Supporting Information

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SI Methods

Electrophysiology. A total of 87 hippocampal slices prepared from 80 male Wistar rats (6- to 7-wk-old) were used for electrophysiological recordings. All procedures were approved by guidelines from the Animal Committee on Ethics in the Care and Use of Laboratory Animals of Technische Universität Braunschweig. Briefly, after anesthetization using CO_2, the rats were decapitated and the brains were quickly removed and cooled in 4 °C artificial cerebrospinal fluid (ACSF). Transverse hippocampal slices (400-μm) were prepared from the right hippocampus by using a manual tissue chopper, and the slices were incubated at 32 °C in an interface chamber (Scientific System Design) (for details, see ref. 1). The ACSF contained the following: 124 mM NaCl, 4.9 mM KCl, 1.2 mM KH_2PO_4, 2.0 mM $MgSO_4$, 2.0 mM $CaCl_2$, 24.6 mM $NaHCO_3$, and 10 mM D-glucose, equilibrated with 95% O_2, 5% (vol/vol) CO_2 (32 L/h). In all experiments, three monopolar lacquer-coated, stainless-steel electrodes (5 MΩ; AM Systems) were positioned at an adequate distance within the stratum radiatum of the CA1 region for stimulating three separate distal synaptic inputs S1, S2, and S3 of one neuronal population (Fig. S1*A*). Pathway independence was tested by a paired-pulse facilitation protocol with an interpulse interval of 30 ms as described previously (2, 3). For recording the field excitatory post synaptic potentials (fEPSP) (measured as its initial slope function), one electrode (5 MΩ; AM Systems) was placed in the CA1 apical dendritic layer, and signals were amplified by a differential amplifier (model 1700; AM Systems). The signals were digitized using a CED 1401 analog-to-digital converter (Cambridge Electronic Design). After a preincubation period of 3 h, an input–output curve (afferent stimulation vs. fEPSP slope) was plotted before the experiments. For setting the test stimulus intensity (biphasic constant-current pulses), an fEPSP of 40% of its maximal amplitude was determined for both synaptic inputs S1 and S2. Late long-term potentiation (L-LTP) was induced using three stimulus trains of 100 pulses ("strong" tetanus, 100 Hz; duration, 0.2 ms per polarity; intertrain interval, 10 min). Early (E-)LTP was induced using a weak tetanization protocol consisting of one 100-Hz train (21 biphasic constant-current pulses; pulse duration per half-wave, 0.2 ms) (4, 5). For inducing depotentiation, low-frequency stimulation was applied 5 min after the induction of primed E-LTP in the same synaptic input using 250 impulses at a frequency of 1 Hz (6). The slopes of the fEPSPs were monitored online. The baseline was recorded for 60 min. Four 0.2-Hz biphasic constant-current pulses (0.1 ms per polarity) were used for baseline recording and testing at each time point (1).

Statistical Analysis. The average values of the slope function of the fEPSP (mV/ms) per time point were analyzed using the Wilcoxon signed-rank test (Wilcox test) when compared within one group or the Mann–Whitney *U* test (*U* test) when data were compared between groups. $P < 0.05$ was considered statistically significantly different. The nonparametric test was used because the sample sizes did not always guarantee a Gaussian normal distribution of the data per series (4, 7). *P* values at 60, 90, 240, 480, and 720 min were calculated in experiments wherever necessary. All statistical results for all measurements are shown in Tables S1–S5, labeled with the corresponding figure.

1. Sajikumar S, Navakkode S, Frey JU (2005) Protein synthesis-dependent long-term functional plasticity: Methods and techniques. *Curr Opin Neurobiol* 15(5):607–613.
2. Sajikumar S, Korte M (2011) Different compartments of apical CA1 dendrites have different plasticity thresholds for expressing synaptic tagging and capture. *Learn Mem* 18(5):327–331.
3. Sajikumar S, Korte M (2011) Metaplasticity governs compartmentalization of synaptic tagging and capture through brain-derived neurotrophic factor (BDNF) and protein kinase Mzeta (PKMzeta). *Proc Natl Acad Sci USA* 108(6):2551–2556.
4. Sajikumar S, Navakkode S, Frey JU (2007) Identification of compartment- and process-specific molecules required for "synaptic tagging" during long-term potentiation and long-term depression in hippocampal CA1. *J Neurosci* 27(19):5068–5080.
5. Sajikumar S, Navakkode S, Sacktor TC, Frey JU (2005) Synaptic tagging and cross-tagging: The role of protein kinase Mzeta in maintaining long-term potentiation but not long-term depression. *J Neurosci* 25(24):5750–5756.
6. Sajikumar S, Frey JU (2004) Resetting of 'synaptic tags' is time- and activity-dependent in rat hippocampal CA1 in vitro. *Neuroscience* 129(2):503–507.
7. Li Q, et al. (2014) Making synapses strong: Metaplasticity prolongs associativity of long-term memory by switching synaptic tag mechanisms. *Cereb Cortex* 24(2):353–363.

Fig. S1. (*A*) Diagrammatic representation of a hippocampal slice with three independent synaptic inputs, S1 (red), S2 (blue), and S3 (green), and one recording electrode located in the CA1 distal region. (*B*) Time duration of L-LTP (12 h) in S1 with two control recordings (S2 and S3) (n = 10). (*C*) Stability and persistence of potentiated inputs of S1 and S2 of the synaptic tagging experiments in Fig. 1*A*. All three inputs showed almost 100% stability at various time points. (*D*) Time duration of E-LTP (red filled circles) with a stable control (blue filled circles) (n = 6). DG, dentate gyrus; mf, mossy fibers; sr, stratum radiatum; STET, strong tetanization; WTET, weakly tetanized pathway. Error bars indicate ±SEM.

Fig. S2. (*A*) Priming of L-LTP by bath appliction of group I metabotropic glutamate receptors (mGluR) agonist (S)-3,5-Dihydroxyphenylglycine (DHPG; 20 μM) for 15 min and then washout for 15 min before the induction of L-LTP in S1 (red filled circles) prevented synaptic competition ($n = 7$). (*B*) Bath application of an irreversible mRNA synthesis inhibitor, actinomycin D (ACD; 25 μM), for 15 min alone and then coapplication with DHPG prevented the rescue effect. This is most likely due to the fact that mGluR stimulation leads to the synthesis of new plasticity-related proteins (PRPs) and this is inhibited under ACD application. This supports the view that PRP synthesis by transcriptional activation can prevent synaptic competition ($n = 5$). The experimental design to study synaptic competition was similar to that of Fig. 1*B*.

Table S1. Statistical analysis for Fig. 1

	Time, min							
	90		240		480		720	
	Wilcox test	*U* test	Wilcox test	*U* test	Wilcox test	*U* test	Wilcox test	*U* test
Fig. 1*A*								
S1	$P = 0.017$	$P = 0.001$	$P = 0.017$	$P = 0.001$	$P = 0.017$	$P = 0.002$	$P = 0.017$	$P = 0.001$
S2	$P = 0.017$	$P = 0.001$	$P = 0.013$	$P = 0.001$	$P = 0.009$	$P = 0.001$	$P = 0.013$	$P = 0.001$
S3	$P = 0.710$	NA	$P = 0.859$	NA	$P = 0.404$	NA	$P = 0.210$	NA
Fig. 1*B*								
S1	$P = 0.027$	NA	$P = 0.027$	NA	$P = 0.345$	NA	$P = 0.345$	NA
S2	$P = 0.027$	NA	$P = 0.018$	NA	$P = 0.176$	NA	$P = 0.456$	NA
S3	$P = 0.027$	NA	$P = 0.181$	NA	$P = 0.600$	NA	$P = 0.260$	NA
Fig. 1*C*								
S1	$P = 0.017$	NA	$P = 0.017$	NA	$P = 0.042$	NA	$P = 0.612$	NA
S2	$P = 0.017$	NA	$P = 0.017$	NA	$P = 0.042$	NA	$P = 0.859$	NA
S3	$P = 0.017$	NA	$P = 0.013$	NA	$P = 0.021$	NA	$P = 0.285$	NA
Fig. 1*D*								
S1	$P = 0.017$	NA	$P = 0.017$	NA	$P = 0.017$	NA	$P = 0.017$	NA
S2	$P = 0.017$	NA	$P = 0.013$	NA	$P = 0.017$	NA	$P = 0.017$	NA
S3	$P = 0.017$	NA	$P = 0.027$	NA	$P = 0.929$	NA	$P = 0.479$	NA

NA, not applicable.

Table S2. Statistical analysis for Fig. 2

	Time, min							
	90		240		480		720	
	Wilcox test	*U* test	Wilcox test	*U* test	Wilcox test	*U* test	Wilcox test	*U* test
Fig. 2*A*								
S1	$P = 0.013$	NA	$P = 0.017$	NA	$P = 0.013$	NA	$P = 0.017$	NA
S2	$P = 0.236$	NA	$P = 0.916$	NA	$P = 0.865$	NA	$P = 0.865$	NA
S3	$P = 0.017$	NA	$P = 0.017$	NA	$P = 0.017$	NA	$P = 0.017$	NA
Fig. 2*B*								
S1	$P = 0.018$	NA	$P = 0.027$	NA	$P = 0.027$	NA	$P = 0.311$	NA
S2	$P = 0.017$	NA	$P = 0.017$	NA	$P = 1$	NA	$P = 0.723$	NA
S3	$P = 0.017$	NA	$P = 0.021$	NA	$P = 0.176$	NA	$P = 1$	NA
Fig. 2*C*								
S1	$P = 0.017$	NA	$P = 0.013$	NA	$P = 0.017$	NA	$P = 0.017$	NA
S2	$P = 0.017$	NA	$P = 0.027$	NA	$P = 0.612$	NA	$P = 0.137$	NA
S3	$P = 0.017$	NA	$P = 0.013$	NA	$P = 0.017$	NA	$P = 0.013$	NA

Table S3. Statistical analysis for Fig. 3

	Time, min							
	90		240		480		720	
	Wilcox test	*U* test	Wilcox test	*U* test	Wilcox test	*U* test	Wilcox test	*U* test
Fig. 3*A*								
S1	$P = 0.022$	NA	$P = 0.079$	NA	$P = 0.043$	NA	$P = 0.5$	NA
S2	$P = 0.043$	NA	$P = 0.043$	NA	$P = 0.043$	NA	$P = 0.224$	NA
S3	$P = 0.043$	NA	$P = 0.043$	NA	$P = 0.079$	NA	$P = 0.445$	NA
Fig. 3*B*								
S1	$P = 0.043$	NA	$P = 0.043$	NA	$P = 0.138$	NA	$P = 0.360$	NA
S2	$P = 0.022$	NA	$P = 0.043$	NA	$P = 0.022$	NA	$P = 0.022$	NA
S3	$P = 0.043$	NA	$P = 0.022$	NA	$P = 0.043$	NA	$P = 0.043$	NA

Table S4. Statistical analysis for Fig. S1

	Time, min							
	90		240		480		720	
	Wilcox test	*U* test	Wilcox test	*U* test	Wilcox test	*U* test	Wilcox test	*U* test
Fig. S1*B*								
S1	$P = 0.017$	$P = 0.001$	$P = 0.017$	$P = 0.001$	$P = 0.017$	$P = 0.001$	$P = 0.017$	$P = 0.001$
S2	$P = 0.463$	$P = 0.742$	$P = 0.236$	$P = 0.492$	$P = 0.865$	$P = 1$	$P = 0.735$	$P = 0.443$
S3	$P = 0.595$	$P = 0.470$	$P = 0.330$	$P = 0.324$	$P = 0.498$	$P = 0.693$	$P = 0.371$	$P = 0.554$
	60		180		360			
	Wilcox test	*U* test	Wilcox test	*U* test	Wilcox test	*U* test		
Fig. S1*D*								
S1	$P = 0.018$	$P = 0.002$	$P = 0.018$	$P = 0.009$	$P = 0.221$	$P = 0.471$		
S2	$P = 0.652$	NA	$P = 0.589$	NA	$P = 0.345$	NA		

Table S5. Statistical analysis for Fig. S2

	Time, min							
	90		240		480		720	
	Wilcox test	*U* test	Wilcox test	*U* test	Wilcox test	*U* test	Wilcox test	*U* test
Fig. S2*A*								
S1	$P = 0.017$	NA	$P = 0.017$	NA	$P = 0.013$	NA	$P = 0.017$	NA
S2	$P = 0.013$	NA	$P = 0.013$	NA	$P = 0.017$	NA	$P = 0.013$	NA
S3	$P = 0.017$	NA	$P = 0.017$	NA	$P = 0.017$	NA	$P = 0.017$	NA
Fig. S2*B*								
S1	$P = 0.022$	NA	$P = 0.022$	NA	$P = 0.047$	NA		
S2	$P = 0.022$	NA	$P = 0.043$	NA	$P = 0.079$	NA		
S3	$P = 0.043$	NA	$P = 0.043$	NA	$P = 0.685$	NA		