Supporting Information

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SI Materials and Methods

Electrophysiology. For electrophysiological experiments, individual slices were transferred from the holding chamber to an interface recording chamber, where they were continuously perfused with oxygenated artificial cerebrospinal fluid (ACSF) maintained at 29 °C and at a flow rate of 1.6–1.8 mL/min. ACSF composition was (in mM): 120 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 26.2 $NaHCO_3$, 1.0 NaH_2PO_4, 11.0 glucose, bubbled with a mixture of 95% O_2/5% O_2, pH 7.4. Extracellular field excitatory postsynaptic potentials (fEPSPs) were recorded in *stratum radiatum* of CA1 or in *stratum lucidum* of CA3 with glass electrodes filled with 3 M NaCl coupled to the input stage of a Warner IE-210 amplifier (Handen, CT). Stimuli were delivered to the Schaffer collateral/commissural afferents (for CA1 recordings) or to the dentate gyrus granule cell layer and hylus (for CA3 recordings) with bipolar concentric stainless steel electrodes. Basal stimulation frequency was either 0.033 Hz (in input–output and PPF experiments), 0.1 Hz (in 100-Hz potentiation experiments), or 0.2 Hz (in 50- and 20-Hz potentiation experiments; see below). Data were collected and analyzed on line (10-kHz sampling rate) by using pClamp software (Clampex, Axon Instruments). The presynaptic fiber volley was measured as the amplitude to the negative peak that appears after the stimulus artifact. The maximum slope of the rising phase of the fEPSP was calculated as a measure of synaptic response. Input–output curves were built by measuring the fiber volley and fEPSP of the responses evoked by stimulating afferent fibers with current intensities ranging from 10–500 μA. Paired pulse facilitation (PPF) was induced by applying pairs of stimuli at the following interstimulus intervals (in ms): 10, 25, 50, 75, 100, 150, 200, 250, and 300. Long-term potentiation (LTP) was induced by three 100-Hz trains of 100 pulses administered at 10-sec intervals. Mossy fibers (MF)-CA3 potentiation experiments were conducted in the presence of the NMDA receptor antagonist D,L-2-amino-5-phosphonovaleric acid (APV; 50 μM) perfused 15 min before HFS to avoid contamination with NMDA-dependent LTP of associational fibers. MF responses were identified by testing paired-pulse facilitation (>200%) at 50-ms interstimulus interval and by the selective reduction of synaptic responses caused by bath application of the group II metabotropic glutamate receptor agonists, DCG-IV [2*S*,2′*R*,3′*R*-2-(2′,3′-dicarboxycyclopropyl)glycine] (2 μM) at the end of the experiment in slices that were not subsequently used for immunocytochemical studies. When indicated, slices were incubated for 60–120 min before and during tetanic stimulation with ACSF containing U0126 to inhibit ERK activation (20 μM in DMSO, Promega). Statistical analysis was done by one-way Anova by using Origin 7.0 software (Origin). To quantify the decay kinetics of post-tetanic enhancement we fitted the time course data with a single exponential decay model, which best fit the time course in the first 6 min after HFS. The equation was as follows: y = A1*exp(−x/t1) + y0, where A1 is the amplitude, t1 is the time constant of decay and y0 is the offset. All data are presented as means ± SEM, with n indicating the number of slices.

Immunocytochemistry. Following electrophysiology, hippocampal slices were fixed in ice-cold paraformaldehyde [4% in 0.1 M phosphate buffer (PB)] with 1 mM sodium orthovanadate to block endogenous phosphatases. After overnight fixation at 4 °C, slices were rinsed, cryo-protected (10, 20, and 30% sucrose), and subsequently cut in 35-μm sections with a cryostat. Free-floating sections were blocked and permeabilized in 10% normal goat serum (NGS) and 0.5% TX-100 in PBS for 1 h, then incubated at room temperature overnight with the following primary antibodies: mouse anti-phospho-ERK (1:500, Sigma); rabbit anti-Syn-I (1:500, Chemicon); rabbit anti-VGAT (1:300, Synaptic Systems); guinea pig anti-VGluT1 (1:5,000, Chemicon). Double immunofluorescence was performed with simultaneous addition of the primary antibodies. After PBS rinsing, sections were incubated with secondary fluorescent antibodies (goat anti-mouse CY3, Jackson Immunoresearch; goat anti-rabbit Alexa 488, Invitrogen; goat anti-guinea pig Alexa 488, Molecular Probes) for 1 h, rinsed extensively, and mounted on gelatin-coated glass slides. No immunoreactivity was detected in control experiments in which primary antibodies were omitted. Moreover, treatment of hippocampal slices with the MEK inhibitor U0126 completely abolished pERK immunolabeling (Fig. 1*D*), thus confirming the specificity of the anti-phospho-ERK antibody used in this study.

Confocal Imaging and Data Analysis. pERK-positive MF axon terminals were distributed throughout the *stratum lucidum* of the CA3 region after HFS. The density of labeled terminals was greater closer to the dentate gyrus and progressively decreased toward the CA2 region of the hippocampal slice (see Fig. 1*B*). Thus, for quantitative analysis of ERK-positive MF terminals, we collected high-magnification confocal images always in the same area of the *stratum lucidum* (see white box in Fig. 1*B*).

To achieve an accurate sampling of pERK-immunopositive profiles throughout the depth of hippocampal slices, each of the 5–8 cryostat sections cut from a single slice was analyzed with a confocal microscope (Zeiss LSM-5 Pascal) by using sequential acquisition of separate channels to avoid fluorescence cross-talk (pinhole: 1.0 airy unit). For quantitative analysis of axon terminals, Z-series stacks of five consecutive confocal sections (512 × 512 pixels) spaced by 0.5 μm were acquired at 100× with a 1.5 digital zoom. Digital zoom 4 was used to resolve individual labeled profiles in hippocampal stratum radiatum of CA1 area. Confocal images were processed with Imaris software (Bitplane). Images from both channels were overlaid and background was subtracted when necessary. Immunolabeled MF terminals were counted manually and separately for each channel; colocalization of signals in overlaid images was confirmed in x, y, and z dimensions. Numerical data obtained from all sections of each slice were averaged. All data are presented as means ± SEM, and n indicates the number of slices. Statistical analysis was done by two- and one-way Anova (Origin 8, OriginLab). For presentation, digital micrographs were processed with the software Imaris (Bitplane). Files were imported into Adobe Photoshop (Adobe Systems), where images were cropped.

Western Blotting. In a series of experiments, hippocampal slices were frozen in liquid nitrogen 5 min after HFS, extracted in boiling SDS (1% plus 1 mM sodium orthovanadate) and subjected to SDS-polyacrylamide gel electrophoresis (SDS/PAGE) and quantitative immunoblotting using the following antibodies: anti-total ERK2 (Promega; 1:5,000); anti-phospho-ERK2 (Santa Cruz Biotechnology; 1:500); mouse anti-total Syn I (mAb 10.22; 1:1,000); rabbit anti-ERK-phosphorylated Syn I (phospho-sites 4,5; G526, 1:5,000). Immunoreactivities were revealed by using horseradish peroxidase-labeled secondary antibodies (BioRad) and the ECL western blotting kit (General Electric). The resulting chemiluminescence signal was quantitated with ImageQuant 400 imager (General Electric).

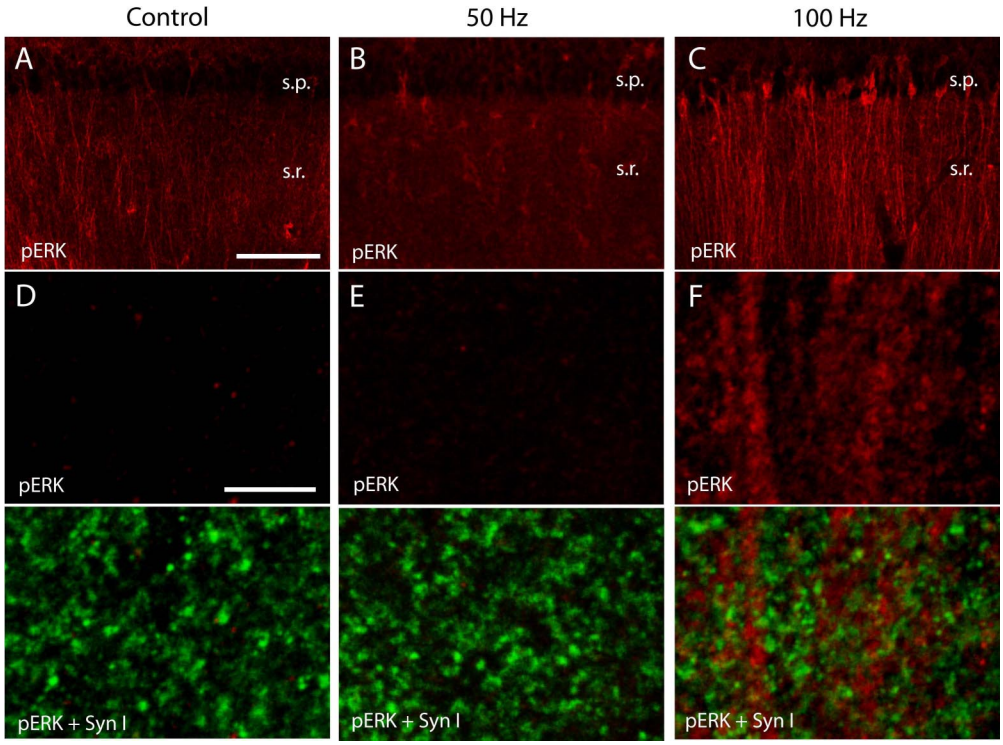

Fig. S1. 100-Hz HFS at the Schaffer collateral-CA1 pathway activates ERK at postsynaptic sites. (*A–C*) Micrographs show the distribution of immunostaining for pERK in CA1 *s. radiatum* of a representative slice that received baseline stimulation (control, *A*), and slices that were harvested 2 min after 50-Hz (*B*) or 5 min after 100-Hz (*C*) tetanization at CA3-CA1 synapse. As illustrated, pERK-IR is robustly increased in the soma and dendritic processes of CA1 pyramidal neurons after 100-Hz tetanization (*C*). (*D–F*) Confocal micrographs show double labeling for pERK (red) and Syn I (green) in CA1-*s. radiatum* of control (*D*) and tetanized (*E* and *F*) slices. In the 100-Hz potentiated slice, syn I-positive puncta are found in close apposition with dendritic segments that are pERK-positive, but no colocalization is visible (*F*). [Scale bar, 100 μm (*A–C*); 5 μm (*D–F*).]