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<th>Novelty-induced phase-locked firing to slow gamma oscillations in the hippocampus: requirement of synaptic plasticity</th>
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Inventory of Supplemental Information

Supplemental Figures and Legends

**Figure S1.** The spatial distribution of GluR1-c-tail expression was examined throughout the dorsal hippocampus and entorhinal cortex. Related to Figure 1.

**Figure S2.** All recording sites were confirmed to be in the GFP-positive dorsal CA1 area. Related to Figures 2-8.

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**Figure S8.** A potential interpretation of Figure 7, regarding how synaptic plasticity regulates phase locking and place field formation. Related to Figure 7.

Supplemental Experimental Procedures

Supplemental References
Figure S1, related to Figure 1. Distribution of transgene expression.

(A) The spatial distribution of GFP-GluR1-c-tail expression was examined in coronal sections throughout the dorsal hippocampus. GFP-positive cells were largely confined to the CA1 area and were sparsely found in the deep layers of the cerebral cortex dorsal to the hippocampus. Importantly, GFP-positive cells were rarely detected in the CA3 area, which sends afferent inputs to the CA1 area. The panel labelled as 0 µm indicates a section containing a trace of the virus injection site; the positive and negative numbers indicate the anterior and posterior distance from the injection site,
respectively. The three images (-720, 0, and 720 µm) are color-merged and shown in Figure 1B. These images were used to construct Figures 1D and 1E.

(B, C) Scarce GFP expression in the entorhinal cortex. (B) Potential retrograde transduction of the viral vector was examined in the entorhinal cortex ipsilateral to the GFP-GluR1-c-tail vector injection. Few GFP-positive cells (arrowhead) were detected. Green, GFP fluorescence; purple, DAPI. (C) GFP-positive cells (arrowheads) were counted in horizontal sections throughout the dorsoventral axis (one in every 10 sections, 400 µm apart), and the proportion of GFP-positive cells in the entorhinal cortex was estimated. One side of the entorhinal cortex contained 145 ± 62 GFP-positive cells ($n = 4$ hemispheres), which corresponds to 0.12% of all neurons in the entorhinal cortex layer III (approximately 250,000 neurons)(Mulders et al., 1997). The yellow boxes indicate the area corresponding to the image in (A).
Figure S2, related to Figures 2-8. Detection of recording sites.

The recording sites were verified with GFP fluorescent images (left) and cresyl-violet stained sections (right). All recording sites (red dots) were confirmed to be in the GFP-positive dorsal CA1 area. Each row represents images from a single rat (for a total of 11 rats). In 2 out of 18 hemispheres, GFP expression was also observed in the dentate gyrus; both of these were control hemispheres. Therefore, the GFP expression in the dentate gyrus is unlikely to affect neuronal activity in the CA1 area.
Figure S3, related to Figures 2-8. Multi-tetrode unit recording and unit classification.

(A) Spike cluster separation. (Left) Pairs of the four different amplitude values recorded in four channels in single tetrodes are plotted in six projections. The axes are labeled with the channel numbers (1-4). Well-separated groups of dots representing individual spikes (indicated by different colors) are classified as single units. (Right) A histogram of the inter-spike intervals from a unit classified as a principal cell (putatively recorded from a pyramidal cell) showing a clear refractory period (< 2 msec) followed by a large number of short inter-spike intervals (2-10 msec) caused by typical bursts of complex firing. Inset, mean spike waveforms (mean ± s.d.). (B) The units were classified into principal cells (red) or interneurons (blue) based on the mean firing rate, the spike width and the occasional presence of bursting (see the Supplemental Experimental Procedures). Each dot represents a single unit. Top and right: the distribution of spike width and mean rate, respectively. The dotted lines indicate the thresholds for unit classification. (C, D) The numbers of units (C) and of units classified as principal cells (D) per tetrode did not differ between the GFP and GluR1-c-tail hemispheres (\(P > 0.5\) for both, two-sided, independent samples \(t\)-test).
Figure S4, related to Figures 2. Additional analysis for gamma oscillations.

Data in Figure 2 were analyzed separately for room A (sessions A1-6) and room B (sessions B1-12). (A) Powers of theta (left), slow gamma (middle) and fast gamma (right) oscillations were indistinguishable between GFP and GluR1-c-tail hemispheres in both rooms ($P > 0.1$ for each two-sided, independent samples t-test). (B) The proportion of sampling time points in $30^\circ$ phase bins for theta (left), slow gamma (middle), and fast gamma (right) oscillations in room A and B. No differences were detected between hemispheres (phase × hemisphere, $P > 0.1$ for each two-way repeated measures ANOVA). Note that all colors of symbols are overlapping.
Figure S5, related to Figure 4. Running speed-independent increase of slow gamma power in a novel environment.

(A) Relationships between running speed and power of theta (left), slow gamma (middle) and fast gamma (right) oscillations. Instantaneous LFP power (defined as the squared absolute value of Hilbert transform of bandpass-filtered LFP signals) within 200-ms time windows was compared with the running speed at the same time window. Power of slow gamma oscillations monotonically decreased as rats run faster (main effect of running speed, $F_{4,1000} = 259, P < 0.001$, two-way repeated measures ANOVA), which was consistent with Ahmed and Mehta, 2012 and Kemere et al., 2013. Pooled analysis of sessions A1-6 and B1-12. (B) Running speed in familiar and novel environments. Running speed showed a significant increase transiently for the first two time points (two minutes) during the B1 session (time × session interaction, $F_{18,270} = 26, P < 0.001$, two-way repeated measures ANOVA; **$P = 0.009$, *$P = 0.023$, compared with the same time points in the session A1, post-hoc Bonferroni test), which would reduce power of slow gamma oscillations if the power was solely regulated by running speed. Thus the observed increase of slow gamma power during B1 session (Figure 4A) cannot be accounted for by the change of running speed. (C) Minute-by-minute
relationship between slow gamma power and running speed during sessions A₁ (left) and B₁ (right). Across wide ranges of running speed, slow gamma power during the first minute of the session B₁ was largest compared with the later time points in both GFP and GluR1-c-tail hemispheres. (D) The same analysis with (C) for fast gamma oscillations.
Figure S6, related to Figure 4. Additional analyses of the strength of phase locking without bias caused by spike numbers.

The values of the resultant vector length can be biased by the numbers of spikes included in the analysis. To exclude the possibility that the difference described in Figure 4D was caused by differences in the numbers of spikes, we performed two additional analyses: the resultant vector length with bootstrapping (Fujisawa et al., 2008) (A) and pairwise phase consistency (Vinck et al., 2010) (B). Both analyses confirmed the between-hemisphere difference in session B_1 described in Figure 4D. (A) The resultant length after bootstrapping with fixed sample size. In session B_1, the resultant length was higher in GFP hemispheres than GFP-c-tail hemispheres (session × hemisphere, $F_{2, 1440} = 6.01$, $P = 0.003$, two-way ANOVA). *$P < 0.05$, post hoc Bonferroni test. (B) Pairwise phase consistency. The pairwise phase consistency was higher in the GFP hemispheres than the GFP-c-tail hemispheres in session B_1 (session × hemisphere, $F_{2, 1440} = 6.07$, $P = 0.002$, two-way ANOVA). *$P < 0.05$, post-hoc Bonferroni test. A, $A_{1-6}$ sessions; B, $B_{2-12}$ sessions.
Figure S7, related to Figure 5. Place field size analysis with different definitions of place fields.

(A) The size of place fields defined as contiguous spatial bins in which their firing rates were above differential thresholds between 10 to 90% of the peak rate (see the Supplemental Experimental Procedures). In sessions A₁ and B₉, the place field size did not differ between the hemispheres for any of the thresholds (between hemispheres, $P > 0.1$; threshold $\times$ hemisphere, $P > 0.1$, two-way repeated measures ANOVA). In sessions B₁ and B₅, the place field size was larger in the GluR1-c-tail hemispheres compared with the GFP controls (threshold $\times$ hemisphere, $P < 0.001$, two-way repeated measures ANOVA; *$P < 0.05$, post hoc Bonferroni test). (B) Place field size with three different definitions (see the Supplemental Experimental Procedures). Significant differences in place field size were observed between hemispheres. * indicates $P < 0.05$ in the session $\times$ hemisphere interaction for both the two-way repeated measures ANOVA and post hoc Bonferroni test.
Figure S8, related to Figure 7. A potential interpretation of Figure 7, regarding how synaptic plasticity regulates phase locking and place cell activity.

In the possibility 1, synaptic plasticity regulates slow gamma phase locking and place field formation independently, but no other mechanistic link exists between the two phenomena (A). In this model, slow gamma phase locking and place field formation can be correlated through independent regulations by the common regulator, synaptic plasticity. The blockade of synaptic plasticity would compromise the inverse correlation between slow gamma resultant length and place field size because there is no other mechanistic link between them (B).

In the possibility 2, slow gamma phase locking and place field formation are mechanistically coupled independently of synaptic plasticity, and synaptic plasticity regulates these two tightly coupled phenomena (C). In this model, the correlation between slow gamma resultant length and place field size would be intact after the blockade of synaptic plasticity because the correlation is mediated by other mechanistic links independent of synaptic plasticity.

As shown in Figure 7B, we found that the correlation was maintained in the GluR1-c-tail hemisphere; this result supports the possibility 2. A similar reasoning can explain the impaired correlation between fast gamma phase locking and place field size (Figure 7C) if we consider an unidentified factor X which links between them. If the link between place field size and X, but not between fast gamma phase locking and X, is dependent on GluR1-dependent synaptic plasticity, the
correlations between fast gamma phase locking and place field size would be compromised by the blockade of GluR1-dependent synaptic plasticity.
Supplemental Experimental Procedures

Plasmids

The rAAV plasmids containing either the eGFP-GluR1-c-tail or the eGFP coding sequence were constructed with conventional molecular biological techniques and were verified through DNA sequencing. The eGFP-GluR1-c-tail plasmid was kindly provided by Dr. Roberto Malinow. The Ca\textsuperscript{2+}/calmodulin-dependent protein kinase II (CaMKII) promoter sequence (1.3 kb) was obtained through PCR amplification from mouse brain genomic DNA using the following primers (Dittgen et al., 2004): 5’-CATCGATCATTATGGCCTTAGGTCACTT-3’, 5’-CGGATCCGCTGCCCCAGAACTAGGGGCCACTCG-3’. The pAAV backbone was provided by Dr. Karl Deisseroth. The rAAV protein expression cassette contains a CaMKII promoter, the woodchuck hepatitis virus post-translational regulatory element (WPRE), and a human growth hormone (hGH) poly A signal flanked with inverted terminal repeat (ITR) sequences. The pXRI plasmid was provided by the Gene Therapy Center at the University of North Carolina at Chapel Hill (Rabinowitz et al., 2002). The pAAV-RC and pHelper plasmids were obtained from the AAV Helper-Free System (Stratagene, La Jolla, CA).

Preparation of recombinant adeno-associated viral vector (rAAV)

rAAVs with chimeric serotype 1/2 were produced via the co-transfection of four plasmids (see below) to AAV293 cells (Stratagene) using the calcium phosphate precipitation method (During et al., 2003; Hauck et al., 2003). The AAV293 cells were grown in culture medium at 37°C in a 5% CO\textsubscript{2} humidified incubator. The culture medium was high glucose Dulbecco’s modified Eagle’s medium (Gibco, Renfrew, UK) supplemented with 10% fetal bovine serum (Gibco), MEM non-essential amino acids (Gibco), sodium pyruvate (Gibco), 100 U/ml penicillin and 100 µg/ml streptomycin (Gibco).

One day prior to transfection, AAV293 cells were seeded into five tissue culture plates with a 15-cm diameter at 1.0-1.5 × 10\textsuperscript{7} cells/dish in 25 ml of the culture medium without antibiotics, resulting in 70-80% confluency at the time of transfection. The transfections were performed with the pAAV-RC plasmid (11.3 µg/plate), the pXRI plasmid (11.3 µg/plate), the pHelper plasmid (22.5 µg/plate) and either the pAAV-CaMKII-eGFP-GluR1-c-tail or the pAAV-CaMKII-eGFP plasmid (22.5 µg/plate).
The four plasmids were mixed with 0.3 M CaCl$_2$ at 2.25 ml/plate, filtered through a sterilizing 0.2-µm-pore filter (13 mm Acrodisc syringe filter, Pall, USA), mixed with 2× HBS buffer, 2.25 ml/plate (Tashiro et al., 2006), and then added to culture plates at 4.5 ml/plate. The medium was replaced 6 hours later with 20 ml/plate of antibiotic-containing fresh culture medium. Forty-eight hours after transfection, the cells were scraped off the dishes, collected via centrifugation at 200 × g for 10 min, washed with Dulbecco’s phosphate-buffered saline (DPBS, Gibco) and re-suspended in 10 ml/plate of 150 mM NaCl-20 mM Tris-HCl (pH 8.0). The cell suspension was incubated at 37°C for 60 min with 0.5% sodium deoxycholate and 50 U/ml benzonase nuclease (Novagen, Nottingham, UK). Cell debris was separated from the supernatant via centrifugation at 3,000 × g for 15 min. The supernatant was heated to 56°C for 15 min. The solution was then frozen in a dry ice/ethanol bath and thawed in a 37°C water bath, centrifuged at 3,000 × g for 15 min to remove cell debris again and filtered through a 0.45-µm-pore filter (32-mm Acrodisc syringe filter, Pall).

The rAAV vector was then purified and concentrated with heparin affinity columns (1 ml HiTrap Heparin HP, GE Healthcare). A pre-equilibrated heparin column with 150 mM NaCl-20 mM Tris-HCl (pH 8.0) was loaded with the rAAV-containing extract at a constant speed with a syringe pump (Harvard Apparatus, Edenbridge, UK) and washed with 20 ml of 100 mM NaCl/20 mM Tris-HCl (pH 8.0). rAAV was eluted from the column with a gradient of NaCl concentration as follows: 200 mM NaCl (1 ml), 300 mM NaCl (1 ml), 400 mM NaCl (1 ml), 450 mM NaCl (2 ml) and 500 mM NaCl (1 ml). Note that all NaCl solutions were buffered with 20 mM Tris-HCl (pH 8.0). The fractions of 300-500 mM NaCl were collected and further concentrated using centrifugal filter devices (Amicon Ultra-4, 100 K normal molecular weight limit, Millipore, Billerica, MA) at 3,000 × g to a final volume of less than 500 µl. The rAAV stock aliquots were stored at -80°C until use. For slice electrophysiology experiments, the aliquots were transported between laboratories on dry ice.

The viral vector titers were determined using an AAV2 titration ELISA system (PROGEN, Heidelberg, Germany). Three batches of vectors were used. The titers of rAAV-CaMKII-eGFP were $1.6 \times 10^{13}$, $3.8 \times 10^{13}$ and $0.4 \times 10^{13}$, and those of rAAV-CaMKII-eGFP-GluR1-c-tail were $2.1 \times 10^{13}$, $3.0 \times 10^{13}$ and $0.6 \times 10^{13}$ assembled physical particles/ml.

**Slice electrophysiology**

Male Long-Evans rats at three weeks of age were stereotaxically injected with rAAV under anesthesia with xylazine (10 mg/kg, *i.p.*) and somnopentyl (40 mg/kg, *i.p.*). The skull was exposed,
and small craniotomies were performed above the hippocampus. For each injection, 1.0 µl of either rAAV-CaMKII-eGFP-GluR1-ctail or rAAV-CaMKII-eGFP vector was injected into the dorsal CA1 area (3.7 mm posterior to the bregma, 2.4 mm lateral to the midline and 2.25-2.35 mm ventral to the cortical surface) using a microsyringe (800 RN, Hamilton, Bonaduz, Switzerland) at a speed of 0.3 µl/min.

At 14-18 days after the viral vector injection, the rats were anesthetized with diethyl ether and decapitated. The brain was immersed in ice-cold modified artificial cerebrospinal fluid (ACSF) consisting of (in mM) 27 NaHCO$_3$, 1.4 NaH$_2$PO$_4$, 2.5 KCl, 0.5 ascorbic acid, 7.0 MgSO$_4$, 1.0 CaCl$_2$ and 222 sucrose saturated with 95% O$_2$ - 5% CO$_2$. Coronal hippocampal slices of 400 µm thickness were cut using a vibratome (Vibratome 3000, Vibratome, St. Louis, MO) and maintained for 30 min at 37°C and then for at least 90 min at room temperature in normal ACSF consisting of (in mM) 126 NaCl, 26 NaHCO$_3$, 3.5 KCl, 1.25 NaH$_2$PO$_4$, 1.3 MgSO$_4$, 2.0 CaCl$_2$ and 10 glucose saturated with 95% O$_2$ - 5% CO$_2$.

The slices were transferred to a recording chamber continuously perfused with ACSF at room temperature. The slices with GFP expression in the dorsal CA1 were selected. The stimuli were delivered through a bipolar tungsten electrode, and the field excitatory postsynaptic potentials (fEPSPs) were recorded using glass pipettes filled with ACSF. The electrode and pipette were carefully placed in the stratum radiatum of CA1 to maintain the same distance (< 100 µm) from the pyramidal cell layer. For test pulses, a 100-µs duration pulse was given every 1 min. The stimulus intensity was set at approximately 30% of maximum response and ranged from 20-50 µA. The theta burst stimulation protocol (8 trains of 4 pulses at 100 Hz separated by 200 ms) was used to induce long-term potentiation (Larson et al., 1986). The electrophysiological data were acquired using pClamp 9 (Molecular Devices, Sunnyvale, CA). The signals were low-pass filtered at 2 kHz and digitized at 20 kHz. The changes in fEPSP were expressed as the percent change in the initial slope relative to the mean of the baseline period.

**Subjects for multi-tetrode unit recording**

Eleven adult male Long-Evans rats were used (500 ± 47 g, 3.4 ± 0.8 months, mean ± s.d. on the day of surgery) for unit recordings. The rats were trained to perform a food foraging task in an open field in room A prior to surgery. Then, in single surgeries, seven of the rats were bilaterally injected with rAAV-CaMKII-eGFP-GluR1-ctail to the CA1 area in one hemisphere and rAAV-CaMKII-eGFP
vector in the other and bilaterally implanted with two microdrives carrying four tetrodes aimed at the transduced CA1 areas. Four of the rats were unilaterally injected with rAAV-CaMKII-eGFP vector and unilaterally implanted in the injected side. After a recovery period, pre-training of the foraging task in room A was resumed and performed daily until the final recording experiments and subsequent perfusion fixation. The rats were housed individually in transparent plastic cages (45 × 30 × 35 cm) and were maintained under 12-h light/dark cycles. Behavioral training and unit recordings were performed during the dark phase. During the training period, the rats were food-deprived to maintain 85-95% of their free-feeding body weight.

**Surgical procedures**

The rAAV microinjection and tetrode implantation were stereotaxically performed in single surgeries while the rats were deeply anesthetized with either equithesin (1 ml/250 g body weight) or isoflurane (Isoba Vet, Intervet/Schering-Plough Animal Health, Lysaker, Norway). Small craniotomies were performed above the central part of the dorsal hippocampus, and the rAAV vectors were injected into the stratum radiatum of the dorsal CA1 area (4.0 mm posterior to the bregma, 2.4 mm lateral to the midline, 2.5 mm ventral from the cortical surface) with a microsyringe (800 RN, Hamilton). A volume of 0.5 µl of vector-containing solution was injected into the CA1 area in each hemisphere at a rate of 0.3 µl/min.

Four tetrodes were assembled on a microdrive and implanted aiming at the vector-injected CA1 areas. The tetrode tips were placed on the cortical surface at AP 3.8-4.2 mm and ML 2.9-3.1 mm and inserted 1.5 mm into the brain at an angle of 10 degrees (medial direction relative to the DV axis). For one microdrive, the tetrodes were placed on the cortical surface at AP 4.0 mm and ML 2.4 mm and inserted 1.5 mm into the brain parallel to the DV axis. The microdrives were fixed to the skull with 7 to 9 stainless steel screws and dental cement (Meliodent, Heraeus Kulzer, Hanau, Germany). Three skull screws above the frontal cortex served as grounds. Each tetrode was constructed from four twisted polyimide-coated 90% platinum/10% iridium wires (17.8 µm diameter, California Fine Wire, Grover Beach, CA). The electrode tips were plated with platinum prior to implantation to reduce impedances to 200-300 kΩ at 1 kHz.

**Data acquisition**
Electrophysiological data from behaving rats were acquired using an Axona DacqUSB recording system (Axona, Herts, UK). The signals from the brain were amplified with a unity gain amplifier connected to the pre-amplifier module of the recording system. To support free rat movement, the weight of the recording cables was counterbalanced with a weight pulling up on the cables.

At least two recovery days after surgery were allowed before the rats resumed the foraging task training. The tetrodes were daily lowered toward the CA1 area in steps of ≤50 µm until we found well separated, theta-modulated, large-amplitude, low-frequency and occasionally bursting units at depths of approximately 2.0 mm. Typical local field potential (LFP) signals in or near the hippocampus, theta oscillations and sharp wave/ripples were used as additional guides to determine the approximate locations of the tetrode tips relative to the CA1 pyramidal cell layer (Ylinen et al., 1995).

For unit recordings, the signals were amplified by a factor of 5,000-10,000 and band-pass filtered between 600 and 6,000 Hz. The signals from each channel of a tetrode were subtracted by the signals from a channel from another tetrode with the lowest noise and the least frequency of large-amplitude events to minimize movement artifacts. Spike time and waveforms were saved as data when their amplitudes were higher than the threshold (typically 50-55 µV) set by the experimenter, which was several-fold greater than the background noise. The spike waveforms were sampled at 48 kHz (50 samples per spike, 8 bits/sample).

For each hemisphere, the LFP signal was recorded against animal ground from a tetrode located at the CA1 area together with unit activity. The signals were amplified by a factor of 1,000-2,000, low-pass filtered at 500 Hz and sampled at a rate of 4,800 Hz (16 bits/sample). A notch filter was applied at 50 Hz.

The rat locations were monitored by tracking two small light-emitting diodes on a headstage connected to a microdrive. Tracking was accomplished through an overhead camera at a sampling rate of 50 Hz. In the obtained image, a pixel represents 3 mm of physical distance.

**Behavioral procedures**

The rats were trained daily to forage for crumbs of chocolate cereal in an open field for 10 days or longer prior to surgery and for 2-4 weeks after surgery. The enclosure used for the training was a black square open field (100 cm × 100 cm; 50 cm high) located in room A. The room light was dim
to facilitate exploring behavior. No curtain was placed around the open field. The training consisted
of two or more 10-minute sessions per day in the open field. Chocolate cereal crumbs were scattered
over the entire enclosure whenever all the previously scattered crumbs had been collected. Between
training sessions, the animals rested for approximately 5 min on a clay pot covered by a towel on a
pedestal. All 11 rats used for unit recording were subjected for the same sets of final recording
experiments performed 30 ± 2 days after viral vector injection as described below.

Familiar room experiments

On the days of the final recording experiments, the tetrodes were not moved to ensure stable
recordings. The rats foraged for six sessions in an open field in the familiar room A (the same
enclosure used during previous training sessions). Each session was 10 min long, and the rats rested
for 5 min between sessions. A total of 119 units were recorded in the experiments. Of these, 67 and
16 principal cells (described below) in the GFP and GluR1-c-tail groups, respectively, were included
for the analysis according to the unit classification criteria described below.

Novel room experiments

The rats foraged in familiar room A and novel room B for a total of 18 sessions. The rats had never
been exposed to room B prior to the experiment. The recordings were performed sequentially in the
order of A-B-B-B-B-A, and each session lasted for 10 min (the rats rested on a pedestal for 5 min
between sessions). This sequence was repeated at 6 and 24 hours after the initial sequence. Between
sequences, the rats were returned to their home cages. The enclosures in rooms A and B were
identical; however, the two recording rooms were distinguishable due to nearby objects within the
room. A partition was placed between the enclosure and the pedestal so that the rats could not see the
enclosures prior to or between the recording sessions. The numbers of principal cells analyzed were
58 and 36 in the 0-hour sessions, 62 and 32 in the 6-hour session and 65 and 36 in the 24-hour
sessions for the GFP and GluR1-c-tail groups, respectively.

Spike sorting and unit classification

The recorded spikes were sorted into different units (corresponding to the activity of individual
neurons) with the graphical cluster-cutting software Tint (Axona Ltd, St. Albans, UK) using spike
amplitudes and waveforms as the criteria. The clusters with a clear refractory period (< 2 msec) in
the auto-correlograms were accepted (Harris et al., 2000). The cluster pairs with large asymmetric
peaks in cross-correlograms were merged because spikes in a burst demonstrate decreasing amplitudes that can cause erroneous sorting of the spikes into separate clusters (Harris et al., 2000). To estimate the quality of cluster separation, the isolation distance was calculated for each cluster (Schmitzer-Torbert et al., 2005). A cluster that was distant from the other recorded spikes in the multidimensional cluster space composed of spike amplitudes and waveforms received a high value, and clusters with an isolation distance of less than 10 were excluded from the analyses. Examples of spike sorting are provided in Figure S3.

The units were classified as principal cells (putatively recorded from pyramidal cells) if they satisfied all of the following criteria: mean spike width (duration from spike peak to trough) greater than 0.2 msec, mean firing rate between 0.1 Hz to 5 Hz and the occasional presence of burst firing. The units were classified as interneurons if the mean firing rate was more than 5 Hz.

**Analysis in the spatial domain**

**Rate maps**

The spatial firing patterns of individual principal cells were examined by constructing a rate map for each session (Leutgeb et al., 2005). A rate map consisted of firing rates at spatial bins \( x \) (bin width, 5 cm), which were calculated as the number of spikes in each spatial bin divided by the duration spent in each spatial bin (both the numerator and denominator were individually smoothed with kernel density estimation, and 0.0001 was added to avoid division by zero):

\[
\lambda(x) = \frac{\sum_{i=1}^{n} g\left(\frac{s_i - x}{h}\right)}{\int_{a}^{b} g\left(\frac{y(t) - x}{h}\right) dt + 0.0001} + 0.0001.
\]

where \( n \) is the number of spikes, \( s_i \) is the location of the \( i \)-th spike, \( T \) is the duration of the recording, \( y(t) \) is the location of the rat at time \( t \), \( h \) is a Gaussian smoothing factor (set to 5 cm) and \( g \) is the two-dimensional Gaussian kernel:

\[
g(x) = \frac{1}{2\pi} \exp\left(-\frac{1}{2} \|x\|^2\right).
\]

The peak rate of each principal cell was defined as the highest firing rate observed in any spatial bin of the rate map in a recording session.

**Detection of place fields**
A place field was defined in a rate map with a peak rate of > 1 Hz as contiguous spatial bins with a total area of ≥ 200 cm² that consisted of a bin with the peak rate and bins with firing rates above a threshold of 20% of the peak rate (adapted from (Fyhn et al., 2004)). This definition was used for all analyses except for those in Figure S7.

In Figure S7, various field size definitions were used as follows.

In Method 1, the firing rate threshold varied between 10 and 90% of the peak rate instead of the fixed threshold of 20%. For each threshold, a place field was determined as contiguous spatial bins, including the bin of peak rate > 1 Hz irrespective of the total area.

In Method 2, multiple place fields were sequentially determined in each rate map. The first place field was defined as described above as contiguous bins including a bin with a peak rate with a total area of ≥ 200 cm² and a threshold of 20% of the peak rate. If the highest rate outside of the first place field was > 1 Hz, the next field was similarly defined as a contiguous area of ≥ 200 cm² with 20% of the highest rate bin exclusively using bins outside of the first place fields. This procedure was repeated until a bin with the highest rate outside the place fields was < 1 Hz. The field sizes were defined as the sum size of all place fields.

In Method 3, all bins with a firing rate higher than 0.5 × the mean rate were defined as place fields irrespective of bin contiguity (McHugh et al., 1996). The place field size was expressed as the sum area of the bins.

In Method 4, all bins with a firing rate higher than 0.1 × peak rate were defined as place fields irrespective of the contiguity (McHugh et al., 1996). The place field size was defined as the summed area of the bins.

**Mean infield rate**

The mean infield/outfield rate was calculated to examine the minute-by-minute development of the spatial firing pattern. Using a pair of tests and the reference sessions listed below, the eventual place field of each principal cell was determined from the rate map of the reference session. The 10-min test session was then split offline into 5 × 2-min blocks (0-2 min, 2-4 min, etc.), and the mean rate within or outside the eventual place field was calculated for every 2-min block. For the novel environment, the B₁ and B₄ sessions were used for the test and reference sessions, respectively. For the familiar environments in Figure 6D, pairs of B₅ and B₈, B₉ and B₁₂, A₁ and A₂, A₃ and A₄, and A₅ and A₆ sessions were used for the test and the reference sessions, respectively.
**Population vector cross-correlation**

The similarity of spatial firing patterns among two sessions was examined with a population vector cross-correlation analysis (Leutgeb et al., 2005). For each session, all rate maps of the principal cells recorded from the GFP or GluR1-c-tail hemispheres of all animals were stacked in a three-dimensional matrix. The $x$ and $y$ spatial axes represent the open field, and $z$ represents the cell numbers. The matrix size was thus $20 \text{ bins} \times 20 \text{ bins} \times \text{number of cells}$, and a population vector was constructed for each bin (the firing rates of all cells in the bin). The cross-correlational values between two sessions were calculated for each bin as a normalized dot product of two population vectors from the two sessions. An overall cross-correlational value was calculated as a mean of the cross-correlational values of all of the bins.

To investigate the minute-by-minute development of spatial firing patterns in a novel environment, session B$_1$ was split into 2-min blocks, and population vector cross-correlations between each block and session B$_4$ were calculated. For the familiar environment in Figure 6E, Sessions A$_1$, A$_3$, A$_5$, B$_5$ and B$_9$ were split into 2-min blocks, and the population vector cross-correlations between each block from sessions A$_1$, A$_3$, A$_5$, B$_5$ and B$_9$ and sessions A$_2$, A$_4$, A$_6$, B$_8$ and B$_{12}$ were calculated.

**Mean spatial information**

Spatial information (bits/sec) was calculated in each session to examine the amount of information regarding spatial location conveyed by a unit per second as follows:

$$\sum_i p_i \lambda_i \log_2 \frac{\lambda_i}{\lambda},$$

where $p_i$ is the proportion of time spent in bin $i$, $\lambda_i$ is firing rate in bin $i$ and $\lambda$ is the mean firing rate (Skaggs et al., 1993). The values of spatial information from the same unit were averaged across repeated recording sessions in the same enclosure to obtain the mean spatial information.

**Spatial correlation**

The spatial firing similarity of the same principal cell among two sessions was estimated using spatial correlation. The spatial correlation was calculated for each cell as a correlation coefficient of the firing rates in corresponding bins of the pair of rate maps for the two sessions. Bins visited for less than 150 ms in either room were excluded to avoid artifacts in the correlation measure (Fyhn et al., 2004).
Analysis in the temporal domain

Phase-locking of spikes to gamma oscillations

To investigate spike timing along gamma oscillations, an acausal band-pass filter was applied off-line to the signals of local field potential (LFP) in the slow (27-48 Hz passband) and fast (65-138 Hz passband) gamma frequency ranges. The low cut-off stopband was the low passband minus 2 Hz; the high cut-off stopband was the high passband plus 2 Hz. The slow and fast gamma phases at every LFP sampling point were calculated as a phase angle of the Hilbert transform of the filtered LFP signals (Matlab, MathWorks). Every recorded spike in each session was assigned a spike phase \( \theta_j \), where \( j \) denotes the \( j \)-th spike. The mean resultant vector \( r \) was calculated as:

\[
r = \frac{1}{N} \sum_{j} \exp(i\theta_j)
\]

where \( N \) is the total number of spikes. The strength of phase locking (resultant length) was defined as \( |r| \). Theoretically this value ranges from 0 to 1. The value is zero if the phases are uniformly distributed along the phases of gamma oscillations, while it is one if all spikes fire at the exactly same phase. In practice, the values for individual cells are distributed mostly in the range of 0-0.2 as shown in Figure 7B, C. The mean firing phase was defined as \( \arg(r) \). The spike phase counts of each cell in each session were sorted into 30° (or 45° for Figure 7) phase bins, normalized so that the sum across bins became 1 and averaged across cells for group comparison. The trough of gamma oscillation was defined as 0/360°. Two-hundred sets of surrogate data were generated by randomly assigning spike phases while keeping the number of recorded spikes. The chance level of resultant length was estimated as the averaged resultant length of the surrogate data.

Bootstrapping with fixed-sample number

The resultant vector length can be positively biased when calculated from fewer spikes (Vinck et al., 2010). To exclude the bias due to the variance of spike numbers recorded, we performed bootstrapping with a fixed number of samples (Fujisawa et al., 2008). For each cell, a resultant length was calculated from 60 spikes (corresponding to 0.1 Hz in a 10-min session), which were randomly sub-sampled from all spikes generated by the cell without replacement. The sub-sampling was repeated 100 times to utilize all available data; then, the 100 resultant length values were averaged to obtain the cell’s bootstrapped resultant length. A numerical simulation with a uniform phase distribution (i.e., resultant length = 0) estimated the chance level of the bootstrapped resultant length as 0.11.
**Pairwise phase consistency**

The strength of phase locking was also estimated with pairwise phase consistency, which has been proposed as another statistical method to reduce bias caused by spike number variance (Vinck et al., 2010). The phases of firing along oscillation were transformed into two-dimensional unit vectors \((\cos \theta, \sin \theta)\). Then, for each cell, the pairwise phase consistency \(\gamma\) was defined as a mean of the dot products of all given pairs of the unit vectors:

\[
\gamma = \frac{2}{N(N-1)} \sum_{j=1}^{N-1} \sum_{k=(j+1)}^{N} \cos(\theta_j)\cos(\theta_k) + \sin(\theta_j)\sin(\theta_k),
\]

where \(N\) is the number of spikes and \(\theta_j\) and \(\theta_k\) are phases of \(j\)-th and \(k\)-th spikes, respectively.

**LFP power**

The power spectral density of LFP signals was estimated through an autoregressive model for either the whole 10-min session or a 1-min block of the session (Fyhn et al., 2002). The order of the autoregressive model was set to 30. Theta (6-10 Hz), slow gamma (25-50 Hz) and fast gamma (65-140 Hz) powers were estimated as the areas under the curve of power spectral density functions in the corresponding frequency ranges.

**Histology**

After the final recording experiments were completed, the rats were perfused intracardially with saline and 4% paraformaldehyde in 0.1 M phosphate buffer after an overdose of isoflurane and pentobarbital. The tetrodes were removed from the brains before the brains were removed from the skulls to leave the electrode tip locations intact. The brains were stored in the same fixative overnight at 4°C and then stored in 30% sucrose in phosphate-buffered saline (PBS) for more than 48 hours at 4°C.

To verify that data from final recording experiments were collected from the virus-transduced CA1 area, the brains were frozen, coronally sectioned into 40-μm thick slices, which were mounted on gelatine-coated slides with a water-soluble mounting medium and cover slips. Every section through the relevant part of the dorsal hippocampus was collected, and the posterior part of the brain, including the entorhinal cortex, was stored for examining possible retrograde viral transduction. The distribution of viral transduction (GFP-positive area) was determined by fluorescent images taken
with an Axio Scope A1 microscope (Zeiss, Oberkochen, Germany) equipped with a 5× objective. Then, after removing the coverslips, the sections were stained with cresyl violet to determine the recording sites. Brightfield images were captured at the same regions of interest with the same microscope. The recording sites were determined as the ventral end of tissue traces caused by the tetrodes. All recording sites were confirmed to be located in or near the pyramidal cell layers of the GFP-positive CA1 areas (Figure S2).

To examine the cell-type selectivity of viral transduction, the 40-µm-thick coronal sections were stained as floating sections for the interneuron marker GAD67 and a neuronal marker, fluorescent Nissl. The sections were blocked with 2% donkey serum without detergent for 30 min, incubated with mouse monoclonal anti-GAD67 antibody (1:500, MAB5406, clone 1G10.2, Millipore) overnight and then incubated with anti-mouse IgG antibody Dylight 549 (1:500, Jackson ImmunoResearch, Suffolk, UK) and NeuroTrace 435⁄455 blue fluorescent Nissl stain (1:50, Invitrogen, Renfrew, UK) for 2 h. All incubation steps were followed by three washes with PBS. The stained sections were mounted on slides with a mounting medium. Fluorescent images at single cell resolution (0.58 µm/pixel) spanning the whole dorsal hippocampus and cortical areas dorsal to the hippocampus were captured using a tile scanning function of an LSM510 confocal microscope equipped with a 20× objective and 405, 488 and 561 nm lasers (Figure 1, Figure S1A). The GAD67-positive and Nissl-positive cells were classified as interneurons, and the GAD67-negative and Nissl-positive cells were classified as excitatory pyramidal neurons. The proportion of GFP-positive cells in each cell type was calculated.

To examine the extent of retrograde transduction in the entorhinal cortex, the posterior part of the brain was cut horizontally into 40-µm thick sections and stained for cell nuclei with DAPI (0.2 µg/ml, Invitrogen). Fluorescent images for eGFP and DAPI were captured through the dorsoventral entorhinal cortex and tiled manually, and then the number of GFP-positive cells was counted. GFP-positive cells were rarely observed in the medial and lateral entorhinal cortex (Figures S1B, S1C), and the fluorescent intensity of GFP-positive cells was several-fold lower than that of the CA1 pyramidal cells around the viral vector-injected areas.

Statistics

Linear statistics were performed with PASW statistics software (IBM SPSS). Circular statistics were performed with the CircStat Matlab toolbox (Bers, 2009). The permutation test described below
was run in a custom-written Matlab code. Test of the difference between two independent correlation coefficients was performed as described previously to examine whether two correlation coefficients obtained from independent samples are equal (Cohen and Cohen, 1983). The values are reported as the mean ± standard error of the mean in all figures and texts unless otherwise specified.

**Permutation test**

A permutation test was performed to compare the strength of phase locking between two populations of neurons. Given the list of normalized spike counts of individual principal cells from the original recording data, the cell labels (GFP or GluR1-c-tail) assigned to each cell’s data were randomly permuted. The averaged normalized spike counts of GFP and GluR1-c-tail groups were then calculated with the permuted assignment. The difference of resultant vector length of the two normalized spike counts was computed as a statistic. This permutation procedure was repeated 100,000 times to estimate a $p$ value.
Supplemental References


