Supplemental Information

Coherent Phasic Excitation
during Hippocampal Ripples

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Figure S1, related to Figure 1 and Experimental Procedures

(A) Detection of SWRs in vivo was performed with a three-step algorithm. Bandpass-filtered (120-300 Hz) extracellular data (top panel, grey trace) were rectified and smoothed with a moving average filter (10 ms window size, black trace). In a second step all events were tentatively considered that exceeded a threshold of 6×SD of noise (middle panel, blue dotted line). Last, a length criterion was used to reject all events of duration below 12 ms at a threshold level of 2×SD of noise (red dotted line). The bottom panel shows all finally detected SWR events in this example marked with a red dot.

(B1) Current-clamp recordings reveal ripple-associated de- and hyperpolarizing synaptic inputs onto CA1 principal neurons in head fixed, awake mice in vivo. Individual LFP ripple events (top row, 120-300 Hz-filtered) are demonstrated with their respective membrane potential counterparts below (initial membrane potentials are indicated). (B2) Population result including the analysis of 241 single ripple events. The top panel displays the averaged ripple. The bottom histogram presents the changes of membrane voltages binned to 10 ms time windows averaged over all included events (means ± SEM). Each intracellular voltage trace was normalized to a reference voltage (“0”) that was defined as the mean membrane potential 60 ms preceding the LFP ripple peak. On average, ripple-associated depolarization is followed by pronounced hyperpolarization.
Figure S2, related to Figure 2

(A1) Single (grey) and averaged (black) ripple-associated voltage traces from a CA1 pyramidal cell recorded in the current-clamp configuration; recording was performed at resting membrane potential. Below, magnification of the boxed averaged trace segment to demonstrate ripple-related intracellular voltage fluctuations.

(A2) Band pass-filtered version (upper trace) and time derivative (bottom) of the membrane voltage average shown in (A1). Note rhythmic fluctuations at ~5 ms in the filtered and derived signals.

(A3) Example time derivatives of membrane potentials from six recordings at resting membrane potential.
Figure S3, related to Figure 3

(A) Coherence analysis of extracellular ripple activity in CA1. Screenshot of an example experiment showing electrode positions (A1). One electrode, positioned at location 1, was not moved throughout the experiment and sampled the reference signal. A second electrode was stepwise moved to more distant positions (2→7) and extracellular SWR coherence values were compared. (A2) Three examples of ripple averages (bandpass-filtered, 120-300 Hz) are shown from the experiment presented in A1. Increased distances (top to bottom traces) and color code as indicated. (A3) LFP-to-LFP peak coherence in the ripple band (120–300 Hz) is displayed as a function of distance (dual recordings in seven slices). Note the significant decline of coherence but also the high coherence at distant recordings.

(B) Two extreme scenarios to explain cPSCs during sharp wave-ripples in CA1 pyramidal cells. (B1) Phasic high-frequency (~200 Hz) inhibitory input during a slower, sharp wave-associated excitatory wave. (B2) Fast ~200 Hz trains of excitatory inputs during a slow inhibitory signal. Red traces represent the resulting signal as would be observed in voltage-clamp recordings. At potentials below the reversal potential of excitatory synaptic transmission, excitatory (inward) currents within cPSCs should display downward slopes (onsets) steeper than their upward slopes (decays). In contrast, at potentials above the reversal potential of inhibitory GABAergic transmission, putative inhibitory (outward) currents should display steeper upward slopes (onsets) followed by shallower downward slopes (decays).
Figure S4, related to Figure 4

**A** The theoretical equilibrium potential of Cl⁻ (Nernst potential) in our recording conditions was experimentally verified. In the presence of blockers of AMPA- and NMDA receptors, CA1 pyramidal cells were voltage-clamped at systematically varied potentials (see A1 for example recording), and stimulated with short current pulses to evoke inhibitory postsynaptic currents (IPSCs). Amplitudes of IPSCs were plotted against the respective holding potentials. A linear fit was applied to determine the Cl⁻ equilibrium potential (i.e., -667.3 mV; A2 represents pooled data from 5 cells).

**B** Inter downward-slope interval (IDI) analysis of ripple-associated cPSCs recorded at -66 mV (i.e., at Cl⁻ reversal potential, revealing excitatory synaptic input) from eight single-cell-and-LFP recordings; all histograms demonstrate that IDI distributions consistently peak at ~5 ms. Black and grey distributions represent 10% and 25% of strongest slopes. See Fig. 4C (main text) for pooled data representing the entire 10%- and 25% datasets.
Figure S5, related to Figure 5 - The peeling reconstruction algorithm

(A1) Highpass-filtered trace (dark grey; 0.5 Hz) and 400 Hz low-pass filtered trace after local baseline removal (light grey). (A2) Deconvolution peaks (discs) marking first three candidate events. The dashed line represents the deconvolution threshold obtained from eventless epochs. (A3) Starting and end points (triangles) for the fit stretch are calculated from the first two deconvolution peaks (magenta discs) on an 800 Hz lowpass-filtered trace. (A4) Least squares fit (orange) to the unfiltered trace. The relative weight for the fit is represented by the dark blue area. (A5) The fit is subtracted from the trace (light grey) and the process continues as in (A2) on the dark grey trace.

(B) Histogram (top) and raster (bottom) of fitted PSC onsets during cPSCs for one example cell (black label in Figure 5B). Dot area is proportional to fitted amplitude.

(C) Histograms of inter onset-intervals and fit parameters of cPSC-embedded PSCs (black) vs. spontaneous PSCs (red) for the same cell as in (B).
(A1) A further control experiment to demonstrate the general reliability of repatch-recordings used for CsF-DIDS experiments. Short electrical pulses were extracellularly delivered to elicit IPSCs; their amplitudes were compared before (black) and following (blue) re-patching the cells with identical intracellular solution (20 µM NBQX and 50 µM D-APV present in the bath solution).

(A2) Summary: control: 226 ± 48 pA; re-patch: 235 ± 45 pA; n = 6 cells; P = 0.9, ranksum test.

(B) Single cell inter-downward slope-interval histograms for the seven experiments shown in Figure 6E. Black bars correspond to control, and blue histograms to CsF-DIDS condition for the 10% strongest slopes in individual cPSCs; grey histograms indicate values for 25% strongest slopes in individual cPSCs.
We generated a short train of overlaid EPSCs (A1, B1) to mimic ripple-coherent synaptic input. This pattern was injected into pyramidal cells to check whether this ripple-like input can account for locking of action potential timing. Panel (A2) shows the overlaid current clamp traces recorded from six cells close to the action potential firing threshold (10, 20, 20, 22, 26, and 30 trials, respectively). Panel (A3) displays the spike timing of these current injections (128 trials altogether) demonstrating an enhancement of spiking during the synthetic ripple-like input (dark yellow area). This enhancement was better observed in the spike count histogram (A4).

(B) Temporal enlargement of the area highlighted in yellow (in A). The observed spiking (B2) was indeed locked to the ‘rippled’ input pattern (B1) as quantified by a high vector strength value, 0.96. This set of experiments demonstrates that a current waveform resembling the input observed during ripples can indeed account for the occurrence and the timing of action potentials. (C) In another set of experiments we slightly depolarized CA1 principal cells to enhance firing probability and analyzed the timing of action potentials relative to spontaneously occurring LFP ripples. We found significant phase-locking of action potentials with respect to the ripple in an interval of 30 ms centered to the peak of the ripple-band-filtered LFP (seven cells; \( P < 0.05 \), Rayleigh test; mean AP phase -56°). These ripple-locked action potentials represent 72% of all observed action potentials in this approach (27 cells analyzed in total). These results show that cPSCs locked to transient ripple oscillations do have the potential to entrain action potential discharge.
To experimentally determine the reversal potential $V_{\text{rev}}$ of excitation in our experimental conditions, a Cs-gluconate-based pipette solution was applied and CA1 pyramidal neurons were voltage-clamped at systematically varied holding potentials. **Panel A1** depicts an overlay of stimulus-evoked inhibitory postsynaptic currents (IPSCs) from one example experiment (50 µM D-APV and 2 µM gabazine present in the ACSF). Each trace represents the average of ten single IPSCs. Voltages indicated on the left represent liquid junction potential-corrected values (12 mV; experimentally determined). **(A2)** IPSC peak amplitudes from six cells were plotted against the respective holding potentials. A linear fit was applied to determine the reversal potential of excitation (i.e., voltage at zero crossing, -6.5 mV).

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**Figure S8, related to Figure 9**

To experimentally determine the reversal potential $V_{\text{rev}}$ of excitation in our experimental conditions, a Cs-gluconate-based pipette solution was applied and CA1 pyramidal neurons were voltage-clamped at systematically varied holding potentials. **Panel A1** depicts an overlay of stimulus-evoked inhibitory postsynaptic currents (IPSCs) from one example experiment (50 µM D-APV and 2 µM gabazine present in the ACSF). Each trace represents the average of ten single IPSCs. Voltages indicated on the left represent liquid junction potential-corrected values (12 mV; experimentally determined). **(A2)** IPSC peak amplitudes from six cells were plotted against the respective holding potentials. A linear fit was applied to determine the reversal potential of excitation (i.e., voltage at zero crossing, -6.5 mV).
Figure S9, related to Discussion – Carbenoxolone suppression of SWRs might involve effects on excitatory and/or inhibitory synaptic transmission

(A1) At 50 µM, carbenoxolone reduces, but does not block sharp wave-ripples entirely. Time plot of averaged and baseline-normalized SWR incidence values. Analysis time windows as marked (grey); absolute values of incidence: Ctrl: 0.95 ± 0.17 Hz vs. CBX: 0.29 ± 0.09 Hz (P = 0.01, two-tailed Mann-Whitney test; 7 slices).

(A2) Representative experiment displaying the suppressive effects of CBX on the incidence of SWRs and the amplitudes of their associated cPSCs (bottom panel, green).

(A3) Power of ripple-associated cPSCs decreases upon CBX application (362 and 103 events analyzed, respectively; P = 6 x 10^{-7}, K-S test; 6 cells).

(B1) Effect of CBX on excitatory synaptic transmission as a function of time, as tested by stimulus-induced field EPSPs (recorded from area CA1, stratum radiatum). (B2) Averages of fEPSPs, each representing 10 single traces.

(B3) Population analysis of fEPSP slopes demonstrating the reduction of excitatory synaptic transmission upon application of 50 µM CBX. Averaged fEPSP slope values: 0.33 ± 0.05 mV/ms vs. 0.28 ± 0.05 mV/ms (P = 0.03, one-tailed Wilcoxon matched-pairs signed-ranks test, 5 slices).

(C) CBX at 50 µM suppresses stimulus-induced IPSCs recorded in CA1 principal cells.

(C1) Effect of CBX on averaged and baseline-normalized IPSC amplitudes as a function of time. (C2) Two example traces (5-10 trials averaged).

(C3) Population analysis: Ctrl: 243.7 ± 47.6 pA vs. CBX: 186.9 ± 36.7 pA; P = 0.02, two-tailed Wilcoxon matched-pairs signed-ranks test, n = 8 cells.
Supplemental Methods
(related to Figure 5 and Figure S5)

Full reconstruction of fast currents with the peeling fit method

The peeling fit approach

As an alternative approach to obtain onset times of compound PSCs we developed a peeling fit algorithm that attempts to fully reconstruct the complex PSCs. The advantage of such an algorithm is that it also provides information about kinetic characteristics and amplitudes of the individual PSCs. The algorithm proceeds iteratively on a filtered cPSC from left to right finding candidate PSCs, fitting them and subtracting them from the trace (Figure S5A). The causal progression enables us to uncover smaller late events that may otherwise appear buried in the joint tail of the events. In detail, we proceed as follows. First, we remove any local baseline shifts that survive a highpass filter at 0.5 Hz (Figure S5A, top panel). The next steps of the algorithm assume a zero baseline throughout. Second, we detect candidate events as peaks in the deconvolution of the lowpass-filtered trace (400 Hz; Butterworth order 2, phase-correct) with a single exponential kernel of decay constant $\tau$ of 4 ms, equal to the average decay time constant of spontaneous events (Figure S5A, second panel). Such single-exponential deconvolution of a signal $y$ is calculated as a linear combination of its amplitude and its derivative:

$$\text{deconv}(y) = y + \tau \frac{dy}{dt}.$$  

Deconvolution peaks are accepted as PSC onset proxies if they are beyond the mean plus four standard deviations of the deconvolution peaks of noise surrounding the event and if the 400 Hz lowpass-filtered trace has both a negative amplitude and a negative derivative there. Third, we identify the starting and end point for the one-PSC fit (Figure S5A, third panel). As reference points we use the first two accepted deconvolution peaks. The starting point is estimated as the zero crossing before the first deconvolution peak (preceding the peak for at most 3 ms) roughly indicating a maximum of the cPSC trace. The endpoint is estimated either as the first zero crossing of the trace after the first peak of the deconvolution or the last maximum before the second peak, whichever occurs first. Maxima and zeros are searched on the 800 Hz lowpass-filtered trace for increased resolution. These choices are adequate in most cases to provide the largest possible fitting window that does not include contributions from upcoming events. If no local maximum can be identified between the first two deconvolution peaks, we label those intervals as double events and handle them specifically (see below). In a fourth step (Figure S5A, fourth panel), we run a weighted least-squares fit for the selected stretch to an alpha function. A gaussian weight function emphasizes the kink of the EPSC around its minimum with an adaptive length constant equal to the distance of the minimum to the end of the fit or to its start, whichever is shorter but in any case
between 1 and 10 ms. In order to avoid artificially long decays, we add a quadratically growing penalty to the fit error whenever a decay constant $\tau_d$ of 8 ms is exceeded. Artificially long decays jeopardise the method, because they subtract too much amplitude from subsequent deconvolution peaks thus making them invisible. About only 5% of spontaneous decays are longer than our chosen penalty threshold (compare Fig. S5C, lower left panel). Finally, with the fit parameters we rebuild the PSC, including the previously missing tail, and subtract it from the unfiltered trace (Figure S5A, bottom panel). We restart the procedure by detecting deconvolution peaks after the endpoint of our previous fit and proceed as described above until no more events are left in the cPSC event window of (-50, 100) ms around the SWR maximum.

**Handling of double events**
An extremely rapid succession of synaptic inputs can lead to composite currents where the decay phase of the initial event is masked in its entirety by the rapid rise of a second, superposed event. Generally this results in traces that do not show any local extremum between the two onsets but at most a concavity change. We tackle these doublets by fitting them simultaneously with a sum of two component alpha functions. After the doublet fit, only the first PSC fit of the sum is subtracted from the cPSC, and the result is fed again through the algorithm as normal.

**Event selection for statistics**
We exclude from our analysis traces whose standard deviation is larger than that of the original cPSC (32 out of 1085). As for the component PSCs, we only consider onsets in the 30 ms surrounding the SPW peak whose amplitude exceeds 5 pA. The robustness of the fitting algorithm has been tested extensively on individual events and at the population level by changing detection constants (deconvolution time constant and threshold), fit constraints (penalty threshold and penalty function) weighting schemes (constant length scale vs. adaptive; flat weighting), by jittering parameter guesses for each PSC, and by comparing its performance on cPSC events with the results on inter event epochs. No relevant changes of the fit statistics were observed.