Brief Communication

Distinct single but not necessarily repeated tetanization is required to induce hippocampal late-LTP in the rat CA1

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The protein synthesis-dependent form of hippocampal long-term potentiation (late-LTP) is thought to underlie memory. Its induction requires a distinct stimulation strength, and the common opinion is that only repeated tetani result in late-LTP whereas as single tetanus only reveals a transient early-LTP. Properties of LTP induction were compared to learning processes where repetition is often the prerequisite for a long-lasting memory. However, also single events can lead to manifested memory. If LTP subserves processes of learning, similar results should be detectable for LTP. Here we show that a single tetanus is sufficient to induce late-LTP requiring dopaminergic co-transmission during induction.

There is growing interest in phenomena of functional plasticity such as long-term potentiation (LTP) the most prominent cellular models of activity-dependent long-lasting changes of synaptic transmission in the vertebrate brain. Bliss and co-workers (Bliss and Gardner-Medwin 1973; Bliss and Lomo 1973) first described LTP in the dentate region of the hippocampus. The best-studied form of LTP as well as the one which can be induced in the intact adult animal has been referred to as an “associative, NMDA-receptor-dependent LTP.” In 1984, Manfred Krug and colleagues (Krug et al. 1984) demonstrated for the first time that the maintenance phase of hippocampal LTP beyond 3–6 h in the dentate gyrus (DG) in vivo can be blocked by anisomycin, a reversible translation inhibitor. The role of protein synthesis for the maintenance or late-LTP has then been replicated by various other laboratories (e.g., Frey et al. 1988; Otani et al. 1989; Fazeli et al. 1993; Osten et al. 1996; Nayak et al. 1998). Taken together, these first results led us to the hypothesis that LTP has stages or phases like memory consolidation and that the synthesis of proteins is required for the long-lasting maintenance of synaptic changes in efficacy (Krug et al. 1984; Frey et al. 1988; Reymann et al. 1988a,b; Matthies et al. 1990; Reymann and Frey 2007; Frey and Frey 2008). Although the multiple-phase model of LTP is now widely accepted, questions were raised whether the different time course and duration of LTP rather reflected only quantitative differences depending on tetanization strength. Indeed, experimental protocols used to elicit consolidation in LTP are usually based on increasing the intensity of inputs. For example, one 100-Hz tetanization is used for the induction of early-LTP, and three or four spaced tetanizations are required to elicit the late-LTP (Reymann et al. 1985; Huang and Kandel 1994). This type of repeated stimulation was then mechanistically taken as a requirement for late-LTP to occur. However, other studies revealed that also a single tetanus can lead to late-LTP if its stimulation intensity and the number of stimuli per tetanus are sufficiently high (Bortolotto and Collingridge 2000; Sajikumar et al. 2005). Obviously, the stimulation strength and pattern determine whether heterosynaptic inputs become activated by field stimulation, which in turn has been shown to be required to induce late-LTP

(for reviews, see Navakkode et al. 2007; Reymann and Frey 2007; Frey and Frey 2008). Here we have re-investigated the stimulus requirements for a long-lasting late form of LTP in apical dendrites of the CA1 and its dependency on heterosynaptic induction processes as well as on protein synthesis, thus resembling late-LTP.

We used 37 transverse hippocampal slices (400 μ m), prepared from 37 male Wistar rats (7-wk-old), as described previously (Frey and Morris 1997; Sajikumar et al. 2007). The animals were killed rapidly by a single blow to the back of the neck using a metallic rod (cervical dislocation) and then decapitated (the whole procedure taking ~3–5 min). Slices were incubated in an interface chamber at 32°C; incubation medium (ACSF) contained 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; carbon consumption, 18–32 L/h (depending on the chamber used); ACSF flow rate, 0.74 mL/min. In all experiments, two monopolar lacquer-coated, stainless-steel electrodes (5 M Ω ; A-M Systems) were positioned within the stratum radiatum of the CA1 region for stimulating two separate independent synaptic inputs, S1 and S2 (see Fig. 1A). For recording the field excitatory postsynaptic potential (Field EPSP, measured as its slope function) and the population spike amplitude (PS), two electrodes (5 M Ω ; A-M Systems) were placed in the CA1 apical dendritic and cell body layer, respectively, of a single neuronal population to investigate plasticity in the apical dendritic branches, and signals were amplified by a custom-made amplifier. The signals were digitized using a CED 1401 A/D converter and analyzed with custom-made software (PWIN). Slices were preincubated for at least 4 h, which is critical for reliable long-time recordings of late-LTP (for more details, see Sajikumar and Frey 2004; Sajikumar et al. 2005).

Following the preincubation period, the test stimulation strength was determined for each input to elicit a PS of 25% for LTP inputs and 40% for control stimulation. Late-LTP was induced using a weaker tetanization protocol than normally used in our studies (Sajikumar et al. 2005) consisting of a single 100-Hz train (100 biphasic constant current pulses for tetanization, 0.2-msec pulse duration per half-wave, stimulus intensity for tetanization: 25% of maximal PS). The difference to other experiments from our group where we have investigated a transient early-LTP is just the number of stimuli within the 100-Hz train: early-LTP can be induced by 17–21 impulses, whereas late-LTP is induced by 100 impulses. The PS and the slope of the field EPSP were

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Figure 1. Properties of late-LTP induced by a single tetanus in apical dendrites of rat hippocampal pyramidal CA1 in vitro. (A) Schematic location of the electrodes to stimulate two separate synaptic inputs S1 and S2 in the stratum radiatum in rat hippocampal slices in vitro. The analog traces near the recording electrodes show representative examples of recorded potentials. Calibration bar for all analog traces: 2 mV/5 msec. (B) Time course of late-LTP induced by a single tetanus ($n = 10$). A single TET applied to synaptic input S1 resulted in late-LTP (B, filled circles) with a duration of 6 h. A control pathway S2 revealed stable electrophysiological responses throughout the experiment (B, open circles). Inserts represent analog traces (field EPSPs) 30 min before (filled line) and 30 min (dotted line) and 6 h (broken line) after TET. (C) When the reversible protein synthesis inhibitor anisomycin was applied (open box) 30 min before TET ($n = 7$) for a total duration of 1 h, maintenance of LTP in S1 (filled circles) was prevented. The control pathway S2 (open circles) remained stable. Analogs as in B. (D) Emetine (open box) similarly affected one-TET LTP in S1 ($n = 7$). Analogs as in B. (E) Here the NMDA-receptor blocker APV (open box) was applied ($n = 6$) instead of the protein synthesis inhibitors as in C and D. Induction of LTP by a single TET in S1 was reduced when APV was present. No later forms of LTP were detected. The control potentials in S2 again remained stable. Analogs as in B. (F) Requirement of dopaminergic D1/D5-receptor function for one-TET LTP in apical dendrites of the hippocampal pyramidal CA1 was investigated ($n = 7$). As seen in F, application of the specific D1/D5-receptor antagonist SCH23390 (open box) prevented late-LTP in S1 (filled circles) induced by a single TET, similarly as after application of the protein synthesis inhibitors in C and D. Analogs as in B.

monitored on-line. For clarity, the field EPSP data are shown because the two recorded parameters demonstrated similar time courses in the experiments. The baseline was recorded for at least 60 min before LTP induction; four 0.2-Hz biphasic, constant-current pulses (0.1-msec per polarity) were used for baseline recording and testing 1, 3, 5, 11, 15, 21, 25, and 30 min post-tetanus and thereafter once every 15 min up to 6 h maximum.

Anisomycin (25 μ M, a concentration that blocked at least 85% of incorporation of [3 H]leucine into hippocampal slices; Sigma) was dissolved in ACSF. Emetine (20 μ M; Tocris Cookson)

was dissolved in ACSF and 0.1% DMSO (a concentration which did not influence baseline recordings, data not shown). Amino-phosphonopentanoic acid (APV, 50 μ M; Sigma) was dissolved in ACSF, and the selective dopaminergic D1/D5-receptor antagonist *R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH23390) was used at a concentration of 0.1 μ M dissolved in ACSF (Tocris Cookson).

The average values of the population spike amplitude (measured in mV) and the slope function of the field EPSP (mV/msec) per time point were analyzed using the Wilcoxon signed-rank

test when compared within one group, or the Mann-Whitney *U* test when data were compared between groups; $P < 0.05$ considered as statistically significant different. Non-parametric tests were used because the analyses of prolonged recordings do not allow the use of parametric tests. Furthermore, the sample sizes did not always guarantee a Gaussian normal distribution of the data per series.

In a first series of experiments we have identified a stimulus protocol which consisted of a single tetanus resulting in a statistically significant potentiation after tetanization (TET) of synaptic input S1 with a duration of at least 6 h (the latest time point studied; Fig. 1B, filled circles) when compared with potentials recorded in a control pathway S2 (Fig. 1B, open circles; *U* test, $P < 0.05$) or when compared to its own before-TET baseline (Wilcoxon, $P < 0.05$). If the reversible protein synthesis inhibitor anisomycin was applied (Fig. 1C, open box; drug application 30 min before tetanus for a total duration of 1 h), maintenance of LTP in S1 beyond 195 min after LTP induction by a single TET was prevented (Fig. 1C, filled circles; potentiation was observed only in S1 up to 195 min after TET when compared to the control input S2 [Fig. 1C, open circles; *U* test, $P < 0.05$]) or up to 225 min after TET if compared with its own before-TET control baseline (Wilcoxon, $P < 0.05$). Baseline control recordings in S2 remained stable. To exclude unspecific effects of anisomycin we used a second, structurally different protein synthesis inhibitor, emetine. Figure 1D shows that emetine similarly affected one-TET LTP in S1 (LTP in S1 was significant up to 210 min after TET when compared with control stimulation of S2 [*U* test, $P < 0.05$] or up to 225 min after TET when compared with its own pre-TET baseline [Wilcoxon, $P < 0.05$]).

In the next set of experiments, we applied the NMDA-receptor blocker APV instead of the protein synthesis inhibitors (Fig. 1E). Here, already the induction of LTP by a single TET in S1 was reduced, and no later forms of LTP were detected (the attenuated LTP in S1 was significantly potentiated up to 60 min after TET when compared with control stimulation of S2 [*U* test, $P < 0.05$] or when compared with its own pre-TET baseline [Wilcoxon, $P < 0.05$]). The control potentials in S2 again remained stable.

In a final set we investigated the requirement of dopaminergic D1/D5-receptor function for one-TET LTP in apical dendrites of the hippocampal pyramidal CA1. As seen in Figure 1F, application of the specific D1/D5-receptor antagonist SCH23390 prevented late-LTP in S1 induced by a single TET, similarly as after application of the protein synthesis inhibitors (LTP in S1 was statistically significantly different up to 210 min after TET when compared with control stimulation of S2 [*U* test, $P < 0.05$] or up to 180 min after TET when compared with its own pre-TET baseline [Wilcoxon, $P < 0.05$]). Taken together, a single TET in synaptic inputs to the apical dendrites of CA1 neurons can result in late-LTP.

LTP is commonly used as the cellular model for memory formation. The induction processes—cooperativity, associativity, and the requirement of heterosynaptic function and protein synthesis for its prolonged maintenance—as well as its different temporal phases (for reviews, see Reymann and Frey 2007; Frey and Frey 2008) and other properties support this assumption. The common view on learning and the subsequent formation of a long-lasting memory trace is that it does normally require repeated training, whereas transient memory traces are stored after a single event, very similar to the common opinion about LTP: “As with memory . . . , LTP has phases. One stimulus train produces an early, short-term phase of LTP (called early LTP) . . . Four or more trains induce a more persistent phase of LTP (called late LTP)” (Kandel et al. 2000). There are also forms, however, of immediate permanent learning (for instance, distinct forms of

one-trial avoidance learning [Izquierdo et al. 2000; Vianna et al. 2000; and others]). Here, we show that LTP mechanistically could also underlie such learning at the cellular level. A single tetanus train with a sufficient number of stimuli as well as a distinct stimulation strength can result in a NMDA-receptor-dependent late-LTP, i.e., requiring heterosynaptic, glutamatergic, and dopaminergic activation during its induction and subsequently protein synthesis in apical dendrites of hippocampal CA1 neurons. These results extend earlier studies (Bortolotto and Collingridge 2000; Sajikumar et al. 2005) describing the following additional requirements for a one-TET late-LTP: NMDA-receptor and dopamine-receptor function and protein synthesis. Whether this form of late-LTP is fully identical with late-LTP induced by repeated tetani remains unclear. To avoid, however, further misinterpretation, we suggest determination of the activated cellular processes as a result of afferent stimulation as the main criteria for whether a LTP form is called “early” or “late” instead of the number of stimulus trains. This includes a sufficiently high depolarization to activate NMDA-receptor function for early-LTP and, in addition, heterosynaptic input activation, synaptic tagging, and protein synthesis for late-LTP to occur (for review, see Reymann and Frey 2007). Future studies will be conducted to investigate if single-TET late-LTP also shows differences when compared with repeated-TET late-LTP with respect to their underlying cellular mechanisms and relevance for the system’s function. It also remains unclear if the single-TET late-LTP described herein is only characteristic for the herein-investigated morphological neuronal functional compartment, i.e., for the apical dendrites of the hippocampal CA1 neurons. It had recently been shown that LTP is compartmentalized under more physiological conditions (Alarcon et al. 2006; Sajikumar et al. 2007) and that these functional compartments are also characterized by compartment- as well as process-specific plasticity molecules (such as tag molecules or plasticity-related proteins; Sajikumar et al. 2007). Therefore, we cannot fully exclude that in other neuronal compartments or brain structures a single TET would also result in late-LTP. So far, however, all of the experiments which supported the “repeated-TET” requirement for late-LTP as well as the “single-train” protocol have been described only for the apical dendrites of hippocampal pyramidal cells.

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