Long-term depression (LTD) is a form of synaptic plasticity that can be induced either by low-frequency stimulation of presynaptic fibers or in an associative manner by asynchronous pairing of presynaptic and postsynaptic activity. We investigated the induction mechanisms of associative LTD in CA1 pyramidal neurons of the hippocampus using whole-cell patch-clamp recordings and Ca\(^{2+}\) imaging in acute brain slices. Asynchronous pairing of postsynaptic action potentials with EPSPs evoked with a delay of 20 msec induced a robust, long-lasting depression of the EPSP amplitude to 43%. Unlike LTD induced by low-frequency stimulation, associative LTD was resistant to the application of d-AP-5, indicating that it is independent of NMDA receptors. In contrast, associative LTD was inhibited by (S)-\(\alpha\)-methyl-4-carboxyphenyl-glycine, indicating the involvement of metabotropic glutamate receptors. Furthermore, associative LTD is dependent on the activation of voltage-gated Ca\(^{2+}\) channels.

Induction is likely to be associative, requiring the temporal coincidence of synaptic activation and backpropagating action potentials (for review, see Linden, 1999). Indeed associative LTD in the hippocampus can be induced by asynchronous pairing of presynaptic and postsynaptic activity (Levy and Steward, 1983; Stanton and Sejnowski, 1989). The opposite was shown for associative LTD in acute slices was reported to be independent of NMDARs (Stanton and Sejnowski, 1989). The opposite was shown for associative LTD in organotypic cell culture (Debanne et al., 1994). Finally, in dissociated hippocampal cell culture, the associative LTD appeared to be dependent on both Ca\(^{2+}\) influx through NMDARs and L-type Ca\(^{2+}\) channels (Bi and Poo, 1998).

Here we investigated the conditions necessary for the induction of associative LTD in acute hippocampal slices by asynchronous pairing of presynaptic and postsynaptic activity at the Schaffer collateral–CA1 pyramidal cell synapse. The results suggest that associative LTD is dependent on both mGluRs and Ca\(^{2+}\) influx through voltage-gated L- and N-type Ca\(^{2+}\) channels. As N-type Ca\(^{2+}\) channels are preferential targets of G-protein-mediated neuromodulation (Hille, 1994), we have tested whether the modulation of postsynaptic N-type Ca\(^{2+}\) channels could affect LTD induction, which would provide a novel mechanism to regulate activity-dependent synaptic plasticity in the hippocampus.

**MATERIALS AND METHODS**

_Slice preparation._ Transverse 300-\(\mu\)m-thick slices were cut from the hippocampus of 11- to 22-d-old Wistar rats with a vibratome (DTK-1000; Dosaka, Kyoto, Japan). For most experiments 14- to 18-d-old animals were used. The animals were killed by decapitation, in accordance with national and institutional guidelines. Slices were kept at 35°C for 30 min after slicing and then at room temperature in physiological extracellular saline containing (in mM): 125 NaCl, 25 NaHCO\(_3\), 25 glucose, 2.5 KCl, 1.25 NaH\(_2\)PO\(_4\), 2 CaCl\(_2\), and 1 MgCl\(_2\) bubbled with carbogen (95% O\(_2\) and 5% CO\(_2\)).

 Electrophysiology. The slices were transferred to the recording chamber and continuously superfused with saline at a flow rate of 5–10 ml/min (chamber volume, ~2 ml). CA1 pyramidal neurons were identified by their
location using infrared differential interference contrast video microscopy and their characteristic firing frequency adaptation during long depolarizing current pulses. Patch pipettes were pulled from borosilicate glass tubing (1.2 mm inner diameter, 0.5 mm outside diameter, 2.5 MΩ resistance when filled with 2 M KCl, Miltenyi Biotec, Darmstadt, Germany) and heat-polished immediately before use. An Axopatch 200A amplifier (Axon Instruments, Foster City, CA) or an EPC-9 amplifier (Heka, Lambrecht, Germany) were used for current-clamp (I-clamp fast) and voltage-clamp (V-clamp) recordings. The I-clamp amplifier used a bridge-balancer circuit for compensation of series resistance in the current-clamp mode, similar to that of the Axopatch 200B. Current and voltage signals were filtered at 5 and 10 kHz, respectively, with a 4-pole low-pass Bessel filter and sampled at 140–200 Hz (Micromax; 1 MHz; Princeton Instruments). For data acquisition and analysis we used self-made and commercial programs (EPC, CED; Pulse, Heka).

For current-clamp recordings the patch pipettes were filled with an internal solution (in mM): 35 KCl, 2 MgCl2, 2 NaATP, 0.3 NaGTP, 0.2–0.5 EGTA, and 10 HEPES (pH was adjusted to 7.3 with KOH). Dendritic recordings were performed as described previously (Boisbouvier and Jonas, 1997). Patch resistance was 5–10 MΩ for somatic and 10–12 MΩ for dendritic recordings. Bridge balance was used to correct for series resistance of 20–60 MΩ.

Presynaptic Schaffer collateral fibers were stimulated using a stimulus isolator (List, Darmstadt, Germany) and a patch pipette with a resistance of 1–3 MΩ when filled with HEPES-buffered Na+-rich solution. The stimulus pipette was placed in the stratum radiatum of the CA1 region 20–50 μm away from the pyramidal cell layer. Two hundred microsecond voltage pulses of 10–80 V were applied to evoke subthreshold EPSPs at a frequency of 0.1 Hz. Orthodromic stimulation was performed in >90% of the experiments with stimuli of 0–80 V, without obvious differences concerning basal transmission and plasticity induction. The latency between the center of the stimulus artifact and the onset of the EPSP was 2.9 ± 0.1 msec (n = 53), indicating monosynaptic transmission. Intermittent stimulation (10–50 Hz) was performed in the course of all current-clamp experiments. In some experiments 10 μM glycine was added to the bath solution, with no obvious differences in the results. To record Ba2+ currents, the recording pipettes (mostly 0.8–2 MΩ) were filled with a Na-free intracellular solution (in mM): 200 KCl, 10 NaATP, 0.3 NaGTP, 10 EGTA, and 10 HEPES (pH adjusted to 7.3 with CsOH). The bath solution contained 140 NaCl, 2 tetraethylammonium chloride (TEACl), 2 MgCl2, 2 BaCl2, 1 μM tetrodotoxin (TTX) and 10 HEPES (pH adjusted to 7.3 with CsOH). Recentrifuged microspheres were used in the stratum radiatum of the CA1 region with the dissociation constant (Kd) for Ca2+ in the final solution, 0.01 M. The dissociation constant for Ca2+ was 470 nM. The concentration was on average 470 nM, with the dissociation constant 0.01 M.

Fluorescence measurements. For the measurement of the intracellular Ca2+ signals we used 0.1 mM fura-2 (Molecular Probes, Eugene, OR) instead of EGTA in the pipette solution. Cells were loaded for at least 15–20 min in the whole-cell configuration before measurements were started. The excitation light source (Polychrome II with 75 W Xenon lamp; TILL Photonics, Munich, Germany) was coupled to the epifluorescent port of the microscope (Axioskop FS2, Zeiss; 60× water immersion objective, Olympus Optical, Tokyo, Japan) via a light guide. To minimize bleaching, the light intensity was reduced to 10%. The filter combination for excitation and emission comprised a beam splitter (BS240) and emission filters (LW420, K7600) from Delta Light & Optics (Lyngby, Denmark). Some experiments were performed with 0.1 mM Oregon Green (Molecular Probes, Eugene, OR)-488 Bapta-1, Molecular Probes) instead of fura-2, using a filter combination from Zeiss (FTS10, LP 520) and an excitation light intensity of 5%.

The fluorescence was measured with a backilluminated frame-transfer CCD camera (EBFT 512; Princeton Instruments). Images with full resolution were taken with exposure times of 5 sec. For high-speed Ca2+ measurements (100 Hz repetition rate) we usually defined three rectangular regions of interest (ROIs) of 5 × 5 μm at the soma and 5 × 20 μm at a proximal and distal part of the apical dendrite. The pixels included in the ROIs were binned on-chip and digitized subsequently by the controller (Micromax; 1 MHz; Princeton Instruments). The fluorescence signals were corrected for background, which was obtained from ROIs shifted by 10–15 μm with respect to the original ROIs (Schiller et al., 1995).

Calibration of [Ca2+]i. To convert the fluorescence signals into Ca2+ concentrations, we used the isosbestic ratioing method (Neher and Augustine, 1992; Schiller et al., 1995). The action potential-induced fluorescence change was recorded on an excitation wavelength of 380 nm. The isosbestic fluorescence was measured immediately before and after this sweep, using an excitation wavelength of 356 nm (the Ca2+-insensitive wavelength in our experimental conditions). The ratio of the background-corrected fluorescence signals R = F356/F380 was calculated and converted into the Ca2+ concentration using the equation (Gryniewicz et al., 1985):

\[
[Ca^{2+}] = \frac{K_{eq}(R - R_{	ext{min}})}{R_{	ext{max}} - R}
\]

where Rmax is the ratio in Ca2+-free solution and Rmin the ratio when the fura-2 is completely saturated with Ca2+. These values were determined by recording from CA1 pyramidal cells with internal solutions containing either 30 mM EGTA (Rmax = 0.70 ± 0.01; n = 5) or 50 mM CaCl2 (Rmax = 6.07 ± 0.06; n = 5). Keq was calculated after background subtraction.

RESULTS
Whole-cell current-clamp recordings from CA1 pyramidal cells were made, and EPSPs were evoked by electrical stimulation of Schaffer collaterals (Fig. 1A). The cells were held at a membrane potential of −68 to −70 mV, near the average resting potential (−68.6 ± 0.3 mV; n = 58). A robust associative LTD was induced by asynchronous pairing of extracellular Schaffer-collateral stimulation with a short postsynaptic current injection generating an action potential 20 msec before the EPSP (Fig. 1A,B). Time intervals of 10–20 msec have been shown to be maximally effective for the induction of associative LTD (Levy and Steward, 1983; Markram et al., 1997; Bi and Poo, 1998). The pairing was repeated 360 times at a frequency of 0.3 or 1 Hz. This asynchronous pairing with the EPSP and the LTD was induced with a probability of 0.001 (n = 17; p < 0.001) of the control value measured 15–20 min after the induction protocol (Fig. 1C,D). As shown in Figure 2, 360 action potentials or EPSPs alone at 1 Hz did not induce significant alterations in EPSP amplitude (action potentials alone: 93.8 ± 5.1%; n = 3; p > 0.5; EPSPs alone: 103.4 ± 4.9% of control EPSP amplitude, n = 3; p > 0.5). Thus, this form of LTD is associative and dependent on the asynchronous activity of both presynaptic and postsynaptic neurons.

Associative LTD is dependent on metabotropic glutamate receptors
To investigate the induction mechanisms of associative LTD, we first examined the contribution of metabotropic glutamate receptors (Fig. 3A). In the presence of 500 μM MCPG, an antagonist of metabotropic glutamate receptors, the induction of associative
LTD was inhibited (105.3 ± 3.6% of control EPSP amplitude, n = 6; p > 0.1). By contrast, the NMDAR antagonist D-AP-5 (50 μM) was without effect on the synaptic depression (Fig. 3B). In the presence of D-AP-5, the EPSP amplitude was depressed to 45.5 ± 3.5% (n = 6; p < 0.001), similar to the control condition. In addition, D-AP-5 had no significant effects on EPSP peak amplitude (8.6 ± 1.2 mV in control solution vs 8.5 ± 1.2 mV in D-AP-5, n = 11; p > 0.5) and EPSP decay time constant (11.0 ± 1.1 ms vs. 11.0 ± 0.9 ms, n = 11; p > 0.05). These results are consistent with a minimal contribution of NMDARs to basal synaptic transmission in CA1 pyramidal neurons near the resting membrane potential (Herron et al., 1986; Cash and Yuste, 1999). Thus, under our experimental conditions the NMDARs do not significantly contribute to both basal synaptic transmission and induction of associative LTD.

A standard protocol for the induction of LTD is the application of prolonged LFS of presynaptic neurons (e.g., 900 pulses at 1 Hz; Dudek and Bear, 1992). Using this protocol we could only induce long-term depression if the postsynaptic cell was slightly depolarized to −62 mV, but not at the resting membrane potential of −68 to −70 mV. As this potential was closer to firing threshold we used smaller initial EPSP amplitudes (range, 1–4 mV at −70 mV) to avoid postsynaptic spiking during LFS induction (Fig. 3C, inset). The LFS protocol induced a depression of the EPSPs to 40.9 ± 2.5% of the control amplitude (n = 8; p < 0.001; Fig. 3C). Application of D-AP-5 (50 μM) inhibited the induction of LFS-induced LTD (EPSP amplitude was 100.7 ± 10.4% after 15 min, n = 4, p > 0.5; Fig. 3D). Thus, LFS induces an NMDAR-dependent LTD, consistent with previous reports (Dudek and Bear, 1992; Oliet et al., 1997). In addition, the voltage dependence of the LFS-induced NMDAR LTD was similar to that described previously (Debanne et al., 1996; Goda and Stevens, 1996; Oliet et al., 1997; Fitzsimonds et al., 1997). In conclusion, associative pairing selectively induces mGlur-dependent LTD, whereas low-frequency stimulation leads to NMDAR-dependent LTD.

**Associative LTD is dependent on activation of voltage-gated Ca²⁺ channels**

Previous studies showed that the induction of both mGluR- and NMDAR-dependent LTD is blocked by the Ca²⁺ chelator BAPTA (Mulkey and Malenka, 1992; Oliet et al., 1997). To examine postsynaptic Ca²⁺ signaling in associative LTD we measured Ca²⁺ transients in the soma and apical dendrites of CA1 pyramidal neurons induced by single backpropagating action potentials using 0.1 mM fura-2. After a single action potential, the dendritic Ca²⁺ concentration increased by 148 ± 14 nm from a resting value of 46 ± 5 nm (distance from soma 40–100 μm; n = 17; Fig. 4A). This transient increase in Ca²⁺ concentration decayed to initial baseline levels with a time constant of 707 ± 41 msec. Application of 1 μM α-conotoxin GVIA, an irreversible blocker of N-type Ca²⁺ channels, reduced the dendritic Ca²⁺ transients by 38.3 ± 4.6% (n = 6; 40–100 μm; Fig. 4A,B). This indicates that N-type Ca²⁺ channels are effectively opened by single backpropagating action potentials. To assess the contribution of L-type Ca²⁺ channels, we examined the effects of 10 μM nifedipine. Because nifedipine is very light-sensitive, we used 0.1 mM Oregon Green instead of fura-2 (see Materials and Methods). A single action potential evoked a transient fluorescence increase of ΔF/F = 108.5 ± 13% (n = 14). Application of 10 μM nifedipine reduced the dendritic Ca²⁺ transients by 19.4 ± 1.6% (n = 5; 40–100 μm; Fig. 4B). Thus, a single backpropagating action potential induces a reliable Ca²⁺ influx through voltage-gated Ca²⁺ channels in the proximal apical dendrite of CA1 pyramidal neurons, with a substantial amount carried by N- and L-type Ca²⁺ channels.

To test the involvement of these channels in LTD induction, we applied the LTD induction protocol in the presence of Ca²⁺ channel antagonists. When 0.5 μM α-conotoxin GVIA was applied during basal synaptic transmission, the EPSP amplitude was reduced from 16.8 ± 2.3 mV to 5.8 ± 1.4 mV (n = 9; Fig. 4C), indicating the inhibition of presynaptic N-type Ca²⁺ channels that mediate neurotransmitter release (Dunlap et al., 1995). As a higher stimulus intensity was used in these experiments, EPSPs in the presence α-conotoxin were sufficiently large to examine the effects of subsequent pairing. Under these conditions the asynchronous pairing protocol failed to induce significant depression of the EPSP amplitude (102 ± 5.0% of control EPSP amplitude, n = 9; p > 0.5). Although we cannot exclude a contribution of presynaptic N-type Ca²⁺ channels, these results suggest that Ca²⁺ influx through postsynaptic N-type channels is required for the induction of associative LTD. Similarly, we tested the involvement of L-type Ca²⁺ channels in LTD induction (Fig. 4D). In contrast to α-conotoxin, nifedipine did not reduce the initial EPSP amplitude. However, nifedipine markedly reduced the amount of LTD (reduction of EPSP amplitude to 89.6 ± 4.3%; n = 7, p > 0.5). Thus, postsynaptic Ca²⁺ influx through voltage-gated Ca²⁺ channels is necessary for the induction of associative LTD.
backpropagating action potentials was reported (Sandler and Ross, 1999). To distinguish between these possibilities, we blocked Na⁺ and K⁺ channels (see Materials and Methods) and examined Ca²⁺ channels in isolation in the whole-cell voltage-clamp configuration using 2 mM Ba²⁺ as charge carrier (Fig. 5B). The application of 1 μM OH-DPAT reduced the Ba²⁺ currents to 72.9 ± 10.9% (n = 8; p < 0.01). In the presence of ω-conotoxin GVIA the Ba²⁺ currents were reduced to 45.8 ± 6.6% (n = 4). Subsequent to the application of ω-conotoxin, the modulation by OH-DPAT was completely absent, indicating a selective modulation of N-type Ca²⁺ channels by 5-HT₁₅ receptors. In the presence of 10 μM nifedipine (84.7 ± 7.0% of control; n = 4), however, there was still a substantial reduction of the Ba²⁺ currents by OH-DPAT (57.0 ± 3.9% of control; p < 0.01).

To examine possible effects of 1 μM OH-DPAT on action potential backpropagation, we made double recordings from the soma and the apical dendrite of CA1 pyramidal cells at distances of 64–192 μm from the soma (Fig. 5C). The shape of the dendritic and somatic action potential in 1 μM OH-DPAT was very similar to control conditions. The resting membrane potential was slightly hyperpolarized by −1.0 ± 0.2 mV at the dendrite and by −0.9 ± 0.2 mV at the soma (p < 0.05; six double recordings). As shown in Figure 5D, the action potential amplitude in the presence of 1 μM OH-DPAT was virtually identical to control conditions (101.2 ± 0.9% of control, n = 6; p > 0.1). Furthermore, no significant change in half width of the dendritic AP (102.1 ± 2.1% of control; p > 0.1) or the propagation velocity (98.3 ± 3.2% of control; p > 0.1; Fig. 5D) was observed. In conclusion, these results indicate that the reduction of action potential-induced Ca²⁺ transients by 1 μM OH-DPAT is attributable to a direct modulation of postsynaptic N-type Ca²⁺ channels and not to an inhibition of dendritic backpropagation.

If postsynaptic N-type Ca²⁺ channels are necessary for induction of associative LTD (Fig. 4C) and if these channels are selective targets for modulation via 5-HT₁₅ receptors (Fig. 5), then OH-DPAT should affect LTD induction. We first tested the effect of 1 μM OH-DPAT on basal synaptic transmission, and we found that the peak EPSP amplitude remained unchanged (Fig. 6A; 95.8 ± 6.4% of control, n = 11; p > 0.5). This allowed us to use OH-DPAT as a tool to inhibit selectively postsynaptic N-type Ca²⁺ channels. In the presence of 1 μM OH-DPAT, application of the asynchronous pairing paradigm failed to induce associative LTD (102.8 ± 4.9% of control, n = 11; p > 0.5; Fig. 6B, C). These results indicate that the Ca²⁺ influx via postsynaptic N-type channels is necessary for induction of associative LTD and that the G-protein-mediated modulation of these channels strongly controls this form of synaptic plasticity.

**DISCUSSION**

Our results show that associative LTD at the Schaffer collateral–CA1 pyramidal cell synapse can be induced reliably by asynchronous pairing of EPSPs with preceding postsynaptic action potentials. The induction was dependent on both mGluRs and postsynaptic voltage-gated Ca²⁺ channels. In particular, we show a direct involvement of N-type Ca²⁺ channels in synaptic plasticity. The modulation of postsynaptic N-type Ca²⁺ channels by 5-HT₁₅ receptors was sufficient to inhibit associative LTD induced by asynchronous pairing.

**Associative LTD is dependent on mGluRs**

The induction of LTD by the associative pairing protocol was blocked by the mGluR antagonist MCPG, similar to the previously described mGluR LTD (Bolshakov and Siegelbaum, 1994; Oliet et al., 1997; Otani and Connor, 1998). Although we did not use subtype-specific antagonists, it is likely that the depression is mediated by mGluR5. Immunocytochemical evidence indicates that mGluR5 is the most abundant metabotropic glutamate receptor present on the postsynaptic CA1 pyramidal cells (Shigemoto et al., 1997). Furthermore, there is evidence for the involvement of the phospholipase C (PLC) signal transduction pathway in mGluR

**Modulation of postsynaptic N-type Ca²⁺ channels inhibits associative LTD**

OH-DPAT, a selective agonist of 5-hydroxytryptamine (5-HT)₅ receptors, is known to inhibit N-type Ca²⁺ channels in cortical pyramidal neurons by activation of a Gₛ₅ protein (Foehring, 1996). Immunocytochemical analysis revealed a high density of 5-HT₁₅ receptors in the hippocampal CA1 region and further suggested an exclusively postsynaptic location (Kia et al., 1996). Thus, we considered OH-DPAT as a selective inhibitor of postsynaptic N-type Ca²⁺ channels. As a first experimental step, we examined the effect of OH-DPAT on the action potential-induced Ca²⁺ transient (Fig. 5A). Application of 1 μM OH-DPAT reduced the dendritic Ca²⁺ transients by 25.6 ± 2.7% (n = 5; 40–100 μM). This is consistent with the previously reported reduction of burst-induced Ca²⁺ transients by 10 μM 5-HT in CA1 pyramidal neurons (Sandler and Ross, 1999).

The reduction in the dendritic Ca²⁺ transient by OH-DPAT could be attributable to a direct inhibition of Ca²⁺ channels or a reduction in the amplitude of the backpropagating action potential, or both. For 10 μM 5-HT a slight reduction of the amplitude of the
LTD, consistent with the activation of group 1 mGluRs (Oliet et al., 1997; Otani and Connor, 1998).

The induction mechanism of associative LTD appeared to be different from that of LFS-induced LTD, which was largely blocked by the NMDAR antagonist D-AP-5 (Fig. 3), consistent with previous studies (Dudek and Bear, 1992; Mulkey and Malenka, 1992). The coexistence of two different forms of LTD in hippocampal pyramidal cells was described in detail by Oliet et al. (1997). In bath solutions containing 2.5 mM Ca\(^{2+}\) and 1.3 mM Mg\(^{2+}\), LFS primarily induced NMDAR LTD. In 4 mM Ca\(^{2+}\) and 4 mM Mg\(^{2+}\), however, an additional NMDAR-independent form of LTD was induced, which was dependent on mGluRs and voltage-gated Ca\(^{2+}\) channels (Oliet et al., 1997). Because associative LTD in acute hippocampal slices is dependent on mGluRs but not on NMDARs,
from these dendrites (Magee and Johnston, 1995). In addition, al., 1997; Magee, 1999) and with cell-attached patch recordings of CA1 pyramidal neurons (Westenbroek et al., 1992; Kavalali et al., 1997; Magee et al., 1998). Postsynaptic Ca transporters (CaV1.2, CaV1.3, CaV1.4) and L-type calcium channels (L-type channels) in CA1 pyramidal neurons. Modulation of CaV1.2 channels was analyzed using 30 mV voltage steps from a holding potential of $-90$ to $10$ mV and $Ba^{2+}$ as a charge carrier in the bath. The traces show currents in control and after application of $1 \mu M$ OH- DPAT (left traces) or OH-DPAT in the presence of $0.5 \mu M$ omega-conotoxin GVI A (right traces), respectively. The bar graph summarizes the mean of $n = 8$ (OH-DPAT), $n = 4$ (omega-conotoxin plus OH-DPAT), and $n = 4$ (nifedipine plus OH-DPAT) experiments. C, Double recording from the soma and the apical dendrite of a CA1 pyramidal neuron (distance between recording sites was $192 \mu m$; inset). Somatic current injection (900 pA, 5 msec) evoked backpropagating action potentials, which did not significantly change after application of $1 \mu M$ OH-DPAT. D, The amplitude of the dendritic action potential is plotted against time (top panel, 6 double recordings, normalized to initial value). The average distance was $110 \pm 22 \mu m$. The bar graph (bottom panel) summarizes the effect of $1 \mu M$ OH-DPAT on somatic and dendritic AP amplitude, on the half width of the dendritic AP and on the propagation velocity relative to control ($n = 6$).

**Figure 5.** Selective suppression of N-type Ca$^{2+}$ channels by 5-HT$\_\text{IA}$ receptors. A, Action potential-induced Ca$^{2+}$ transients recorded from the CA1 pyramidal neuron shown on the left (0.1 mM fura-2), in control conditions, and in the presence of the 5-HT$\_\text{IA}$ receptor agonist OH- DPAT (1 \mu M). B, Whole-cell voltage-clamp recordings from CA1 pyramidal neurons. Modulation of Ca$^{2+}$ channels was analyzed using 50 mV voltage steps from a holding potential of $-90$ to $10$ mV and Ba$^{2+}$ as a charge carrier in the bath. The traces show currents in control and after application of $1 \mu M$ OH-DPAT (left traces) or OH-DPAT in the presence of $0.5 \mu M$ omega-conotoxin GVI A (right traces), respectively. The bar graph summarizes the mean of $n = 8$ (OH-DPAT), $n = 4$ (omega-conotoxin plus OH-DPAT), and $n = 4$ (nifedipine plus OH-DPAT) experiments. C, Double recording from the soma and the apical dendrite of a CA1 pyramidal neuron (distance between recording sites was $192 \mu m$; inset). Somatic current injection (900 pA, 5 msec) evoked backpropagating action potentials, which did not significantly change after application of $1 \mu M$ OH-DPAT. D, The amplitude of the dendritic action potential is plotted against time (top panel, 6 double recordings, normalized to initial value). The average distance was $110 \pm 22 \mu m$. The bar graph (bottom panel) summarizes the effect of $1 \mu M$ OH-DPAT on somatic and dendritic AP amplitude, on the half width of the dendritic AP and on the propagation velocity relative to control ($n = 6$).

the asynchronous pairing protocol might be the most physiological way to selectively induce the mGluR-dependent LTD.

Thus, two mechanistically distinct forms of LTD coexist in hippocampal pyramidal cells, which can be induced selectively, depending on pyramidal cell firing during network activity (O’Keefe and Reece, 1993). Prolonged presynaptic activity without any postsynaptic spiking may decrease the EPSP amplitude via nonassociative LTD dependent on NMDARs, whereas EPSPs that occur repeatedly at a certain time delay with respect to postsynaptic action potentials will be depressed by associative LTD dependent on mGluRs.

**Associative LTD is dependent on activation of postsynaptic Ca$^{2+}$ channels**

The mGluR LTD induced by associative pairing could be blocked by the inhibition of either L- or N-type Ca$^{2+}$ channels (Fig. 4). These channels were reliably activated during single backpropagating action potentials (Fig. 4B). This is consistent with the localization of L- and N-type Ca$^{2+}$ channels on soma and apical dendrites of CA1 pyramidal neurons (Westenbroek et al., 1992; Kavalali et al., 1997; Magee, 1999) and with cell-attached patch recordings from these dendrites (Magee and Johnston, 1995). In addition, other types of Ca$^{2+}$ channels are expressed in CA1 pyramidal cells, including P-, R-, and T-type channels (Kavalali et al., 1997). They may be responsible for the omega-conotoxin- and nifedipine-resistant Ca$^{2+}$-influx.

It may seem surprising that a small (20–40%) reduction of the spatially averaged dendritic Ca$^{2+}$ transient was sufficient to substantially reduce or block the associative LTD. However, the Ca$^{2+}$ concentration at Ca$^{2+}$-dependent effector molecules may be very different from the measured Ca$^{2+}$ transients. The peak amplitude of the Ca$^{2+}$ transient in submembrane cytoplasmic compartments could be much higher because of clustering of Ca$^{2+}$ channels and local saturation of Ca$^{2+}$ buffering (Helmchen et al., 1996). If, for example, N-type Ca$^{2+}$ channels were colocalized with molecules involved in LTD induction, then our data would represent a lower estimate for the contribution of these channels to local Ca$^{2+}$ signals near these effector molecules. Such a colocalization could occur in dendritic spines, where action potential-induced Ca$^{2+}$ transients have larger amplitudes than in nearby parent dendrites (Majewska et al., 2000).

Our results and previous reports (Bolshakov and Siegelbaum, 1994; Oliet et al., 1997; Otani and Connor, 1998) converge on the conclusion that postsynaptic Ca$^{2+}$ influx is essential for LTD induction. However, the target molecules for Ca$^{2+}$ remain to be identified. A Ca$^{2+}$-dependent phosphatase is unlikely to be involved, because the phosphatase inhibitor microcystin does not affect mGluR LTD (Oliet et al., 1997). An involvement of Ca$^{2+}$-dependent isoforms of PKC is more likely, because PKC inhibitory peptide blocks mGluR LTD (Oliet et al., 1997; Otani and Connor, 1998). Because some PKC isoforms are activated by both diacylglycerol and Ca$^{2+}$ (Nishizuka, 1992), they could operate as molecular coincidence detectors, onto which the activation of voltage-gated Ca$^{2+}$ channels and group 1 mGluRs converge. This would explain the need for both postsynaptic action potentials and the release of glutamate for induction of associative LTD.

**Modulation of N-type Ca$^{2+}$ channels and LTD**

The involvement of N-type Ca$^{2+}$ channels in synaptic plasticity is difficult to assess because of the inhibition of basal synaptic trans-
Modulation of associative LTD by backpropagating action potentials

We have shown that N-type Ca\(^{2+}\) channels are direct targets of neuromodulation via G-proteins. However, the activation of N-type channels could be also regulated indirectly by modulation of action potential backpropagation. A 1 \(\mu\)M concentration of OH-DPAT, which is thought to activate selectively 5-HT\(_{1A}\) receptors, did not affect the properties of the backpropagated spike within the first 200 \(\mu\)m of the apical dendrite. In contrast, higher concentrations (30 \(\mu\)M) of OH-DPAT and 5-HT induce a marked hyperpolarization of CA1 pyramidal cells by 5 and 14 mV, respectively (Andrade and Nicoll, 1987), which slightly decrease the amplitude of the backpropagated spike (Sandler and Ross, 1999). Furthermore, activation of muscarinic and adrenergic receptors regulates dendritic excitability via modulation of fast dendritic Na\(^{+}\) and K\(^{-}\) channels (Johnston et al., 1999).

Both the amplitude of the backpropagated spike and the evoked dendritic Ca\(^{2+}\) transients decrease with distance from the pyramidal cell soma (Spruston et al., 1995; Magee and Johnston, 1997). Thus, in stratum lacunosum moleculare we would not expect any associative LTD at all, unless backpropagation of action potentials will be enhanced by activation of muscarinic or adrenergic receptors. This will lead to different learning rules for distal and proximal synapses. In general, action potential backpropagation can be very different in different types of neurons (for review, see Magee, 1999). Both CA1 and neocortical pyramidal neurons show decremental spike backpropagation (Magee and Johnston, 1997; Markram et al., 1997). Hipocampal oriens-aleuves interneurons and olfactory bulb mitral cells, however, show nondecremental backpropagation of action potentials into the dendrites (Bischofberger and Jonas, 1997; Martina et al., 2000). It would be interesting to know whether glutamatergic synapses on these neurons show LTD, and if so, whether LTD has associative properties over the entire dendritic tree.

**Physiological significance of associative LTD**

Both associative LTD and LTP in the hippocampus may be important for the dynamical shaping of new place fields during spatial learning and theta-phase associated pyramidal cell firing (O’Keefe and Recce, 1993; Wilson and McNaughton, 1993). In particular, they may contribute to the learning of temporal sequences in the hippocampus (Skaggs and McNaughton, 1996; Mehta et al., 1997). Whereas associative LTP will strengthen the synapses that precede subsequent spike discharge of the postsynaptic cell (Magee and Johnston, 1997), associative LTD will depress EPSPs that occur too late with respect to the postsynaptic spiking, thus leading to temporally asymmetric learning rules. Such rules appeared also to be very effective for the formation of neuronal cell assemblies in artificial neural networks (Sejnowski, 1999), which was shown to be of critical importance for encoding of spatial information in the hippocampus (Wilson and McNaughton, 1993).

In conclusion, we suggest that the induction of associative LTD is a powerful mechanism to depress out-of-phase synaptic input. Thus, it may be important to have a direct gain control of associative synaptic depression, provided by the G-protein-mediated modulation of the voltage-gated N-type Ca\(^{2+}\) channels.

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