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RESEARCH ARTICLE

CA3 place cells that represent a novel waking experience are preferentially reactivated during sharp wave-ripples in subsequent sleep

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Abstract

A popular model of memory consolidation posits that recent memories stored in the hippocampus are reactivated during sleep and thereby transferred to neocortex for long-term storage. This process is thought to occur during sharp wave-ripples (SWRs) in nonrapid eye movement sleep (NREM). However, whether the hippocampus consolidates all recent memories in the same manner remains unclear. An efficient memory system may extract novel information from recent experiences for preferential consolidation. In the hippocampus, memories are thought to be stored initially in CA3. Therefore, CA3 place cells that encode novel experiences may be preferentially reactivated during SWRs in subsequent sleep. To test this hypothesis, we recorded CA3 place cells in rats during exposure to a familiar and a novel environment and during subsequent overnight sleep. We found that CA3 place cells that preferentially coded a novel environment showed larger firing rate increases during SWRs in NREM than place cells that preferentially coded a familiar environment. Moreover, CA3 place cell ensembles replayed trajectories from a novel environment during NREM with higher fidelity than trajectories from a familiar environment. Together, these results suggest that CA3 representations of novel experiences are preferentially processed during subsequent sleep.

KEYWORDS

CA3, hippocampus, place cells, replay, sharp wave-ripples, sleep

1 | INTRODUCTION

Damage to the hippocampus impairs the ability to form new episodic memories while sparing the ability to recall remote memories (Scoville & Milner, 1957; Squire, 1992; Squire, Stark, & Clark, 2004). This selective memory impairment inspired the proposal that recent memories are initially stored in the hippocampus and then later consolidated in neocortex for long-term storage (McClelland, McNaughton, & O'Reilly, 1995). This memory consolidation process is thought to occur during sleep (Diekelmann & Born, 2010). Recordings of hippocampal "place cells", neurons that exhibit spatially selective firing (O'Keefe, 1976; O'Keefe & Dostrovsky, 1971), have provided support for the idea that hippocampal memories are consolidated during sleep. Place cell ensemble activity during nonrapid eye movement sleep (NREM) has been shown to reflect activity from previous awake experiences (Kudrimoti, Barnes, & McNaughton, 1999; A. K. Lee & Wilson, 2002; Nádasdy, Hirase, Czurkó, Csicsvari, & Buzsáki, 1999; Skaggs, McNaughton, Wilson, & Barnes, 1996; Wilson & McNaughton, 1994), and such recurring ensemble activity has thus been termed "replay". Replay of hippocampal firing patterns during NREM tends to occur during sharp wave-ripples (SWRs; A. K. Lee & Wilson, 2002; Nádasdy et al., 1999), the dominant hippocampal network pattern during NREM (Buzsáki, 1986). Brief disruption of hippocampal activity after the onset of SWRs during post-learning sleep and rest has been reported to impair spatial memory retrieval (Ego-Stengel & Wilson, 2010; Girardeau, Benchenane, Wiener, Buzsáki, & Zugaro, 2009), providing causal evidence that SWR-related replay supports memory consolidation.

However, the hippocampus may not consolidate all recent memories equally. Memories of novel experiences may be preferentially reactivated and consolidated, perhaps to reduce redundancy in memory

storage. Indeed, CA1 place cells that represent novel experiences have been shown to be preferentially reactivated during ripples and high-frequency ripple-like events (Cheng & Frank, 2008; O'Neill, Senior, Allen, Huxter, & Csicsvari, 2008). However, memory consolidation requires output from hippocampal subregion CA3 (Nakashiba, Buhl, McHugh, & Tonegawa, 2009), and most studies have focused on replay in CA1 place cells (Csicsvari, O'Neill, Allen, & Senior, 2007; Diba & Buzsáki, 2007; Foster & Wilson, 2006; A. K. Lee & Wilson, 2002). Previous studies indicate that SWRs originate from CA3 (Buzsáki, 2015, for a review), patterns of forward and reverse replay in CA3 have been shown to be similar to those in CA1 (Diba & Buzsáki, 2007), pair-wise signatures of replay are similar in CA3 and CA1, and joint firing patterns of CA3–CA1 cell pairs reactivate after a novel experience (O'Neill et al., 2008). Based on these studies, one would expect preferential reactivation and replay of novel experiences to occur in CA3 in a similar manner as in CA1. However, an earlier study investigating CA1 reactivation of novel and familiar environments did not directly compare reactivation of novel and familiar conditions in CA3 (O'Neill et al., 2008). Moreover, there is some evidence to suggest that place cell representations of novel experiences in CA1 and CA3 may be processed differently during overnight sleep. Experience-dependent changes in CA1 place cell firing are transient in CA1 but long-lasting in CA3. Specifically, CA1 place fields shift backward with experience in an environment each day, regardless of whether the environment is familiar or novel (Roth, Yu, Rao, & Knierim, 2012). In contrast, CA3 place fields shift backward in a novel environment but then these backward shifts are maintained across days once an environment is familiar (Roth et al., 2012). One theory of SWR function is that reactivation during SWRs in sleep serves to transfer memories out of the hippocampus to long-term storage sites in the neocortex (Buzsáki, 2015, for a review). Therefore, the finding that place cell firing patterns that emerge in a novel environment persist after overnight sleep in CA3 but not in CA1 raises the possibility that place cell reactivation during SWRs in overnight sleep following a novel experience differs between CA3 and CA1. Potentially different reactivation patterns in CA3 and CA1 during SWRs in overnight sleep could possibly be related to the recently discovered role of CA2 in CA1 SWR production (Alexander et al., 2018; Oliva, Fernández-Ruiz, Buzsáki, & Berényi, 2016), considering that CA2 cells primarily project to CA1 (Dudek, Alexander, & Farris, 2016). Thus, the question of whether CA3 place cells that represent novel experiences are selectively reactivated during SWRs in overnight sleep deserves further investigation.

Moreover, decreases in firing rates during rapid eye movement sleep (REM) compared to NREM have been reported previously for both CA1 and CA3 (Grosmark, Mizuseki, Pastalkova, Diba, & Buzsáki, 2012; Mizuseki, Royer, Diba, & Buzsáki, 2012). These firing rate decreases during REM may reflect homeostatic processes that reset hippocampal activity levels after memories have been consolidated. However, it remains unclear whether such firing rate decreases during REM occur preferentially for cells encoding novel experiences.

To address the above questions, we recorded place cells in area CA3 of rats navigating both familiar and novel environments and during subsequent overnight sleep. We compared firing patterns of CA3 place cells that coded familiar and novel locations to assess whether novelty-dependent activity changes were observed during NREM and REM. Moreover, we investigated whether replay events from CA3 place cell ensembles during NREM preferentially reflected novel trajectories, as expected if replay in CA3 plays a role in consolidation of newly formed memories. The results suggest that CA3 place cells that code novel experiences are preferentially recruited during SWRs and display higher fidelity replay than CA3 place cells that code familiar experiences.

2 | MATERIALS AND METHODS

2.1 | Subjects

Five male Long-Evans rats weighing from ~400 to 600 g were used in this study. The age of the rats ranged from 20 weeks to 60 weeks old at the time of behavioral testing. Data from four of these rats were included in a previous study (Trettel, Trimper, Hwaun, Fiete, & Colgin, 2017). Rats were housed in custom-built acrylic cages (40 cm × 40 cm × 40 cm) on a reverse light cycle (Light: 8 p.m. to 8 a.m.). The cages contained enrichment materials (e.g., plastic balls, cardboard tubes, and wooden blocks). Active waking behavior recordings were performed during the dark phase of the cycle, and overnight sleep recordings were performed during the light phase of the cycle (i.e., from 8 p.m. to 8 a.m.). Rats recovered from surgery for at least 1 week prior to the start of behavioral testing. During the data collection period, rats were placed on a food-deprivation regimen that maintained them at no less than ~90% of their free-feeding body weight. All experiments were conducted according to the guidelines of the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals under a protocol approved by the University of Texas at Austin Institutional Animal Care and Use Committee.

2.2 | Surgery and tetrode positioning

Recording drives with 14 independently movable tetrodes were surgically implanted above the right hippocampus (anterior–posterior [AP] 3.8 mm, medial-lateral [ML] 3.0 mm, dorsal-ventral [DV] 1 mm). Bone screws were placed in the skull, and the screws and the base of the drive were covered with dental cement to affix the drive to the skull. Two screws in the skull were connected to the recording drive ground. Before surgery, tetrodes were built from 17 μm polyimide-coated platinum-iridium (90/10%) wire (California Fine Wire, Grover Beach, CA). The tips of tetrodes designated for single unit recording were plated with platinum to reduce single channel impedances to ~150–300 kΩ. Over the course of ~1 month following surgery, tetrodes were gradually lowered into the hippocampus (1–4 tetrodes targeting CA1 with final DV depth of ~2 mm, and 8–11 tetrodes targeting CA3 with final DV depth of ~3 mm for each rat). One tetrode was designated as the reference for differential recording and remained in a quiet area of the cortex above the hippocampus throughout the experiments. This tetrode was moved up and down until a quiet location was found and was continuously recorded against ground to ensure that it remained quiet throughout data collection. Another tetrode was placed in the CA1 apical dendritic

layers to monitor local field potentials (LFPs) in the hippocampus during placement of the other tetrodes.

2.3 | Data acquisition

Data were acquired using a Digital Lynx system and Cheetah recording software (Neuralynx, Bozeman, MT). The recording setup has been described in detail previously (Hsiao, Zheng, & Colgin, 2016; Zheng, Bieri, Hwaun, & Colgin, 2016). Briefly, LFPs from one randomly chosen channel within each tetrode were continuously recorded in the 0.1–500 Hz band at a 2000 Hz sampling rate. Input amplitude ranges were adjusted before each recording session to maximize resolution without signal saturation. Input ranges for LFPs generally fell within ±2,000 to ±3,000 μV.

To detect unit activity, signals from each channel in a tetrode were bandpass filtered from 600 to 6,000 Hz. Spikes were detected when the signal on one or more of the channels exceeded a threshold set daily by the experimenter, which ranged from 55–65 μV. Detected events were acquired with a 32,000 Hz sampling rate for 1 ms.

Signals were recorded differentially against a dedicated reference channel (see "Surgery and tetrode positioning" section above). Video was recorded through the Neuralynx system with a resolution of 720 × 480 pixels and a frame rate of 29.97 frames/s. Animal position and head direction were tracked via an array of red and green light-emitting diodes (LEDs) on an HS-54 headstage (Neuralynx, Bozeman, MT) attached to a recording drive.

2.4 | Behaviors and overnight sleep

Rats were trained to run unidirectionally on a circular track (diameter of 100 cm, height of 50 cm, and width of 11 cm) to retrieve food rewards (i.e., pieces of Froot Loops) at opposite ends of the track for at least 3 days in a familiar room. Four 10 min sessions were conducted daily, separated by 10 min rest sessions. Once tetrodes reached their target recording locations (i.e., CA3 and CA1), rats performed the circular track task in the later part of the day (from ~6 p.m. to 8 p.m.), and then were placed in a square box (60 cm × 60 cm with 50 cm high walls) in the familiar room for overnight sleep recording (from 8 p.m. to 8 a.m.). On the next day, rats ran the same circular track task in the familiar room in the first and fourth sessions. However, on this day, the rats ran on a different circular track in a novel room (i.e., a room where the rats had not been previously) during the second and third sessions (Figure 1a). Small raised circles of various colors were adhered to the novel circular track to make its texture and appearance distinct from that of the familiar circular track in the familiar room. However, the dimensions of both novel and familiar circular tracks were the same. Upon completion of the circular track task, the rats again were put in the square box in the familiar room for overnight recording (again from 8 p.m. to 8 a.m.). Putative sleep episodes were identified by finding extended periods of immobility (≥60 s) in the box using custom code written in MATLAB (MathWorks, Natick, MA). Only sleep episodes that were also visually confirmed (i.e., from video recordings) by two independent researchers were included for further analysis. The same behavioral protocol including recordings in the familiar and novel rooms was repeated the next morning

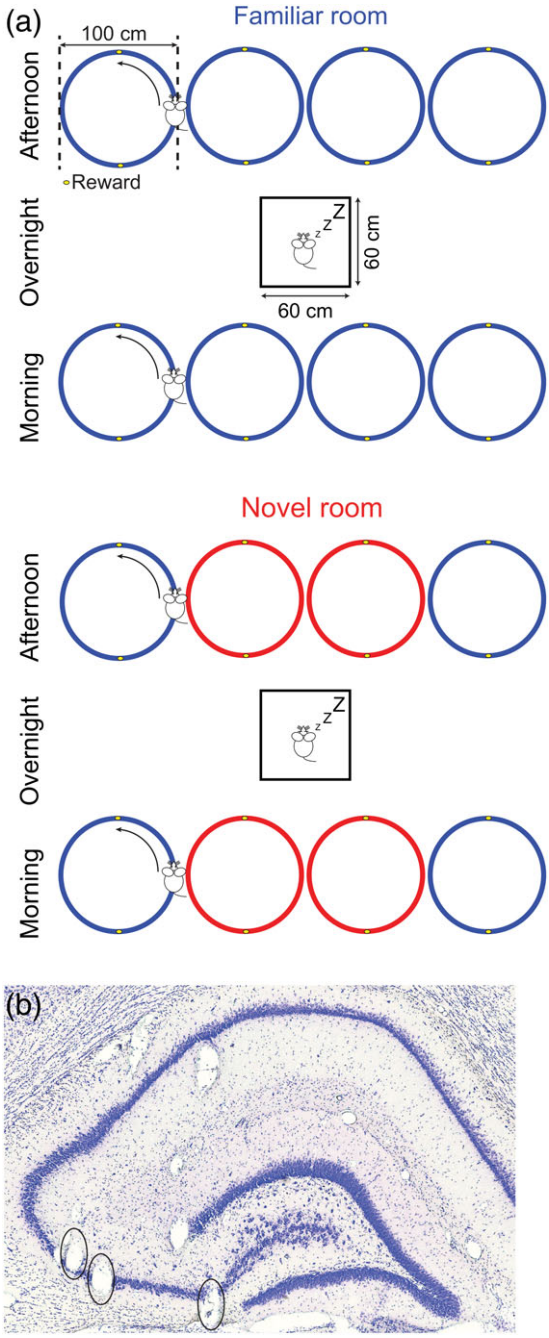

FIGURE 1 Behavioral protocol and verification of recording locations. (a) A schematic of the experimental setup shows the sequence of behavior recordings. Rats were trained to run unidirectionally on a circular track to retrieve food rewards in a familiar room (in blue) for four sessions. After completing the circular track task, rats were placed in a square box for recordings of overnight sleep. On the next morning, the same behavioral protocol was repeated. Later the same day, the same behavioral training protocol was repeated except the middle two sessions took place on a novel track in a novel room (in red; this color scheme is used consistently throughout the article). These behavioral sessions were again followed by overnight sleep recording. The same behavioral training sequence was then repeated the next morning to check whether place cells remained stable across overnight recordings. Note the novel room in the morning was the same novel room as the previous afternoon. (b) Example histologically verified CA3 recording sites are shown (indicated by black ovals)

(from 9 a.m. to 11 a.m.) to check whether cell ensemble recordings remained stable overnight.

2.5 | Identifying periods of REM and NREM

Periods of REM were detected offline using the ratio of the theta band (6–10 Hz) to delta band (2–5 Hz) wave amplitude (Csicsvari, Hirase, Czurkó, Mamiya, & Buzsáki, 1999) using LFPs recorded from a tetrode placed in the CA1 apical dendritic layers. The ratio was smoothed by a moving average time window of 5 s. Sleep periods with a ratio greater than 2 were categorized as REM. Any successively detected periods with an intervening time interval less than 3 s were combined. Detected REM periods with a duration of less than 10 s were excluded. Detected REM was verified by visually determining that there was a sustained increase in theta band amplitude in time-resolved amplitude spectrograms, and boundaries were adjusted manually if necessary. The spectrogram was estimated using a complex Morlet wavelet transform with a width parameter of six periods. The remainder of identified sleep periods that were not classified as REM were categorized as NREM. NREM periods with a duration of less than 5 s were excluded. Total recording time and percentage of time spent in each sleep state for each overnight session are shown in Supporting Information Figure S1A, and mean power spectral density measurements during the different sleep states are shown in Supporting Information Figure S1B and C.

2.6 | Identifying SWRs in NREM

LFPs recorded from tetrodes with CA1 place cells were used to detect SWRs in NREM. The detection method used in this study has been described in detail previously (Cheng & Frank, 2008). Briefly, LFPs were first bandpass filtered between 150 and 250 Hz. A Hilbert transform was implemented to estimate the instantaneous wave amplitude of the filtered LFPs by taking the absolute value of the complex signal. The amplitude was then smoothed with a Gaussian kernel with a standard deviation of 25 ms. A SWR event was detected if the amplitude exceeded 5 standard deviations (*SD*s) above the mean for at least 50 ms. Detected SWR events were bounded by first crossings of the mean amplitude. Overlapping SWR events were combined across CA1 tetrodes, so events could extend beyond a SWR detected on a single tetrode (Karlsson & Frank, 2009). Any SWR event detected during identified REM periods was excluded from further analysis (422 out of 22,343 and 394 out of 21,855 detected events were excluded during the sleep before and after novel experiences, respectively). Consecutive SWR events with intervening time intervals of less than 400 ms were excluded for the analyses to ensure that SWRs were absent during the baseline period (i.e., 400 ms before SWR onset).

2.7 | Spike sorting and unit classification

We performed spike sorting offline using graphical cluster-cutting software (MClust; A.D. Redish, University of Minnesota, Minneapolis, MN). Spikes were clustered manually using two-dimensional projections of three different features of spike waveforms (i.e., energies, peaks, and peak-to-valley differences) from four channels. We excluded putative fast-spiking interneurons (units with mean firing rates >5 Hz). We measured the quality of clusters using L ratio and isolation distance measures (Schmitzer-Torbert, Jackson, Henze, Harris, & Redish, 2005). The two measures were calculated using energies, peaks, and peak-to-valley differences on each channel. We categorized single units into four different groups based on whether they were active only in the novel room (Novel), only in the familiar room (Familiar), in both rooms (Both), or in neither room but active during sleep (Neither). A unit was considered to be active during waking behaviors if it exhibited a peak firing rate of at least 1 Hz (see "Unit firing analyses" section below). Units that were not active during waking behaviors, but had valid clusters from sorting of spikes during sleep, were considered active during sleep. Recordings were stable across experimental days, and no differences in measures of cluster quality were observed across the different groups (Supporting Information - Figure S2). Because we were unable to determine whether units in the "Neither" category were place cells with place fields in another environment (e.g., an area of their home cage) or a different type of cell, units in the Neither category were not analyzed further.

We also quantified how selective a unit was for the novel versus the familiar room using a selectivity index. The selectivity index was computed using the following formula: $(\mu_{novel} - \mu_{familiar})/(\mu_{novel} + \mu_{familiar})$, where μ was the mean firing rate in either the familiar or novel room. The selectivity index ranged from −1 to 1, with −1 indicating that a unit was exclusively active in the familiar room and 1 indicating that a unit was exclusively active in the novel room.

2.8 | Unit firing analyses

We calculated one-dimensional position tuning for each unit by binning the spikes into 5° bins and dividing the number of spikes in each bin by the amount of time spent in each bin. We estimated the position tuning for each lap on the circular track. To exclude out-of-field spikes during the immobile state, we only included times when rats were moving at least 5 cm/s (or equivalently 5.7°/s) to estimate position tuning. We then smoothed the position tuning curves through convolution with a Gaussian kernel (*SD* = 15°). Units with peak firing rates of at least 1 Hz on the circular track in either room were considered active during waking behaviors (as described above).

We constructed a population vector containing firing rates of all units for each position bin in each lap from the normalized position tunings of all simultaneously recorded active units (Leutgeb et al., 2005). The position tuning for each unit across laps was normalized by dividing each unit's maximal firing rate across laps. The population vectors for each position bin within each lap were further concatenated into a lap vector. We computed the Pearson's correlation coefficient between pairs of lap vectors to assess how similar population activities from a pair of laps were as a function of lap number difference. A larger lap number difference indicated that a pair of laps were further apart in time. We computed population vector correlations separately for pairs of laps in novel and familiar environments (Figure 2b).

To investigate unit firing during SWRs in NREM, we aligned the onset of all detected SWRs and averaged firing rates across all SWRs for each unit. We obtained the firing rate around SWRs by binning the spikes into 1 ms time bins. We smoothed the firing rate with a Gaussian

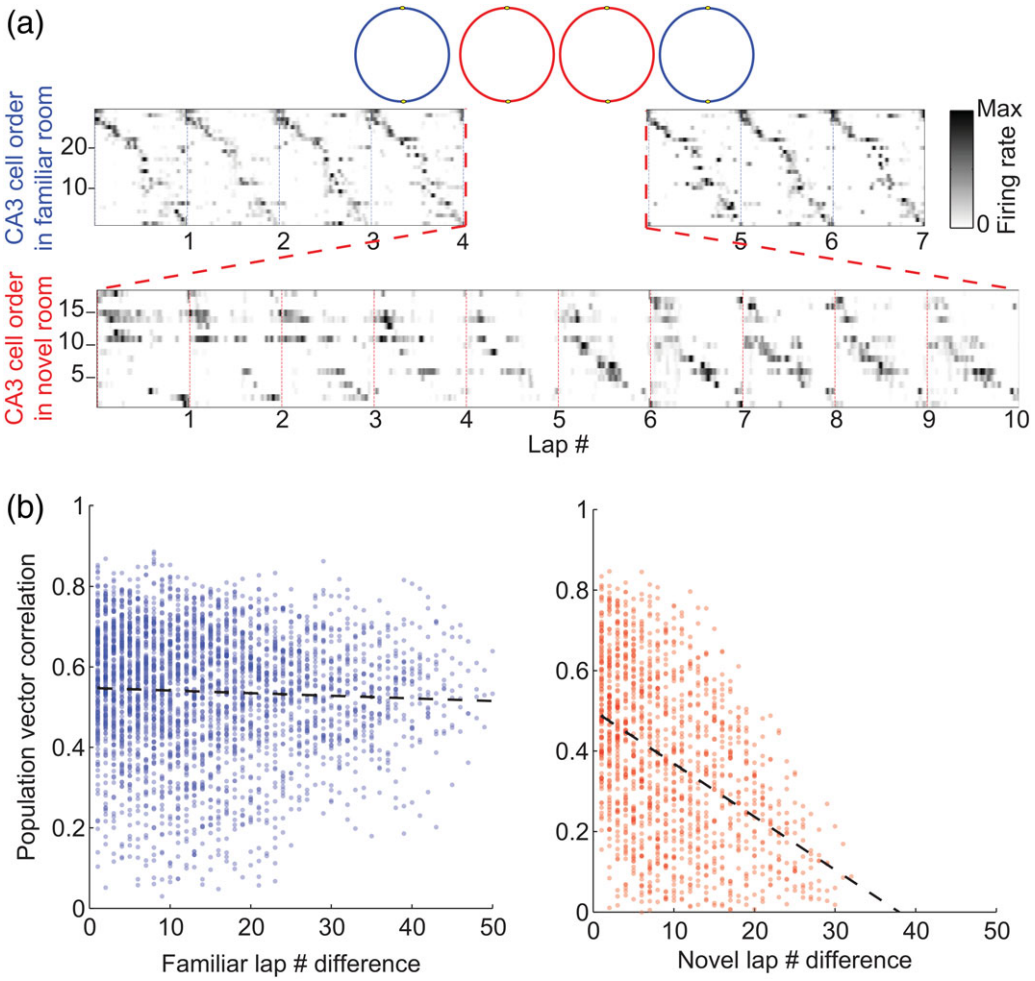

FIGURE 2 CA3 representations of a novel track develop across laps. (a) Examples of simultaneously recorded CA3 place cells on the circular track in the familiar room (blue) and in the novel room (red) are shown. Vertical dashed lines separate each lap on the track. Angular positions for each lap on the circular track were unwrapped on the *x*-axis (shown for each lap in between lap numbers). CA3 place cells active in either the familiar or the novel room were sorted on the *y*-axis according to their peak firing positions. Note that ensembles of CA3 place cells active in the familiar and novel rooms were different. Grayscale indicates normalized firing rate, with the darkest color representing maximal firing rate. In this example, most of the CA3 place cells that were active on the familiar track fired consistently at a specific position along the track, as shown by a roughly diagonal pattern of place cell ensemble activity that appears stable across laps. In contrast, most of the CA3 place cells that were active on the novel track changed their firing locations and/or firing rates across laps. (b) CA3 ensemble activity across laps was more similar in the familiar room than in the novel room. Population vector correlations were employed to quantify similarity between place cell ensemble representations for pairs of laps (see Materials and Methods). Lap number differences reflected how far apart pairs of laps were in time (e.g., laps 1 and 10 would have a lap number difference of 9), and a pair of laps were further apart in time if their lap number difference was higher. Each dot represents a correlation value for a pair of laps in the same room. Black dashed lines show the linear fits to the data

kernel (*SD* = 5 ms). To account for baseline firing rate differences, we normalized the firing rates in two different ways. The first way (Figure 3c–f) was to divide the firing rate of each CA3 place cell by its associated mean firing rate during the period before ripple onset (i.e., from 0.4 to 0.1 s prior to ripple onset). The second way (Figure 5c–f and Supporting Information Figure S3) was to divide the firing rate of each CA3 place cell by its mean firing rate during SWRs in NREM recorded the night before the novel experience. Since SWR firing rates declined across overnight sleep (Figure 4), we examined SWR firing rates during the first hour of sleep (Figure 5).

To examine unit firing during REM (Figure 8), we normalized the duration of each sleep episode (i.e., 100% corresponds to entire duration of each episode) that contained REM periods to obtain a mean firing rate across time for each unit (Grosmark et al., 2012). Each normalized REM and NREM period was divided into equal thirds. Since individual units had different baseline firing rates, we additionally normalized the firing rates by dividing by the mean firing rate during the NREM periods that preceded the REM periods to facilitate comparisons between different groups of units (see "Spike sorting and unit classification" section above). The minimum normalized firing rate was defined as the lowest value across all three-time bins within REM periods (Figure 8e–f).

2.9 | Decoding accuracy analysis

We used a Bayesian decoding algorithm (Zhang, Ginzburg, McNaughton, & Sejnowski, 1998) to compute the probability of a rat's angular position given ensemble activity of recorded CA3 place cells. The position with the highest probability was taken as the decoded position. To ensure stable

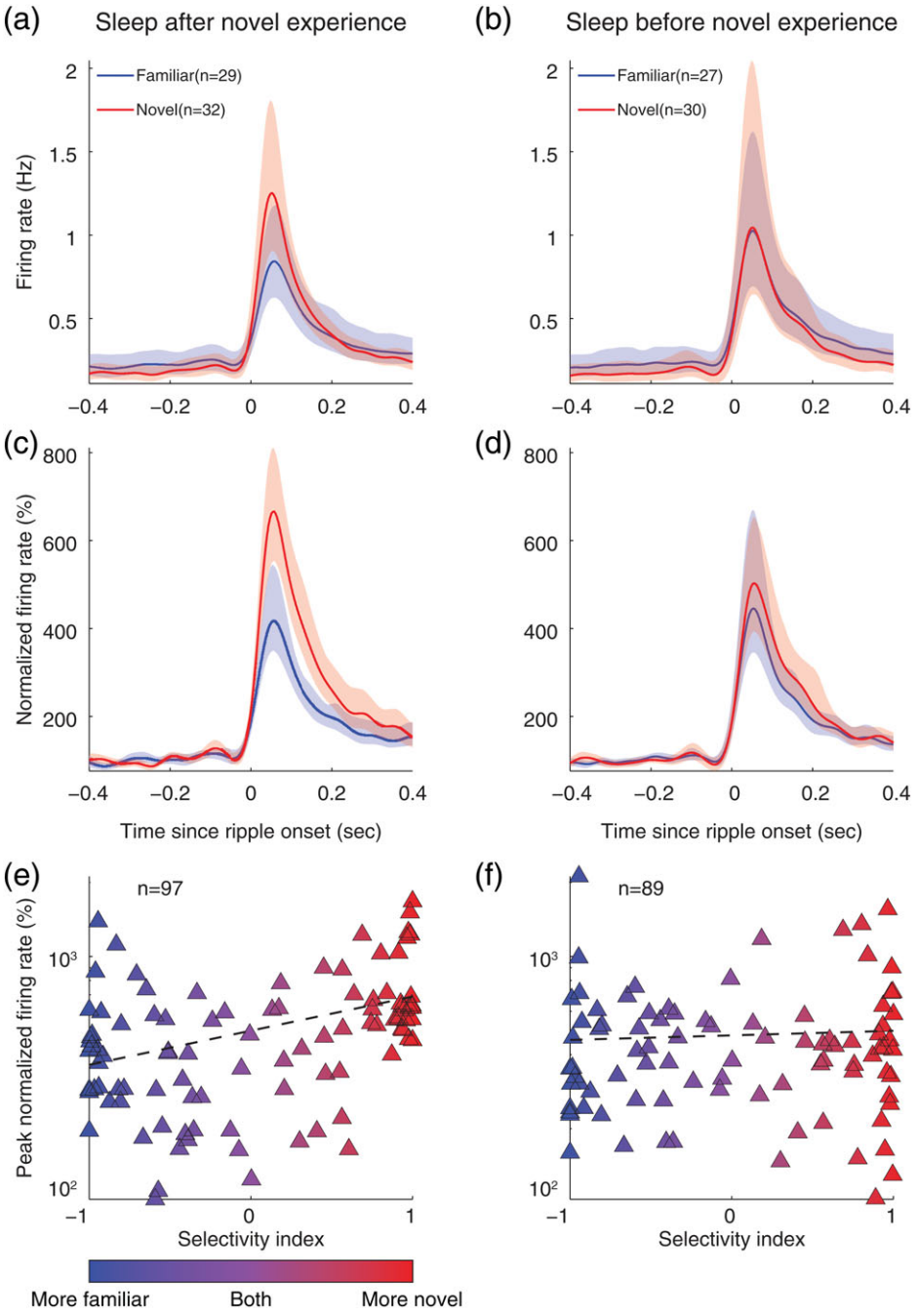

FIGURE 3 CA3 place cells that preferentially represented a novel environment showed stronger increases in normalized firing rates during SWRs following a novel experience. (a,b) Mean firing rates in CA3 increased after the onset of SWRs during sleep following (a) and preceding (b) a novel experience. Firing rates for each CA3 place cell were averaged across detected SWRs. Shaded areas in a–d represent 95% confidence intervals. (c,d) Normalized firing rates of CA3 place cells in the novel group increased more after the onset of SWRs than CA3 place cells in the familiar group for sleep following (c) but not preceding (d) a novel experience. Mean firing rates near SWRs were normalized by the mean firing rates during the period before ripple onset (i.e., from 0.4 to 0.1 s prior to ripple onset). (e,f) Peak normalized firing rates during SWRs were positively correlated with a selectivity index during sleep following (e) but not preceding (f) a novel experience. The selectivity index quantified how selective activity of a CA3 place cell was for the familiar room (selectivity index = −1 for maximal selectivity for the familiar room) or the novel room (selectivity index = 1 for maximal selectivity for the novel room). Both color scale and horizontal position indicate the degree of selectivity. The black dashed line shows the linear fit to the data

spatial representations across overnight sleep, we used the position tunings of each CA3 place cell during active behaviors on the circular track before sleep to decode rats' positions during active behaviors on the circular track after sleep. Positions were decoded across 500 ms time windows using a time step of 100 ms. Only time windows when rats were running more than 5 cm/s were included.

We quantified decoding accuracy measures for each rat using two different measures that have been previously described (Davidson, Kloosterman, & Wilson, 2009). First, decoded probability distributions were averaged across every pass through each position on the track (Figure 6b and 6c, top panels). For each rat, 50% of the total probability density was required to be less than 20° away from the actual position

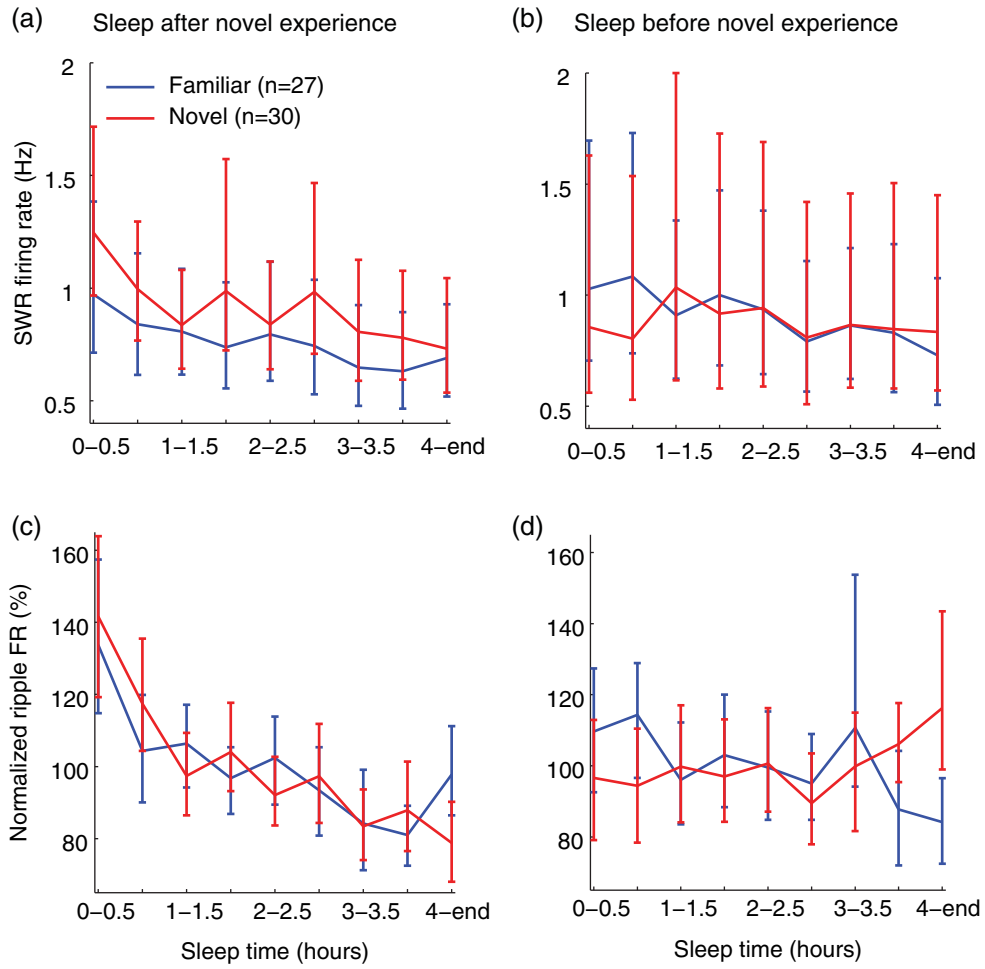

FIGURE 4 SWR firing rates in CA3 declined across overnight sleep. (a,b) CA3 place cell raw firing rates during SWRs in NREM sleep after (a) and before (b) a novel experience are shown. Firing rates decreased across time during overnight sleep. (c,d) To account for differences in baseline firing rates, CA3 place cell firing rates during SWRs across different time periods were divided by their mean SWR firing rates across time. Normalized SWR firing rates of CA3 place cells after (c) and before (d) a novel experience declined across overnight sleep. Error bars represent 95% confidence intervals

to be included in further analyses (Rat 100's familiar session and Rat 140's familiar and novel sessions were excluded based on this criterion). The second measure took the differences between decoded positions and actual positions as errors, and cumulative distributions of errors were determined (Figure 6b and 6c, bottom panels). Only rats with an error distribution reaching 50% at error values less than 20° were included for further analysis (Rat 140's familiar and novel sessions did not meet this criterion but had already been excluded, see above).

2.10 | Sleep replay analysis

We defined candidate replay events during NREM as the periods of time when the population firing rate of a subset of CA3 place cells exceeded 3 *SD*s above the mean, bounded by first crossings of the mean (Pfeiffer & Foster, 2013). Only candidate events with a minimum of five different active cells and a duration ranging from 50 to 500 ms were included for further analysis.

To evaluate whether a familiar or a novel experience was replayed during NREM, we applied the Bayesian decoding method described above to successive 20 ms time windows within a candidate replay event using a time step of 10 ms. We decoded all candidate events using position tunings in both the familiar and novel room. Thus, novelty was an additional feature in the decoding results (similar to the feature of direction tuning in Davidson et al., 2009). A standard method was used to assess the replay fidelity of familiar and novel trajectories separately, which involved fitting a line to decoded distributions across time and then using associated r^2 values as a measure of replay fidelity (Davidson et al., 2009; Karlsson & Frank, 2009). r^2 values range from 0 to 1, with values closer to one corresponding to higher fidelity replay. Each candidate event had a pair of r^2 values that correspond to familiar and novel trajectories. Since the behavioral apparatus was a circular track, we employed circular–linear regression to obtain r^2 values (Kempter, Leibold, Buzsáki, Diba, & Schmidt, 2012).

2.11 | Histology

At the end of the experiments, rats were sacrificed by injecting (i.p.) a lethal dose of pentobarbital. Rats then received intra-cardiac perfusion with phosphate-buffered saline followed by formalin. Brains were then sliced into 30 μm coronal sections and stained with cresyl-violet to confirm final tetrode positions in CA3 (Figure 1b).

2.12 | Experimental design and statistical analyses

Due to a limited number of recording rooms available at the time of data collection, we were only able to expose most of the rats to one novel room (except Rat 143 who was exposed to two different novel rooms). Rats were exposed to the novel room on the recording day when we estimated that our yield of simultaneously recorded CA3 place cells was maximal. The number of simultaneously recorded CA3 cells for each recording session is reported in Table 1. Note that the set of CA3 cells recorded in the second novel room for Rat 143 was different than the set of CA3 cells recorded in the first novel room. In Table 2, the number of CA3 cells was further divided into different cell groups for each overnight recording session following exposure to a novel room. In all five rats, multiple CA3 cells were included in all four groups (i.e., Novel, Familiar, Neither, Both; Table 2). However, CA1 place cells were included in both Novel and Familiar groups in only two rats (Table 3). Thus, CA1 place cells were not included in this study.

Pearson's correlations, ANOVAs, repeated measures ANOVAs, and generalized linear models were performed using *corr*, *fitlm*, *fitrm*, and *fitglm* functions in Matlab, respectively. Multiple comparisons were performed only when a significant interaction was found between variables included in ANOVAs. The permutation test used in this study shuffled cell groups (i.e., Familiar and Novel) 5,000 times to obtain a null distribution for mean SWR firing rate changes and r^2 values (see Supporting Information Figure S4 and Figure 7b, respectively). Monte Carlo P-values were calculated using the formula: $(N_{subset} + 1)/(N_{shuffle} + 1)$, where N_{subset} is the number of shuffles with values greater than or less than the observed value (two-tailed test) and $N_{shuffle}$ is the total number of shuffles.

Variability within our data is shown by 95% confidence intervals. Confidence intervals were computed by bootstrapping using the *bootci* function in MATLAB. We used 5,000 bootstrapped samples drawn with replacement to estimate each confidence interval.

2.13 | Code and data accessibility

MATLAB scripts were custom written for the analyses in this article. Scripts and data are available upon request.

3 | RESULTS

3.1 | CA3 place cell ensemble representations of a novel track emerged across laps

A previous study reported that stable representations of novel spatial environments emerge in CA3 place cell ensembles after approximately 20–30 min of experience (Leutgeb, Leutgeb, Treves, Moser, & Moser, 2004). Therefore, we expected that CA3 place cell ensembles would exhibit initially unstable representations of a novel track that would gradually stabilize across multiple exposures. To confirm this, we examined the dynamics of position tuning from CA3 place cell ensembles across successive laps on a novel circular track and compared results to CA3 place cell firing across laps on a familiar track. As is apparent in example recordings (Figure 2a), firing patterns of CA3 place cells remained stable across laps on the familiar circular track but changed across laps on the novel circular track. We used a population vector analysis (Leutgeb et al., 2005) to quantify the change in place cell ensemble activity between pairs of laps on the familiar or the novel circular track. For each lap on the track, firing rates from all simultaneously recorded CA3 place cells were combined (see Materials and Methods). We then assessed the similarity of CA3 place cell population activity for a pair of laps as a function of lap number difference. The degree of familiarity of the track (i.e., novel or familiar) significantly affected how much ensemble activity correlations decreased as the lap number difference increased. In general, ensemble activity across laps on the familiar track was significantly more similar than was ensemble activity across laps on the novel track (Main effect of track type in multiple regression analysis: $F[1,4372] = 1{,}180$, $p = 1.93 \times 10^{-229}$). Moreover, ensemble activity correlations on the novel and familiar tracks were differentially affected by the amount of time between laps (Figure 2b; significant interaction between track type and lap number difference: $F[1,4372] = 350$, $p = 3.71 \times 10^{-75}$). Correlation values for the familiar track decreased slightly as time between laps increased (Pearson's $r = -0.0502$, $p = 0.0057$), whereas correlation values for novel lap pairs decreased rapidly as time between laps increased (Pearson's $r = -0.448$, $p = 2.47 \times 10^{-67}$). These results confirm that CA3 place cell ensemble activity patterns change in a novel environment more than in a familiar one, suggesting that plasticity occurs in the CA3 network during learning of a novel environment.

3.2 | CA3 place cells that represented a novel track were selectively reactivated during SWRs in NREM

Presumably, newly formed place cell representations of novel places must be consolidated so that memories of these places can be stably retrieved in the future. As explained above, memory consolidation is thought to occur during NREM. Thus, we examined whether novelty-dependent changes in CA3 place cell ensemble activity during active awake behaviors persisted into subsequent NREM. To assess novelty-dependent effects, we separated CA3 place cells into different categories according to whether they were active on a novel track, a familiar track, or both (i.e., Novel, Familiar, and Both groups; see Materials and Methods). In previous studies, CA1 place cells that represented novel locations during awake behaviors were more likely to be reactivated during high-frequency ripples (Cheng & Frank, 2008), and CA3 and CA1 place cells that represented the same novel locations were shown to reactivate simultaneously during subsequent sleep (O'Neill et al., 2008). Thus, we hypothesized that CA3 place cells representing novel environments would be preferentially reactivated during SWRs in subsequent NREM compared to CA3 place cells representing familiar environments. We found that both CA3 place cells that were active on a novel track ($n = 32$) or a familiar track ($n = 29$) showed firing rate increases near the onset of SWRs in NREM (Figure 3a,b). In order to compare firing rate changes relative to baseline firing across the two groups, we then normalized the firing rate of each CA3 place cell by dividing by its mean baseline firing rate in the period before SWR onset. We observed that place cells that represented a novel environment increased their normalized firing rates during SWRs more so than place cells that represented a familiar environment (Figure 3c, d; significant interaction between cell group and time since ripple onset: repeated

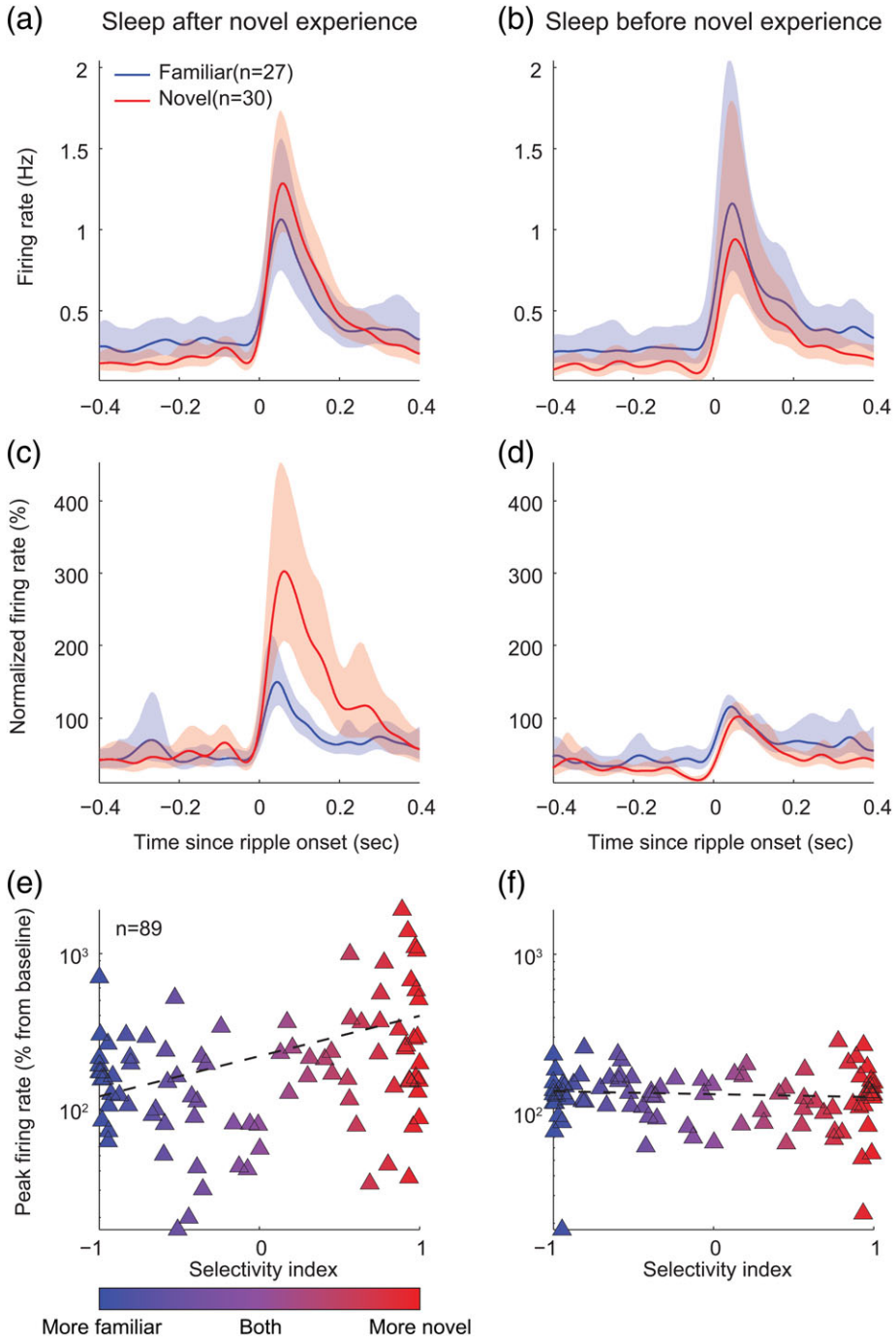

FIGURE 5 Comparison of firing rates during SWRs in the first hour of overnight sleep for CA3 place cells that preferentially represented novel or familiar environments. In this figure, firing rates were normalized according to firing rates during SWRs recorded during sleep prior to the novel experience. Only CA3 place cells that remained stable and thus could be tracked across two consecutive overnight recordings were included in this figure, which is why cell counts are slightly lower than those in Figure 3. (a,b) CA3 place cell mean firing rates increased during SWRs recorded during sleep following (a) and preceding (b) a novel experience. Firing rates for each CA3 place cell were averaged across detected SWRs. Shaded areas in a–d represent 95% confidence intervals. (c,d) Normalized in-ripple firing rates of CA3 place cells in the novel group increased more than those in the familiar group during sleep following (c) but not preceding (d) a novel experience. (e,f) peak normalized firing rates of CA3 place cells during SWRs were positively correlated with the selectivity index during sleep following a novel experience (e) but not during sleep preceding a novel experience (f)

measures ANOVA, $F(1,55) = 5.34$, $p = 0.0246$; Tukey post hoc test, Novel vs. Familiar across all time points: $p = 0.029$). However, these differences in SWR-associated firing between Familiar and Novel cells following a novel experience were only found when firing rates were normalized to the pre-SWR baseline. This raises the possibility that place cells representing novel environments selectively decrease their firing rates outside of SWRs following a novel experience rather than selectively increasing their firing during SWRs. To test this possibility, we compared baseline firing rates (i.e., firing rates 0.4–0.1 s prior to SWR onsets) between Novel and Familiar groups

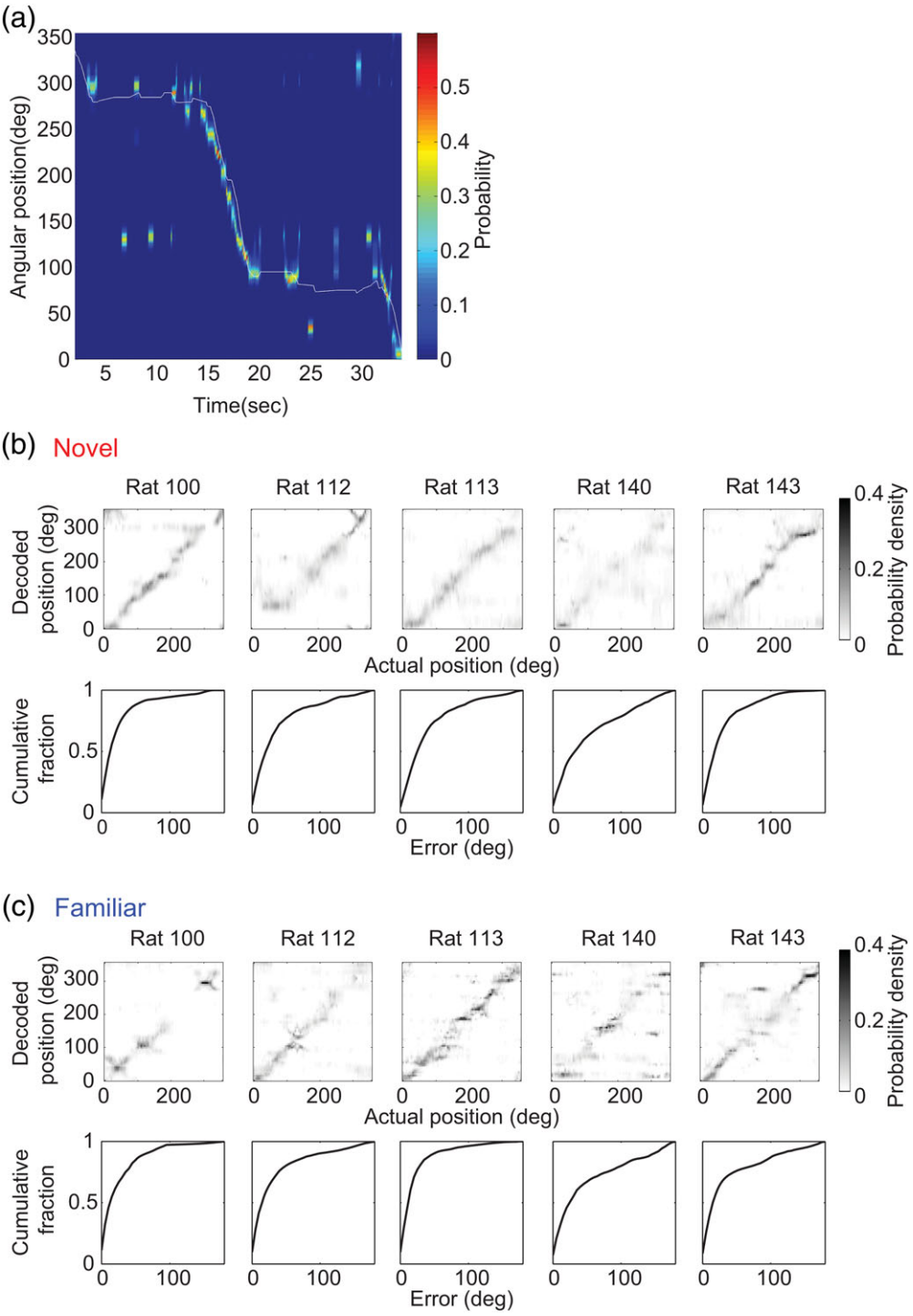

FIGURE 6 Decoding accuracy across active waking behaviors. (a) An example Bayesian decoded spatial probability distribution for a CA3 place cell ensemble recorded on the circular track shows that a rat's actual position could be decoded accurately, especially during active movement. The color scale indicates the decoded probability distribution of angular position, with warmer colors representing higher probability. The white line shows the actual position of the rat. (b,c) (Top) Confusion matrices show mean decoded probability distributions across rats' actual positions at times when movement speeds on the circular track >5 cm/s. A continuous diagonal stripe in a confusion matrix corresponds to accurate decoding. (Bottom) Cumulative distributions of decoding errors are presented for each rat. Decoding errors were defined as the difference between a rat's actual position at a given time and the position with the highest decoded probability at that time. Panel b shows data from the novel environment, and panel c shows data from the familiar environment

and found no significant differences (Main effect of cell group within baseline period prior to SWRs: repeated measures ANOVA, $F(1,55) = 0.74$, $p = 0.39$; see also overlapping confidence intervals for baseline firing rates in Figure 3a,b).

However, the analyses above did not consider place cells that were active in both novel and familiar environments but showed preferential activation for one environment over the other (i.e., CA3 place cells in the Both category, which showed a range of preferential firing

TABLE 1 Number of simultaneously recorded CA3 cells in each recording session

Animal ID	Run session in afternoon	Overnight sleep session	Run session in next morning
Rat 100	27	49	25
Rat 112	23	48	19
Rat 113	40	59	45
Rat 140	21	28	23
Rat 143 first set	37	57	39
Rat 143 second set	17	36	18

selectivity for the novel and familiar environments). Thus, we implemented a selectivity index to quantify on a numerical scale how selective each CA3 place cell's firing was for the novel or familiar environment (see Materials and Methods). We found that peak normalized firing rates within SWRs were positively correlated with selectivity for the novel environment only for SWRs recorded the night following the novel experience but not for SWRs recorded the night before the novel experience (Figure 3e,f; significant interaction between selectivity index and novelty: $F[1,87] = 8.63$, $p = 0.0042$; significant correlation between selectivity index and peak normalized firing rate for sleep after novel experience: $r = 0.361$, $p = 5.16 \times 10^{-4}$; no significant correlation between selectivity index and peak normalized firing rate for sleep before novel experience: $r = 0.0463$, $p = 0.667$).

We next sought to address the question of whether cells that represent novel experiences undergo changes in SWR firing after the novel experience. It is possible that place cells that fire in a novel environment represent a special subset of neurons that already show enhanced excitability during SWRs prior to experience in a novel environment. To investigate this possibility, we compared SWR firing rates of CA3 place cells that represented a novel or familiar environment, normalized to their mean SWR firing rates during NREM the night before the novel experience. Since we only included CA3 place cells that could be tracked across two consecutive overnight recordings, the cell yields during sleep after a novel experience ($n = 27$ Familiar and $n = 30$ Novel) were slightly lower than those in our previous analysis (i.e., $n = 29$ Familiar and $n = 32$ Novel for Sleep after novel experience in Figure 3). A previous study reported that CA1 firing rate increases during SWRs declined within the first 30 min of sleep following active waking behaviors (Kudrimoti et al., 1999). Similarly, we found that SWR firing rates of CA3 place cells declined across the first hour of overnight sleep for place cells that represented both familiar and novel environments during sleep both preceding and following a novel experience (raw SWR firing rates in Figure 4a,b; repeated measures ANOVA; main effect of time: $F[1,55] = 16.2$, $p = 1.75 \times 10^{-4}$; normalized SWR firing rates in Figure 4c,d; main effect of time: $F[1,55] = 15.2$, $p = 2.63 \times 10^{-4}$). Thus, when comparing SWR firing rates of cells that were normalized to mean SWR firing rates the night before the novel experience, we focused on the first hour of sleep (see Supporting Information Figure S3 for results when the entire duration of overnight sleep was included). We again found that both CA3 place cells that were active on a novel track ($n = 30$) and a familiar track ($n = 27$) increased their firing rates near the onset of SWRs in NREM both preceding and following a novel experience (Figure 5a,b). However, when we normalized the firing rate of each CA3 place cell by dividing by its mean SWR firing rate during NREM preceding a novel experience, we found that place cells in the Novel group increased their relative SWR firing rates more so than place cells in the Familiar group during NREM following (Figure 5c) but not preceding (Figure 5d) a novel experience (Repeated measures ANOVA; significant interaction between time point, cell group, and novelty: $F[1,55] = 7.90$, $p = 0.0068$; Tukey post hoc test, Familiar vs. Novel during sleep after novel experience across all time points: $p = 0.0140$, Familiar vs. Novel during sleep before novel experience across all time points: $p = 0.183$). We assessed whether mean SWR firing rates between Familiar and Novel cell groups were different during sleep prior to novel experience and found no significant difference (Permutation test, 5,000 shuffles of cell group identity, $p = 0.47$). Moreover, when we included all place cells that were active in both familiar and novel environments (as in Figure 3e,f), we found that place cells' preferences for a novel environment were positively correlated with SWR-associated firing rate increases for NREM following but not preceding a novel experience (Figure 5e,f; significant interaction between selectivity index and novelty: $F[1,87] = 10.3$, $p = 0.0019$; significant correlation between selectivity index and peak normalized firing rate for sleep after novel experience: $r = 0.337$, $p = 0.0012$; no significant correlation between selectivity index and peak normalized firing rate for sleep before novel experience: $r = -0.0776$, $p = 0.470$). Taken together with the results described above, these results suggest that CA3 place cells that preferentially coded a novel environment were more strongly reactivated during SWRs in subsequent NREM than were CA3 place cells that preferentially represented a familiar environment.

However, not all CA3 place cells that preferentially fired in a novel environment increased their firing rates during SWRs in subsequent sleep (see Figure 5e). It is possible that some cells achieved their maximal possible firing rate increases during SWRs prior to the novel experience and were thus unable to further increase their firing during SWRs that followed the novel experience. To investigate this possibility, we used the median SWR firing rate of the Familiar group during sleep before novel experience to divide the cells into those with SWR firing rates that were relatively low (i.e., Below median) or relatively high (i.e., Above median) prior to the novel experience. A significant difference in the change in SWR firing rates following the novel experience (i.e., mean SWR firing rates in sleep after novel experience—mean SWR

TABLE 2 Number of CA3 cells in each cell group from each overnight sleep session

Cell group	Rat 100	Rat 112	Rat 113	Rat 140	Rat 143 first set	Rat 143 second set
Novel	9	1	5	2	11	4
Familiar	3	2	11	2	5	6
Neither	32	35	33	19	37	24
Both	5	10	10	5	4	2

TABLE 3 Number of CA1 cells in each cell group from each overnight sleep session

Cell group	Rat 100	Rat 112	Rat 113	Rat 140	Rat 143 first set
Novel	5	0	3	0	0
Familiar	2	1	2	0	0
Neither	2	9	15	1	1
Both	8	3	14	1	3

firing rates in sleep before novel experience) was observed between the Familiar and Novel cell groups for cells with relatively low SWR firing rates prior to the novel experience (Supporting Information Figure S4A, lower panel; Permutation test on firing rate changes following novel experience, 5,000 shuffles of cell group identity, $p = 0.018$). However, an analogous difference between Novel and Familiar cells' SWR firing rate changes was not observed for cells that exhibited relatively high SWR firing rates prior to the novel experience (Supporting Information - Figure S4A, upper panel; Permutation test on firing rate changes following novel experience, 5,000 shuffles of cell group identity, $p = 0.261$). The unchanging subset of cells may be related to "rigid" place cells that have been previously reported in CA1 (Grosmark & Buzsaki, 2016). In this earlier study, CA1 rigid cells showed high firing rates in SWRs preceding exploration of a novel environment and did not tend to increase their firing rates much more during SWRs following exploration of a novel environment. CA1 rigid cells also showed low spatial specificity. Thus, we assessed whether similar effects were observed for "rigid" CA3 place cells that fired in novel environments. We found a positive correlation between changes in peak firing rate during SWRs in subsequent NREM and spatial information for place cells representing the novel environment (Supporting Information Figure S5A) but no such correlation for place cells representing the familiar environment (Supporting Information Figure S5B; significant interaction between cell type and peak firing rate change: $F[1,45] = 13.2$, $p = 7.17 \times 10^{-4}$; significant correlation for Novel group: Pearson's $r = 0.516$, $p = 0.0059$; no correlation for Familiar group: Pearson's $r = -0.143$, $p = 0.527$). These results suggest that CA3 place cells that showed relatively low spatial specificity on the novel track also tended not to change their firing rates much during SWRs following the novel experience, similar to previously reported CA1 rigid cells.

We next investigated whether differential firing rate changes during SWRs following the novel experience for cells with relatively low and relatively high SWR firing rates prior to the novel experience were restricted to periods of SWRs. Specifically, we tested whether differential

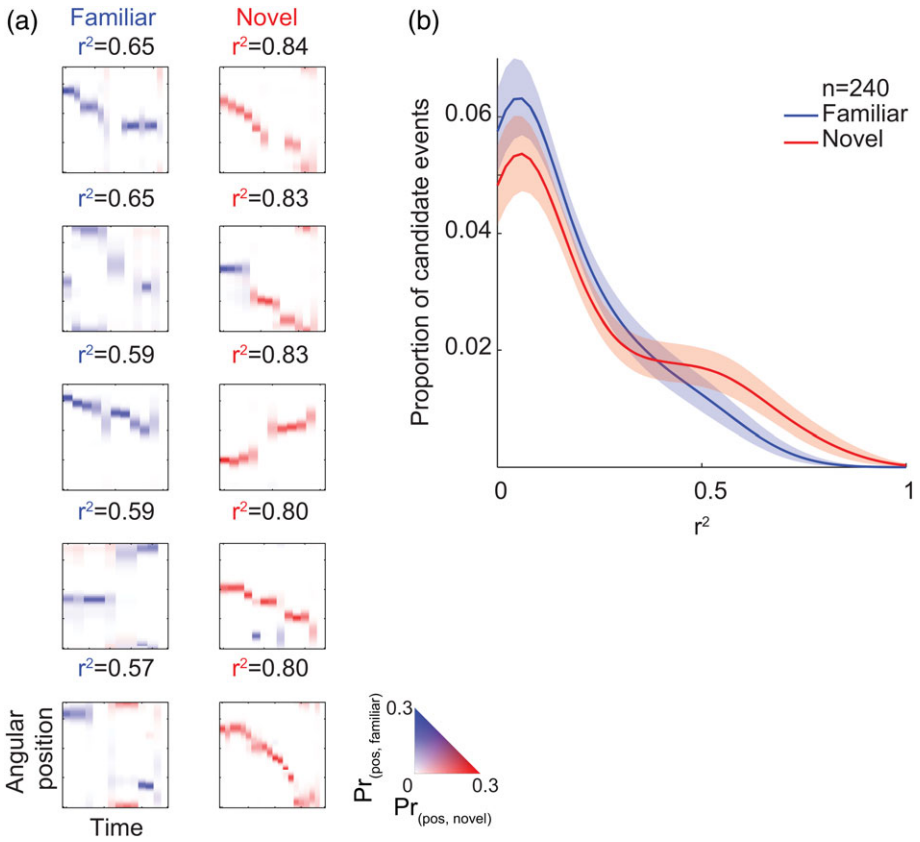

FIGURE 7 Replay fidelity was higher for CA3 place cell ensembles representing a novel environment than for ensembles representing a familiar environment. (a) Candidate replay events with the top five r^2 values for familiar and novel trajectories are shown. The color scale corresponds to the position probability from Bayesian decoding on the novel track (red) and on the familiar track (blue). Circular-linear regressions were computed between angular positions and time to obtain a r^2 value for each candidate replay event. (b) Distributions of r^2 values for novel and familiar trajectories are shown

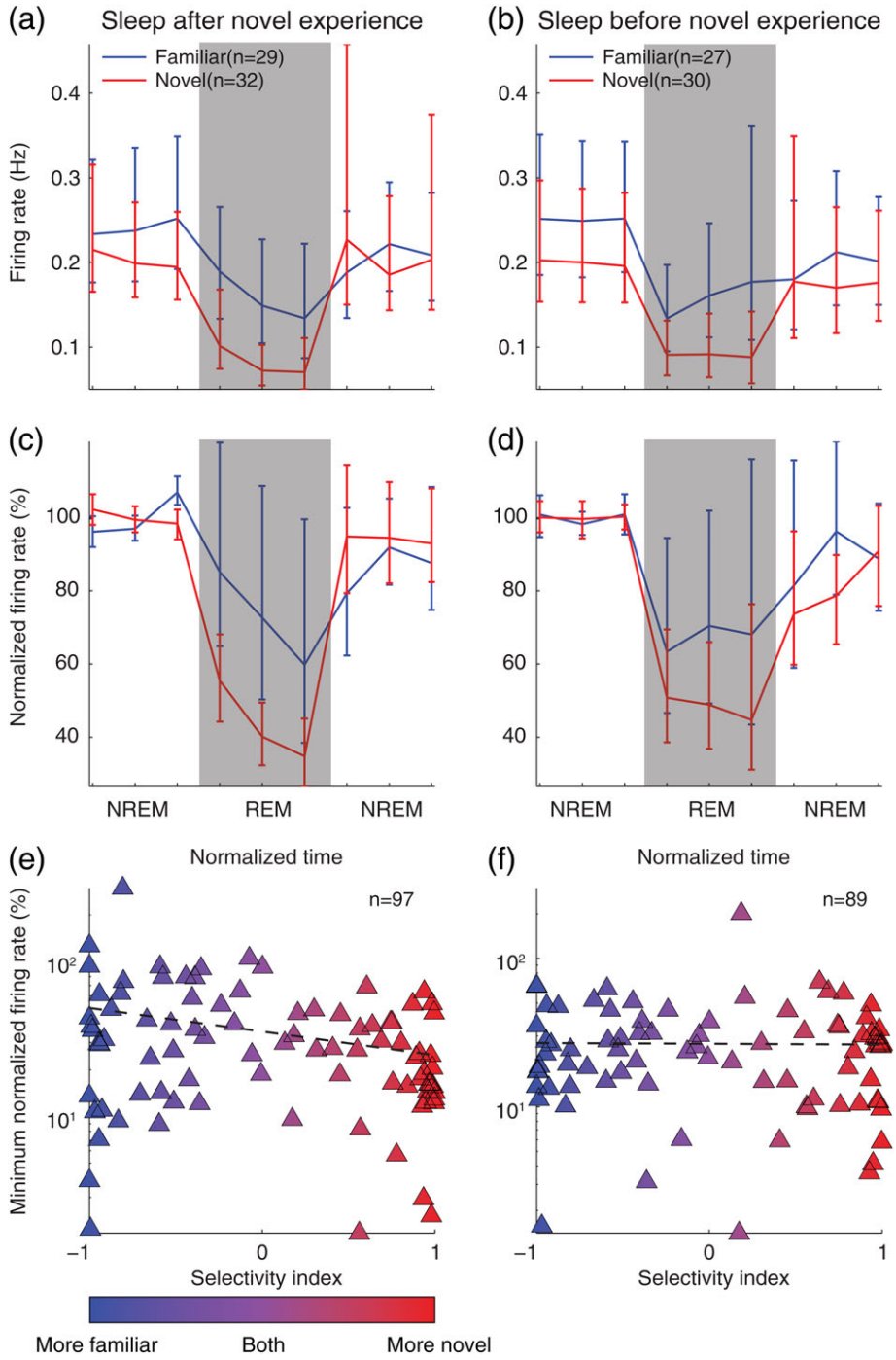

FIGURE 8 CA3 place cells decreased their firing rates during REM sleep. (a,b) Firing rates of CA3 cells decreased from NREM to REM during sleep following (a) and preceding (b) a novel experience. The firing rate for each CA3 cell was averaged across the sleep episodes that contained REM periods. Error bars in a–d indicate 95% confidence intervals. (c,d) Normalized firing rates of CA3 place cells in novel and familiar groups decreased similarly from NREM to REM during sleep following (c) and preceding (d) a novel experience. The firing rate for each CA3 cell was normalized by the mean firing rate in the NREM period that immediately preceded the REM period. Durations of NREM and REM periods in sleep episodes were normalized (see Materials and Methods). (e,f) Individual place cells' minimum normalized firing rates during REM (i.e., minimum values across the three REM time bins) were negatively correlated with the selectivity index during sleep following (e) but not preceding (f) a novel experience. The black dashed line shows the linear fit to the data

firing rate changes were also observed in pre-SWR baseline firing rates (i.e., 0.4–0.1 s prior to SWR onsets) when cells were separated into the same Above median and Below median categories described above (i.e., in Supporting Information Figure S4A). We found that pre-SWR baseline firing rates of Novel and Familiar cells with both relatively high and relatively low SWR firing rates prior to novel experience were not differentially changed by novel experience (Supporting Information - Figure S4B; Permutation test on firing rate changes following novel experience, 5,000 shuffles of cell group identity, $p = 0.217$ for Below median category and $p = 0.263$ for Above median category).

3.3 | CA3 place cells preferentially replayed representations of recently learned trajectories during SWRs in NREM

The enhanced SWR firing of CA3 place cells representing the novel track raises the possibility that CA3 place cell ensembles preferentially replay novel trajectories during SWRs in NREM. To test this hypothesis, we first set out to establish the viability of assessing replay in our overnight recordings by determining whether CA3 place cell representations remained stable across overnight sleep. To assess the extent to which CA3 place cell representations of familiar and novel trajectories remained stable, we used the position firing rate maps before sleep to reconstruct rats' positions from ensemble spiking activity on the circular track after sleep using a probabilistic approach based on Bayes' rule (Zhang et al., 1998). An example Bayesian decoded spatial probability distribution that corresponded to a lap on the circular track in the novel room is shown in Figure 6a. The peak of the decoded probability distribution closely followed the rat's actual position, especially when the rat was moving. Decoding accuracy using this approach is shown for each rat in the novel (Figure 6b) and familiar (Figure 6c) environments. Most rats' recordings (Rats 100, 112, 113, and 143 in the novel room and Rats 112, 113, and 143 in the familiar room) surpassed our criterion for sufficient decoding accuracy (see Materials and Methods) and were thus included in subsequent replay analyses during NREM.

To evaluate the extent to which familiar or novel experiences were replayed during NREM, we estimated spatial representations coded by CA3 place cell ensembles during candidate replay events using Bayesian decoding (see Materials and Methods). Similar to previous findings (Pfeiffer & Foster, 2013), detected SWR events coincided with candidate events (Supporting Information Figure S6). Candidate events were decoded for familiar and novel trajectories separately (see Materials and Methods). Following standards set by previous studies, we assessed the fidelity of replay by fitting a line to the decoded distributions across time and then using corresponding r^2 values to represent the fidelity of replay (Davidson et al., 2009; Karlsson & Frank, 2009). r^2 values closer to 1 correspond to higher fidelity replay. The candidate events with the top five r^2 values for novel and familiar trajectories are shown in Figure 7a. As can be seen in these selected events, reconstructed positions of novel trajectories were more continuous and linear than reconstructed positions of familiar trajectories. The distributions of r^2 values across all candidate replay events for novel and familiar environments are shown in Figure 7b. Overall, the r^2 values for novel trajectories were significantly higher than r^2 values for familiar trajectories (n = 240, Permutation test, 5,000 shuffles of trajectory type, $p = 2.0 \times 10^{-4}$). To account for the difference in decoding accuracy of familiar and novel trajectories, we used a generalized linear model with a log link function to predict r^2 values from decoding errors for each session and trajectory type (i.e., novel or familiar). Including trajectory type increased prediction accuracy while including decoding errors had no effect in the model (Trajectory type, $\beta = 2$, $p = 1.1 \times 10^{-8}$; Decoding errors, $\beta = 0.25$, $p = 0.57$), suggesting the difference in r^2 values was unlikely due to differences in decoding accuracy. These results suggest that ensembles of CA3 place cells preferentially replay novel experiences during NREM.

3.4 | CA3 place cells reduced their firing rates during REM

A previous study reported that putative pyramidal cells in CA1 increase their firing rates during NREM and decrease their firing rates during REM, suggesting that REM may serve to prevent accumulating increases in firing rates across successive NREM episodes (Grosmark et al., 2012). Another related study reported that firing rates of CA3 putative pyramidal cells reduced their firing rates more in REM relative to NREM compared to CA1 cells (Mizuseki et al., 2012). These findings, taken together with the increased firing rates during SWRs that we observed for CA3 place cells preferentially representing a novel environment (Figures 3 and 5), suggest that particularly strong firing rate decreases may occur during REM for CA3 place cells that preferentially code novel environments. On the other hand, place field expansion, which develops with experience in novel environments, persists following overnight sleep for CA3 but not for CA1 (Roth et al., 2012). This finding raises the possibility that increased SWR-associated firing of CA3 place cells representing novel environments may not be offset by particularly strong firing rate decreases occurring during REM sleep. To investigate whether novelty-dependent reductions in CA3 firing rates occurred during REM, we compared normalized firing rates of CA3 place cells with fields on only the novel track or only the familiar track across NREM and REM episodes. Both groups of CA3 place cells (i.e., Novel and Familiar) decreased their firing rates from NREM to REM periods of sleep on the night preceding the novel experience and the night following the novel experience (Figure 8a–d; repeated measures ANOVA; main effect of normalized time: $F[1,55] = 702$, $p = 5.33 \times 10^{-33}$; no significant interaction between neuronal group and normalized time, $F[1,55] = 1.47$, $p = 0.230$). This pattern of results suggests that CA3 place cell firing rates decrease during REM regardless of novel experience. However, a small effect of novel experience on firing rate decreases during REM was observed when all CA3 place cells were included and categorized according to their selectivity for the novel or the familiar environment. CA3 place cells showed slightly greater firing rate reductions during REM as their preference for coding the novel track increased, and this correlation was only observed during REM after the novel experience (Figure 8e,f; significant interaction between selectivity index and night: $F[1,87] = 4.30$, $p = 0.0410$; significant correlation between selectivity index and minimum normalized firing rate for night after novel experience: $r = -0.246$, $p = 0.0202$; no significant correlation between selectivity index and minimum normalized firing rate for night before novel experience: $r = -0.00930$, $p = 0.931$). A previous study showed that firing rate decreases over sleep correlate with the rate of SWRs in NREM (Miyawaki & Diba, 2016). However, the weak relationship between preferential coding of the novel environment and CA3 place cell firing rate decreases during sleep after the novel experience is unlikely to be explained by this effect considering that rates of SWRs did not differ during sleep preceding and following the novel experience (Supporting Information Figure S7). However, the extent to which these weak changes during REM in the activity of cells representing novel experiences would significantly impact processing of novel memories during sleep remains unclear. Although SWR-associated firing rates decreased over the course of overnight sleep for both Novel and

Familiar cell classes (Figure 4), such decreases during SWR periods represent a small portion of the total NREM duration and were insufficiently strong to yield significant differences in overall NREM firing rates across the course of overnight sleep. That is, overall firing rates during NREM epochs following REM were not significantly lower than overall firing rates during the NREM periods preceding REM (Figure 8a, b; Tukey post hoc test, NREM before REM vs. NREM after REM: $p = 0.0819$). From this pattern of results, we are unable to draw strong conclusions about whether firing rate decreases during REM counteract particularly strong SWR firing rate increases in the CA3 cells that represent novel environments.

A previous study in CA1 reported shifts in the preferred theta phase of spikes during REM relative to active waking behaviors that occurred for place cells that represented familiar but not novel experiences (Poe, Nitz, McNaughton, & Barnes, 2000). It was suggested that such shifts during REM allow recently acquired memories to be strengthened during REM while familiar memories are weakened. We investigated whether analogous shifts in the preferred theta phase of CA3 place cell spikes representing familiar but not novel environments occurred between active waking behaviors and REM. No significant theta phase shifts between track running and REM were observed for CA3 place cells that were active in either familiar or novel environments (Supporting Information Figure S8). Thus, the results shown in Figure 8e,f are unlikely to be due to shifts in place cells' preferred theta phases during REM.

4 | DISCUSSION

A major question in sleep research is how new memories are consolidated during overnight sleep. Memories involving the hippocampus are thought to be initially stored in the CA3 recurrent network (Marr, 1971; Steffenach, Sloviter, Moser, & Moser, 2002; Treves & Rolls, 1992), and CA3 is required for consolidation of hippocampal-dependent memory (Nakashiba et al., 2009). Yet, most in vivo studies of hippocampal neuronal activity during active exploratory behaviors (i.e., when memories are formed) and subsequent sleep (i.e., when recently formed memories are thought to be consolidated) have focused on CA1 (Kudrimoti et al., 1999; A. K. Lee & Wilson, 2002; Nádasdy et al., 1999; Pavlides & Winson, 1989; Wilson & McNaughton, 1994). Although previous studies have shown that CA1 place cell reactivation during SWRs is stronger for cells representing novel places compared to familiar places (Cheng & Frank, 2008; O'Neill et al., 2008), supporting a role for SWRs in the consolidation of new memories, analogous studies focusing exclusively on CA3 place cells were lacking. Here, we investigated changes in CA3 place cell firing patterns during overnight sleep after experiences in both novel and familiar environments. We found that CA3 place cells that represented novel environments were preferentially recruited during SWRs (Figures 3 and 5) and that CA3 place cell ensembles replayed representations of novel environments during NREM sleep with higher fidelity than representations of familiar environments (Figure 7).

Taken together with previous results (Cheng & Frank, 2008; O'Neill et al., 2008), the present results imply that memory consolidation during sleep may be an active process that selects newly encoded information rather than a passive process that reflects the duration of recent experiences. If the latter were the case, one would not expect preferential reactivation of CA3 representations of novel experiences during sleep since rats spent equal amounts of time in familiar and novel environments before sleep. However, some CA3 place cells did not change their firing rates during SWRs following exploration of a novel environment (Supporting Information Figure S4) and may relate to a "rigid" subset of place cells that has been reported previously in CA1 (Grosmark & Buzsaki, 2016). It is interesting to hypothesize that those place cells that increase their firing rates during SWRs following a novel experience represent aspects of an experience that are more strongly remembered later. We were unable to investigate this hypothesis, though, because the circular track in the present study had no measurable memory component.

However, previous results support the hypothesis that enhanced firing rate increases observed during SWRs for CA3 place cells that preferentially represent novel environments would relate to behavioral performance in newly learned memory tasks. A previous study showed that contextual fear conditioning increased firing rates of CA1 neurons during subsequent sleep in mice (Ognjanovski et al., 2014). However, no place cells were reported in this previous study. Instead, the cell ensembles in the study by Ognjanovski and colleagues appeared to primarily contain fast-spiking interneurons, based on the reported firing rates (i.e., ~5–10 Hz mean firing rates in their figure 3), which were considerably higher than the sparse mean firing rates that are characteristic of place cells (e.g., ~0.1–1 Hz mean firing rates in Figures 3a,b and 5a,b of the present study; see also Jung, Wiener, & McNaughton, 1994). Consistent with this assumption, the same group later reported increases in firing rates of fast-spiking interneurons during SWRs following contextual fear conditioning (Ognjanovski et al., 2017) and furthermore linked changes in interneuron activity during NREM to consolidation of contextual fear memory (Ognjanovski, Broussard, Zochowski, & Aton, 2018). However, the recordings in the present study were targeted toward place cells and did not contain fast-spiking interneurons. Thus, how the present place cell results relate to previous results involving firing rate changes in fast-spiking interneurons during sleep (Ognjanovski et al., 2017) remains a question for future study.

Although rats in the present study did not perform a memory task to directly demonstrate how well they remembered the novel environment after sleep, CA3 place cell ensemble representations of novel environments remained stable after sleep (Figure 6), suggesting successful retrieval of spatial representations that were formed before sleep. CA1 place cell firing during SWRs has been reported to be important for stabilization of place fields (Roux, Hu, Eichler, Stark, & Buzsáki, 2017). However, this study involved sharp waves during waking behavior, and analogous findings were not observed in another study that silenced pyramidal cell activity during SWRs in post-experience sleep (Kovács et al., 2016). Still, previous studies have shown that disrupting SWR-related neuronal activity during both NREM and wakefulness impairs memory performance (Ego-Stengel & Wilson, 2010; Girardeau et al., 2009; Jadhav, Kemere, German, & Frank, 2012). Moreover, SWR silencing during awake rest was found to corrupt spatial representations of a novel, but not a familiar, environment (van de Ven, Trouche, McNamara, Allen, & Dupret, 2016). Therefore, a plausible hypothesis is that novel information is preferentially

consolidated during SWRs in part due to enhanced reactivation of CA3 cells that encode novel experiences (Figure 3). Together, these results are consistent with a central role for SWRs in consolidation of new memories.

However, the synaptic mechanisms that allow SWRs during NREM to preferentially reactivate cells that code novel experiences remain unknown. There are at least two possible ways to selectively increase the relative strength of synaptic connections that store a novel memory. A direct way is to strengthen synaptic connections between neurons that encode novel information. In support of this idea, an in vivo study showed that stimulation of CA1 putative pyramidal cells during a transient period consisting of 250 detected SWRs increased neuronal activity during subsequent SWRs (King, Henze, Leinekugel, & Buzsáki, 1999). Furthermore, stimulating CA1 pyramidal cells and Schaffer collaterals in vitro using stimulation protocols patterned after CA3 and CA1 place cell firing during SWRs in vivo has been shown to potentiate CA1 cells' responses to Schaffer collateral stimulation (Sadowski, Jones, & Mellor, 2016). Thus, preferential recruitment of CA3 place cells during SWRs that coded earlier novel experiences may selectively strengthen their synaptic connections. On the other hand, a second possible way to selectively increase the relative strength of synaptic connections underlying newly encoded memories is to weaken synaptic connections between neurons that do not store novel information. Several studies have suggested that SWRs can weaken synaptic strength (Bukalo, Campanac, Hoffman, & Fields, 2013; Colgin, Kubota, Jia, Rex, & Lynch, 2004; Norimoto et al., 2018). In particular, a recent study reported that silencing SWR-related neuronal activity during NREM prevents spontaneous weakening of synapses and impairs subsequent learning of new memories (Norimoto et al., 2018). This study further showed that CA1 place cells active in a novel environment maintained their firing rates during SWRs across NREM while other hippocampal cells' firing rates gradually declined during SWRs across the course of NREM. Firing rates of cells representing novel environments may be affected by both of these mechanisms. The two potential roles of SWRs in synaptic plasticity are not mutually exclusive as long as strengthening and weakening of synaptic connections occur in different subsets of synapses.

However, although the above hypotheses assume that firing rate changes are related to changes in synaptic strength, it is important to note that firing rate changes do not directly reflect changes in synaptic strength (Cirelli, 2017). The reported effects could also reflect changes in intrinsic excitability, particularly considering that intrinsic excitability in CA1 pyramidal neurons has been shown to be increased by hippocampal-dependent learning (Mckay, Matthews, Oliveira, & Disterhoft, 2009). Thus, more studies are needed to directly investigate potential changes in intrinsic excitability and synaptic plasticity in CA3 pyramidal neurons following experience in a novel environment.

The present results in CA3, taken together with previous results in CA1 and CA3, shed light on similarities and differences between hippocampal subregions with regard to formation of novel spatial memories. In the present study, stable CA3 place cell representations of a familiar environment were present as soon as the animal was placed in the environment, whereas CA3 place cell representations of a novel environment developed gradually (Figure 2), consistent with prior results (Leutgeb et al., 2004). This earlier study further showed that CA3 place cell representations of a novel environment develop more slowly than CA1 representations (Leutgeb et al., 2004). While spatial memories may stabilize more slowly upon initial exposure to a novel environment, CA3 place cell representations may be more stable across days than CA1 representations. In a study that repeatedly exposed rats to an initially novel room, the population firing rate of CA3 cells remained consistent while the CA1 population firing rate gradually declined across multiple days of exposure (Karlsson & Frank, 2008). Moreover, during learning of new goal locations, many CA1, but not CA3, place cells shifted their firing fields to new goal locations (Dupret, O'Neill, Pleydell-Bouverie, & Csicsvari, 2010). In addition, CA3 place fields expand backward during the first traversals of the first day in a novel environment and then maintain this backward expansion across all traversals on subsequent days of exposure as the environment becomes familiar. In contrast, CA1 place cells expand backward during the first traversals of each and every day, even in familiar environments (I. Lee & Knierim, 2007; Roth et al., 2012).

The above-described differences between CA3 and CA1 raise important questions about how exactly novel memories are stored in synapses in the two subregions and the extent to which changes in CA3 firing patterns drive changes in CA1. A previous study in mice reported that CA1 place cells encoding new experiences maintain their firing rates during SWRs across NREM, while other CA1 place cells decrease their SWR firing rates across NREM (Norimoto et al., 2018). In the present study, CA3 place cells active in both novel and familiar environments decreased their SWR firing rates across the course of NREM (Figure 4). It is possible that CA3 to CA1 synapses are potentiated during a novel experience and remain potentiated during NREM sleep following a novel experience, allowing CA1 cells to continue to respond robustly to gradually decreasing CA3 inputs, at least for some time (e.g., ~75 min in Norimoto et al., 2018). This possibility is quite speculative, however, and remains a question for future study. The CA1 place cell yield in the present study was too low to permit comparisons across subregions. It is possible that the differences between the Norimoto et al. and present studies are unrelated to subregion differences but instead due to other differences between the studies, such as the use of different species and behavioral paradigms.

Another open question relates to how memories represented in CA3 and CA1 are consolidated. The reactivation of memory representations during sleep is traditionally thought to promote consolidation of memories in neocortex (Squire & Alvarez, 1995). However, while CA1 projects directly to extrinsic cortical areas, including the retrosplenial cortex and the medial prefrontal cortex, CA3 does not (Cappaert, Van Strien, & Witter, 2015). Thus, such a consolidation process would be expected to rely on CA1, with consolidation of memory representations stored in CA3 requiring transmission through CA1. Yet, the extent to which CA3 and CA1 place cell ensembles replay representations of the same sequences of spatial locations at the same time remains largely unknown. In summary, more work remains to be done to understand how replay of representations of novel experiences by CA3 and CA1 place cells relates to consolidation of spatial memories.

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