Supporting Information

Bukalo et al. 10.1073/pnas.1210735110

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 $NaHCO_3$, 25 glucose, 4 KCl, 1.25 NaH_2PO_4, 1 $CaCl_2$, and 3 $MgCl_2$, 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 $CaCl_2$/1.5 mM $MgCl_2$, 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–moleculare were performed (Fig. S6). To obtain clear isolation of responses evoked by activation of temporoamonic input to dendrites in stratum lacunosum–moleculare, 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. Stimulations were 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. 2*A* and Fig. S4*A*) 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. 5*I* 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. S7*B*) or antidromic (Fig. S7*C*) origin correspondingly. Due to different origins, these spikes exhibit a distinct waveform (5). Two components were clearly present on antidromic population spikes (Fig. 5*I* 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. 5*I* and Fig. S7 *A* and *D*). In addition, the amplitude of the somato-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. 2 *B* and *C* and 4 *A* and *B*).

Intracellular alkalinization, which enhances gap-junctions coupling (6), was achieved by adding ammonium chloride (NH_4Cl; 10 mM) to ASCF. General gap-junction blocker carbenoxolone (100 μM) was added to the bath with other drugs as indicated. L-type Ca^{2+}-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. $GABA_A$ 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 (1,000 ms, 100–200 pA). The amplitude was selected to induce ~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 $P < 0.05$.

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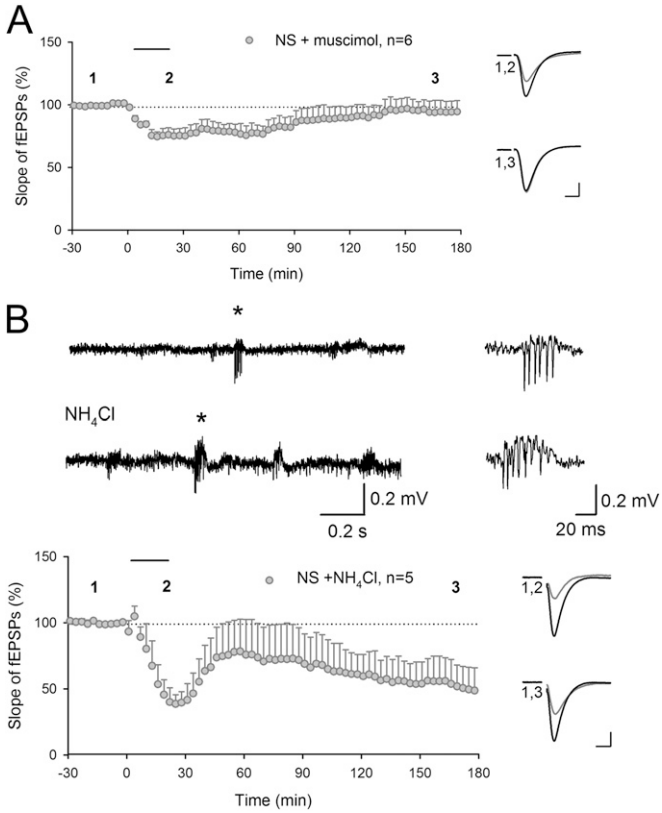

Fig. S1. Transient and long-lasting depression of synaptic strength by facilitation of spontaneous SPW-Rs. (*A*) Application of muscimol (10 μM) to the border of stratum oriens/alveus induced transient depression of synaptic responses (75.6 ± 6.0%, $P < 0.01$, at the end of drug application), but was not sufficient to induce long-lasting changes (94.5 ± 9.3%, $P = 0.60$ at 160–180 min). (*B*) Opening gap junctions with NH_4Cl (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.

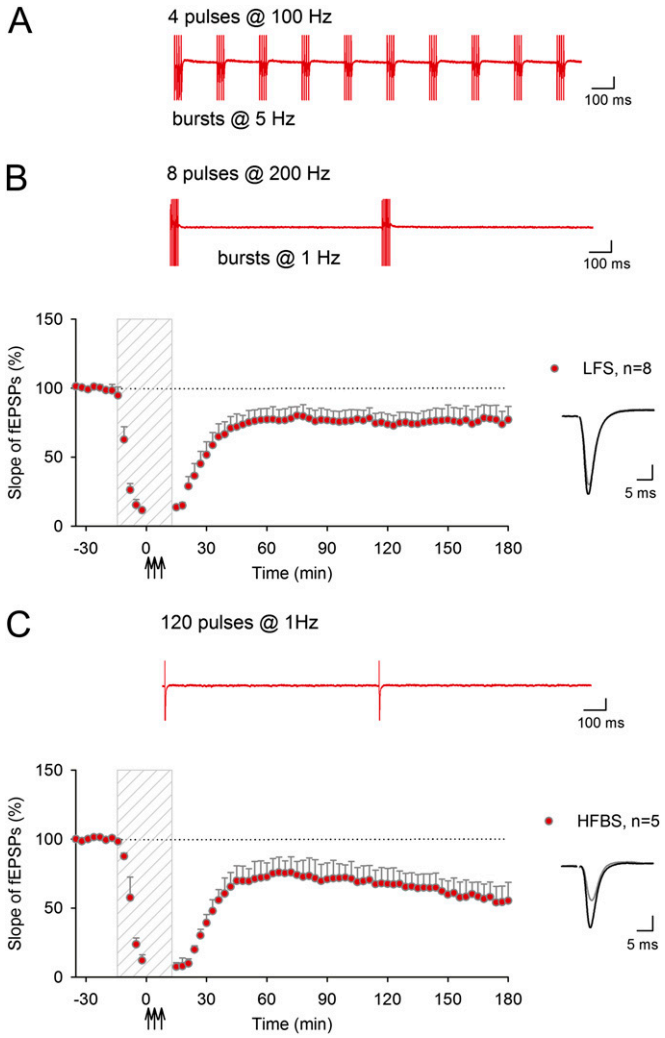

Fig. S2. Axonal stimulation in different patterns is capable of inducing AP-LTD. (*A*) Representative fEPSP trace recorded during antidromically delivered TBS in the presence of glutamatergic antagonists (as for induction of AP-LTD shown on Fig. 2*B*). Note that this pattern resembles short (40 ms) high-frequency (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.)

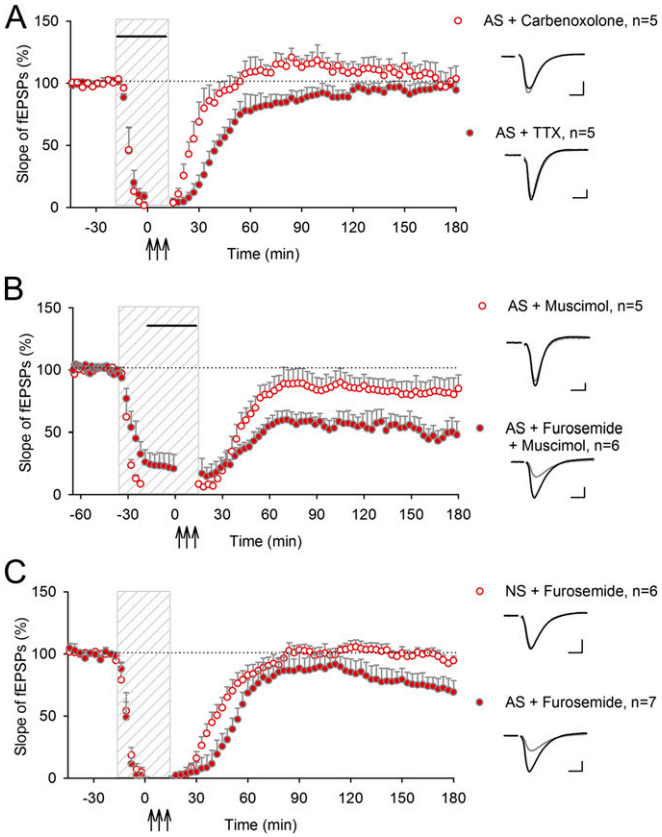

Fig. S3. Mechanisms for AP-LTD induction by AS are common with action potential firing during SPW-Rs. (*A*) AP-LTD is prevented by AS delivered in the presence of TTX (500 nM; injected to the axons, 94.6 ± 4.7%, $P < 0.01$) or carbenoxolone (100 μM; bath applied, 100.2 ± 10.8%, $P < 0.01$). (*B*) AP-LTD 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.)

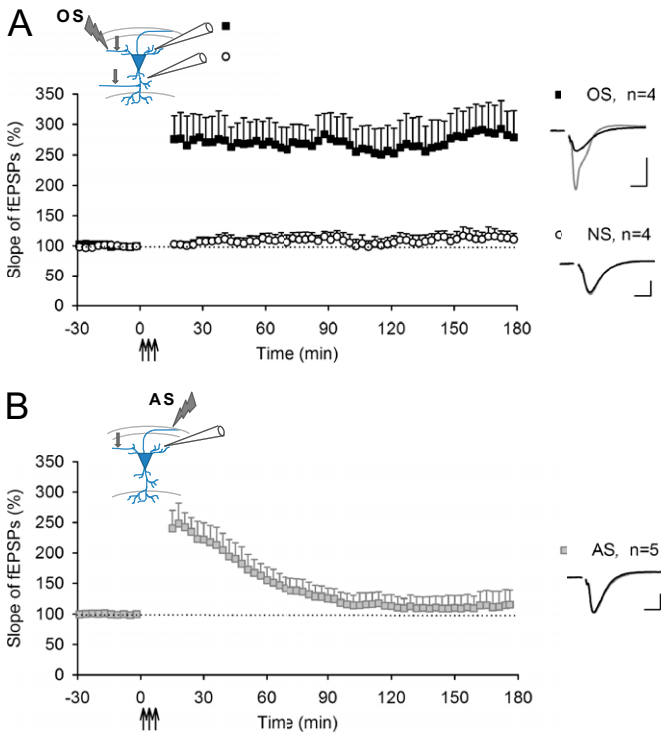

Fig. S4. Synaptic changes induced in stratum oriens. (*A*) Repeated OS of basal dendrites induced robust L-LTP in the stratum oriens (287.2 ± 57.2%, $P < 0.05$), but no changes were detected in nonstimulated (NS) synapses recorded simultaneously in the stratum radiatum (111.8 ± 15.0%, $P = 0.38$), confirming 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.)

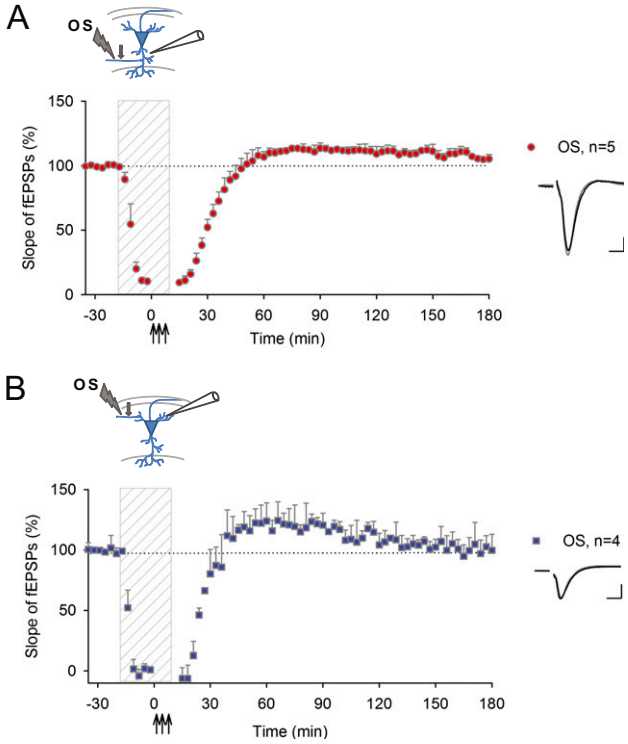

Fig. S5. L-LTP induced by OS required activation of glutamatergic receptors. (*A* and *B*) No LTP or LTD could be induced in stratum radiatum (*A*, 105.2 ± 3.5%, $P = 0.56$) or stratum oriens (*B*, 101.2 ± 9.4%, $P = 0.87$) in response to OS while blocking glutamatergic transmission with kynurenic acid (3 mM), APV (50 μM), and MCPG (250 μM) (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.)

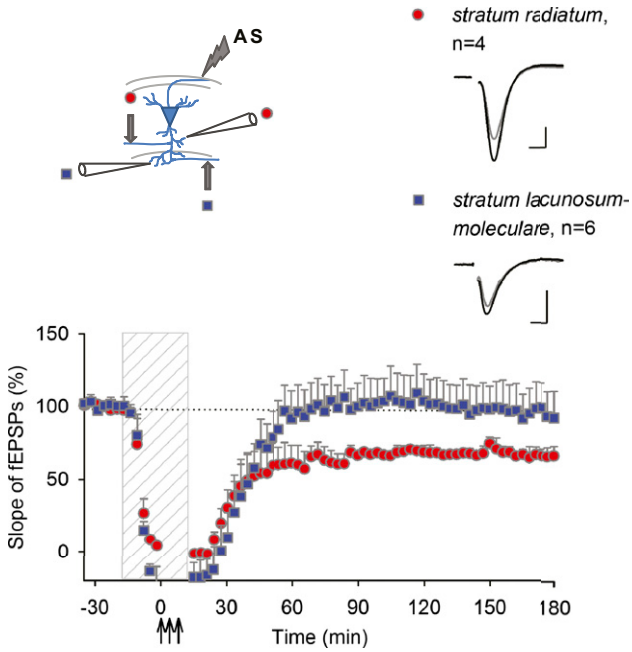

Fig. S6. EC–CA1 synapses exhibit no AP-LTD. Although AS of axons induced robust AP-LTD in the stratum radiatum (66.1 ± 5.4%, $P < 0.01$), no changes were detected in synapses recorded simultaneously in the stratum lacunosum–moleculare (95.8 ± 18.1%, $P = 0.82$), suggesting special restriction of AP-LTD 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.)

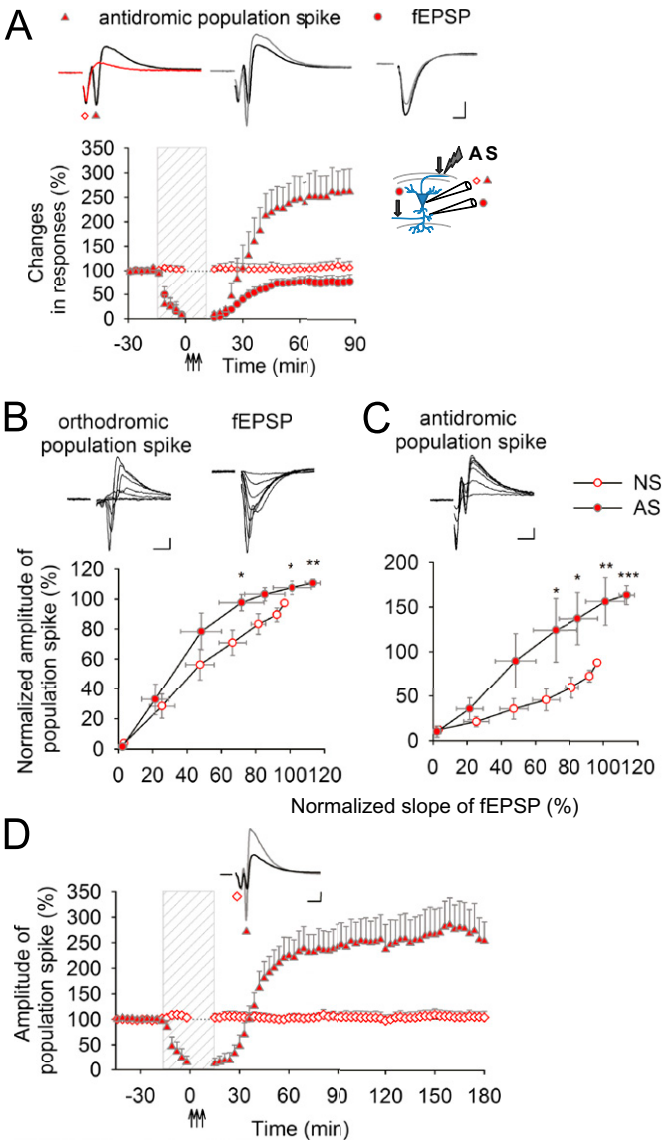

Fig. S7. Antidromically induced increase in excitability. (*A*) Synaptic depression induced by AS (75.7 ± 12.2%, $P < 0.05$, $n = 7$) is accompanied by a long-lasting increase in the population spike amplitude of antidromic origin (256.1 ± 47.2%, $P < 0.01$, $n = 7$). Representative traces of the antidromic 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.)