trajectory on the novel track from ensemble place cell activity during the sleep/rest session before novel track exploration (i.e., prerun sleep/rest) in one naïve rat (20-ms bins, animal velocity <1 cm/s). The white lines show the linear fit maximizing the likelihood along the virtual trajectory. (G) Distribution of correlation values between spiking events and the place cell sequence for all events occurring during the prerun sleep/rest in three animals. Open bars, spiking events vs. the original (unshuffled) templates; filled black bars, spiking events vs. 500 shuffled templates scaled down 500 times; red bars, distribution of preplay (i.e., significant) events. The *P* value reflects the minimum significance level (i.e., the largest *P* value) of the difference between the original set (open bars) and any of the 500 sets of shuffled (black bars) correlation values using the rank-sum test. (H) Distribution of decoding scores for the virtual novel track trajectory for all events occurring during the prerun sleep/rest in three rats. Open bars, scores during all spiking events; filled black bars, scores of 500 time-bin shuffles scaled down 500 times; red bars, scores of significant events exceeding the 99th percentile of both time-bin and position-bin shuffle distributions. The *P* value reflects the minimum significance level (i.e., the largest *P* value) of the difference between the original set (open bars) and any of the 500 sets of shuffled (black bars) scores using the rank-sum test. (*Inset*) Enlarged display of the distribution for score values between 0.5 and 1. (I) Cross-correlation between preplay events and ripple occurrence in CA1 during prerun sleep/rest in the three rats (the distribution of the time of ripple occurrence with reference to the time of occurrence of the significant preplay events).

during sleep/rest. We modeled this relationship as a polynomial function to extrapolate the number of potential novel tracks that could be preplayed by individual subsets of temporal sequences from the larger repertoire expressed during sleep/rest that would be specifically correlated with only one track (Fig. 3C). We estimate that during a given sleep/rest session, the hippocampal network of the recorded neurons has the capacity to preplay with high specificity at least 15 distinct novel tracks of similar distinctiveness (>90% temporal sequence specificity on a pair of tracks) and level of complexity (Fig. 3C). The average proportion of preplay events per novel track, and implicitly our estimate of network capacity, does not change significantly when the minimum number of cells simultaneously active during a spiking event changes from 6 to 10 ($P > 0.05$, rank-sum test; Fig. 3C, *Inset*).

Discussion

Our findings show that the specificity of the representation of a particular novel spatial experience in the rat is achieved during the experience via the “selection” of subsets of corresponding temporal sequences from a larger repertoire of preconfigured temporal sequences rather than from a dominant cluster of temporal sequences preplaying the very next experience (5). The corresponding temporal firing sequences are rapidly bound to the specific novel experiences in the form of specific place cell sequences. The selection of specific sequences from a larger preconfigured repertoire confers the hippocampal network with the capacity to rapidly encode multiple parallel novel experiences. The preplay events described in our study were recorded exclusively in the sleep/rest box with high opaque walls during the sleep/rest session before the linear tracks were first introduced into the room. We can thus exclude the possibility that during these events the animals “mentally traveled” within the room, having view of the linear tracks. Moreover, the fact that more than 90% of all the recorded CA1 place cells are active on more than one track indicates that individual CA1 cells participate in multiple cellular assemblies encoding different spatial experiences and that individually they cannot accurately distinguish the identity of multiple linear tracks. Instead, the sequences of CA1 place cells in our data accurately distinguish across multiple linear tracks. Given that the subsets of preplay sequences devoted to the representation of each of the three tracks represent 6–7% of all detected spiking events with similar proportions across tracks, we estimate that for a similar experimental setting and based on the activity of the same group of CA1 neurons, the hippocampal network has the capacity to simultaneously encode at least 15 different novel spatial experiences of similar distinctiveness and complexity. These estimates are based on an average of 6–7% of all temporal sequences preplaying each track and ~90% novel track specificity between pairs of tracks. If a smaller proportion of events preplays each track or if a greater overlap is tolerated for the distinction of a pair of experiences, then the total encoding repertoire of the hippocampal network will be greater. Similarly, if the activity of all of the hippocampal cells were to be taken into account in multiple contexts (14), the hippocampal capacity to encode different novel experiences would be expected to be higher. The capacity of the hippocampal network for a rich repertoire of temporal preplay sequences may contribute to the role of the hippocampus in prospective coding (15), rapid learning (16), and imagining (17, 18).

Materials and Methods

Surgery and Experimental Design. Electrophysiological recordings were performed on three adult Long-Evans male rats. All animals were implanted under isoflurane anesthesia with either 22 independently movable tetrodes (rats 1 and 3) or 64-channel 8-shank NeuroNexus linear silicone octatetrodes (rat 2) aiming for area CA1 of the right hippocampus (4 mm postbregma, 1.5–3 mm lateral to midline). The reference electrode was implanted posterior to lambda over the cerebellum. During the following week of

recovery, the electrodes were advanced daily while animals rested in a high-wall opaque sleeping box [30 × 45 × 40 (h)-cm]. The animal's position was monitored via two infrared diodes attached to the headstage.

The experimental apparatus consisted of a 150 × 150-cm rectangular elevated linear track maze. All tracks were 6.25-cm-wide and 50 cm above the floor. Experimental sessions were conducted while the animals explored for chocolate sprinkle rewards placed always at the ends of the corresponding linear tracks (one sprinkle at each end of the track on each lap). Neuronal activity was recorded in naïve animals during the prerun sleep/rest session in the sleep box for ~1 h, after which the linear maze was brought into the room and installed, followed by the recording of an additional ~1 h of sleep/rest. Subsequently, the animals were transferred onto the linear maze for the first time and allowed to explore a 150-cm-long linear track whose ends were blocked by 20-cm-high, 10-cm-wide barriers (track 1). The animals were familiarized with the linear track via repeated run-sleep/rest sessions. Finally, while the animals were on the linear track, the two end barriers were lifted, allowing the animals to explore for the first time two additional 150-cm-long linear tracks attached to the ends of track 1 (tracks 2 and 3) to form the shape of the letter “U.” After completion of all experiments, the brains of all the rats were perfused, fixed, sectioned, and stained using cresyl violet for electrode track reconstruction. The rats were kept on a 12-h light/dark cycle and cared for in accordance with the standards of the Massachusetts Institute of Technology Committee on Animal Care and in compliance with National Institutes of Health guidelines.

Recordings and Single-Unit Analysis. A total of 114 neurons were recorded from hippocampal area CA1 in the three rats (31, 27, and 56 neurons) during the sleep/rest and run sessions. Single cells were identified and isolated using the manual clustering method Xclust (19). Pyramidal cells were distinguished from interneurons based on spike width, average rate, and autocorrelations (20).

Place fields were computed as the ratio between the number of spikes and the time spent in 2-cm bins along the track, smoothed with a Gaussian kernel with an SD of 2 cm. Bins where the animal spent a total of less than 0.1 s and periods during which the animal's velocity was below 5 cm/s were excluded. Place field length and peak rate were calculated after separating the direction of movement and linearizing the trajectory of the animal. Linearized place fields were defined as areas with a localized increase in firing rate above 1 Hz for at least five contiguous bins (10 cm). The place field peak rate and location were given by the rate and location of the bin with the highest ratio between spike counts and time spent. Place field borders were defined as the points where the firing rate became less than 10% of the peak firing rate or 1 Hz (whichever was bigger) for at least 2 cm.

Local Field Potential Analysis. Ripple oscillations were detected during sleep/rest periods in the sleep box. The EEG signal was filtered (120–200 Hz) and the ripple-band amplitude was computed using the Hilbert transform. Ripple epochs with maximal amplitude higher than 4 SDs above the mean, beginning and ending at 1 SD, were detected. The time of ripple occurrence (Fig. 1f) was the time of its maximal amplitude.

Preplay Analyses Using Template-Matching Procedure. To analyze the preplay process, spiking events were detected during prerun sleep/rest periods in the sleep box (velocity <1 cm/s). Only the spiking events detected in the sleep/rest session before the naïve animals first ran on track 1 were used throughout this study. A spiking event was defined as a transient increase in the multiunit firing activity of a population of at least six different pyramidal cells within a temporal window preceded and followed by at least 100 ms of silence that delimited the beginning and end of the event. The spikes of all the place cells active on the novel track that were emitted during the prerun sleep in the box were sorted by time and further used for the detection of the spiking events. All three naïve animals exhibited a significant number of spiking events in the prerun sleep/rest session. The time of the spiking events used to compute the cross-correlation with the ripple epoch occurrence (Fig. 1f) was the average time of all spikes composing the individual spiking events. Place cell sequences (templates) were calculated for each direction of the animal's movement and for each run session for each track by ordering the spatial location of the place field peaks that corresponded to the peak firing rate for each cell above 1 Hz on that track. In the three-track maze design, the spiking events were detected during sleep/rest using the spiking activity of all of the recorded CA1 pyramidal cells regardless of them later becoming place cells on the linear tracks or not. For place cells with fields above 1 Hz on more than one track, only the place field corresponding to the peak firing rate of the place cell on a particular track was considered for the construction of the template of that particular track; individual cells