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Learning and other cognitive tasks require integrating new experiences into context. In contrast to sensory-evoked synaptic plasticity, comparatively little is known of how synaptic plasticity may be regulated by intrinsic activity in the brain, much of which can involve nonclassical modes of neuronal firing and integration. Coherent high-frequency oscillations of electrical activity in CA1 hippocampal neurons [sharp-wave ripple complexes (SPW-Rs)] functionally couple neurons into transient ensembles. These oscillations occur during slow-wave sleep or at rest. Neurons that participate in SPW-Rs are distinguished from adjacent nonparticipating neurons by firing action potentials that are initiated ectopically in the distal region of axons and propagate antidiromically to the cell body. This activity is facilitated by GABA_A-mediated depolarization of axons and electrotonic coupling. The possible effects of antidromic firing on synaptic strength are unknown. We find that facilitation of spontaneous SPW-Rs in hippocampal slices by increasing gap-junction coupling or by GABA_A-mediated axon depolarization resulted in a reduction of synaptic strength, and electrical stimulation of axons evoked a widespread, long-lasting synaptic depression. Unlike other forms of synaptic plasticity, this synaptic depression is not dependent upon synaptic input or glutamate receptor activation, but rather requires L-type calcium channel activation and functional gap junctions. Synaptic stimulation delivered after antidromic firing, which was otherwise too weak to induce synaptic potentiation, triggered a long-lasting increase in synaptic strength. Rescaling synaptic weights in subsets of neurons firing antidromically during SPW-Rs might contribute to memory consolidation by sharpening specificity of subsequent synaptic input and promoting incorporation of novel information.

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transform this depression to a long-lasting change. Alternatively, activation of nonaxonal GABAA receptors by muscimol could result in hyperpolarizing response (22, 23), and thus limit axonal depolarization and action potential propagation.

We therefore tested the effect of increasing SPW-Rs using a different method. Electrototoxic coupling through gap junctions located between axons of hippocampal projection cells are crucial for very fast oscillations, including neuronal synaptogenesis located between axons of hippocampal projection cells. Through gap junctions is sufficient to induce long-lasting reduction of synaptic strength. A mechanism of ectopic spike propagation requiring opening gap junctions is crucial for very fast oscillations, including neuronal synaptogenesis located between axons of hippocampal projection cells.

Fig. 1. Facilitation of spontaneous SPW-Rs leads to reduction in synaptic strength. (A) Representative field potential recording of spontaneous SPW-Rs recorded in stratum pyramidale of area CA1 (Top). Opening gap junctions with NH4Cl (10 mM) added to perfusate in the presence of glutamatergic antagonists kynurenic acid (3 mM), DL-2-Amino-5-phosphono pentanoic acid (APV) (50 µM), and (RS)-α-Methyl-4-carboxyphenylglycine (MCPG) (250 µM) increased the number and duration of spontaneous SPW-Rs in CA1 (Middle), which is prevented by gap-junction blocker carbenoxolone (100 µM) (Bottom). Corresponding expanded events are shown on the right. (B) LTD can be induced by NH4Cl in the presence of glutamatergic antagonists (dashed vertical bar) induced by addition of carbenoxolone (82.5 ± 9.1%, P < 0.05) and is blocked by addition of carbenoxolone (100 µM) (Bottom). Carbenoxolone and NH4Cl application is indicated by horizontal bar. Representative synaptic responses evoked and recorded in the stratum radiatum before (black) and after (gray) treatment are shown on the right.

Antidromic Stimulation of Axons Induces LTD. To determine whether LTD that develops after increasing SPW-Rs is mediated by synchronous antidromic action potential firing, we applied electrical stimulation to axons in the alveus to induce antidromic firing in CA1 neurons directly. Repeated theta-burst stimulation (TBS) delivered antidromically to axons evoked AP-LTD (LTD induced by antidromic action potentials (APs)) in CA1 stratum radiatum (Fig. 2A). When antidromic stimulation (AS) was delivered in the presence of glutamatergic antagonists, AP-LTD was not blocked (Fig. 2B), indicating that excitatory synaptic

Fig. 2. Widespread depression is induced by antidromic stimulation (AS). (A) Recordings in the stratum radiatum demonstrating L-LTP in response to orthodromic stimulation (OS) (182.5 ± 11.0%, P < 0.001), but LTD was induced by AS of axons in the alveus (AS, 53.6 ± 15.3%, P < 0.05). There were no changes in nonstimulated (NS) slices (103.5 ± 5.0%, P = 0.42). (B and C) Antidromic stimulation delivered to the alveus during application of glutamatergic antagonists (dashed vertical bar) induced AP-LTD in stratum radiatum (B, 67.3 ± 3.6%, P < 0.001) and in stratum oriens (C, 64.1 ± 9.2%, P < 0.05). Transient blockade of synaptic transmission had no long-lasting effect on synaptic responses in either stratum radiatum or stratum oriens (99.7 ± 5.9%, P = 0.15 and 96.3 ± 2.3%, P = 0.77, correspondingly). The insets show electrode placement (arrow indicates position of the test stimulation electrode) and representative synaptic responses before (black) and after (gray) repetitive stimulation. (Calibration, 0.5 mV, 5 ms.)

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transmission is not required for AP-LTD. It has been suggested that repetitive high-frequency discharges during TBS resemble SPW-Rs (25). To test other stimulation patterns also resembling spontaneously occurring SPW-Rs (26), the same total number of pulses as used in TBS was delivered antidromically in different patterns, as described in Fig. S2. Repeated high-frequency burst stimulation (HFBS) and low-frequency stimulation (LFS) delivered to the alveus induced robust AP-LTD in stratum radiatum (Fig. S2 B and C).

Antidromic stimulation in the presence of carbenoxolone prevented AP-LTD induction (Fig. S3A), consistent with the known role of electrotonic coupling in promoting synchronous firing of axons (13, 27, 28). AP-LTD was facilitated by AS in the presence of muscimol and furosemide to better depolarizing effects of GABA receptor activation to axons (Fig. S3B). Furosemide application did not affect synaptic responses in nonstimulated (NS) slices or the magnitude of AP-LTD (Fig. S3C). However, AP-LTD was not facilitated, but rather prevented, by AS delivered during application of muscimol in the absence of furosemide (Fig. S3B). Together the results demonstrate that increasing firing of antidromic action potentials associated with SPW-Rs evoked long-lasting depression of synaptic strength, which is independent of activation of glutamatergic receptors, requires gap junctions, and is facilitated by GABA<sub>A</sub>-mediated depolarization of axons.

**LTD Induced by AS Is Widespread Within the Dendritic Tree.** A hallmark of most forms of synaptic plasticity is the change in synaptic strength are specific to the activated synapse (29, 30), but since intracellular signaling would originate from the axon during antidromic action potential firing, we hypothesized that antidromic firing might induce changes in synaptic strength that are cell-wide. TBS of Schaffer collaterals, which provide synaptic input to apical dendrites of CA1 neurons, produced late form of long-term potentiation (L-LTP) in CA1 stratum radiatum as expected (Fig. 2A), which is shown to be input-specific (29). Similarly, stimulation of inputs to basal dendrites in stratum oriens reliably induces t-LTP restricted to this compartment, since no changes were observed in simultaneously recorded responses from stratum radiatum (Fig. S4A). Furthermore, when AS was delivered in the presence of glutamatergic antagonists, AP-LTD developed in two dendritic compartments of the CA1 region—stratum radiatum and stratum oriens—demonstrating wide-spread weakening of synapses following axonal stimulation (Fig. 2B and C). As expected, and in contrast to AP-LTD, no changes in synaptic strength in either of these dendritic compartments could be produced by orthodromic stimulation (OS) in the presence of glutamatergic antagonists (Fig. S5). We also recorded synaptic responses in the direct entorhinal cortex–CA1 pathway in response to AS. Although AS reliably induced AP-LTD in the stratum radiatum, no significant changes in synaptic responses recorded simultaneously in the stratum lacunosum–moleculare were observed (Fig. S6). These data suggest that a set of extrahippocampal inputs to the distal compartment of CA1 dendrites respond differently to AS from intrahippocampal inputs to CA1 from CA3 (31). In summary, we demonstrate that unlike orthodromically induced input-specific LTP, which depends on glutamate receptor activation, the alterations in synaptic strength induced by AS were widespread throughout the dendrite and did not require glutamatergic transmission.

**Mechanisms for AP-LTD Induction.** Local application of the sodium channel blocker TTX to axons during AS blocked AP-LTD in the presence of glutamatergic receptor antagonists (Fig. 3B and Fig. S3A), indicating that actively propagated action potentials are required for this form of plasticity. This is consistent with observations showing TTX-sensitive ectopic action potential firing during high-frequency oscillations in hippocampus (13, 32). We tested possible involvement of L-type voltage-dependent Ca<sup>2+</sup> channels (L-VDCCs) in AP-LTD induction. As in the presence of L-VDCCs antagonist nifedipine failed to evoke AP-LTD, whereas pharmacologically activating L-VDCCs with the agonist Bay K 8644 was sufficient to induce LTD (Fig. 3A). Combining AS with L-VDCC agonist treatment did not further depress synaptic strength beyond that induced by AS alone (Fig. 3A), suggesting common mechanisms acting upstream of L-VDCC activation. Together these data indicate that induction of AP-LTD is independent on glutamate receptor activation, but is facilitated by GABA<sub>A</sub>-mediated depolarization, and requires active propagation of action potentials, functional gap junctions, and L-VDCC activation. Thus, AP-LTD was induced by mechanisms implicated in SPW-R propagation and antidromic firing during SPW-Rs.

**Interactions Between AP-LTD and t-LTP.** The cell-wide scope of AP-LTD would have a significant influence on information processing in the subset of neurons experiencing antidromic firing, by down-scaling all synaptic weights in these neurons. We tested whether this would include depression of previously potentiated synapses and observed that AS depotentiated previously established t-LTP (Fig. 4A). As a result of decreasing strength of all synaptic inputs to the neuron after antidromic firing, weaker synapses would become subthreshold, and neurons providing these inputs would become functionally removed from the circuit. Such tuning of receptive fields and responses to sensory experience are a common feature of learning (33–35).

**Fig. 3.** Mechanism of AP-LTD induction. (A) Either 10 μM nifedipine or 10 μM Bay K 8644 were added to block or activate t-VDCCs. Application of nifedipine abolished AP-LTD (92.2 ± 19.7%, p < 0.01). In contrast, Bay K 8644 did not affect the magnitude of AP-LTD (65.9 ± 8.0%, p = 0.89) but was sufficient to induce long-lasting EPSP depression (76.1 ± 7.7%, p < 0.05, comparing to NS slices). Indicated drugs were applied to NS or antidromically stimulated slices (AS) during perfusion with glutamatergic antagonists (dashed vertical bar). Representative synaptic responses evoked and recorded in the stratum radiatum before (black) and after (gray) repetitive stimulation are shown on the right. (Calibration, 0.5 mV, 5 ms.) (B) Summary data of changes in synaptic strength 160–180 min after LTD induction in the presence of indicated drugs (*p < 0.05, comparing to AS slices; #p < 0.05, comparing to NS slices; +p < 0.05 and n.s. p > 0.05, comparing NS and AS slices treated similarly; Student t-test).
Another feature of learning is an increased sensitivity to novel stimuli and more facile incorporation of novel experiences into memory (36). Consistent with this idea, AP-LTD lowered the threshold for subsequent synaptic potentiation. Weak TBS of Schaffer collaterals in the stratum radiatum produces only a transient potentiation, but the same stimulus delivered after AS induced stable long-lasting LTP (Fig. 4B). As a consequence, synaptic strength is potentiated by subsequent synaptic input preferentially in ensembles of neurons that had fired antidromically.

The lower threshold for LTP induction after AP-LTD could be due to the increased probability of action potential firing induced by AS. To test this hypothesis, we performed whole-cell recordings, which demonstrated an increase in neuron firing rate, decrease in spike threshold, and leftward shift in the input-output function curve (Fig. 5A–F). This was further confirmed by extracellular recordings of the population spike of antidromic origin simultaneously with changes in synaptic responses. AP-LTD was accompanied by a long-lasting increase in the population spike amplitude of antidromic origin (Fig. 5B and Fig. S7A and D). There was a clear leftward shift of the EPSP–spike (E–S) curve for both orthodromic and antidromic spikes following AS, indicative of E–S potentiation (Fig. S7 B and C). Since the axonal (first) component of antidromic action potential (Fig. 5F and Fig. S7A and D) remained unchanged after AS, we would exclude that this potentiation is due to increased axonal excitability. Our extracellular and intracellular recordings rather support the view in which AS results in an increase of somatic excitability, thereby lowering the threshold for subsequent LTP induction (Fig. 4B). Thus, despite the decreased synaptic efficacy, the pyramidal cells firing antidromic action potentials have a higher probability of synchronous discharge, thus facilitating induction of stable long-lasting LTP.

Discussion

The results show that antidromic action potential firing that occurs during intrinsic high-frequency network oscillations (11, 12) induces a form of synaptic plasticity that reduces the strength of synapses in a cell-wide manner. From a neuronal information-processing perspective, action potential firing is a most reasonable reference for regulating synaptic strength in a neuron globally since the action potential threshold is the ultimate binary output of the neuron that results from all synaptic integration that is provided by thousands of afferent inputs to it (37). In these respects, a cellular process of regulating synaptic strength globally that is based not on glutamate receptor activation but instead on voltage-dependent sodium and calcium channels activated by axonal firing seems reasonable.

Despite decades of research, it is still unclear how postsynaptic action potential firing regulates strengthening or weakening of synapses (38). Our results show that whether the action potential is generated antidromically or orthodromically is a critical factor in neuronal plasticity that has not been considered previously. These findings are not in conflict with synapse-specific mechanisms of synaptic plasticity. Previous studies of spike-timing–dependent plasticity (STDP) have in general not explored the consequences of ectopic action potentials, and the studies typically examine synaptic plasticity on the temporal domain of tens of milliseconds from action potential firing (39). The comparatively slow time course of AP-LTD, developing tens of minutes after action potential firing, is typically outside the scope of STDP studies.

Axonal properties and ectopic action potential generation can be modified concomitantly with, or in response to, neuronal plasticity. The generation of SPW-Rs in hippocampus is facilitated by LTP (26) and after learning (40). Putative ectopic spikes occur in CA1 in vivo during exploration (41) and in the distal axon of interneurons in response to natural firing patterns in vitro (27). Similarly, as we observed for AP-LTD, previous reports show antidromic spiking persisted in the presence of glutamate receptor blockers (13, 27), is facilitated by GABAA-mediated depolarization of axons (11), and relies on electrotonic coupling through gap junctions (13, 27). Interestingly, activity-dependent down-regulation of the K+–Cl– cotransporter (KCC2) is a common mechanism for the reduction of GABAergic inhibition in certain physiological states (42), and following LTP induction (43), suggesting that GABA-mediated facilitation of ectopic action potential firing may be enhanced by a positive shift in ECl.

Membrane depolarization and t-VDCC activation can occur subsequently to GABA_A receptor activation (44), and involvement of t-VDCCs in different types of LTD is well documented in the literature (45–48). It has been suggested that a transient influx of Ca^{2+} into the postsynaptic cell is a sufficient trigger for inducing LTD (48). Prolonged/sustained mild depolarization provided by LTD-inducing stimulation could be optimal for relatively slow activation kinetics of t-VDCC (49). Our data are well in line, demonstrating dependence of AP-LTD induction on t-VDCC. We suggest that firing of action potentials of axonal origin during SPW-Rs may provide the optimal depolarization necessary for LTD induction.

Our data suggest that the widespread rescaling of synaptic weight in a neuron by SPW-R–associated antidromic firing would have multiple consequences on neuronal circuits. First, it would prevent saturation of synapses strengthened according to synapse-specific rules of learning that are based on coincident activity of the pre- and postsynaptic membrane (50). In agreement
uncoupling of neuronal ensembles to allow the emergence of new potentials associated with SPW-Rs may become subthreshold, thus removing these inputs functionally within a neuron is that weak synaptic inputs to the neuron would other important consequence of reducing synaptic weights globally with that, we found that antidromic firing is capable of downscaling synaptic weights in previously potentiated synapses. Another important consequence of reducing synaptic weights globally within a neuron is that weak synaptic inputs to the neuron would become subthreshold, thus removing these inputs functionally from the neuronal circuit. AP-LTD induced by antidromic action potentials associated with SPW-Rs may reflect a process of active uncoupling of neuronal ensembles to allow the emergence of new ensembles, which may be necessary to proceed from one cognitive state to another (51).

In support of this we observed that weak synaptic input to the neuron that would otherwise fail to induce long-lasting potentiation results in L-LTP after antidromic firing. These results showed that subsequent synaptic potentiation could be facilitated in neurons that had experienced antidromic firing. On a neuronal circuit level, these results suggest that neurons in functional assemblies that have participated in coordinate antidromic firing would become sensitized to subsequent weak synaptic input. As a consequence, new functional input would more easily strengthen synapses and become preferentially incorporated into existing circuits or schema (3, 52). Remarkably, the cellular mechanism for the increased sensitivity of neurons to subsequent weak synaptic input is also global, rather than a synapse-specific process—namely, increased excitability of the neuron after firing antidromic action potentials in coherent oscillations with other neurons. Altogether, our data suggest that active and dynamic circuitry modifications, induced by antidromic firing, may ensure the plastic state of synapses, which may underlie cognitive flexibility.

We suggest that firing of antidromic action potentials in subsets of neurons that participate in coherent activity during SWR at sleep and periods of quite restfulness would provide a cellular basis for SPW-R–associated memory replay and reactivation (3, 53). The reduced sensory input during such periods, when the brain is not processing external input, would be favorable for replay of neuronal assemblies induced intrinsically by antidromic firing and thus contribute to memory consolidation. Whereas, activation of sensory-evoked input to the CNS transmitted through orthodromic firing during exploratory activity is necessary to engage specific ensembles of CA1 neurons in a relatively labile form (54). The importance of two behavior stages in memory consolidation as well as their physiological sequences has been emphasized before (1). Our results expand this view and exploit concepts of frequency-dependent multiplexing to separate forms of plasticity induced by different modes of firing.

The synaptic plasticity induced by antidromic action potentials described here may have practical significance for human therapy. For example, synaptic depression induced by antidromic firing may provide insight on the neurological mechanisms by which deep brain stimulation, used as a treatment for Parkinson disease, obsessive–compulsive disorder, depression, and addiction (55), exerts effects on brain tissue.

Materials and Methods
For full details, see SI Materials and Methods.
All experiments were conducted in accordance with animal study protocols approved by the National Institutes of Child Health and Human Development Animal Care and Use Committee. Intracellular recording of intrinsic excitability and extracellular recordings of spontaneous SWR-Rs, field excitatory postsynaptic potentials (fEPSPs), and population spike were done in CA1 region of hippocampal slices prepared from adult (7–10-wk-old) male Sprague–Dawley rats.

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Supporting Information

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SI Materials and Methods

Preparation of Hippocampal Slices. Hippocampal slices (400 μm) were cut in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) 250 sucrose, 25 NaHCO3, 25 glucose, 4 KCl, 1.25 Na2HPO4, 1 CaCl2, and 3 MgCl2, pH 7.3. The slices were then kept at room temperature in a holding chamber filled with carbogen-bubbled ACSF, containing 125 mM NaCl instead of 250 mM sucrose and 2.5 mM CaCl2/1.5 mM MgCl2, for at least 2 h and then transferred to the interface recording chamber. In the recording chamber, slices were continuously superfused with carbogen-bubbled ACSF (2–3 mL/min) and maintained at 32 °C. For sharp-wave ripple complexes (SPW-Rs), recordings slices were kept at modified conditions (1): they were allowed to recover in interface recording chamber at 35 °C before starting the experiments and were superfused with ACSF at a higher rate (6 mL/min).

Stimulation Protocols and Extracellular Recordings. Spontaneous SPW-R recordings were performed from CA1 pyramidal layer. In the majority of experiments, field excitatory postsynaptic potentials (fEPSPs) were evoked by test stimulating electrode in the CA1 stratum radiatum and recorded in the same layer with glass pipettes filled with ACSF. In some experiments, additional recordings of fEPSP in the stratum oriens or population spike in the stratum pyramidale were performed. In these experiments, two recording electrodes were placed in a line perpendicular to the cell body layer so that each would tend to record responses from the same neuronal population. In addition, simultaneous recordings of fEPSP in stratum radiatum and stratum lacunosum–molecular were performed (Fig. S6). To obtain clear isolation of responses evoked by activation of temporoammonic input to dendrites in stratum lacunosum–molecular, dentate gyrus and the CA3 region were dissected in slices as described before (2).

Basal synaptic transmission was monitored at 0.1 Hz with pulses of 100 μs. Stimulation was delivered through a concentric bipolar stimulating electrode. Test stimulation strength was set to evoke orthodromic fEPSPs with an amplitude of 40–50% of the subthreshold maximum and to evoke a population spike of 70–80% of maximum amplitude. Repetitive theta-burst stimulation (TBS) was applied either orthodromically to the stratum oriens and stratum radiatum, or antidromically to the border of stratum oriens/alveus. Using the same pattern for antidromic stimulation (AS) that induces long-term potentiation (LTP) when delivered orthodromically (Fig. 24 Fig. S4A) provides the best comparison of different effects of ortho- and antidromic firing on synaptic strength. Orthodromically and antidromically applied TBSs are referred as orthodromic stimulation (OS) and AS correspondingly in all figure legends. TBS consisted of 10 bursts delivered at 5 Hz. Each burst consisted of four pulses delivered at 100 Hz. Duration of pulses was 0.2 ms. Three TBSs were applied every 30 s (weak TBS, ↑) and repeated three times in 5 min intervals (repetitive stimulation, ↑↑). To test other stimulation patterns resembling spontaneously occurring SPW-Rs (3), the same total number of pulses (360) as used in TBS was delivered antidromically, as described in Fig. S2: high-frequency burst stimulation (HFBS) and low-frequency stimulation (LFS). HFBS consisted of five bursts delivered at 1 Hz. Each burst consisted of eight pulses delivered at 200 Hz. Three HFBSs were applied every 30 s and repeated three times in 5 min. LFS consisted of 120 pulses delivered at 1 Hz and replicated 3 times in 5 min.

For Fig. 5I and Fig. S7 we recorded population spikes, which reflect the extracellular summation of action currents during synchronous action potential firing. In the hippocampal CA1 region, active dendritic invasion of action potentials (APs) ensures mixed contribution of somatic and dendritic currents to any extracellular location. The customary hippocampal population spike is considered a reliable index for the number of synchronously firing neurons (4) and so has been used to test changes in average neuron excitability in countless studies of physiological phenomena, such as synaptic plasticity. The population spikes were recorded by placing recording electrode in stratum pyramidale, and test stimulation was delivered either to stratum radiatum or alveus, resulting in spikes of orthodromic (Fig. S7B) or antidromic (Fig. S7C) origin correspondingly. Due to different origins, these spikes exhibit a distinct waveform (5). Two components were clearly present on antidromic population spikes (Fig. 5I and Fig. S7): fast axonal component, due to the activation of axon initial segment (was present during glutamatergic antagonists application, but blocked by injection of tetrodotoxin (TTX) to the axons) and larger and slower somatic component sensitive to glutamatergic blockade. Changes in amplitude of both antidromic population spike components were measured in response to AS (Fig. 5I and Fig. S7A and D). In addition, the amplitude of the soma-dendritic component of orthodromic and antidromic population spikes were plotted against the fEPSP slope recorded in the stratum radiatum at a range of stimulation currents, before and 90 min after AS. Resulting curves reflect changes in probability of action potential firing.

Pharmacological Treatments. In most experiments (as indicated by dashed vertical bar), AS was delivered in the presence of glutamate receptor blockers: AMPA/kainate receptors antagonist kynurenic acid (3 mM), NMDA receptor antagonist DL-2-Amino-5-phosphonopentanoic acid (APV, 50 μM), and group I/group II metabotropic glutamate receptor antagonist (RS)-α-Methyl-4-carboxyphenylglycine (MCPG; 250 μM) for 25–35 min (dashed vertical bar). After cessation of stimulation, this drug mixture was washed away. In nonstimulated slices, incubation with glutamatergic antagonists did not affect synaptic strength (nonstimulated, NS, P = 0.91, Kruskal–Wallis test; Figs. 2B and C and 4A and B).

Intracellular alkalinization, which enhances gap-junctions coupling (6), was achieved by adding ammonium chloride (NH4Cl; 10 mM) to ASCF. General gap-junction blocker carbamoloxide (100 μm) was added to the bath with other drugs as indicated. L-type Ca2+-channel agonist Bay K 8644 (10 μM) and L-type calcium channel blocker nifedipine (10 μM) were bath applied 10 min after the start of perfusion with glutamate antagonists and washed out in 30 min. GABA<sub>A</sub> receptor agonist muscimol (10 μM) and voltage-gated sodium channels blocker tetrodotoxin (TTX, 500 nM) were applied locally to the alveus/stratum oriens for 25 min by leakage from a large glass pipette. Fast Green FCF dye (1%) was included to the pipette to monitor the spatial restriction of drug application. Slices were allowed to equilibrate in ASCF containing loop diuretic furosemide (100 μM), which is known to block the function of the K+–Cl– co-transporter (KCC2) and reduce Cl− efflux (7, 8), for 40–60 min before recording of baseline. The depolarizing effect of GABA on axons is explained by higher intracellular chloride concentration in this cellular compartment due to decreased chloride efflux (7). Shifting a GABA-mediated response to more depolarizing values by blocking KCC2 function with furosemide may
therefore restrict the effects of muscimol to axons. Furosemide remained in the bath solution throughout the duration of the recording (9).

**Whole-Cell Recordings.** Whole-cell recordings were obtained from CA1 pyramidal cells. The recording electrodes (4–8 MΩ resistance) were filled with an internal solution containing (in mM): 125 K-gluconate, 20 KCl, 10 Hepes, 4 NaCl, 0.5 EGTA, 4 Mg ATP, 0.3 GTP, and 10 phosphocreatine (pH 7.2, 290 mOsm). Throughout the recording, intrinsic excitability was measured every 10 s using a constant amplitude depolarizing current step (~20 spikes during the baseline period and then remained constant throughout the recording. Measures of intrinsic excitability in response to antidromic TBS included spike rate, first spike latency, voltage threshold and after hyperpolarization. To construct the input/output function, depolarizing current steps were injected (1 s, 50–400 pA in 50 pA increments). The membrane potential $V_m$ was monitored during recording. Resting input resistance was calculated by measuring the steady-state voltage deflection in response to a hyperpolarizing pulse (~30 pA, 50 ms).

**Statistical Analysis.** Values are expressed as mean ± SEM of n (number of slices) experiments.


Values of long-term depression (LTD) or LTP were calculated as the changes in mean slopes of fEPSPs measured 160–180 min after stimulation in most cases and 70–90 min after stimulation for Fig. 4 A and B and Fig. S7 A. Normality of distributed data were verified by Kolmogorov–Smirnov test if $P > 0.15$. When data did not meet the normality test, a nonparametric Mann–Whitney test was applied for two group comparisons and Kruskal–Wallis test for comparison between more than two groups. Paired Student $t$ test was applied to normally distributed data to compare changes in synaptic strength within the same slice before and after treatment/stimulation. Unpaired Student $t$ test was applied to normally distributed data to compare changes in synaptic strength between stimulated and nonstimulated slices treated similarly.

Within- and between-cell comparisons were done as follows: each measurement of excitability was extracted from each test pulse and the average calculated over a 5-min period both immediately before and 30 min after AS. A two-tailed paired Student $t$ test or nonparametric Wilcoxon rank test was run to compare the response 30 min after the induction stimulus to the response before the induction stimulus for individual neurons. A difference was considered statistically significant if $P < 0.05$.
Fig. S1. Transient and long-lasting depression of synaptic strength by facilitation of spontaneous SPW-Rs. (A) Application of muscimol (10 μM) to the border of stratum oriens/alveus induced transient depression of synaptic responses (75.6 ± 6.0%, *P < 0.01, at the end of drug application), but was not sufficient to induce long-lasting changes (94.5 ± 9.3%, *P = 0.60 at 160–180 min). (B) Opening gap junctions with NH4Cl (10 mM) added to ACSF increased the frequency of spontaneous SPW-Rs in the CA1 and induced depression of synaptic responses (39.0 ± 11.4%, *P < 0.05, at the end of drug application), which was transformed to slow-developed LTD after NH4Cl washout (59.3 ± 19.9%, *P < 0.05 at 160–180 min). Representative field potential recording of spontaneous SPW-Rs recorded in stratum pyramidale of area CA1 are shown on the top. Representative fEPSPs recorded and evoked in stratum radiatum before (black) and after (gray) treatment at the time points indicated are demonstrated on the right. Time of drug application is shown by horizontal bar.
Axonal stimulation in different patterns is capable of inducing AP-LTD. (A) Representative fEPSP trace recorded during antidromically delivered TBS in the presence of glutamatergic antagonists (as for induction of AP-LTD shown on Fig. 2B). Note that this pattern resembles short (40 ms) high-frequency (100 Hz) bursts observed during SPW-Rs. (B) HFBS consisted of the same number of total pulses delivered at higher frequency (200 Hz), but at a lower occurrence rate (1 Hz). Antidromically delivered HFBS induced robust AP-LTD in stratum radiatum (55.5 ± 11.8%, P < 0.05). (C) Repeated continuous AS at LFS (1 Hz) was sufficient to induce AP-LTD in stratum radiatum (76.7 ± 10.1%, P < 0.05). (B and C) Antidromic stimulation was applied in the presence of glutamatergic antagonists (dashed vertical bar). The insets show representative traces recorded in stratum radiatum during alvear stimulation (red) or evoked by Schaffer collateral test stimulation before (black) and after (gray) AS. (Calibration, 0.5 mV, 100 ms or 5 ms.)
Fig. S3. Mechanisms for AP-LTD induction by AS are common with action potential firing during SPW-Rs. (A) AP-LTD is prevented by AS delivered in the presence of TTX (500 nM; injected to the axons, 94.6 ± 4.7%, \( P < 0.01 \)) or carbenoxolone (100 μM; bath applied, 100.2 ± 10.8%, \( P < 0.01 \)). (B) AP-LTD induction was facilitated by co-application of furosemide (100 μM, bath applied throughout the duration of recording) and muscimol (10 μM, local application to axons) (44.3 ± 7.8%, \( P < 0.001 \)). Application of muscimol during AS did not result in AP-LTD (83.6 ± 11.3%, \( P = 0.23 \)). (C) Furosemide did not affect synaptic responses in nonstimulated slices (95.1 ± 3.1%, \( P = 0.17 \)) or magnitude of AP-LTD (73.8 ± 8.4%, \( P = 0.42 \)). (A–C) Application of TTX, carbenoxolone, and muscimol is indicated by horizontal bar. Antidromic stimulation was delivered in the presence of glutamatergic antagonists (dashed vertical bar). The insets show representative traces recorded in stratum radiatum in response to Schaffer collateral activation before (black) and after (gray) AS. (Calibration, 0.5 mV, 5 ms.)
Fig. S4. Synaptic changes induced in stratum oriens. (A) Repeated OS of basal dendrites induced robust L-LTP in the stratum oriens (287.2 ± 57.2%, \( P < 0.05 \)), but no changes were detected in nonstimulated (NS) synapses recorded simultaneously in the stratum radiatum (111.8 ± 15.0%, \( P = 0.38 \)), confirming input specificity of induced plasticity. (B) Only a transient potentiation immediately after TBS (240.5 ± 30.3%, \( P < 0.01 \), 2 min after TBS), but no L-LTP (112.1 ± 25.2%, \( P = 0.67 \), 160–180 min after TBS) was detected in stratum oriens after AS of axons in the alveus (AS). The insets show electrode placement (arrow indicates position of the test stimulation electrode) and representative synaptic responses before (black) and after (gray) stimulation. (Calibration, 0.5 mV, 5 ms.)

Fig. S5. L-LTP induced by OS required activation of glutamatergic receptors. (A and B) No LTP or LTD could be induced in stratum radiatum (A, 105.2 ± 3.5%, \( P = 0.56 \)) or stratum oriens (B, 101.2 ± 9.4%, \( P = 0.87 \)) in response to OS while blocking glutamatergic transmission with kynurenic acid (3 mM), APV (50 \( \mu \)M), and MCPG (250 \( \mu \)M) (dashed vertical bar). The insets show electrode placement (arrow indicates position of the test stimulation electrode) and representative synaptic responses before (black) and after (gray) stimulation. (Calibration, 0.5 mV, 5 ms.)
Fig. S6. EC–CA1 synapses exhibit no AP-LTD. Although AS of axons induced robust AP-LTD in the stratum radiatum (66.1 ± 5.4%, \( P < 0.01 \)), no changes were detected in synapses recorded simultaneously in the stratum lacunosum–moleculare (95.8 ± 18.1%, \( P = 0.82 \)), suggesting special restriction of AP-LTD to intrahippocampal inputs. The insets show electrode placement (arrow indicates position of the test stimulation electrode) and representative synaptic responses before (black) and after (gray) stimulation. (Calibration, 0.5 mV, 5 ms.)
Fig. S7. Antidromically induced increase in excitability. (A) Synaptic depression induced by AS (75.7 ± 12.2%, P < 0.05, n = 7) is accompanied by a long-lasting increase in the population spike amplitude of antidromic origin (256.1 ± 47.2%, P < 0.01, n = 7). Representative traces of the antidromic population spike before (black) and after (red) application of glutamatergic antagonists indicate the pronounced axonal component (diamond), which remained unaltered by drug application and AS (106.4 ± 12.2%, P = 0.38). (B and C) Representative traces of population spike in response to test stimulation of Schaffer collaterals (B) or alveus (C) and fEPSPs in response to increasing stimulus intensity. The EPSP–spike (E–S) curve obtained from the same slices (n = 7) shown in A before (nonstimulated) and 90 min after the AS. Note the leftward shift of E–S curve, indicating that AS caused E–S potentiation. *P < 0.05, **P < 0.01, ***P < 0.001, Mann–Whitney test. Data are expressed as a percentage of the response elicited during baseline recordings by the maximal stimulus intensity. (D) Potentiation of antidromic population spike (filled triangle) induced in the presence of glutamatergic antagonists (dashed vertical bar) lasts at least for 3 h (261.4 ± 42.8%, P < 0.01, n = 8). Note that the first antidromic spike (diamond) was unaltered by drug application and remained stable over the recording duration (104.6 ± 11.3%, P = 0.71, n = 8). Schematic of experimental design showing electrode placement (arrow indicates position of the test stimulation electrode) and representative traces of the antidromic population spike and fEPSP before (black) and after (gray) stimulation. (Calibration, 0.5 mV, 5 ms.)