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Correspondence and requests for materials should be addressed to M.H. (e-mail: mark@neuro.mpg.de).

Synaptic tagging and long-term potentiation

Uwe Frey & Richard G. M. Morris*

Federal Institute for Neurobiology, Gene Regulation and Plasticity, PO Box 1860, Brenneckestrasse 6, 39008 Magdeburg, Germany

* Centre for Neuroscience, University of Edinburgh, Crichton Street, Edinburgh EH8 9LE, UK

Repeated stimulation of hippocampal neurons can induce an immediate and prolonged increase in synaptic strength that is called long-term potentiation (LTP)—the primary cellular model of memory in the mammalian brain¹. An early phase of LTP

(lasting less than three hours) can be dissociated from late-phase LTP by using inhibitors of transcription and translation^{2–8}. Because protein synthesis occurs mainly in the cell body^{9–12}, whereas LTP is input-specific, the question arises of how the synapse specificity of late LTP is achieved without elaborate intracellular protein trafficking. We propose that LTP initiates the creation of a short-lasting protein-synthesis-independent ‘synaptic tag’ at the potentiated synapse which sequesters the relevant protein(s) to establish late LTP. In support of this idea, we now show that weak tetanic stimulation, which ordinarily leads only to early LTP, or repeated tetanization in the presence of protein-synthesis inhibitors, each results in protein-synthesis-dependent late LTP, provided repeated tetanization has already been applied at another input to the same population of neurons. The synaptic tag decays in less than three hours. These findings indicate that the persistence of LTP depends not only on local events during its induction, but also on the prior activity of the neuron.

Suppose that induction of early or late LTP on one input (S1) causes the transient activation of a local synaptic tag, but only repeated tetanization leading to late LTP initiates protein synthesis. Provided the creation of the putative tag is independent of protein synthesis, and plasticity-related proteins activated by LTP travel nonspecifically in the neuron, the proteins sequestered by a tag do not need to have been induced by the same event that sets the synaptic tag (Fig. 1).

To explore this idea, two independent synaptic inputs to the same neuronal population were stimulated in the CA1 region of hippocampal slices *in vitro* (Fig. 2a). Repeated tetanization of S1 (ref. 13) resulted in the establishment of late LTP (Fig. 2b, open circles) which lasted for at least 8 h, with minimal effect upon the second control input (Fig. 1a; Fig. 2b, S2, squares). A separate control experiment showed that consecutive induction of late LTP to S1 and S2 (1-h interval) was prevented by inhibitors of protein synthesis applied from 25 min before tetanization of input S1 until 1 h after initiation of LTP in S2 (Fig. 2c). The potentiation of the field

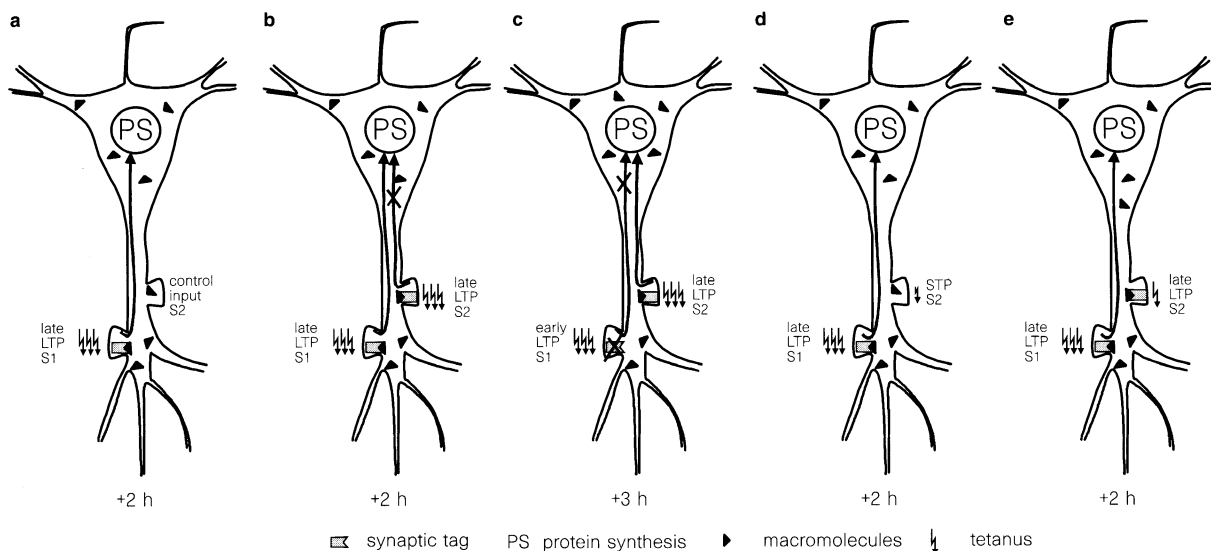


Figure 1 Possible occurrence of the synaptic tag (flag symbol) and proteins (triangles) after distinct stimulation of the two inputs at various time points after tetanization of synaptic inputs S1. **a**, Situation two hours after the induction of late LTP in S1. Intracellular arrows indicate the initiation of protein synthesis. Low-frequency control stimuli to S2 do not create a tag at this synaptic site. **b**, Late LTP is induced in S1 followed by application of a protein synthesis inhibitor that prevents the synthesis of macromolecules normally initiated by induction of late LTP in S2. Late LTP is nevertheless seen at S2 because its tag hijacks proteins synthesized by S1. The cross represents inhibition of protein synthesis. **c**,

Tetanization of S1 during protein synthesis inhibition transiently creates a tag that lasts for less than 3 h. Initiation of late LTP in S2 three hours after tetanization of S1 (and the removal of anisomycin) fails to rescue late LTP in S1. The tag created by tetanization of S1 must have decayed by the time the macromolecules associated with tetanization of S2 are available. **d, e**, Possible lack of a tag after STP-inducing stimulation and its association with early LTP after the induction of late LTP in S1. Very weak tetanization (small symbol in **d**) only leads to STP, whereas stronger single tetanization of S2 (**e**) results in late LTP.

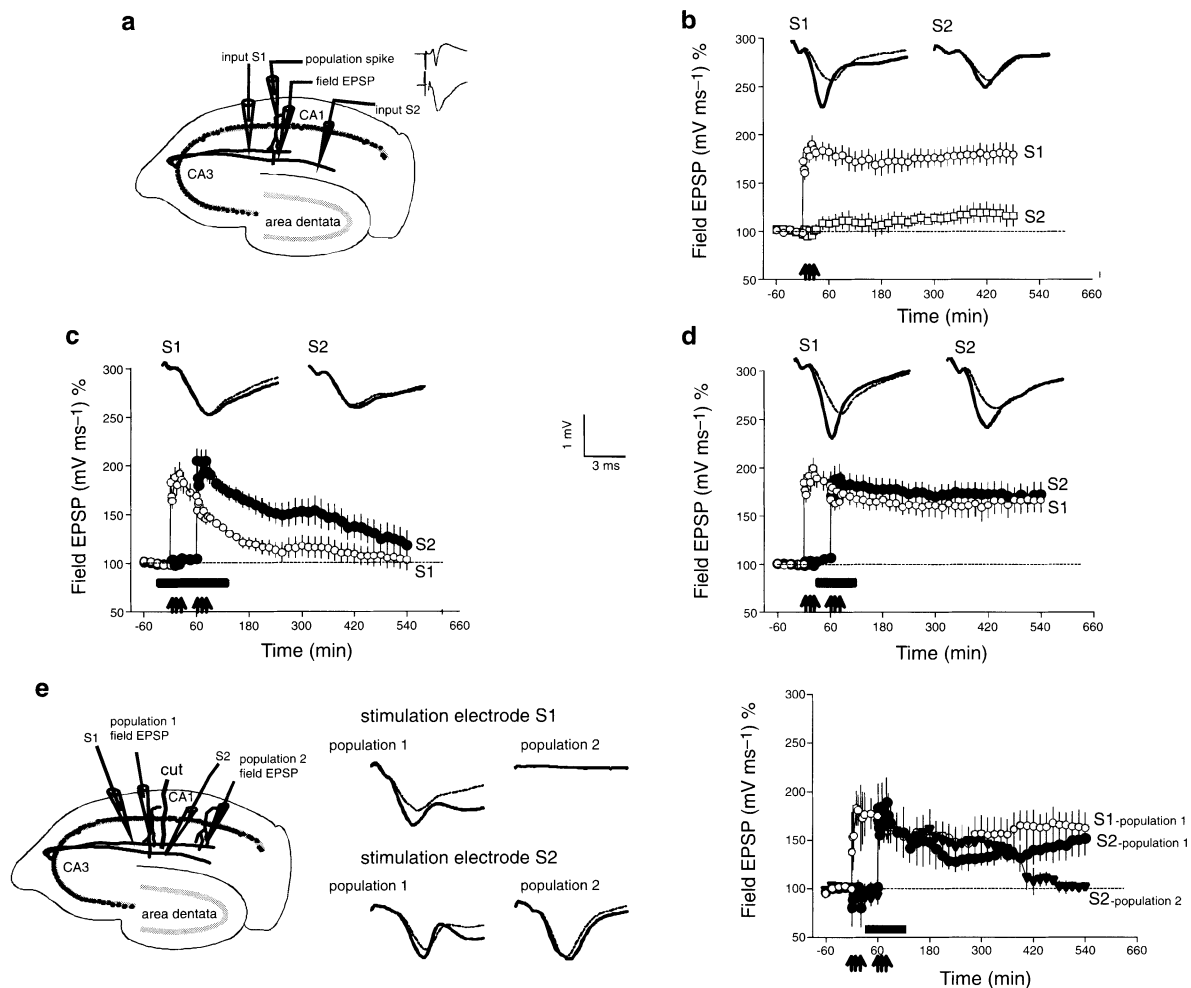


Figure 2 Induction of protein-synthesis-dependent LTP in the presence of a protein-synthesis inhibitor. **a**, Transversal hippocampal slice showing the positioning of the electrodes. The two independent inputs S1 and S2 to the same neuronal population, and the recording sites for the population spike amplitude and the field EPSP are shown. **b**, Tetanization of S1 (arrows) by a repeated-stimulation protocol revealed the establishment of late LTP (per cent change of field EPSP; open circles, 179.5 ± 10.7 and of the population spike amplitude $296.6 \pm 16.3\%$ of baseline after 8 h, $n = 6$; latter not shown). Low-frequency stimulation of S2 revealed stable recordings for the full 8 h after induction of LTP in S1 ($120.6 \pm 18.0\%$; $115.9 \pm 11.4\%$ respectively). Insets show representative recorded potentials immediately before (dotted line) and 7 h after tetanization (the same combination of potentials is shown for each experiment, calibration 1 mV per 3 ms). **c**, Induction of early LTP in two separate inputs after inhibition of protein synthesis revealed no late LTP in both measured parameters (field EPSP: in S1, $103.9 \pm 10.9\%$; S2, $118.1 \pm 14.3\%$ after 8 h, both statistically significantly different compared to control LTP in **b**; $n = 8$). **d**, LTP was induced in

S1 without drug application (open circles, $n = 7$). 35 min after tetanization of S1, anisomycin was added (bar), and 1 h after LTP of S1, input S2 was tetanized, but when protein synthesis was inhibited (filled circles). Paradoxically late LTP on S2 was still observed. **e**, Within-slice experiment showing the time course of LTP simultaneously in two different neuronal populations without or during protein synthesis inhibition. Left, positioning of the electrodes and the location of the microsurgical cut made to stimulate and record from two different neuronal populations 1 and 2. As for **d**, LTP was induced in S1 of population 1 (open circles, $n = 5$; field EPSP after 8 h $166.0 \pm 12.8\%$). Anisomycin (bar) was then applied and LTP was induced in S2 of population 1 and S1 of population 2. Late LTP in S2 of population 1 (filled circles; 8 h after tetanization: $145.2 \pm 15.9\%$) was rescued by LTP in S1 of population 1, whereas LTP in S2 of population 2 decayed as in **c** (filled triangles; $102.0 \pm 5.6\%$). The slightly different time course of LTP in S2 population 1 compared to **d** might be due to suboptimal stimulation parameters for this particular input (see Methods).

excitatory postsynaptic potential (EPSP) (and population spike; not shown) declined in both inputs as described previously³.

In the first key set of experiments, LTP in input S1 was induced (Fig. 2d, open circles) and anisomycin added to the bath medium 35 min later (a time when the protein-synthesis inhibitor is ineffective in influencing late LTP in this input⁵). Twenty-five minutes later, S2 was tetanized, but this time during the inhibition of protein synthesis (Fig. 2d, filled circles). The establishment of late LTP in S2 was normal, suggesting that proteins synthesized by LTP in S1 also allow the induction of late LTP in S2 (Fig. 1b). Similar results were obtained using a structurally different inhibitor of macromolecule synthesis (emetine; see Methods).

The paradoxical induction of protein-synthesis-dependent LTP during inhibition of protein synthesis was supported by a further within-slice experiment. Stimulating electrodes were positioned in stratum radiatum to activate simultaneously two independent inputs in one neuronal population and one input to a second, independent neuronal population (Fig. 2e). One electrode was positioned to evoke only potentials in S1 of population 1 (Fig. 2e, right panel, open circles) by means of a microsurgical cut through most of the Schaffer collaterals between the two populations recorded. The second electrode was positioned so that it simultaneously stimulated population 2 (Fig. 2e, filled triangles) and input S2 of population 1 (filled circles in Fig. 2e). Late LTP was first

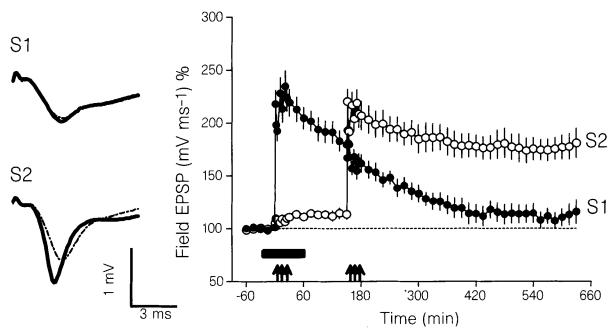


Figure 3 Transient occurrence of the putative synaptic tag. Experimental conditions were the same as for Fig. 2a. When anisomycin (bar) was applied 25 min before induction of LTP on S1 until 1 h afterwards (filled circles; $n = 6$), late LTP was prevented. After removal of the drug, tetanization of S2 (open circles) was made 2 h and 30 min after tetanization of S1. The persistence of late LTP on S2 was unaffected (field EPSP in S2: $166.2 \pm 12.3\%$ after 8 h). The tag created by tetanization of S1 must have decayed by the time the macromolecules associated with tetanization of S2 are available.

induced in input S1 of population 1 (Fig. 2e, open circles) and anisomycin applied 35 min later. Subsequent tetanization through stimulation electrode 2 resulted in late LTP in input S2 of the already activated neurons in population 1, but induced only a decaying early LTP in the independent population 2. This result strongly supports our proposition of a localized presence of proteins synthesized by LTP of input S1 in population 1 which could be hijacked by the proposed synaptic tag of input S2 to that population, but which are absent in the 'virgin' neuron population 2 because of the simultaneous inhibition of protein synthesis.

Is the putative synaptic tag permanently or transiently created after LTP induction? If transient, a time window should exist for the coincidence of the activated synaptic tag and newly synthesized proteins. To investigate this possibility, S1 was tetanized in the presence of anisomycin (Fig. 3, filled circles). The drug was then washed out for 2.5 h before S2 was tetanized (Fig. 3, open circles). Late LTP was induced in S2 and maintained for 8 h, but the LTP in S1 continued to decline to baseline. This finding suggests that proteins synthesized by tetanization of S2 about 3–4 h after LTP induction in S1 are unable to stabilize S1's potentiation. The putative synaptic tag is therefore transient, lasting no more than 2–3 h (Fig. 1c). This time window resembles the phase of LTP during which activated synapses are unable to respond with additional potentiation following a second tetanus¹⁴. Further tetanization can only induce additional potentiation during late LTP. The ability to react with further synaptic plasticity after prior induction of LTP may depend on the resetting of the proposed synaptic tag.

If the input specificity of late LTP is determined by the synaptic tag and its persistence by the availability of relevant proteins, the question arises whether short-term potentiation (STP) can be transformed into late LTP by prior induction of late LTP in another input to the same neuronal population. As late LTP normally requires repeated tetanization¹⁵, a weak single tetanus was used (see Methods) to induce a potentiation lasting for about 1–2 h (STP; Fig. 4a). Prior induction of late LTP in a first input S1 (Fig. 4b, open circles) did not prolong the potentiation of STP in S2 (Figs 4b, filled circles, and 1d). In the second protocol, a stronger but still single tetanus was applied to S2 that normally elicits early LTP lasting for 3–5 h (Fig. 4c) and corresponding to the stage named 'LTP1' in the model of ref. 1. It could be transformed into late LTP by prior induction of late LTP in another input (Figs 4d and 1e).

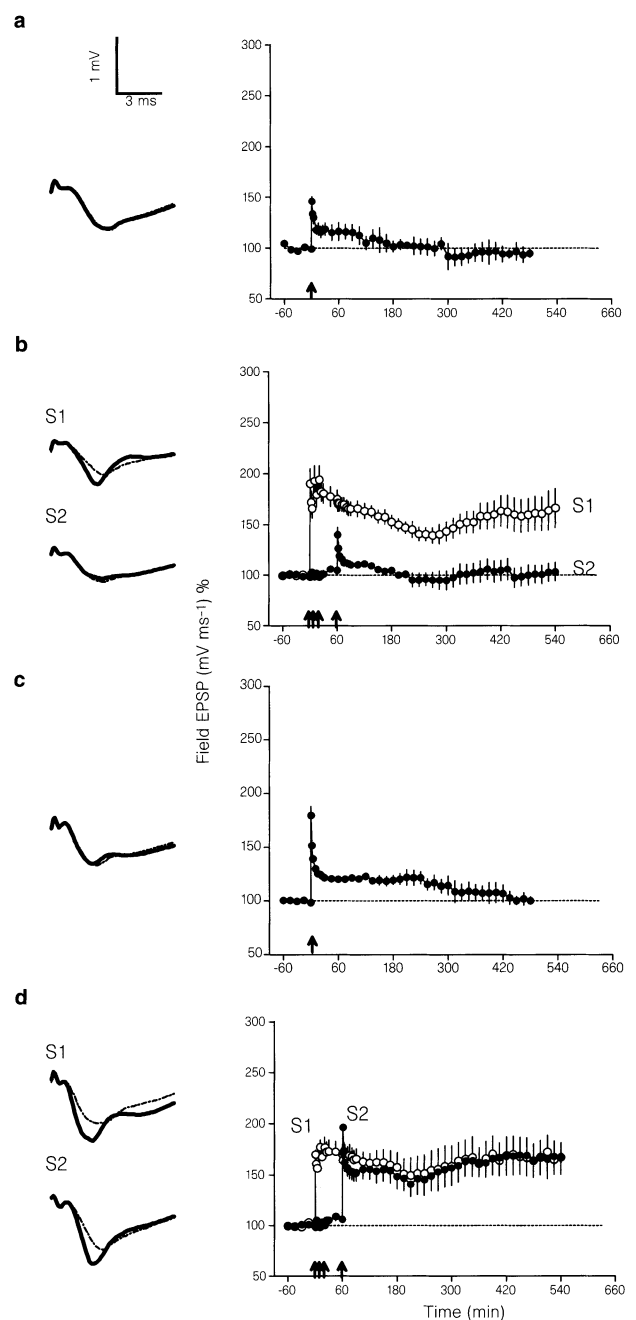


Figure 4 conversion of early-LTP into late-LTP. **a**, A weak tetanization protocol was used which elicited a short-term potentiation of the field EPSP for ~1 to 2 h ($n = 4$; symbols as in Fig. 1). **b**, Prior induction of late LTP in S1 (open circles; $n = 6$) did not influence the short potentiation in S2 (filled circles) induced 1 h after LTP on S1. **c**, A stronger single tetanization revealed early LTP lasting ~4 to 6 h ($n = 5$). **d**, When repeated tetanization and subsequent induction of late LTP in input S1 (open circles; $n = 5$) preceded the stronger single tetanization in S2 (filled circles), early LTP was transformed into late LTP in that input (field EPSP in S2: $167.7 \pm 17.6\%$ after 8 h).

To summarize, the consolidation of LTP involves the commitment of cellular proteins but this is insufficient to induce synapse-specific late LTP. A synaptic tag must also be transiently activated during early LTP. Our data provide the first experimental evidence for the selective catching of proteins by activated synapses, enabling late LTP to have input-specific properties without elaborate protein trafficking. They also establish that the putative synaptic tag

involves neither somatic nor local¹⁵ dendritic protein synthesis. Tetanization need not involve multiple trains, but must reach some critical threshold for the tag to be formed. Constitutively active kinases at activated synapses^{1,16} are appropriate candidates for fulfilling this function, particularly in the light of our findings that a tag lasts at most for 3 h and must be reset for further potentiation to occur. It remains to be determined whether the synaptic tag can also be reset in an activity-dependent way, for example by stimulation that induces depotentiation¹⁷.

These novel determinants of long-lasting potentiation have intriguing implications for the concepts of input specificity and persistence of LTP. According to the Hebbian model¹⁸, pre- and postsynaptic neurons have to be coactive within a distinct time to modify synaptic strength. Our results extend this hypothesis in one important respect: whereas the time interval for coactivity to create the synaptic tag must be short (<300 ms; refs 19, 20), the persistence of LTP can be influenced heterosynaptically by tetanization of other synaptic afferents over a period of 2–3 h. The establishment of late LTP appears to be determined by more than the local degree of NMDA-receptor activation²¹ in one synaptic input. The processing of incoming signals at a given time may alter the persistence of short-term changes in plasticity induced later at different synaptic inputs to the same neuron. Studies investigating the long-term modification of synaptic inputs should therefore take into account the prior and, possibly, the subsequent activity state²² of hippocampal neurons, considering them as integrative units over time²³. Processes other than potentiation-like events, such as long-term depression, might also influence different activated inputs in a similar way. These findings also suggest a new way in which protein synthesis can influence memory consolidation²⁴. A further, and speculative implication is that, if late-LTP-like synaptic changes are involved in long-term memory storage in the mammalian brain, our findings could provide a mechanistic explanation of why inconsequential events are typically remembered for much longer if they occur around the same time as well-remembered events. The vividness of 'flashbulb' memories²⁵ may derive, in part, from this cellular mechanism. □

Methods

53 transversal hippocampal slices (400 μm) were prepared from 53 male Wistar rats (7 weeks old) as described^{3,8,14}. Slices were incubated in an interface chamber at 32 °C and at a flow rate of artificial cerebrospinal fluid (ACSF, containing 124 mM NaCl, 4.9 mM KCl, 1.2 mM KH₂PO₄, 2.0 mM MgSO₄, 2.0 mM CaCl₂, 25.6 mM NaHCO₃, 10 mM D-glucose; carbogen consumption: 34 litres per hour) of 1 ml min⁻¹. In most of the experiments, two monopolar, lacquer-coated, stainless-steel electrodes (A-M Systems) were positioned in the stratum radiatum of the CA1 region for stimulation. For recording, 2 electrodes were placed in the CA1 dendritic and cell-body layer of a single neuronal population, except for the within-slice experiments (Fig. 2e), in which the two recording electrodes were positioned in the striatum radiatum at a distance that guaranteed—in combination with a microsurgical cut through a part of the CA1 region immediately after preparation—the stimulation of two independent neuronal populations. In the within-slice series, one stimulation electrode 1 was positioned only to evoke potentials in S1 of population 1. The second stimulation electrode simultaneously elicited potentials in S2 of population 1 and S1 of population 2 (Fig. 2e). In all other experiments, 2 stimulation electrodes were positioned at an adequate distance to stimulate separate inputs of one neuronal population (Fig. 2a). Slices were preincubated for at least 4–5 h, a period that is critical for a stable long-term recording for at least 8 h. This may be due to the requirement for a distinct stable level of activity to be reached after nonspecific synaptic activation during preparation¹⁴. After the preincubation period, the stimulation strength was determined for further testing by eliciting a population spike of 25% of its maximal amplitude. The stimulation intensity in the within-slice experiments was adjusted to obtain a field EPSP of 50% of its maximal slope in input S1 of populations 1 and 2, respectively. For S1 in population 1 and S2 in population 2, this intensity was the same strong synaptic activation as in experiments where the stimulus intensity was

determined by the mean of the population spike. Therefore, the stimulation intensity of S2 in population 1 varied between different experiments. The baseline was recorded for at least 60 min before LTP induction. Four 0.2-Hz biphasic, constant-current pulses (0.1 ms per polarity) were used for testing 1, 3, 5, 11, 15, 25, 30 min post-tetanus, and then once every 15 min. Late LTP was induced using three stimulus trains of 100 pulses (100 Hz, duration 0.2 ms per polarity) with 10 min intertrain intervals. This schedule of tetanization was used to produce very stable long-term maintenance of potentiation *in vitro* for longer than 8 h. In experiments in which a weaker induction of LTP was investigated, a short-term potentiation was induced using a single tetanus consisting of one train of 11 pulses (100 Hz, duration 0.1 ms per polarity, population spike threshold stimulus intensity for tetanization); and induction of early LTP alone using a single tetanus with 21 pulses (100 Hz, duration 0.2 ms per polarity, population spike threshold stimulus intensity for tetanization). The first weak tetanus resulted in a potentiation which lasted for ~1–2 h, whereas the second weak tetanization procedure caused a potentiation for ~3–5 h, unless preceded by late LTP to an independent pathway.

The responses were averaged and analysed on-line using a CED 1401 A/D converter 486/33 computer. The population spike amplitude (mV) was evaluated by taking the voltage difference between onset and peak. The slope function (mV per ms) of the field EPSP was calculated from 8 successive points of the steepest 400-μs segment.

Anisomycin, a reversible inhibitor of protein synthesis, was used at 25 μM, a concentration that blocks at least 85% of incorporation of ³H-leucine into hippocampal slices³. In addition to anisomycin, emetine, a second structurally different inhibitor, was used at 10 μM²⁶ to verify results in Fig. 2c, d (data not shown). Results were similar after the application of anisomycin either when emetine was applied throughout LTP induction of S1 and S2 (*n* = 3, field EPSP after 8 h in S1: 102.3 ± 7.3%; S2: 98.9 ± 15.8%) or only during induction of LTP on S2 (*n* = 3, field EPSP after 8 h in S1: 144 ± 23.2%; S2: 165.3 ± 32.0). This result supports a specific action of the protein synthesis inhibitors. Drugs were added to the bath medium 25 min before LTP induction and were washed out 1 h after tetanization in the corresponding experiments. All drugs were dissolved in modified ACSF immediately before application.

Statistical analysis (Figs 2 and 3b, c) compared the time course of potentials between control LTP in input S1 and the low-frequency control input S2 in b or between control LTP in b and the potentiation of the corresponding groups using the two-tailed Mann-Whitney *U*-test (*P* < 0.05). In Fig. 3a the time course of the potentiation of population 1 was compared to the potentials of population 2 (Mann-Whitney *U*-test; *P* < 0.05). In Fig. 4, the time course of potentiation between LTP in input S1 and the potentiation in S2 was compared using the two-tailed Mann-Whitney *U*-test (*P* < 0.05).

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Correspondence and requests for materials should be addressed to U.F. (e-mail: frey@ifn-magdeburg.de).