Methods

All experiments were performed on organotypic hippocampal slice cultures. Briefly, hippocampi were dissected from 5-6 day-old, CO₂-anesthetized rat pups and cut into 375-μm thick transverse slices using a tissue chopper. Slices were plated onto polylysine-coated glass coverslips in 20 μl of chicken plasma, which was then coagulated with thrombin. The coverslips were placed into culture tubes with 750 μl of serum-containing media, and incubated in a roller-drum at 36°C. In order to reduce the proliferation of glial cells, slices were X-irradiated at the time of explantation and, antimitotics were applied at 3 μM after 3-5 days in vitro, followed by replacement with fresh culture media 24 hrs later. Slice cultures were incubated for ≥14 days in vitro in all experiments to allow for synaptic maturation.

Electrophysiology. EPSCs were recorded from CA1 pyramidal cells in response to stimulation of Schaffer collateral inputs from area CA3. For all experiments except augmentation, cultures were perfused with control extracellular saline containing (in mM): 137 NaCl, 2.8 KCl, 2.5 CaCl₂, 2.5 MgCl₂, 11.6 NaHCO₃, 0.4 NaH₂PO₄, and 5.6 glucose at approximately 1 ml/min at room temperature. The extracellular saline used for augmentation experiments was identical except the concentrations of CaCl₂ and MgCl₂ were changed to 1 mM and 4 mM respectively. Extracellular stimuli (−10 to −100 μA for 25 to 100 μsec) were delivered in stratum radiatum at the border between area CA3 and CA1 using a 2 MΩ patch pipette filled with extracellular saline. Postsynaptic responses were recorded using whole-cell recording techniques with an Axopatch 200B amplifier (Axon Instruments, Union City, CA), low pass filtered at 2 kHz, and digitized at 10 kHz. Patch pipettes (5 - 7 MΩ) were filled with (in mM): 140 KF, 10 KCl, 0.4 HEPES, 2 MgCl₂ and 1.1 EGTA (pH 7.2). Whole-cell recordings during which the access resistance exceeded 30 MΩ were discarded. The presence of
fluoride ions in the pipette solution blocked GABAergic inhibitory currents in the recorded cell\textsuperscript{3}. The omission of ATP had no adverse affects on the stability EPSC slope during the course of an experiment. NMDA receptors were blocked in all experiments with 40-80 µM DL-2-amino-5-phosphono-pentanoic acid (AP5) in all experiments to isolate non-NMDA receptor-mediated excitatory responses and to prevent the induction of long-term synaptic plasticity.

**Calcium imaging.** For Ca\textsuperscript{2+} imaging experiments, the internal solution was modified as follows (in mM): 140 K-gluconate, 10 KCl, 0.4 HEPES, 2 MgCl\textsubscript{2} and 0.012 CaCl\textsubscript{2} (pH 7.2). The internal solution also contained the Ca\textsuperscript{2+} indicator fluo-4 (100 µM) and fluorescent dye Alexa 568 (20 µM)(Molecular Probes, Eugene, OR). Pyramidal cells in area CA3 were filled by passing brief (1 s) hyperpolarizing current pulses under voltage-clamp ($V_m = -75$ mV). After 15-20 min of dye loading, axonal segments were located in area CA1 by searching for Alexa using a rhodamine filter set, 60x 1.0 n.a. water immersion Nikon objective, and imaged with a Hamamatsu ORCA-ER camera and Simple PCI software (C-Imaging). Once a well filled axon segment with multiple swellings, presumably corresponding to axonal boutons (see ref. 4), in a single focal plane was located, the recording was switched to current-clamp ($V_m = -70$ mV). Images were collected every 2 s with a FITC filter set and 0.5 s exposure time to minimize photobleaching. After collecting 10 images to establish the baseline, a train of action potentials (100 Hz for 4.5 s) was elicited by injecting 1.5 - 2 nA, 2 ms depolarizing current pulses. Ca\textsuperscript{2+} signals were measured in 3 - 6 regions of interest centered over the boutons on each axonal segment. The average intensity within each region of interest was measured, and the average value for each segment was used in the analysis. For each axonal segment, tissue fluorescence in an area of the background adjacent to the axon of interest was also measured and this value was subtracted from the axonal fluorescence at
each time point. To correct for photobleaching, the baseline values after background subtraction were fit with an exponential function and these values were subtracted from the background corrected data at each time point.

**Data Analysis.** Responses from 3 - 5 trials per cell were averaged. Data for each cell were normalized to the mean amplitude of EPSCs evoked at 0.2 Hz for 1 min prior to tetanic stimulation. Data are expressed as mean ± SEM. The maximum increase in EPSC amplitude was compared using repeated-measures ANOVA, Student’s paired, or unpaired t-tests where appropriate. Spontaneous EPSC frequency and amplitude distributions from individual cells were compared using the Kolmogorov-Smirnov (K-S) test. Data sets were considered significantly different if $P < 0.05$ for all tests except K-S, for which $P < 0.005$. PPR was calculated as mean EPSC$_2$/mean EPSC$_1$ $^5$. Phorbol 12,13 diacetate (PDAc) (Sigma, St. Louis, MO) was prepared as a 100x aqueous stock solution, and diluted in extracellular saline. BIS and STR were prepared in DMSO at 1000x the final concentration. STR, BIS, and CHEL (Tocris, Ballwin, MO) were added directly to the culture media in the test tubes and incubated for 1-3 hrs$^6$.

**References**


