Supporting Information

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SI Materials and Methods

Animals. We included data from 18 (nine saline-injected and nine CPP-injected) implanted male Long Evans rats (Charles River; 3–9 mo old; 300–450 g at time of testing). After surgery, the rats were given a recovery period lasting 1 wk, following which the rats were food restricted to 85–90% of their free-feeding weight. All procedures were carried out in accordance with the Oregon Institutional Animal Care and Use Committee (IACUC) and National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH publications no. 80-23).

Surgery. Rats were anesthetized with 1–4% isoflourane mixed with oxygen and implanted with a microdrive containing six tetrodes moveable as a bundle. The tetrode bundle was aimed over the CA1 region of the left hemisphere (3.5 mm posterior from Bregma; 2.5 mm lateral from midline) and lowered to ∼250 μM above the CA1 pyramidal layer. Either two or four stainless steel anchoring screws were set into the skull. The microdrive was bonded with the skull using Grip Cement (Dentsply).

Behavioral Training. Rats were screened and familiarized in the testing apparatus. The rats were trained to freely forage for scattered food pellets dropped from an overhead feeder. Pellets landed in both the inner and outer box. During this period, the rats were restricted to a clear inner box (50 × 50 cm) within an outer box area (130 × 130 cm) as shown in Fig. 1 (I1). The position of a red and a green LED attached to the headstage was recorded by an overhead camera. Rats were familiarized to the environment for at least six sessions lasting at least 10 min. At the end of each familiarization session the bundle of tetrodes was advanced 25–75 μM and the rat was returned to its home cage for at least 8 h. The floor paper was changed and the floor and walls of the chamber were wiped down with 90% ethanol after every session. This procedure was continued until large-amplitude, well-isolated place cells were present (number of exposures ranged from 7 to 29, median was 15, with no differences between groups). The six-session experiment illustrated in Fig. 1 and described in the main text was then initiated.

Electrophysiology. Tetrodes were made from 17 μM platinum 10% iridium wire (California Fine Wire) twisted together. Wires were plated with platinum (Technic) to a final impedance of 250–750 kΩ. Spiking activity was filtered from 600 to 6,000 Hz and sampled at 32 kHz online and local field potential (LFP) was filtered from 1 to 475 Hz and continuously sampled at 30 kHz using the Cheetah-32 system (Neuralynx). Clusters were cut in MClust (A. D. Redish, University of Minnesota, Twin Cities, MN) and SpikeSort 3D (Neuralynx). Single units were judged to be the same if similar cluster boundaries could be applied across sessions. We allowed the cluster boundaries to be stretched or contracted between sessions, as necessary, to account for changes in size and shape of the cluster resulting from dramatic increases or decreases in firing rate during remapping. Finally, the waveforms of each cell were compared across sessions.

In total, 934 well-isolated CA1 putative pyramidal neurons were analyzed (471 CPP; 397 saline) with a mean number of 77 cells/session in the CPP group and 66 cells/session in the saline group. Cells that were not held across the entire testing sequence were used for single-session statistics and for comparisons where similar cluster boundaries could be applied.

Data Analysis. ***Spiking activity.*** The spiking activity of single units was associated with the rat's position in space at the time of the spike. Cells with high mean firing rates (>10 spikes/s) were classified as interneurons. All data were filtered for epochs of walking by removing any data points where the rat's instantaneous running speed was less than 3 cm/s. The position of rat and the spikes were then binned into 4 × 4 cm bins. The binned spikes were then divided by the binned occupancy to create an unsmoothed rate map. A smoothed rate map was created by convolving the rate map with a 3 × 3 Gaussian kernel.

Correlation scores based on smoothed rate maps were generated for session pairs by correlating the two maps. A Pearson's correlation score was calculated between equivalent bins, with unvisited and common-zero bins ignored. A cell was eligible for the measure only if it was judged to be the same between the two sessions and showed a place field in either of the two sessions being compared. In addition, the rat must have occupied >85% of the bins in the rate maps and the majority of fields needed to follow the rotation of the cues. These three requirements (recording stability, rotation, and coverage) reduced the number of rats to six saline (48 cells) and six CPP (51 cells) rats for the critical O1–O2 comparison. Data from the other rats were included in single-session statistics and other comparisons when similar cluster boundaries could be applied. For the O1–O2 comparison, we divided the environment into an inner and outer box area and computed a separate stability score for the two regions. To do this, we first found the place fields (defined as a contiguous 80-cm^2 region where the cell fired above 20% of its peak firing rate for the whole environment) of a cell. If a place cell had a field centered in the inner box in either session, then a stability score was taken for that cell in the inner box. This procedure was then repeated for the outer box, thus creating an inner and outer group. Some cells contributed to both the inner and the outer box groups, as one in five cells showed fields in both compartments (e.g., Fig. 2, cell 3). In total, 21 saline and 26 CPP cells contributed to the inner box correlation, and 30 saline and 36 CPP cells contributed to the outer box comparison.

Mean firing rate was taken as the number of spikes divided by the total length of the session. Coherence was the z-transformed Pearson's correlation score between a pixel and its eight nearest neighbors in the unsmoothed rate map. Peak firing rate was the highest firing rate bin in the smoothed rate map. A field was identified as above. Single session statistics were compared between sessions and across groups (Table S1). All analyses were performed using custom-written MATLAB (MathWorks) code.

Ripple identification and analysis. The LFP signal was band pass filtered between 150 and 250 Hz, and the envelope was determined by Hilbert transform (1). Events were considered sharp wave ripples (SWRs) if the envelope exceeded a threshold conservatively set at +6 SD above the mean for at least 15 ms. For each cell, spikes were counted in the window from −50 ms to +50 ms around the center of each SWR (2). We analyzed spiking activity during the 559 detected SWRs in the two standard sessions preceding exposure to the novel space (I1 and I2) in six saline-injected rats. The SWR mean firing rate of a cell was defined as the total number of spikes for the cell divided by the total time in the SWR state. To explore whether cells showing place fields in the directly experienced inner box and the observed outer box areas were active during SWRs, we separately analyzed cells with fields in the inner box and cells that were considered off in the inner box, as defined above, but showed fields in the outer box area during session O1 (Fig. S3).

Histology. Following completion of the experiment, a brief pulse of current (~25 μA) was passed through the wire that yielded the best recordings. The rat was then killed with Euthasol (100 mg/kg, i.p) and perfused transcardially with 10% formaldehyde. The brain was sliced into 50-μM thick coronal sections and stained with cresyl violet and the final electrode position was compared with a standard atlas of the rat brain (3). Only data from recording locations confirmed to be in the CA1 region of the dorsal hippocampus were included in the present study (Fig. S4). Recording locations were considered either proximal or distal by drawing an arbitrary line that bisected CA1 in the proximal–distal axis. O1–O2 correlation scores were then separately computed for both recording locations (Fig. S5).

1. Singer AC, Frank LM (2009) Rewarded outcomes enhance reactivation of experience in the hippocampus. *Neuron* 64:910–921.
2. Foster DJ, Wilson MA (2006) Reverse replay of behavioural sequences in hippocampal place cells during the awake state. *Nature* 440:680–683.
3. Paxinos G, Watson C (1998) *The Rat Brain in Stereotaxic Coordinates.*

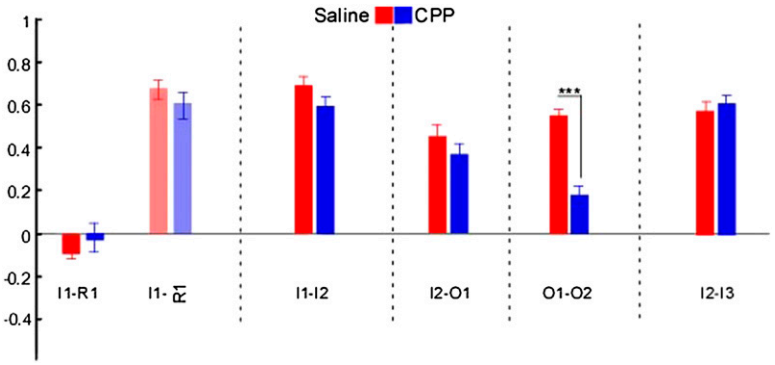

Fig. S1. Correlation scores for session pairs. Height of bars gives the mean correlation score. Error bars are SEM. Sessions being compared are given underneath the bars. Solid bars in the first group are the I1 to R1 comparison. Light bars in that same group are I1 to a clone of the R1 map rotated counterclockwise 90° to offset rotation of the cues. The third group shows lower means than the other groups due to the effect of barrier removal described in Fig. S2. The only significant difference between CPP and saline was seen in the O1–O2 comparison. In contrast to the figure in the main text, the correlation score shown here is for the entire environment and not broken into inner and outer box areas.

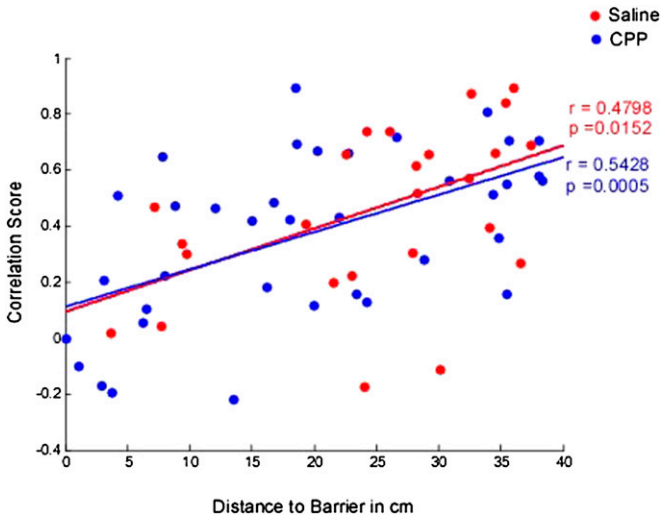

Fig. S2. Effect of barrier removal on place fields. Correlation scores for the I2–O1 comparison are plotted against the distance to the barrier. A linear relationship between the two variables suggests that place fields near the removed barrier were preferentially destabilized. No differences were seen between the saline and CPP groups.

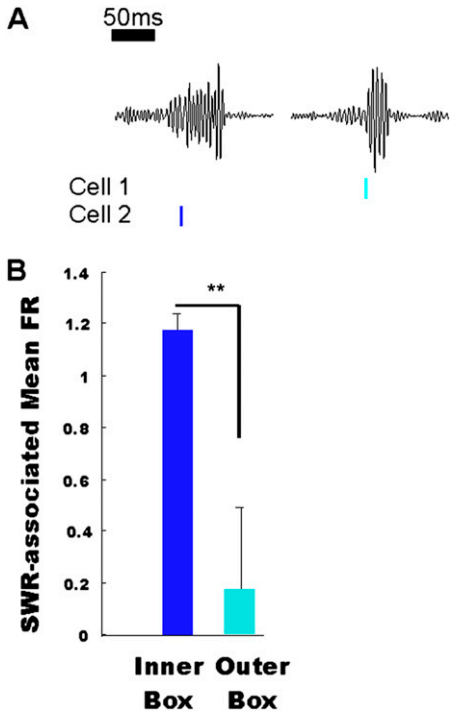

Fig. S3. Inner box place fields are preferentially reactivated during sharp wave ripples before exploration of the outer box (sessions I1 and I2; *SI Materials and Methods, Data Analysis* for details) in saline-injected rats. (*A*) At top are the filtered CA1 LFP (150–250 Hz) of two example ripples. Below are spikes of an outer box place cell (cell 1) and an inner box place cell (cell 2) during the same windows. (*B*) Mean firing rate of cells with inner box place fields (dark blue) and cells that turned on only in the outer box area (light blue). Inner box place cells were significantly more active during sharp waves (error bars are SEM; *t* test $t(40) = 3.3$, $P = 0.008$).

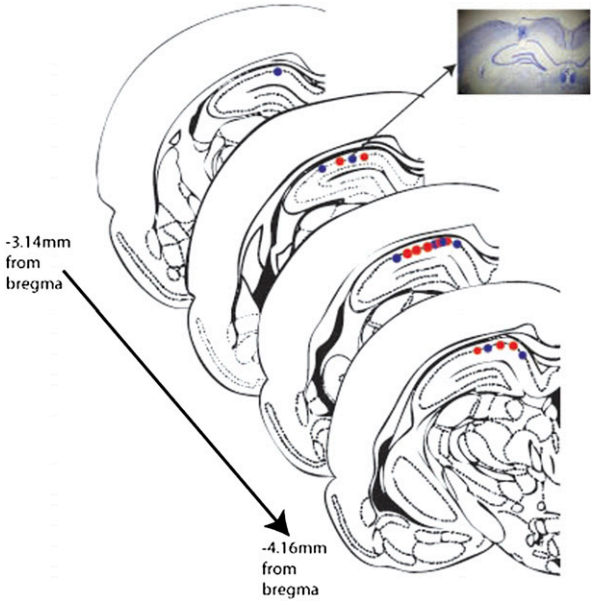

Fig. S4. Recording locations. Final electrode positions are given as red (saline) and blue (CPP) dots. *Inset* shows an example animal. Images were traced in from ref. 3 using Adobe Illustrator.

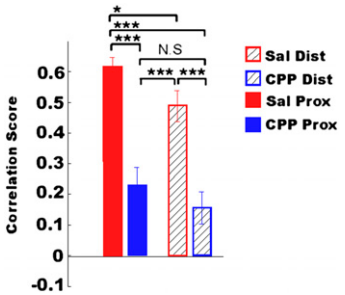

Fig. S5. Comparison of place field stability along the proximal–distal axis. Cells were grouped by distance from the CA3 field (proximodistal axis, *SI Materials and Methods, Histology*). The mean correlation score for the O1–O2 session is given by the height of the bars. Error bars are SEM. An ANOVA revealed a significant difference between groups $F(4,109) = 17.97$, $P < 0.001$). Post hoc tests showed that the field stability was significantly lower for the cells recorded more distal to CA3 in the saline-injected rats, but all scores from the saline-injected rats were higher than scores from the CPP-injected rats. NS, not significant; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

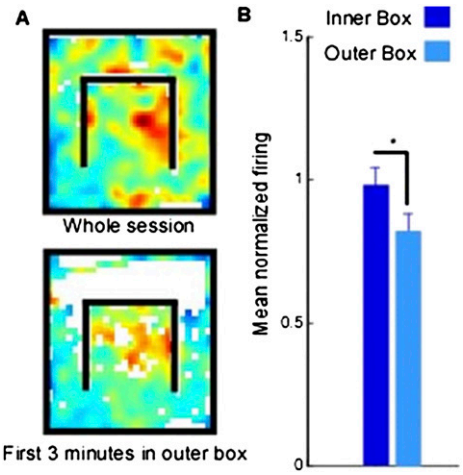

Fig. S6. Suppressed activity of putative interneurons in the outer box area compared with the inner box area for saline-injected animals. (*A*) Rate maps showing activity of a putative interneuron over the whole session (*Upper*) and the first 3 min of experience in the outer box area (*Lower*). Note the suppression of activity in the outer box. (*B*) Group data. Bars show the mean normalized activity of cells ($n = 12$) in the first 3 min of experience in the familiar inner box compared with the first 3 min of experience in the outer box area (error bars are SEM; paired t test, $P = 0.0262$).

Table S1. Single session statistics

	I1	R1	I2	O1	O2	I3
Number of cells ^	*74*	*69*	*80*	*92*	*89*	*67*
	73	79	78	78	86	75
Mean rate						
Inner	*0.53 ± 0.11*	*0.56 ± 0.11*	*0.54 ± 0.18*	*0.82 ± 0.15*	*0.68 ± 0.08*	*0.54 ± 0.11*
	0.71 ± 0.09	0.78 ± 0.10	0.63 ± 0.08	0.78 ± 0.72	0.64 ± 0.78	0.51 ± 0.06
Outer				*0.68 ± 0.12*	*0.84 ± 0.11*	
				0.83 ± 0.75	0.84 ± 0.77	
Peak rate						
Inner	*6.69 ± 0.43*	*5.99 ± 0.36*	*5.90 ± 0.46*	*4.83 ± 0.56*	*5.18 ± 0.63*	*5.81 ± 0.31*
	7.73 ± 1.09	7.82 ± 0.99	7.19 ± 0.95	7.24 ± 1.04	4.93 ± 1.54	4.94 ± 0.49
Outer				*6.62 ± 0.84*	*10.53 ± 0.97*	
				8.35 ± 1.48	10.89 ± 0.92	
Coherence						
Inner	*0.56 ± 0.09*	*0.51 ± 0.07*	*0.52 ± 0.09*	*0.55 ± 0.04*	*0.44 ± 0.04*	*0.56 ± 0.07*
	0.62 ± 0.05	0.62 ± 0.05	0.63 ± 0.05	0.44 ± 0.04	0.68 ± 0.04	0.58 ± 0.05
Outer				*0.41 ± 0.03*	*0.43 ± 0.03*	
				0.53 ± 0.03***	0.66 ± 0.03***	
Field size (pixels)						
Inner	*25.84 ± 5.33*	*25.84 ± 5.54*	*20.94 ± 7.61*	*35.5 ± 5.75*	*25.15 ± 3.11*	*26.96 ± 4.25*
	35.07 ± 3.90	36.12 ± 3.89	35.07 ± 3.89	40.65 ± 4.08	30.78 ± 4.31	37.47 ± 4.38
Outer				*30.79 ± 5.49*	*23.99 ± 2.56*	
				38.76 ± 6.11	31.36 ± 2.90	
Median no. of fields						
Inner	*1*	*1*	*1*	*1*	*1*	*1*
	1	1	1	1	1	1
Outer				*2*	*2*	
				2	2	

Single session statistics described in the *SI Materials and Methods* for saline and CPP (italics) groups. Measures are further broken down into inner and outer box areas. The outer box measures are blank for I1, R1, I2, and I3 because the outer box area was inaccessible to the rat during those sessions. ± denotes SEM. Each measure was compared between groups for every session using a *t* test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. ^, not all cells recorded for a session were included in every measure; for example, a field size can only be calculated if a field existed for that cell (*SI Materials and Methods, Data Analysis*). CPP did not significantly alter any parameter in the standard environment (I2), but did significantly reduce the coherence of outer box fields in the expanded environment.