Cellular correlate of assembly formation in oscillating hippocampal networks in vitro

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Neurons form transiently stable assemblies that may underlie cognitive functions, including memory formation. In most brain regions, coherent activity is organized by network oscillations that involve sparse firing within a well-defined minority of cells. Despite extensive work on the underlying cellular mechanisms, a fundamental question remains unsolved: how are participating neurons distinguished from the majority of nonparticipants? We used physiological and modeling techniques to analyze neuronal activity in mouse hippocampal slices during spontaneously occurring high-frequency network oscillations. Network-entrained action potentials were exclusively observed in a defined subset of pyramidal cells, yielding a strict distinction between participating and nonparticipating neurons. These spikes had unique properties, because they were generated in the axon without prior depolarization of the soma. GABA_A receptors had a dual role in pyramidal cell recruitment. First, the sparse occurrence of entrained spikes was accomplished by intense perisomatic inhibition. Second, antidromic spike generation was facilitated by tonic effects of GABA in remote axonal compartments. Ectopic spike generation together with strong somatodendritic inhibition may provide a cellular mechanism for the definition of oscillating assemblies.

Results

CA1 Pyramidal Cells Form Two Functionally Distinct Subgroups During SPW-Rs. Spontaneously occurring field events in mouse hippocampal brain slices resemble characteristic properties of SPW-Rs in vivo (10). We recorded such field potentials in the CA1 pyramidal cell layer together with intracellular potentials of 153 CA1 pyramidal neurons (Materials and Methods and SI Materials and Methods). At resting membrane potential, network-correlated action potentials were observed in 41% of the cells. Each participating cell fired only on a minority of SPW-Rs (Figs. L/4 and A/B). Median discharge probability was one action potential on 1.6% of sharp waves (median; P25 = 0.3% and P75 = 6%), corresponding to a firing rate of 0.038 Hz (median; P25 = 0.01 Hz and P75 = 0.15 Hz). This value is more than fivefold higher than the firing rate outside sharp wave episodes (0.007, 0.001, and 0.022 Hz) (Fig. 2B). SPW-R-associated action potentials were strongly phase-coupled to the ≈200-Hz network ripples (Fig. 1, A, C, and E). During single ripple cycles, 50% of action potentials occurred within 1.06 ms (0.98–1.26 ms), corresponding to 26.4 ± 2.4% of a ripple cycle (n = 13 cells). This temporal precision (9) is in marked contrast to the reported large temporal jitter of excitatory postsynaptic potential (EPSP)-evoked spikes in CA1 pyramidal cells (17).

Passive membrane properties were not different between participating and nonparticipating cells (Fig. S1). Interestingly, nonparticipating cells could not be recruited into SPW-Rs by somatic depolarization (Fig. 2C). Although injection of positive current enhanced basal firing rates of both participating and

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Information processing in neuronal networks has been proposed to rely on coordinated patterns of activity in transiently stable neuronal assemblies (1). Such patterns underlie different cognitive or behavioral tasks including motor patterns (2), perception (3), and spatial cognition (4). The functional coupling of neurons within distributed assemblies is believed to be organized by network oscillations that cover multiple frequency bands and follow distinct mechanisms (5). However, it is still unclear how neurons within an activated assembly are distinguished from the majority of nonparticipating cells. This distinction is essential for maintaining sparse and stable neural representations (6).

Spatial memory formation in rodents has become an important model system for studying neuronal representations within networks. Place-selective neurons of the hippocampus are sequentially activated during exploration of an environment and reactivated during subsequent resting periods (7), indicating the formation of stable assemblies. During reactivation, temporal and spatial precision of pyramidal cell firing is organized by propagating sharp waves with superimposed high-frequency network oscillations [sharp wave ripple complexes (SPW-Rs)] (8, 9). While traveling through the CA1 area, each SPW-R recruits only a few selected cells to fire action potentials (8), whereas the majority of nonparticipating cells is silent, ensuring clear signal to noise separation (10). The mechanisms underlying this functional distinction between participating and nonparticipating cells are, however, unclear. Recordings in vivo (11) and in vitro (12) have provided strong evidence for a major role of inhibitory interneurons in the temporal organization of cortical oscillations. In addition, electrical coupling between axons has been suggested to


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Afterpotential that decayed with afterhyperpolarization (Fig. 3a). Slow depolarization and were followed by a fast and medium.

Data show that CA1 pyramidal cells form two clearly distinct subgroups during SPW-Rs (Fig. 1). Firing patterns of CA1 pyramidal cells during SPW-Rs. (A) Original recording from a cell that participates in SPW-Rs. (B) Basal firing rates are plotted over firing rates during SPW-Rs for both participating (red circles) and nonparticipating (black circles) neurons. Note that most participating cells fire at a much higher rate during ripples (red circles on the right side of the dotted line). (C) Firing rate of nonparticipating cells at different membrane potentials. Depolarization increases firing (black curve) but does not induce any SPW-R–coupled spikes (red curve), indicating high signal to noise separation during SPW-Rs. (D) Firing rate of participating cells at different membrane potentials. Although basal action potential frequency increases on depolarization, spiking during ripples is largely independent of somatic membrane potential.

Nonparticipating cells, SPW-R–coupled spikes were independent of membrane potential (Fig. 2D and Fig. S24). Together, these data show that CA1 pyramidal cells form two clearly distinct functional groups with respect to their participation in SPW-Rs.

**SPW-R–Coupled Action Potentials Are Antidromic Spikes.** Action potentials outside and inside SPW-Rs had strikingly different waveforms. Ripple-associated spikes arose abruptly from a presumably GABA_A receptor-mediated fast hyperpolarization (reversal potential $= -72.3 \pm 1.0$ mV; $R = -0.83 \pm 0.04; n = 5$ cells) (Fig. 3 A and B and Fig. S2F). They were followed by a depolarizing afterpotential that decayed with $\tau = 4.9 \pm 0.3$ ms ($n = 16$ cells). In contrast, noncoupled action potentials emanated from a slow depolarization and were followed by a fast and medium afterhyperpolarization (Fig. 3 A and B) (18). Firing threshold of SPW-R–coupled spikes was $-66.4 \pm 1.2$ mV ($n = 17$ cells), which is about 19 mV more negative than the threshold for action potentials occurring outside ripples ($-47.8 \pm 1.4$ mV, $n = 14$ cells; $P = 5.6 \times 10^{-11}$, unpaired $t$ test) (details on spike properties in Fig. S3). Together, this waveform is strongly reminiscent of antidromically generated action potentials (16, 19–21).

Action potentials are usually generated in the initial segment (IS spike) before back-propagating into the somatodendritic compartment (SD component) (22). The depolarizing postsynaptic potentials preceding spike generation precharge the somatodendritic capacitor before reaching the initial segment. This precharging is missing in antidromic action potentials, thus enhancing the latency between spike generation (IS spike) and the somatodendritic component (SD). IS-SD latency can, therefore, be used as a marker for antidromic spikes (20, 22).

In contrast, orthodromic spikes could not be elicited by somatic electrical stimulation in stratum radiatum or the alveus, respectively. As expected, Schaffer collateral-evoked orthodromic action potentials matched properties of spontaneous spikes outside SPW-Rs, whereas antidromically evoked spikes showed the characteristic features of ripple–entrained action potentials (Fig. S5). Interestingly, antidromic spikes could be evoked during and outside spontaneous network events ($n = 6$) (Fig. S5). In contrast, orthodromic spikes could not be elicited by somatic current injection during SPW-Rs (Fig. 2C and Fig. S5F).

**Fig. 1.** Firing patterns of CA1 pyramidal cells during SPW-Rs. (A) Original recording from a cell that participates in SPW-Rs. Upper shows intracellular recording with two action potentials, and Lower shows field sharp wave ripple complexes. (B) Original recording from a cell (Upper) that does not fire during SPW-Rs (Lower). (C) Expanded trace from the cell in A showing an action potential during the field SPW-R (marked with an asterisk in A). (D) Expanded trace from the nonparticipating cell shown in B. The sharp wave is accompanied by a subthreshold synaptic potential. (E Left) Event cross-correlogram showing precision of firing during SPW-Rs (data from the cell shown in A and C). (Left inset) Cumulative plot of spikes within one ripple cycle (red box around the central peak of the cross-correlogram). In this cell, 50% of action potentials occurred within 23% of a full ripple cycle, indicating strong phase-coupling to ripples. (Right) Loss of phase coupling on shuffling of spikes and ripples. Cross-correlogram was constructed between spikes and ripples from the subsequent SPW-R event. Cumulative plot (Right Inset) shows loss of coupling.

**Fig. 2.** CA1 pyramidal cells form two functionally distinct subgroups during SPW-Rs. (A) Raster plot of action potentials from participating cells (1–6) and nonparticipating cells (7–12). Red ticks indicate phase-coupled action potentials during ripples, and black ticks are noncoupled action potentials. (B) Basal firing rates are plotted over firing rates during SPW-Rs for both participating (red circles) and nonparticipating (black circles) neurons. Note that most participating cells fire at a much higher rate during ripples (red circles on the right side of the dotted line). (C) Firing rate of nonparticipating cells at different membrane potentials. Depolarization increases firing (black curve) but does not induce any SPW-R–coupled spikes (red curve), indicating high signal to noise separation during SPW-Rs. (D) Firing rate of participating cells at different membrane potentials. Although basal action potential frequency increases on depolarization, spiking during ripples is largely independent of somatic membrane potential.
Antidromic spikes can be elicited by blocking axonal potassium channels (23). Local application of 4-aminopyridine (4-AP; 100 μM) into the alveus increased firing frequency during SPW-Rs in five of eight cells (control: median = 0.0% of SPW-Rs with spikes, P25 = 0.0%, and P75 = 0.0%; 4-AP: median = 1.0%, P25 = 0.0%, and P75 = 10.8%) (Fig. 3 D and E). More than 98% of the induced antidromic spikes were entrained by the network rhythm. Together, these data suggest that SPW-R–coupled action potentials are generated in the axon without prior somatic depolarization. Antidromic action potentials are a distinguishing feature of CA1 pyramidal cells that participate in SPW-Rs.

**Discharge Probability of All Pyramidal Cells Is Controlled by Strong Perisomatic Inhibition During Ripples.** Juxtacellular recordings from putative interneurons revealed high-frequency firing at earlier phases of sharp waves than pyramidal neurons (n = 19 cells) (Fig. 4), similar to findings in vivo (9, 11). In contrast to pyramidal cells, interneurons were activated on the majority of SPW-Rs (83.6 ± 3.9%) and typically emitted multiple spikes (1.9 ± 0.2 spikes per SPW-R). Spikes were strongly coupled to individual ripple cycles (coupling strength = 25.1 ± 2.0%; not different from participating pyramidal cells, P = 0.67, unpaired t test). Light microscopy of juxtacellularly labeled cells revealed somata within or close to the pyramidal cell layer, radially oriented dendritic trees, and axonal ramifications in the somatic and proximal dendritic layers, indicative of perisomatically inhibiting interneurons. Four cells with axonal projection patterns in basal portions of the pyramidal cell layer were analyzed at the ultrastructural level. All examined postsynaptic contacts were confined to the perisomatic region or proximal dendrites (Fig. 4A), and the cells were, therefore, classified as basket cells. These data are in line with recent findings from in vivo recordings indicating that perisomatically inhibiting neurons are highly active during SPW-Rs, whereas axoaxonic cells do not participate (11). In addition, we performed whole-cell recordings from eight fast-spiking interneurons (Fig. 4 D–F) (24). Recordings in both current and voltage clamp indicated that these cells receive excitatory inputs at ripple frequency, which causes multiple orthodromic spikes during each network event (Fig. 4 E and F).

The strong perisomatic inhibition during SPW-Rs was reflected by a conductance increase of participating and nonparticipating pyramidal cells (153 ± 7.6% and 156.3 ± 6.6% of baseline conductance, respectively; n = 9 cells each; P = 0.75, unpaired t test) (Fig. 5A). Reversal potential of subthreshold SPW-R–coupled potentials was −70.2 mV (−73.6 to −64.8 mV; n = 9) in participating and −68.4 mV (−69.1 to −65.5 mV) in nonparticipating cells (n = 9; P = 0.26, Mann–Whitney u test) (Fig. 5A). Thus, participating and nonparticipating cells receive comparably strong somatodendritic GABAergic input during SPW-Rs, consistent with data from in vivo recordings (8, 11). The inhibitory role of GABAergic hyperpolarization became apparent in recordings with chloride-filled electrodes that shifted the GABAergic reversal potential to more positive values (n = 14 cells) (Fig. 5B). Under these conditions, all pyramidal cells fired at high frequency during most of the SPW-R events (66.8 ± 8.2% of SPW-Rs, firing frequency during SPW-Rs = 4.920 ± 0.918 Hz). Tight coupling of neurons to the network rhythm was maintained [50% of action potentials within 1.21 ms (1.12–1.28 ms) of a ripple cycle; coupling strength = 25 ± 2.3%].

This notion was confirmed by recordings with intracellular application of the GABA_A receptor blocker picrotoxin (200 μM) (25). Basal activity outside SPW-Rs was not affected by the drug, whereas 11 of 24 cells showed a continuous increase in ripple-coupled action potentials during diffusion of the drug into somatodendritic compartments (control: median = 0.0% of SPW-Rs with spikes, P25 = 0.0%, and P75 = 0.0%; picrotoxin: median = 0.0%, P25 = 0.0%, and P75 = 0.6%; P = 10^{-3}, Wilcoxon matched-pairs signed-ranks test) (Fig. 5 C and D). Cells that were recorded without the addition of picrotoxin did not show such a time-dependent increase in SPW-R–associated firing (n = 7). Thus, efficient perisomatic inhibition is crucial.
for maintaining a sparse firing mode of selected pyramidal cells during SPW-Rs (see also Fig. S6).

**Tonic Activation of Axonal GABA_A Receptors Is Necessary for the Generation of Antidromic Action Potentials During SPW-Rs.** High-frequency discharges of inhibitory interneurons in CA1 elevate ambient GABA concentrations and cause tonic activation of GABA_A receptors (26). In stratum oriens, this mechanism induces high-frequency network activity by increasing axonal excitability (27), whereas basal dendrites in the same layer are hyperpolarized by activation of GABA_A receptors (28). In the absence of classical somatic EPSP spike sequences, we reasoned that similar mechanisms could contribute to antidromic spike generation during SPW-Rs. We, therefore, locally applied muscimol (5 μM) to stratum oriens. Indeed, this agent induced phase-coupled action potentials in six of seven nonparticipating...
cells and increased SPW-R–related firing rates in three of three participating cells (Fig. 6 A and B). As a group, spike occurrence during SPW-Rs increased from 0% (median; 0.0–0.1%) to 0.4% (0.1–0.7%; n = 10; P = 4 × 10−3, Wilcoxon matched-pairs signed-ranks test). Similar to 4-AP, muscimol did not induce a significant increase in firing outside SPW-Rs.

α2-Subunit–containing GABA_A receptors have been reported to be enriched in pyramidal cell axons (29–31). We made use of GABA_A-R α2H101R mice in which this receptor isoform is selectively insensitive to benzodiazepines (32). In WT mice, bath application of diazepam (20 μM) increased SPW-R–associated firing in three of three participating neurons, whereas firing outside SPW-Rs remained unchanged (Fig. 6C and Fig. S7). In addition, diazepam recruited three of five nonparticipating cells into SPW-Rs. Together, spike occurrence during SPW-Rs increased from a median value of 0% (0.0–0.9%) to 9% (0.9–10.6%; n = 8; P = 0.03, Wilcoxon matched-pairs signed-ranks test) (Fig. 6C). At the network level, diazepam did not change properties of individual SPW-R events (Fig. S7), whereas it decreased the frequency of occurrence of SPW-Rs, which was expected from a general reduction in excitability (from 1.60 ± 0.28 Hz to 1.23 ± 0.24 Hz; n = 8; P < 0.05). In slices from α2H101R mice, sharp wave–associated spikes had the same antidromic waveform as observed in control tissue. Again, diazepam reduced the occurrence of SPW-Rs. In contrast with WT mice, CA1 pyramidal cells from slices of α2H101R mice could not be recruited into SPW-Rs by application of diazepam (n = 5 nonparticipating cells). In all seven cells tested, SPW-R–coupled firing was not increased by diazepam, supporting the facilitating role of axonal GABA_A receptors in spike generation (Fig. 6D) (median increase in α2H101R mice = 0.0%, P25 = 0.0%, and P75 = 0.2%; P = 0.95, Mann–Whitney u test; different from increase in WT mice; P = 0.04, Mann–Whitney u test).

Finally, the differential GABAergic effects at axonal vs. proximal somatodendritic sites were validated by local application of GABAergic modulators (Fig. 7). Spatial restriction of drug applications was verified in experiments where we locally applied the fluorescent dye fluorescein (1 mM) (SI Materials and Methods and Fig. S8). Infusion of diazepam into stratum oriens increased ripple–associated firing in participating cells and induced SPW-R–associated action potentials in previously nonparticipating neurons (control: median = 0.1% of SPW-R with spikes, P25 = 0.0%; and P75 = 0.4%; diazepam: median = 0.8%, P25 = 0.1%, and P75 = 2.3%; data from eight participators and seven nonparticipators; P = 0.03, Mann–Whitney test) (Fig. 7 A and B). In contrast, application of diazepam into stratum radiatum did not exert an effect on firing during ripples (control: median = 0.00%, P25 = 0.0%, and P75 = 0.1%; diazepam: median = 0.2%, P25 = 0.0%, and P75 = 0.6%; n = 4 participators and 7 nonparticipators; P = 0.22, Mann–Whitney test) (Fig. 7A). Conversely, suppression of GABAergic transmission by local application of gabazine into stratum oriens completely abolished ripple–associated spiking in participators (n = 4) (Fig. 7A and B) in a reversible fashion. Local application of gabazine into stratum radiatum did not reduce but rather, increased the ripple–associated firing of participating cells (n = 4) (Fig. 7B).

These data indicate that axonal, α2-subunit–containing GABA_A receptors potentiate the generation of SPW-R–coupled ectopic action potentials in both participating and previously nonparticipating neurons. In contrast, perisomatic GABAergic inhibition reduces firing probability of all pyramidal cells during SPW-Rs.

**Simulation Results.** Strong, rhythmic inhibition of pyramidal cells has been consistently observed in our experiments and in vivo (9, 11), and it has been proposed as the main mechanism for the
How do we reconcile these observations (particularly vii)? One solution is to suppose that the fast oscillation is, indeed, generated in the axonal plexus but that spike generation and coupling occur in fine axonal branches (21, 38). We postulate that these branches are connected to main axonal trunks with a low safety factor (21, 39), and therefore, only a small fraction of the plexus spikes have a chance of producing antidromic action potentials (SI Materials and Methods and Fig. S9A). If these braches were, instead, all located on the main axon, then a mixture of full spikes and large (often >5 mV) spikelets would occur at the soma (contrary to experiment). The sequence of events in the model (Fig. 8) can be visualized in this way: when axonal gap junctions are open, the fine axonal plexus generates a high-frequency network oscillation that does not (by itself) require chemical synapses (35, 40). The orthodromic output of the axonal plexus induces rhythmic high-frequency EPSPs in both pyramidal cells and interneurons, and interneurons follow this input at high frequency (27) (Fig. S9B). The net synaptic input to pyramidal cells will then be alternating compound EPSPs and inhibitory postsynaptic potentials (IPSPs) (Fig. 8). In addition, some of the fine-plexus spikes are able to invade the main axon and lead to antidromic spikes—the participating spikes. The hyperpolarization before a participating spike is also supported by a burst of spikes in axoaxonic interneurons (similar to what is observed in vivo) (11). In the model, blocking synaptic inhibition in a particular pyramidal cell can (in at least some cells) convert a nonparticipating cell into a participating cell (Fig. S6). Although inhibition indeed plays a role in controlling antidromic discharges of pyramidal cells during SPW-Rs (as also observed during experiments) (Fig. 5 C and D), the dominant effect in the model is failure of spike propagation (e.g., branch-point failures). We were not able to configure any inhibitory circuitry that would by itself allow sparse SPW-R-associated firing while not resulting in large spikelets at the same time.

Thus, the model not only reproduces our experimental findings but provides a coherent understanding of the network interactions involved in assembly formation during SPW-Rs.

**Discussion**

We show that CA1 pyramidal cells fall into two functionally distinct classes with respect to the formation of transient oscillating cell assemblies. All recorded pyramidal cells had similar intrinsic properties and received strong somato-dendritic inhibition during SPW-R events. Participating cells, however, expressed antidromic action potentials with precise coupling to ripple oscillations. Interestingly, the occurrence of putative ectopic spikes in vivo has recently been reported for CA1 pyramidal cells (14). Ectopic spikes and inhibitory potentials have also been described during SPW-Rs in vitro (15). Our present work suggests an unexpected mechanism of pyramidal cell recruitment during fast network oscillations. In our proposed model, somatic spikes during SPW-Rs originate in distal axonal compartments of selected pyramidal cells forming an axonally connected cluster. After invading the main axonal trunk, action potentials will propagate into both directions, exciting downstream target neurons and causing antidromic somatic spikes that might invade the dendrite and facilitate synaptic plasticity. Another surprising finding is the dual role of GABA<sub>A</sub> receptors, which suppress background activity and promote axonal spike generation in participating neurons.

**Ripple-Associated Spikes in Pyramidal Cells Are Antidromic Spikes.** Cells participating in high-frequency oscillations are distinguished by ectopically generated action potentials that are entrained with millisecond precision. Ripple-associated action potentials are generated in the axon and invade the somatic recording site antidromically, which was shown by their typical waveform, IS-SD interval, notches, and resistance to hyperpo-

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Fig. 6. Pyramidal cell recruitment is modulated by a subunit-specific tonic GABAergic input to the axonal compartment. (A Left) Application of muscimol into stratum oriens increases SPW-R-coupled firing in 9 of 10 cells (7 non-participating cells and 3 participating cells). (Right) Experimental setup showing the intracellular recording pipette in stratum pyramidale and the application pipette in stratum oriens, respectively. (B) Raster plot of action potentials from a representative cell. Note generation of SPW-R-associated spikes by local application of muscimol into stratum oriens. Right shows a ripple-associated antidromic action potential from that cell. (C) Increased sharp wave ripple-associated firing of eight pyramidal cells (three participators and five non-participants) in the presence of diazepam. Firing outside SPW-Rs was not changed. (D) Absolute change in SPW-R-associated firing before and after diazepam in WT (Left) and GABA<sub>A</sub> R<sub>2</sub>α<sub>2</sub>101/11 (Right). Note the absence of diazepam effect in cells with benzodiazepine-insensitive α2-subunits.

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selection of pyramidal cells during fast network oscillations (8, 33, 34). However, this model is unable to explain the existence of antidromic action potentials. Two alternative models have suggested that ectopic spikes originating in either the axon (35) or the basal dendrites (36) are crucial for the organization of ripple oscillations. We, therefore, used a modeling approach to tackle these questions. A network model of the CA1 region should explain these features in a self-consistent manner.

i) The field potential oscillates at more than ~150 Hz, waxing and waning in amplitude.

ii) Somatic pyramidal cell action potentials are rare during SPW-Rs (Figs. 1 and 2).

iii) Participating spikes are, on average, phase-locked to the local SPW-R field potential (Fig. 1 C and E).

iv) Participating spikes arise from an initial hyperpolarization and show characteristics of antidromic spikes (Fig. 3).

v) Participating spikes are more apt to occur in pyramidal cells in which perisomatic synaptic inhibition has been reduced (Fig. 5 C and D).

vi) Ripple frequency EPSPs occur in both interneurons (Fig. 4 E and F) and pyramidal cells (37), indicating that the pyramidal cell axonal plexus is indeed generating a high-frequency output.

vii) Spikelets occur very rarely in pyramidal cells during SPW-Rs, which contrasts previous network simulations (35).
larization (16, 19–23). As an alternative mechanism, ectopic spikes have been suggested to originate in dendrites (41), and dendritic spikes have been reported during SPW-Rs in vivo (42). However, the vast majority of SPW-R–coupled action potentials in our in vitro model must be generated in the axon. Somatic spikes of dendritic origin have normal action potential waveform and threshold (43), are blocked by somatic hyperpolarization (43, 44), and should not be abolished by disinhibition with gabazine (42). In line with these results, we were unable to model the present observations using dendritically generated action potentials (SI Materials and Methods).

**Dual Role of GABA<sub>A</sub> Receptors.** The distinction between participating and nonparticipating cells depends on two different GABAergic mechanisms. First, all pyramidal cells receive equally strong perisomatic and dendritic GABAergic input during SPW-Rs, ensuring sparse firing and high signal to noise ratio. Second, release of GABA within active networks facilitates spike generation in remote axonal compartments (23, 45). Collective evidence for this notion comes from the positive modulation of spike by muscimol and diazepam, the counterintuitive block of increased firing of eight participants and four of seven nonparticipating cells on application of diazepam into stratum oriens (Upper). There was no significant effect with application of diazepam into stratum radiatum (Lower; four participators and seven nonparticipating cells). (Right) SPW-R–associated firing ceases with gabazine application into stratum oriens (Upper). Firing persists with gabazine in stratum radiatum (Lower). (B) Raster plot of action potentials from representative cells. (Upper) Recruitment of a nonparticipating cell with diazepam in stratum oriens. (Lower) Participating cell application of gabazine into stratum oriens. Right shows typical coupled (red) and noncoupled (black) action potentials from the respective cells.

**Modeling Provides a Mechanistic Understanding of Pyramidal Cell Recruitment.** To put our cellular observations into a network context, we used multicompartment modeling of the CA1 network. According to the model, the very fast rhythm is generated within the axonal plexus, predominantly in smaller branches (38). Some of these spikes succeed in invading the main axonal trunk and also propagate antidromically to the soma, where they can be recorded as participating action potentials. Most of the plexus spikes do not, however, invade the main axonal trunk (39). The output of CA1 axons is also recurrently fed back onto local interneurons as well as CA1 basal dendrites (37), resulting in high-frequency synaptic currents in these structures. In addition, somatic inhibitory potentials at ripple frequency (8, 9) narrow the time window during which successfully propagating ripple-associated spikes do occur (46). In the model, however, synaptic inhibition is not the sole factor that controls somatic firing. Branch-point failures were even more important. Several other models have proposed that neuronal synchronization during fast oscillations can result from nonlinear dendritic integration (36) or excitatory–inhibitory feedback loops (33, 34). Although the present observations show a crucial role for excitatory–inhibitory interactions, they also provide clear evidence for antidromic spikes that are not captured by other models.

The proposed mechanism of spike generation has several implications for signal processing in the network. The occurrence of a participating spike in the soma indicates that the action potential has reached the main axonal trunk. Only then will it propagate to downstream structures (such as the subiculum) as well as back-propagate into the dendritic tree. A participating spike could, thereby, link plastic changes of excitatory synaptic inputs to a pyramidal cell with the main axonal outputs—although the antidromic spike is not caused by the synaptic inputs in question. This concept would be a variation on classical Hebbian synaptic plasticity, in which synaptic inputs cause the action...
much smaller amplitude (lines 4 and 5). In the model, perisomatic inhibition
was mediated by inhibitory inputs that alternate with rhythmic excitatory inputs of
3 Hz (Fig. S9). Rhythmic synaptic conductances of pyramidal cells are domi-
nated by gap junctions between pyramidal cell axons that
are connected by chemical and electrical synapses (
Materials and Methods).

A computer model of SPW-Rs reproduces cellular recruitment during
ripples. Multicompartment modeling of the CA1 network was used to gain
a mechanistic understanding of pyramidal cell recruitment during SPW-Rs.
The model includes CA1 pyramidal cells and three interneuron subtypes
connected by chemical and electrical synapses (Materials and Methods and
SI Materials and Methods). Generation of ~200-Hz oscillations (lines 1 and 2) is
based on gap junctions between pyramidal cell axons that fire at ripple
frequency during network events (bottom trace). Although basket cells fire
during most ripple cycles (~2 ms after pyramidal cell somata), only a minority
of pyramidal cells participate in individual network events (line 6). Thus, only
a few of the axonal spikes successfully invade the soma antidromically (line 3) (Fig. S9). Rhythmic synaptic conductances of pyramidal cells are domi-
nated by inhibitory inputs that alternate with rhythmic excitatory inputs of
much smaller amplitude (lines 4 and 5). In the model, perisomatic inhibition
potentials that, then, can strengthen those inputs. Interestingly, activity-dependent plasticity of ectopic spike generation has recently
been reported for hippocampal interneurons (21), indicating that axons might be more important for signal integration than previously thought.

In summary, we show that principal hippocampal neurons fall into two distinct classes with respect to local network activity. Members of oscillating assemblies show an unusual mechanism of
tropic action potential generation, whereas somatodendritic inhibition suppresses background activity in the network during SPW-R events. Our findings provide a mechanism of assembly formation in oscillating networks. They may be relevant for the study of SPW-R-associated memory consolidation in the hippocampus.

Materials and Methods
The study was conducted in compliance with German law and with the
approval of the state governments of Baden-Württemberg and Berlin. Experiments were performed on adult male C57Bl6 mice (4–12 wk). In experiments using α2H101R mice (ref. 32 has additional information on the
generation of this mouse line), animals of both sexes were used.

Slice Preparation. Brains of ether-anesthetized mice were removed and
cooled to 1–4 °C in artificial cerebrospinal fluid (ACSF) containing 124 mM NaCl, 3 mM KCl, 1.8 mM MgSO4, 1.6 mM CaCl2, 10 mM glucose, 1.25 mM NaH2PO4 and 26 mM NaHCO3 saturated with 95% O2/5% CO2 (pH 7.4 at
37 °C). After removal of the cerebellum and frontal brain structures, we cut
horizontal slices of 450 μm on a vibratome (VT 1000 S; Leica). Slices were
allowed to recover for at least 2 h in a Haas-type interface recording chamber at 35 ± 0.5 °C before starting the experiments. Most slices used for
recordings were from the middle part of the hippocampus. The procedures
differed slightly for slices that were used for recordings in a sub-
merged chamber (SI Materials and Methods) (24).

Electrophysiology and Labeling. Extracellular recordings from CA1 pyramidal
layer were performed with glass electrodes (tip diameter > 5 μm; filled with
ACSF). Potentials were amplified 100 times with an EXT 10–2F amplifier
(NPI Electronic), low pass-filtered at 3 kHz, and digitized at 5–10 kHz for offline analysis (1401 interface; CED). These parameters slightly differed
for extracellular recordings in the submerged condition (SI Materials and Methods) (24).

Whole-cell recordings were performed using a Multiclamp 700B amplifier
(Axon Instruments). Borosilicate glass electrodes (2–5 MΩ) were filled with
135 mM K-glucuronate, 10 mM Hepes, 2 mM Mg-ATP, 20 mM KCl, and 0.2 mM
EGTA, pH 7.2 (KOH). Cells were identified using infrared differential inter-
ference contrast video microscopy and characterized by de- and hyper-
polarizing current steps (200–1,000 ms). We included only cells showing
typical fast-spiking discharge characteristics of interneurons. Series re-

cistance Rs was monitored continuously throughout experiments; cells were
rejected if Rs was >20 MΩ or varied more than ±30% during recordings.

Potential indicated are liquid junction potential-corrected (calculated at
−14 mV). Intracellular (sharp electrode) and juxtacellular recordings were
performed in the vicinity of the extracellular electrode with a SEC-05 LX
amplifier (NPI Electronic) in bridge mode. Potentials were amplified 10 times, low pass-filtered at 8 kHz, and sampled at 20 kHz. Sharp electrodes for in-
tracellular recordings (OD at 1.5 mm) were filled with either 2 M KAc (pH
7.35; n = 153) or 1 M KCl (n = 14) and had a resistance of 60–110 MΩ. After
impalement, we injected negative current until the cell stabilized and then,
gradually reduced the current injection to zero. Bridge balance was re-
peatedly adjusted by optimizing the voltage response to small (~200 pA)

square current pulses. Offset potentials were determined and subtracted
for subsequent analysis (1401 interface; CED). These parameters slightly differed
for extracellular recordings in the submerged condition (SI Materials and Methods) (24).

Fig. 8. A computer model of SPW-Rs reproduces cellular recruitment during
ripples. Multicompartment modeling of the CA1 network was used to gain
a mechanistic understanding of pyramidal cell recruitment during SPW-Rs.
field
soma average
(inverted)
cross
-20 -15 -10 -5 0 5 10 15 20 20 mV
2 mV
participating soma
AMPA
GABA(A)
# participating cells
5 somata
axons
1000 axons
50 ms

Material and Methods.

# participating cells
5 somata
axons
1000 axons
50 ms

Material and Methods.

# participating cells
5 somata
axons
1000 axons
50 ms

Figure 8. A computer model of SPW-Rs reproduces cellular recruitment during
ripples. Multicompartment modeling of the CA1 network was used to gain
a mechanistic understanding of pyramidal cell recruitment during SPW-Rs.
The model includes CA1 pyramidal cells and three interneuron subtypes
connected by chemical and electrical synapses (Materials and Methods and
SI Materials and Methods). Generation of ~200-Hz oscillations (lines 1 and 2) is
based on gap junctions between pyramidal cell axons that fire at ripple
frequency during network events (bottom trace). Although basket cells fire
during most ripple cycles (~2 ms after pyramidal cell somata), only a minority
of pyramidal cells participate in individual network events (line 6). Thus, only
a few of the axonal spikes successfully invade the soma antidromically (line 3) (Fig. S9). Rhythmic synaptic conductances of pyramidal cells are domi-
nated by inhibitory inputs that alternate with rhythmic excitatory inputs of
much smaller amplitude (lines 4 and 5). In the model, perisomatic inhibition
helps to control discharge probability and spike timing during SPW-Rs (Fig.
56). Inset shows cross-correlation of the participating cell signal with the
field. Note that increased antidromic spike rates caused by tonic GABAergic
currents in stratum oriens (as suggested by our experimental findings) are
also fundamental for the organization of SPW-Rs in the model.
Electrical stimulation was performed with bipolar platinum/iridium wire electrodes that were located in the alveus or the Schaffer collaterals, respectively. Monopolar square pulses of 100 μs duration were delivered at a strength calibrated to evoke reliable action potential firing. Juxtacellular recordings were performed with glass electrodes (15–25 MΩ) filled with 3% (wt/vol) Biocytin (Sigma) in 0.5 M NaCl. After unit recording, individual cells were filled using the juxtacellular labeling method (47).

Drugs and Chemicals. Drugs were purchased from Sigma and RBI/Tocris, and the fluorescent dye fluorescein was purchased from Invitrogen. Pharmacological agents (stock solutions dissolved in ACSF) were either added to the bath solution (20 μM diazepam) or were applied locally by leakage from large extracellular glass electrodes (tip diameter ~ 15 μm). Local application electrodes were placed on the surface of the slice in stratum radiatum, stratum oriens, or the alveus of CA1 (20 μM diazepam, 10 μM gabazine, 5 μM muscimol, 100 μM 4-AP). In one experiment, picrotoxin (200 μM) was added to the intracellular solution for the selective manipulation of impaled cells.

Histological Procedures. Neurobiotin- or biocytin-filled cells were visualized using standard procedures (fluorescence and 3–3’ diamino benzidine tetrahydrochloride staining). Detailed methods regarding tissue processing are in SI Materials and Methods. Samples were analyzed using an Olympus BX61 microscope (Olympus). One cell (Fig. 4A) was reconstructed with the aid of Neurolucida software (Microbrightfield) using a 100x: 0.7 objective. Subsequent EM was carried out on four cells as described in detail elsewhere (48). Serial sections were cut with an ultramicrotome (EM UC6; Leica) and analyzed for synaptic contacts of biocytin-labeled axon terminals using a Phillips CM 100 electron microscope.

Data Processing and Analysis. Data were sampled with the Spike2 and Signal program (CED) and analyzed offline using custom written routines in Matlab (MathWorks). A detailed description is in SI Materials and Methods.

Statistical Quantities. The statistical quantities are given as mean ± SEM or as median and the first and third quartiles (P25 and P75). Parametric tests were used if groups passed a normality test, and otherwise, nonparametric statistics were used. A P value < 0.05 was regarded as significant.

Simulation Methods. We constructed a CA1 network model to explore possible physiological mechanisms for the generation of SPW-Rs and associated participating somatic action potentials. At this preliminary stage, we were interested only in basic cellular, synaptic, and gap junctional contributions to general features of the events, such as ripple shape, action potential timing, and site of origin of the participating action potentials; we did not attempt to make quantitative predictions concerning the precise values of synaptic and intrinsic membrane conductances or anatomical features. The style of modeling is as in the works by Traub et al. (49) and Roopun et al. (50) (a detailed description of the model is in SI Materials and Methods). The network model contains CA1 pyramidal neurons with branching axons (4000), fast-spiking basket cells (200), bistratified (dendrite-contacting, fast-spiking) interneurons (100), and axoaxonic cells (40); orioles-lacunomus molecular interneurons were omitted, because in vivo data suggest their silence during SPW-Rs (11), and other interneurons were omitted for the sake of simplicity. Preliminary simulations were performed, varying the synaptic and gap junctional conductances, synaptic connectivity, axonal branching patterns, and sites of axonal gap junctions.

The field potential was approximated using two methods. First, field potentials were computed using a weighted average of pyramidal cell transmembrane currents [details in the work by Traub et al. (49)]. Second, average values of pyramidal cell somatic voltages were inverted.

The simulation program, CA1Netx, was written in Fortran with mpi instructions for operating in a parallel environment and run on 20 processors of an AIX cluster at the IBM T. J. Watson Research Center. A fixed integration time step of 2 μs was used. Simulation of 250 ms of activity required about 1.25 h of central processing unit time. Copies of the code are available on request from R.D.T. (rrtraub@us.ibm.com).

Quantities Saved by the Simulation Program for Plotting and Analysis. The program saves a number of signals, including average values of pyramidal cell somatic voltages (inverted to resemble a field potential), somatic, axonal, and dendritic potentials in selected pyramidal cells and interneurons, average somatic potentials of interneuron populations, and the number of over-shooting pyramidal cell distal axons and somata.

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Supporting Information

Bähner et al. 10.1073/pnas.1103546108

SI Materials and Methods

Recordings Under Submerged Conditions. Submerged recordings were performed as described recently (1). Mice (both sexes, ages 4–8 wk) were anesthetized with an isoflurane vaporizer and decapitated. Brains were transferred to cooled (1–4 °C) standard artificial cerebrospinal fluid (ACSF) containing 119 mM NaCl, 2.5 mM KCl, 1.3 mM MgSO4, 2.5 mM CaCl2, 10 mM glucose, 1.0 mM NaH2PO4, and 26 mM NaHCO3 gassed with 95% O2/5% CO2 (pH 7.4 at 37 °C; 290–310 mosmol/L). Horizontal slices of 400 μm were cut on a microslicer (Vibroslice; Leica), stored in a modified Haas-type interface chamber, and allowed to recover for at least 2 h. Recordings were performed in standard ACSF at 32 °C in a submerged modified recording chamber perfused at high rate (5–6 mL/min). Glass microelectrodes (tip diameter ∼5–10 μm) were filled with ACSF before use. Extracellular signals were amplified 1,000-fold, filtered (Bessel; 2 or 4 kHz), and digitized at 5 or 10 kHz.

Intracellular (Sharp Electrode) Recordings. Input resistance and membrane time constant were obtained from a series of −200-pA pulses. Rm was measured as the ratio of the voltage deflection during the last 100 ms of the current pulse and the amount of injected current. Membrane time constant was determined using a monoexponential fit between pulse onset and 10 ms. Neurons were regarded as pyramidal cells if they showed an accommodating firing pattern on injection of positive current pulses and had typical action potential waveforms (Fig. S1). Some cells were filled with Neurobiotin (1% wt/vol; Vector Laboratories). Of these cells, 12 were nonparticipating and 5 were participating neurons. There were no obvious differences in cell morphologies between nonparticipating and participating neurons. In addition, three cells were filled during recording with KCl-containing electrodes. All of these neurons were classified as CA1 pyramidal cells by light microscopy.

Electrical Stimulation. Electrical stimulation was performed with bipolar platinum/iridium wire electrodes that were located in the alveus or the Schaffer collaterals, respectively. Monopolar square pulses of 100 μs duration were delivered at a strength calibrated to evoke reliable action potential firing. In a separate set of experiments, antidromically elicited spikes during and outside sharp wave ripple complexes (SPW-Rs) were compared (n = 6 cells) (Fig. S5E). Electrical stimulation during SPW-Rs was initiated when the local field potential in the CA1 region exceeded 0.25 mV. For antidromic stimulation outside SPW-Rs, pulses were applied 60 ms after the local field potential exceeding 0.25 mV (i.e., during the time when, normally, no spontaneous SPW-Rs occur).

Data Processing and Analysis. Data were sampled with the Spike2 and Signal program (CED) and analyzed offline using custom written routines in Matlab (MathWorks).

Analysis of field potentials. Sharp waves were detected after low-pass filtering of raw data at 50 Hz and finding local maxima with amplitude >120 μV within 30-ms time windows. This value corresponds to four SDs of event-free baseline noise (2), yielding stable and reliable detection of SPW-Rs (as confirmed by visual inspection of traces and detected events). Subsequently, SPW-Rs were analyzed with a continuous wavelet transform (complex Morlet wavelet) starting 33 ms before and ending 67 ms after the peak of the detected sharp wave. From this spectrogram, we extracted the leading ripple frequency of the oscillation at frequencies higher than 140 Hz. Ripple energy was defined as the area under the spectrogram at this detected frequency. Borders were set at 2 × SD of event-free baseline. In juxtacellular recordings, units were detected offline after high-pass filtering at 500 Hz and setting a threshold at 6 × SD of event-free baseline. Analysis of intracellularly recorded action potentials. Action potentials were analyzed using the following parameters: amplitude, maximal rate of rise, maximal rate of repolarization, full duration at half-maximal amplitude (FDHM), action potential threshold, and initial segment-SPW-R antidromic latency (IS-SD) latency. Amplitude was measured as the difference of the potential value at the peak of the spike and the resting membrane potential. Spike threshold was defined as the membrane potential at which the first temporal derivative of the potential exceeds 10 V/s (3). IS-SD latency was measured as the latency between the two peaks of the second temporal derivative of the action potential (4, 5).

Reversal potential of the negative prepotential. The reversal potential of the negative prepotential was calculated from amplitudes measured at five or more different membrane potentials. The intersection of a linear fit with the x axis (displaying the membrane potential) yielded the value of the reversal potential (example in Fig. S2H).

Synaptic input at the peak of SPW-Rs. Similarly, the reversal potential of intracellular correlates of SPW-Rs was obtained from the intersection of the linear fit of the peak amplitudes (at five different holding potentials) with the x axis (displaying the membrane potential). The increase in conductance during SPW-Rs was assessed by measuring subthreshold intracellular potentials before and during SPW-Rs and comparing the slopes of linear fits to current–voltage plots (6).

Synaptic conductance measurements. Intracellular potentials were recorded at five different holding potentials. For each SPW-R event examined, the following parameters were extracted: intracellular potential at SPW-R peak (Erest), mean membrane potential 80–40 ms before the SPW-R (Erest), and injected current amplitude (Iinj). Current was then plotted over time and fitted to linear functions for each current step and analyzed only sharp waves with amplitude values more than P10 (first quartile) and less than P90 (third quartile) for conductance measurements.

Erest is defined as the peak membrane potential. The increase in conductance during SPW-Rs was assessed by measuring subthreshold intracellular potentials before and during SPW-Rs and comparing the slopes of linear fits to current–voltage plots (6).

Discharge probability. The discharge probability of pyramidal cells and interneurons during SPW-Rs (mean ± SEM) was computed as follows. Event cross-correlograms of spikes and the peak of the sharp wave were constructed (±150 ms, 5 ms binning) for each cell. The y value of each bin was expressed as the percentage of spikes occurring in the entire 300-ms window centered on the sharp wave peak multiplied by the mean firing probability.
frequency. The latter was defined as the ratio of the number of all spikes recorded and the total recording time multiplied by 0.3. **Assessment of coupling strength.** Coupling strength of pyramidal cell firing to the field ripple was computed from event cross-correlograms of spikes and ripple troughs (+40 ms, 0.2 ms binning) (7). We calculated the percentage of a ripple cycle during which 50% of spikes occurred. Stable temporal correlations between cell firing and ripple oscillation phase (cycle length ~ 5 ms) result in prominent peaks in cross-correlograms (Fig. 1E). To quantify coupling strength, we constructed cumulative diagrams of action potentials with respect to one full ripple cycle (using the central peak of the cross-correlogram). A random distribution of action potentials with respect to ripples would result in 50% of spikes occurring within 50% of the full cycle time (i.e., 2.5 ms for a 200-Hz rhythm). Lower values (50% of spikes occurring in less than 50% of the ripple cycle) indicate a temporal correlation between spikes and individual ripples (Fig. 1E). Shuffled data from cross-correlograms between the nth spike and the (n + 1)th SPW-R resulted in a smooth Gaussian distribution, confirming the validity of the coupling index (Fig. 1E).

**Firing rates.** Firing rates were obtained from ≥10-min recordings, which were started at least 10 min after the cell had stabilized after impalement. Firing frequencies during or outside ripples were determined as the number of spikes that were phase-coupled to or occurred outside ripples, respectively, divided by the total current-free recording time. In some cells, we also evaluated the firing rate at different membrane potentials (Fig. 2 C and D and Fig. S2). The firing frequency during ripples was also expressed as the percentage of SPW-Rs on which spikes occurred (all ripple-entrained spikes divided by the number of SPW-Rs recorded). Most interneurons emitted more than one action potential during individual sharp waves. In these cases, a burst was counted as one spike to avoid overrating of the percentage value.

In experiments where we bath-applied diazepam, the effects of field potential parameters and firing rates were evaluated 30 min after wash in of the drug (≥10-min data trace). In the case of local applications, we binned the raw data (sections of 100 s) after positioning of the application pipette. We then analyzed firing during and outside ripples for each of these bins. If a consistent change (at least two subsequent 100-s intervals) of the firing rate was recognized, we measured the drug effect by determining the firing rate from a 10-min data trace starting from the first changed bin. Otherwise, we used the last 10 min of the recording in the presence of the drug to evaluate the firing rate.

**Tissue Processing. Fluorescence staining.** Slices containing cells filled with neurobiotin (intracellular recordings) were fixed in 4% paraformaldehyde (PFA) in phosphate buffer (PB) for at least 48 h (4 °C). Slices were embedded in 4% agar, sectioned at 70 μm on a vibratome (VT 1000 S; Leica), mounted on superfrost microscope slides (Menzel-Gläser), and stored at −20 °C. For staining, slices were permeated in methanol (−20 °C) for 10 min and then rehydrated in PBS at room temperature. Background fluorescence was minimized by 0.3 M glycine in PBS (30 min). After three washing steps, unspecific antibody binding sites were blocked with 1% BSA, 10% FCS, and 1% Triton-X in PBS. Cells were stained with an avidin-Alexa Fluor 488 conjugate (Invitrogen) at 4 °C overnight. Finally, slices were embedded in Moviol (Polyvinylalkohol 4–88; Fluka).

3-3′ Diaminobenzidine tetrahydrochloride staining. After juxtacellular labeling with biocytin, slices were fixed in 0.1 M PB containing 1% PFA and 2% glutaraldehyde for at least 24 h (4 °C). Subsequently, slices were rinsed five times in PB (10 min each). To block endogenous peroxidases, slices were transferred into phosphate-buffered 3% H2O2 in 0.1 M PB for 15–25 min. After washing in PB, sections were incubated in 2% Triton-X in 0.1 M PB for 1 h.

Thereafter, sections were incubated overnight at 4 °C in 1% Triton-X and biotinylated horseradish peroxidase conjugated to avidin (Vectastain Elite ABC-Kit; Linaris) in 0.1 M PB. Triton-X was omitted in sections to be processed for electron microscopic analysis. After incubation, sections were washed four times in PB and developed with the chromogen 3–3′ diaminobenzidine tetrahydrochloride (avidin biotin peroxidase method) under visual control using a bright-field microscope (BX51; Olympus) until all processes of the cell appeared clearly visible. The reaction was stopped by transferring the section into 0.1 M PB. After washing in the same buffer, slices were kept at 4 °C overnight in the same solution while shaking. To enhance the staining contrast, slices were postfixed for 1 h in phosphate-buffered osmium tetroxide (Merek) and counterstained in 1% uranyl acetate. After several rinses in PB, sections were flattened between a glass slide and coverslip and dehydrated through an ascending series of ethanol. After two 10-min washes in propylene oxide (Merek), slices were flat-embedded in Epon (Fluka) between coated glass slides.

**Fluorescence Microscopy of Local Applications.** Experiments with fluorescein were performed to assess the spread of locally applied substances in stratum radiatum or oriens, respectively (Fig. S8). Slices were imaged through a dry objective (PlanNe C 4×/0.1 NA; Olympus) using a custom-built microscope. Fluorescence was excited by a high-power light-emitting diode (LED) (470 nm; Rapp Optoelectronic) and recorded by a high-end charge-coupled device (CCD) camera (ImageEM C9100-13; Hamamatsu). Basal fluorescence (without dye) was subtracted from the measured intensity values (measured after 30 min of local application).

**Model. Properties of model CA1 pyramidal neurons.** The 88-compartment axon soma dendritic architecture was similar to the architecture described by Traub et al. (8), but we now use a 24-compartment axon with a 16-compartment initial segment and main trunk (total of 480 μm) and two 4-compartment side branches (80 μm each attached at 90 and 180 μm from the soma). The radius of the more distal axonal branch was different in each cell (0.225–0.245 μm), whereas the radius of the proximal branch was always 0.25 μm. We also performed an extensive series of simulations in which a nonbranching axon was used. We could not find synaptic connectivity parameters for which the action potential patterns matched the experimental findings (sparse pattern of antidromic action potentials and absence of large spikelets).

There was a branching apical dendrite and four equivalent basal dendrites. Each basal dendrite had two levels of symmetrical binary branching; hence, each basal dendrite contained seven (1 + 2 + 4) compartments, and there were 16 distal compartments.

The simulation of membrane conductances was modified from that simulation used for pyramidal neurons in the work by Traub et al. (9). Some specific parameters include Cm = 0.8 μF/cm², Rin = 50,000 Ω·cm² for soma/dendrites, Rm = 1,000 Ω·cm² for the axon, R1 = 200 Ω·cm for soma/dendrites, and R2 = 100 Ω·cm for the axon. Equilibrium potential for the leak current, VLE, was −70 mV, and equilibrium potential was −75 mV for GABA A (currents with modifications in some simulations); for K T, it was −85 mV, and for Na +, it was 50 mV. Bias currents of 0.2 nA were applied to somata and 0.006–0.021 nA were applied to axonal compartments (introducing axonal heterogeneity).

For the sake of simplicity, certain membrane conductances were removed completely or nearly completely: the slow after-hyperpolarization conductance, high-threshold gca (so that gK(C) also becomes small), axonal gK(MD), persistent gNa, and the K2 potassium conductance. The anomalous rectifier conductance was present at 0.1–0.2 mS/cm² in soma/dendrites, with equilibrium potential at −35 mV. Pyramidal cell behavior was, thus, dominated by transient gNa(F), the delayed rectifier gK(DR), and gK(A) (10).
Maximum $g_{Na}(V)$ conductance densities were 400 and 250 mS/cm$^{-2}$ in the soma and axon, respectively, 75 mS/cm$^{-2}$ in the most proximal dendrites, and 2 mS/cm$^{-2}$ elsewhere. Kinetics were modified from those kinetics used by Martina and Jonas (11): $m_{\infty} = (1 + \exp((-V - 32)/10))^{-1}$; $r_m = 0.025 + 0.14 \exp((V + 24)/10)$ ms (if $V < -24$ mV) and $\tau_m = 0.02 + 0.145 \exp((-V - 24)/10)$ ms (otherwise); $h_{\infty} = (1 + \exp((-V + 31)/15))^{-1}$; $\tau_h = 0.15 + 1.15 \times (1 + \exp(V + 31)/15)^{-1}$. Maximum delayed rectifier density was 700 mS/cm$^{-2}$ in the soma, 350 mS/cm$^{-2}$ in the proximal dendrites, and 10 mS/cm$^{-2}$ elsewhere. Kinetics were like the kinetics used by Traub et al. (9). Maximal $g_{K(A)}$ densities were 12.5-150 mS/cm$^{-2}$ in various portions of the axon, 4-5 mS/cm$^{-2}$ in the soma, 1.6-2.0 mS/cm$^{-2}$ in proximal dendrites, 2.5 mS/cm$^{-2}$ elsewhere [again with kinetics like the kinetics used by Traub et al. (9)]. The density of the $A$ type of $g_K$ along the main axonal trunk also was different between axons, varying by up to 50% (12, 13).

**Properties of model CA1 interneurons.** Individual basket bistratified cells and axoaxonic cells each had 59 compartments (for soma + dendrites + axon), and were simulated with the code used for fast-spiking interneurons in the work by Traub et al. (9). Bias currents of 0.00-0.02 nA were applied to somata to produce heterogeneity. Axoaxonic cells received a 10-ms, 0.6-nA somatic pulse before the beginning of a simulated SWP-R.

**Chemical synaptic connectivity:** How many synapses, where they are located, and time course of synaptic conductances. For the sake of simplicity, we modeled the CA1 network alone. Moreover, it is known that the isolated CA1 region is able to generate SWP-Rs (2, 14). The connectivity map was generated in such a way that connections were random and subject to constraints: (i) Each cell had a defined number of presynaptic inputs from every other type of cell. (ii) The inputs arrived at a defined subset of membrane compartments. (iii) There was a single release site per connection. Pyramidal cells received input from 10 other pyramidal cells onto distal basal compartments; this connectivity is somewhat less than that connectivity observed in the rat slice in vitro (one-way connection probability of 0.25% vs. ~0.5% in the literature) (15) because of the large number of pyramidal cells in this network. Recurrent excitatory postsynaptic currents in this model are, in any case, small (Fig. 8) and have little influence on network dynamics. Pyramidal cells also received input from 10 basket cells (to soma or proximal dendrites), 40 bistratified cells (to midapical or midbasilar compartments), and axoaxonic cells (to the axon initial segment).

Connectivity to each basket, each bistratified cell, and each axoaxonic cell is comparable: from 40 pyramidal cells (to middle dendrites), from 20 basket cells (to middle dendrites), and from 20 bistratified cells (to middle dendrites).

**AMPAR receptor-mediated unitary synaptic conductances have time course scaling factor $x \times \exp(-t/\tau)$, where $t$ is the time in milliseconds, $\tau = 1.2$ ms for contacts on pyramids and 0.8 ms for contacts on interneurons, and the scaling factor is 0.1 nS for contacts on pyramidal cells. The scaling factor is 2 nS for contacts on basket cells and 1 nS for contacts on bistratified cells and axoaxonic cells.**

**GABAA receptor-mediated unitary synaptic conductances** have time course scaling factor $x \times \exp(-t/\tau)$, where $\tau = 5$ ms for interneuron/pyramidal contacts (values up to 15 ms were tried) and $\tau = 2$ ms for interneuron/interneuron contacts (16). The scaling factor was 0.3 nS for basket/pyramid connections, 0.1 nS for bistratified/pyramid, and 0.05 nS for interneuron/interneuron (except that axoaxonic cells do not connect to interneurons).

**NMDA conductances in these simulations are small enough to be negligible.**

**Gap junction connectivity, locations, and properties:** interneuron–interneuron and pyramid–pyramid. Each basket cell is electrically coupled to a mean of 2.5 other basket cells, randomly chosen, at sites in proximal dendrites, with nonrectifying gap junctions of conductance of 1 nS. Likewise, each bistratified interneuron is connected to a mean of four others by gap junctions with corresponding properties. A xoaxonic cells did not have gap junctions.

Gap junctions were placed between homologous compartments of the more distal axonal branch. Connectivity was random (17). Each axonal gap junction was nonrectifying and when open, had a conductance of 9 nS, enough to allow a spike in one axon to induce a spike in another axon (18, 19). One axon coupled to a mean of 2.25 others, making the size of the large cluster ~89% of the population or about 3,560 neurons [as explained in the work by Traub et al. (17)]. The variations in $g_{K(A)}$ density and axonal geometry (see above) contribute to the variable patterns of retrograde conduction of action potentials that are initiated at the gap junction site. In baseline conditions, axonal gap junctions were shut; to evoke an SWP-R, all gap junctions were opened for 50 ms.

**Basal dendrites.** In addition, we performed an extensive series of simulations in which the basal dendrites of CA1 pyramidal cells were electrically active and presumed to be the source of participating spikes (as proposed in ref. 20). Basal dendrites seem more probable than apical dendrites, because the preponderance of recurrent synaptic excitation is in the basal dendrites (15). In these latter simulations, extremely large values of dendritic $Na^+$ conductance and excitatory synaptic conductances were required, and therefore, this avenue was not pursued.

**Additional details on axons.** Pyramidal cell distal axons generate spontaneous action potentials that are randomly and independently between axons and Poisson-distributed at a mean frequency of 2 Hz in each axon. Axon-triggered synaptic release cannot occur at rates >250 Hz.

Fig. S1. Intrinsic properties of CA1 pyramidal cells. (A) Discharge properties of a participating cell (Left) and a nonparticipator (Right). (B) Intrinsic properties are not different between both groups. (Left) Input resistance (participating cells: \( n = 23 \) cells; nonparticipating cells: \( n = 24 \) cells). (Center) Resting membrane potential (participating cells: \( n = 22 \); nonparticipating cells: \( n = 25 \)). (Right) Membrane time constant (participating cells: \( n = 20 \); nonparticipating cells: \( n = 19 \)).
Fig. S2. SPW-R–coupled spikes are not abolished by somatic hyperpolarization. (A) Example traces of an SPW-R–participating cell at different membrane potentials (Left, full trace; Center, enlarged view of the asterisk-marked spike; Right, first and second derivative of the asterisk-marked spike shown as dotted and solid lines, respectively). Spikes are present at resting potential (Top) as well as during mild (Middle) and strong (Bottom) hyperpolarization. Note polarity reversal of prepotential on hyperpolarization. The latency between the IS and SD component of the spike (as represented by the two peaks in the second derivative of the spike) increases as a function of membrane hyperpolarization. Somatic hyperpolarization, thus, delays but does not prevent somatodendritic invasion (SD component) of the axonal spike (IS component). (B) Plot of prepotential amplitude vs. membrane potential of the cell shown in A (reversal potential = −75.2 mV, R = −0.91). (C) Plot of IS-SD latency vs. membrane potential for spikes during (red circles) and outside (black circles) SPW-Rs of the cell shown in A.
Fig. S3. Action potential parameters for SPW-R–associated action potentials and spikes occurring outside network events. (A) Amplitude (SPW-R–coupled spikes are in red: 78.3 ± 1.9 mV, n = 20 cells; action potentials outside SPW-Rs are in black: 69.5 ± 2.2 mV, n = 17 cells; *P < 0.01 unpaired t test, Welch-corrected). (B) Full duration at half-maximal amplitude (spike width; not different). (C) Action potential threshold is shifted to negative values for SPW-R–associated spikes (in the text). (D) Maximal rate of rise (not different). (E) Maximal rate of repolarization (SPW-R–coupled spikes: 79.6 ± 2.7 V/s; action potentials outside SPW-Rs: 64.6 ± 4.3 V/s; *P < 0.01 unpaired t test, Welch-corrected).
Fig. S4. Action potentials during ripples show typical features of antidromic spikes. (A) Hyperpolarization of a participating cell reveals unchanged SPW-R–coupled spikes (Left), IS-SD breaks (Center, arrow), and partial spikes (Right). The latter are typical markers of antidromic action potentials. Note that the peak of the partial spike matches the potential of the notch in the same cell. Corresponding first and second derivatives are displayed below. (B) Corresponding phase plots of the spikes shown in A. (C) Overlay of the initial part of the three phase plots shown in B. Note identical potential trajectories of the initial part (which correspond to the IS component) 1, 2 that are independent from successful soma invasion.

Fig. 55. Characterization of evoked orthodromic and antidromic action potentials. (A) Evoked orthodromic (black) and antidromic (red) action potentials from a nonparticipating pyramidal cell that resembles spontaneously occurring spikes outside and during SPW-Rs, respectively. (B) Phase plot (dV/dt vs. V) of action potentials shown in A. The blue line represents resting membrane potential. (Left) Full action potential. (Right) Expanded trace of action potential initiation. The dotted line indicates threshold, defined as dV/dt = 10 V/s. Note the negative shift of threshold for the antidromic action potential. (C) Action potential parameters for evoked orthodromic and antidromic action potentials (n = 6 cells). (D) Plot of IS-SD latency for spikes evoked at different membrane potentials (same cell as in A). Action potentials fall into two separate clusters. Note that the IS-SD latency (Fig. S2) of antidromic spikes increases as a function of membrane hyperpolarization. (E Left) Evoked antidromic spikes in CA1 pyramidal cells during (red) and outside (pink) SPW-Rs and spikes after somatic depolarization or synaptic input (black). Note similar waveform of antidromic spikes during and outside SPW-Rs. (Center) Corresponding phase plots of antidromic spikes during (red) and outside (pink) SPW-Rs and orthodromic spikes (black). (Right) Spike threshold is equally shifted to negative values in antidromic spikes during (−74.8 ± 2.2 mV) and outside (−77.7 ± 2.2 mV) SPW-Rs compared with spikes elicited by somatic depolarization (−56.5 ± 1.5 mV; P < 0.05; n = 6). (F) Inhibition of somatic depolarization-induced spikes during SPW-Rs. Representative example (same cell as in E).
Fig. S6. Effect of perisomatic inhibition on antidromic spike invasion in the computer model. The computer model reproduces the experimental finding that blockade of perisomatic inhibitory conductances in individual pyramidal cells results in increased antidromic firing during SPW-Rs. In the model, pyramidal cells axons do fire at ripple frequency, but only few of the spikes invade the soma (Fig. S9). Note rhythmic EPSPs in participating and nonparticipating pyramidal cell.

A

control  diazepam

20 mV

-65 mV

0.4 mV

2 s

B

frequency (Hz)

20 ms

33

10

0

Fig. S7. Differential effects of diazepam at the cellular and field potential level. (A) Original traces of a participating cell (Upper) and field potential (Lower) before application of diazepam (Left) and 30 min after application (Right). Note enhanced ripple-associated spike rate in the presence of diazepam. (B) Averaged time frequency plots of SPW-Rs before and after the addition of diazepam from one experiment show similar characteristics of the events.
Fig. S8. Spatial distribution of locally applied fluorescein. (A) Fluorescein (1 mM) was applied for 30 min using glass electrodes with the same properties as those electrodes used during local drug applications (tip diameter ~ 15 μm) (Figs. 3, 6, and 7). Electrodes were removed before the fluorescence picture was recorded. Background fluorescence was removed by subtracting a picture that was taken before the application of fluorescein. (B) Fluorescence intensity along a line as indicated in (A). Blue indicates the application in stratum radiatum (two slices), and red indicates application in stratum oriens (three slices). Full width of half-maximum was 262 ± 55 μm (n = 5). Inset shows typical pyramidal cell morphology in realistic spatial relationships with areas of fluorescein diffusion.
Fig. S9. Firing behavior of pyramidal neurons and basket cells in a computer model of ripple oscillations. (A) During simulated SPW-Rs, the opening of gap junctions located on fine branches of the axonal plexus of CA1 pyramidal cells causes high-frequency firing of these structures (red). Propagation of this signal into the soma (black) most of the time fails at axonal branching points, leading to sparse somatic firing. Green indicates propagation of action potential through distal axon to subiculum. Blue indicates invasion of initial segment before somatic spike. (B) Basket cells receive strong, rhythmic excitatory conductances (line 3) originating from the CA1 axonal plexus (line 2). These conductances lead to high-frequency firing of basket cells (line 4) during ripples (line 1).