METHODS

Surgery and experimental design. Electrophysiological recordings were performed on four C57BL/6 mice (strain NR1-floxed[27]) with ages between 18 and 22 weeks. All animals were implanted under Avertin anaesthesia with six independently movable tetrodes aiming for the CA1 area of the right hippocampus (1.5–2 mm posterior to bregma and 1–2 mm lateral to the midline; Supplementary Fig. 1). 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 small, walled sleeping box ($12 \times 20\,cm^2$, 35 cm high). The animal position was monitored by means of two infrared diodes attached to the headstage.

The experimental apparatus consisted of a $90 \times 65\,cm^2$ rectangular, walled, linear track maze. All tracks were 4 cm wide at the bottom and 8–9 cm wide at the top, and all linear track walls were 10 cm high. 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 naive animals (four mice) during the sleep/rest session in the sleep box immediately preceding the first experience on linear tracks, and continued (Fig. 4) during the first run session on a novel track. After familiarization with the linear track, the animals went through a recording session of 15–60 min (Fam session), and the recordings continued for the next 34–42 min (Contig session) while the animals explored an L-shaped track for the first time. In this track, the familiar arm and the novel arm were made contiguous by removing the barrier that had separated them (Fig. 1). For the purpose of analysing the recording data, the Fam session was further divided into Fam-Run, in which the animals ran through the track (velocity of animal's movement was higher than $5\,cm\,s^{-1}$), and Fam-Rest, where the animals took awake rests at the ends of the track (velocity of animal's movement was less than $2\,cm\,s^{-1}$). During resting periods, the animals consumed the chocolate sprinkle and groomed, but mostly they were still until they self-initiated the next lap of run on the linear track. After completion of the experiments, the brains of all mice were perfused, fixed, sectioned and stained using nuclear fast red (Supplementary Fig. 1) or cresyl violet for electrode track reconstruction.

Recordings and single-unit analysis. A total of 87 neurons were recorded from the CA1 area of the hippocampus in four mice during the Fam and Contig sessions (Supplementary Tables 1–3). A total of 69 CA1 neurons were recorded from the four mice in the *de novo* condition (26, 20, 10 and 13 cells, respectively). Single cells were identified and isolated using the manual clustering method Xclust[2] and the application of cluster quality measurements[28]. Pyramidal cells were distinguished from interneurons on the basis of spike width, average rate and autocorrelations[22].

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 a standard deviation 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 $5\,cm\,s^{-1}$ 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 and during rest periods at the ends of the tracks. The electroencephalography signal was filtered (120–200 Hz) and ripple-band amplitude was computed using the Hilbert transform. Ripple epochs with maximal amplitude more than 5 s.d. above the mean, beginning and ending at 1 s.d. were detected. The time of ripple occurrence (Figs 2c and 4C) was the time of its maximal amplitude. The proportion of ripples with which cells with place fields on the novel arm of the L-shaped track fired in the preceding session (Supplementary Fig. 3) was calculated for each qualifying cell as the ratio between the number of ripples during which the cell fired at least one spike and the total number of ripples during the corresponding exploratory session.

Preplay and replay analyses. To analyse the preplay and replay processes, spiking events were detected during Pre-Run sleep/rest periods in the sleep box (*de novo* condition; velocity, $<1\,cm\,s^{-1}$) or during awake rest periods at the ends of the running tracks (Contig condition; velocity, $<2\,cm\,s^{-1}$). A spiking event was defined as a transient increase in the firing activity of a population of at least four different place cells within a temporal window preceded and followed by at least 50 ms of silence. Overall, similar results were obtained using 50-, 60-, 75- and 100-ms time windows. The spikes of all the place cells active on the novel track that were emitted during the Pre-Run sleep/rest in the box for the *de novo* condition as well as the spikes of all the place cells active on the familiar track or the novel arm that were emitted during Fam-Rest session at the two ends of the familiar track for Contig condition were respectively sorted by time and further used for the detection of the spiking events.

All four animals exhibited a significant number of spiking events in the Pre-Run session of the *de novo* condition. Three of the four animals (mice 1–3) exhibited a significant number of spiking events in the Contig condition, the remaining animal (mouse 4) having a below-threshold number of simultaneously active CA1 place cells. The time of the spiking event used to compute the cross-correlation with ripple epoch occurrence (Figs 2c and 4C) was the average time of all spikes comprising the spiking event. The place cell sequences (templates) were calculated for each direction of the animal's movement and for each run session (*De novo*-Run, Fam-Run and Contig-Run) by ordering the spatial location of the place field peaks that were above 1 Hz. For place cells with multiple place fields above 1 Hz on a particular arm or track in the Contig condition (six of 52 place cells active on the novel arm in the two directions, or 12%: two for each direction in mouse 1, one in mouse 2 and one in mouse 3), only the place field corresponding to the peak firing rate of the place cell on that arm or track was considered for the construction of the template of that particular arm or track, to be consistent with all the previous studies that used spatial templates to demonstrate replay during sleep or awake rest[3,4,10]. Place cells with fields on both the novel arm in the Contig-Run session and the familiar track in the Fam-Run session participated in the construction of both the novel arm and familiar track templates.

Statistical significance was calculated for each event by comparing the rank-order correlation between the sequence of cells' firing during the event (that is, event sequence) and the place cell sequence (template), on the one hand, and the distribution of correlation values between the event sequence and 200 surrogate templates obtained by shuffling the order of place cells, on the other[4] (Fig. 2a). The significance level was set at 0.025 to control for multiple comparisons (two directions of run). The proportions of significant events (preplay novel track, preplay novel arm (Fig. 2b), replay novel arm and replay familiar track) were each calculated as the ratio between the number of significant events and the total number of spiking events in which at least four corresponding place cells were active[4]. Corresponding familiar track templates (Fig. 2h) were constructed by ordering the location of peak firing on the familiar track during Fam-Run (no minimum threshold of firing) of all place cells that subsequently fired on the novel arm. Cells comprising the corresponding familiar track templates are the same as those comprising the novel arm templates. We note that these corresponding familiar track templates are different from the ones used in Figs 1 and 2a–g, which were constructed by ordering the peak firing of all place cells active on the familiar track >1 Hz.

The overall significance of the preplay (Fig. 2a) or replay process was calculated by comparing the distribution of correlation values of all events relative to the original template with the distribution of correlation values relative to the shuffled surrogate templates, using the Kolmogorov–Smirnov test[3]. Quantification of the replay versus preplay events during the Fam-Run session (Fig. 2f, g) was performed as described above using different spatial templates for the familiar track and the novel arm. All spiking events were correlated with both the novel arm and the familiar track templates. Events significantly correlated only with familiar track or with novel arm templates were considered pure replay and pure preplay, respectively. The template specificity index was calculated for each event as the difference between the absolute value of the event's correlation with the novel arm template (preplay, high positive index) and the event's correlation with the familiar track template (replay, high negative index). For the purpose of displaying the template specificity index, events correlated with the novel arm but not with the familiar track templates were considered preplay and events correlated with the familiar track but not with the novel arm templates were considered replay (Fig. 2g). Additionally, events correlated with both the familiar track and the novel arm templates formed a third group, preplay/replay events, displayed in yellow in the inset of Fig. 2g.

Correlations between pairs of familiar track and novel arm templates (Fig. 2h) were performed using modified familiar track templates that were constructed using the location of peak firing (>0 Hz) of only those cells that had place fields on the novel arm (peak rate, >1 Hz). The lack of significant correlation in this case demonstrates that the novel arm place cell sequence is not simply a transposition of a familiar track place cell sequence on the novel arm.

We also identified neurons that did not fire during Fam-Run, that activated during Fam-Rest events and that corresponded to trajectories on the novel arm during Contig-Run (silent cells). We calculated the correlation between the order in which they fired during Fam-Rest events and their spatial sequence as new place cells on the novel arm during Contig-Run, as previously explained. Owing to the low absolute number of silent neurons, only triplets of cells were available for further analysis ($n = 24$). The proportion of events perfectly matching the spatial template was compared with the proportion of by-chance perfect matching (0.33).

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Stability of place cell maps. Stabilities of place cell firing on the familiar track before and after barrier removal as well as on the novel track (*de novo* condition) and the novel arm (Contig condition) in the beginning versus the end of the run session were assessed by calculating, for each place cell and each direction, a correlation between the spatial firing in the corresponding paired situations (before versus after barrier removal for the familiar track or the first four laps versus the last four laps of the *De novo*-Run or Contig-Run session for the novel track or arm, respectively). The place cell activity was not partitioned in place fields; rather, the whole activity on the particular track or arm was considered separately for each cell and direction (average correlations are shown in Figs 2e and 4D, blue bars). In addition, we calculated the same type of correlation after shuffling the identity of the cell in one member of the correlation (once for each different cell; average correlations are in Figs 2e and 4D, black bars). Shuffle results (Figs 2e and 4D, black bars) were computed as correlation between spatial tuning of cells on the familiar track during Fam-Run and spatial tuning of all other simultaneously recorded cells on the familiar arm during Contig-Run (familiar track group; Fig. 2e, left), or correlation between spatial tuning of cells on the novel arm (or novel track) during the beginning of Contig-Run (or *De novo*-Run) and spatial tuning of all the other simultaneously recorded cells on the novel arm (or novel track) during the end of Contig-Run (novel arm group; Fig. 2e, right) or *De novo*-Run (Fig. 4D). Original and shuffled correlations were compared using the rank-sum test. The average number of laps (traversal of the novel track in both directions) per session was 20.5 in *De novo*-Run (21, 16, 27 and 18 in the four mice) and 16.3 in Contig-Run (13, 14 and 22 in the three mice).

Bayesian reconstruction of actual and virtual trajectories. For each cell, we calculated a linearized spatial tuning curve on the familiar track during the Fam-Run session and a linearized spatial tuning curve on the novel arm during the Contig-Run session. The tuning curves were constructed in 2-cm bins from spikes emitted in both run directions at velocities higher than 5 cm s^{-1}, and were smoothed with a Gaussian kernel with a standard deviation of 2 cm. We constructed a joint spatial tuning curve for each cell by juxtaposing the spatial tuning curve on the familiar track during the Fam-Run session and the spatial tuning curve on the novel arm during the Contig-Run session. We also detected for each cell all the spiking activity emitted at velocities below 5 cm s^{-1} during the Fam-Rest session, where replay and preplay events where shown to occur using the rank-order correlation method. We used a Bayesian reconstruction algorithm[6,18] to decode the virtual position of the animal from the spiking activity during Fam-Rest (Fig. 3b) in non-overlapping, 20-ms bins using the joint spatial tuning curves. We then extracted epochs of reconstructed trajectory matching the time of the spiking events as detected using multiunit activity of place cells from the familiar track and novel arm (rank-order correlation method; see 'Preplay and replay analyses', above).

We used two shuffling procedures to measure the quality of the Bayesian decoding. In the first shuffling procedure, for each event, the original time-bin columns of the probability distribution function (PDF) were replaced with an equal number of time-bin columns randomly extracted from a pool containing the time-bin columns of all PDFs of all detected events[6]. The shuffling procedure was repeated 500 times. In the second shuffling procedure, the identity of the place cells was randomly shuffled 100 times and new PDFs were calculated for all events. For all original and shuffled PDFs, a line was fitted to the data using a previously described line-finding algorithm[6]. Lines fitted to the original and shuffled data were compared using slope, spatial extent, location on the track and probability score. We defined replay and preplay as the epochs of Fam-Rest in which the reconstructed trajectory was located on the familiar track or the novel arm, respectively. The trajectory was defined across a set of position estimates during the corresponding epoch (Fig. 3c). Only epochs that lasted at least 60 ms (three bins) and which contained reconstructed trajectories spanning at least 10 cm were considered for further analysis. Trajectories for which 75% or more of their length was located on the familiar track were considered to represent replay of an animal's trajectory on the familiar track (Fig. 3c, middle), and trajectories for which 75% or more of their length was located in the novel arm were considered to represent preplay of the animal's future trajectory on the novel arm (Fig. 3c, top). The remaining events were considered preplay–replay (Fig. 3c, bottom).

An epoch was considered significant if the new line was less than 75% contained in the familiar track for replay or novel arm for preplay in at least 95% of the shuffled cases. For each epoch that was significant for replay or preplay using the reconstruction method, we retrieved the value of the rank-order correlation between the neuronal firing sequences and the familiar track and novel arm spatial templates as calculated using the rank-order correlation method. We compared the absolute correlation values between the epoch's firing sequences and familiar track templates with the absolute correlation values between the same epoch's firing sequences and novel arm templates. We also reconstructed the trajectory of the animal on the familiar track from the spiking activity during the Fam-Run session at velocities above 5 cm s^{-1} in 250-ms bins using the spatial tuning curves on the familiar track[6,18] (Fig. 3a) to validate the decoding procedure.

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