Associative Long-Term Depression in the Hippocampus Is Dependent on Postsynaptic N-Type Ca\(^{2+}\) Channels

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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 \(\alpha\)-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 by postsynaptic action potentials. Both nifedipine, an L-type Ca\(^{2+}\) channel antagonist, and \(\alpha\)-conotoxin GVIA, a selective N-type channel blocker, abolished the induction of associative LTD. 8-hydroxy-2-dipropylaminotetralin (OH-DPAT), a 5-HT\(_{1A}\) receptor agonist, inhibited postsynaptic Ca\(^{2+}\) influx through N-type Ca\(^{2+}\) channels, without affecting presynaptic transmitter release. OH-DPAT also inhibited the induction of associative LTD, suggesting that the involvement of N-type channels makes synaptic plasticity accessible to modulation by neurotransmitters. Thus, the modulation of N-type Ca\(^{2+}\) channels provides a gain control for synaptic depression in hippocampal pyramidal neurons.

Key words: associative long-term depression; hippocampus; N-type Ca\(^{2+}\) channels; NMDA receptors; metabotropic glutamate receptors; asynchronous pairing

Long-term changes in synaptic strength at glutamatergic synapses are thought to underlie complex functions of neuronal networks, such as learning and memory (Bliss and Collingridge, 1993). The frequency of synaptic stimulation determines both the extent and the duration of the change in synaptic efficacy; high-frequency stimulation (HFS) leads to long-term potentiation (LTP), whereas low-frequency stimulation (LFS) results in long-term depression (LTD) (Dudek and Bear, 1992). Whereas several molecular steps of the induction and expression of LTP have been identified, the mechanisms that lead to LTD are less clear.

In the hippocampus, LFS induces two distinct forms of LTD, which depend either on the Ca\(^{2+}\) influx through NMDA receptors (NMDARs) (Mulkey and Malenka, 1992) or on the activation of metabotropic glutamate receptors (mGluRs) (Bolshakov and Siegelbaum, 1994; Oliet et al., 1997; Otani and Connor, 1998). The mGluR LTD appears to be the predominant form in young animals (postnatal days 3–8; Bolshakov and Siegelbaum, 1994), whereas NMDAR LTD and mGluR LTD coexist in older animals (Heynen et al., 1996; Oliet et al., 1997; Otani and Connor, 1998).

Although LTD induces robust changes in synaptic strength, the situation that leads to the induction of hippocampal LTD \textit{in vivo} could be more complex. CA3 and CA1 pyramidal neurons generate action potentials in a precise temporal relationship, depending on the behavioral context (O’Keefe and Reece, 1993), i.e., pyramidal cells in the center of their place field fire action potentials more early in the theta cycle than neurons with adjacent place fields (Skaggs et al., 1996). Thus, the natural paradigm for LTP and LTD 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; Stanton et al., 1991). However, the induction mechanisms of associative LTD have remained controversial. 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-μm-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 NaHPO\(_4\), 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 (1.5 mm outer diameter, 0.5 mm inner diameter, 30° bevel, drawn in an airfield, 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 provided a bridge-balance 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 lowpass Bessel filter at 10 and 20 kHz, respectively (1410 filter; Monitcor, Cambridge, UK). 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): 130 CsCl, 5 KCl, 0.5 CaCl 2, 2 Na 2ATP, 0.3 NaGTP, 0.2–0.5 EGTA, and 100 HEPES (pH adjusted to 7.3 with KOH). Dendritic recordings were performed as described previously (Bischoffer and Jonas, 1997). Patch resistance was 5–10 MΩ for somatic and 10–12 MΩ for dendritic recordings. Bridge balance was used in all recordings where resistance was >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 stimulation pipette was placed in the stratification 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 and heterosynaptic stimulation in the rest 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. Voltage-clamp measurements were performed in the presence of current-clamp experiments. In some experiments 10 μM glycine was added to the bath solution, with no obvious differences in the results.

To record Ba 2+ currents, the recording pipettes (mostly 0.8–2 MΩ) were filled with a solution containing (in mM): 140 CsCl, 2 MgCl 2, 2 Na 2ATP, 0.3 NaGTP, 0.2 NaCl, 2 MgCl 2, 2 Na 2ATP, 0.3 NaGTP, 0.2–0.5 EGTA, and 100 HEPES (pH adjusted to 7.3 with KOH). Dendritic recordings were performed as described previously (Bischoffer and Jonas, 1997). Patch resistance was 5–10 MΩ for somatic and 10–12 MΩ for dendritic recordings. Bridge balance was used in all recordings where resistance was >20–60 MΩ.

For fluorescence measurements, the intracellular Ca 2+ signals were 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 (Polychrom 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 was selected (LWP420, KP600) from Delta Light & Optics (Lyngby, Denmark). Some experiments used 10 μM Bapta-1, Molecular Probes) instead of fura-2, using a filter combination tailored to the Ca 2+ signal (LWP420, KP600) from Delta Light & Optics (Lyngby, Denmark). Some experiments used 10 μM Bapta-1, Molecular Probes) instead of fura-2, using a filter combination tailored to the Ca 2+ signal (LWP420, KP600) from Delta Light & Optics (Lyngby, Denmark).

For data acquisition we used self-made and commercial programs (EPC, CED, Pulse, Heka).

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 protocol (100 Hz) reduced the EPSP amplitude by 18% (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% decrease, 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 Ca**²⁺**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 ω-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 ω-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 ω-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 ω-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.
Modulation of postsynaptic N-type Ca\(^{2+}\) channels inhibits associative LTD

OH-DPAT, a selective agonist of 5-hydroxytryptamine (5-HT)\(_{1A}\) receptors, is known to inhibit N-type Ca\(^{2+}\) channels in cortical pyramidal neurons by activation of a G\(_{i/o}\) protein (Foehring, 1996). Immunocytochemical analysis revealed a high density of 5-HT\(_{1A}\) 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 Ca\(^{2+}\) channels. As a first experimental step, we examined the effect of OH-DPAT on the action potential-induced Ca\(^{2+}\) transient (Fig. 5A). Application of 1 \(\mu\)M OH-DPAT reduced the dendritic Ca\(^{2+}\) transients by 25.6 \pm 2.7\% \((n = 5; 40–100 \mu\)M). This is consistent with the previously reported reduction of burst-induced Ca\(^{2+}\) transients by 10 \(\mu\)M 5-HT in CA1 pyramidal neurons (Sandler and Ross, 1999).

The reduction in the dendritic Ca\(^{2+}\) transient by OH-DPAT could be attributable to a direct inhibition of Ca\(^{2+}\) channels or a reduction in the amplitude of the backpropagating action potential, or both. For 10 \(\mu\)M 5-HT a slight reduction of the amplitude of the 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 Ca\(^{2+}\) channels in isolation in the whole-cell voltage-clamp configuration using 2 mM Ba\(^{2+}\) as charge carrier (Fig. 5B). The application of 1 \(\mu\)M OH-DPAT reduced the Ba\(^{2+}\) currents to 72.9 \pm 10.9\% \((n = 8; p < 0.01)\). In the presence of \(\alpha\)-conotoxin GVIA the Ba\(^{2+}\) currents were reduced to 45.8 \pm 6.6\% \((n = 4)\). Subsequent to the application of \(\alpha\)-conotoxin, the modulation by OH-DPAT was completely absent, indicating a selective modulation of N-type Ca\(^{2+}\) channels by 5-HT\(_{1A}\) receptors. In the presence of 10 \(\mu\)M nifedipine (84.7 \pm 7.0\% of control; \(n = 4)\), however, there was still a substantial reduction of the Ba\(^{2+}\) currents by OH-DPAT (57.0 \pm 3.9\% of control; \(p < 0.01\)).

To examine possible effects of 1 \(\mu\)M 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 \(\mu\)m from the soma (Fig. 5C). The shape of the dendritic and somatic action potential in 1 \(\mu\)M OH-DPAT was very similar to control conditions. The resting membrane potential was slightly hyperpolarized by –1.0 \pm 0.2 mV at the dendrite and by –0.9 \pm 0.2 mV at the soma \((p < 0.05; \text{six double recordings})\). As shown in Figure 5D, the action potential amplitude in the presence of 1 \(\mu\)M OH-DPAT was virtually identical to control conditions (101.2 \pm 0.9\% of control, \(n = 6; p > 0.1\)). Furthermore no significant change in half width of the dendritic AP (102.1 \pm 2.1\% of control; \(p > 0.1\)) or the propagation velocity (98.3 \pm 3.2\% of control; \(p > 0.1\); Fig. 5D) was observed. In conclusion, these results indicate that the reduction of action potential-induced Ca\(^{2+}\) transients by 1 \(\mu\)M OH-DPAT is attributable to a direct modulation of postsynaptic N-type Ca\(^{2+}\) channels and not to an inhibition of dendritic backpropagation.

If postsynaptic N-type Ca\(^{2+}\) channels are necessary for induction of associative LTD (Fig. 4C) and if these channels are selective targets for modulation via 5-HT\(_{1A}\) receptors (Fig. 5), then OH-DPAT should affect LTD induction. We first tested the effect of 1 \(\mu\)M OH-DPAT on basal synaptic transmission, and we found that the peak EPSP amplitude remained unchanged (Fig. 6A; 95.8 \pm 6.4\% of control, \(n = 11; p > 0.1\)). This allowed us to use OH-DPAT as a tool to inhibit selectively postsynaptic N-type Ca\(^{2+}\) channels. In the presence of 1 \(\mu\)M OH-DPAT, application of the asynchronous pairing paradigm failed to induce associative LTD (102.8 \pm 4.9\% of control, \(n = 11; p > 0.5\); Fig. 6B,C). These results indicate that the Ca\(^{2+}\) 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 Ca\(^{2+}\) channels. In particular, we show a direct involvement of N-type Ca\(^{2+}\) channels in synaptic plasticity. The modulation of postsynaptic N-type Ca\(^{2+}\) channels by 5-HT\(_{1A}\) 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 (Bolschakov 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...
Fig. 3. Associative LTD is dependent on activation of metabotropic glutamate receptors. A. The mean EPSP amplitude is plotted against time. In the presence of 500 µM MCPG, an antagonist of metabotropic glutamate receptors, the associative LTD was inhibited \( n = 6 \). B. The NMDAR antagonist D-AP-5 (50 µM) was without effect on both basal synaptic transmission and the induction of associative LTD \( n = 6 \). C. Nine hundred EPSPs at a membrane potential of \(-62\) mV (inset), evoked at a frequency of 1 Hz (indicated by the arrow) reliably depressed the EPSP amplitude \( n = 8 \). D. LFS-induced LTD was blocked by the application of 50 µM D-AP-5 \( n = 4 \). Insets in A and C represent the first three sweeps of the induction paradigm of a representative experiment. Horizontal bars indicate the presence of the MCPG and D-AP-5, respectively.

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,

Fig. 4. Associative LTD is dependent on Ca\(^{2+}\) influx via both, voltage-gated N- and L-type Ca\(^{2+}\) channels. A, Fluorescence image of a CA1 pyramidal neuron filled with 0.1 mM fura-2 (380 nm excitation). Rectangles indicate somatic and dendritic ROIs from where Ca\(^{2+}\) transients were recorded with high time resolution (100 Hz, traces on the right). The action potential-induced Ca\(^{2+}\) transients were reduced in the presence of 1 µM \(\omega\)-conotoxin GVIA (\(\omega\)-con, N-type blocker). B, The bar graph summarizes the mean inhibition of the Ca\(^{2+}\) transient by \(\omega\)-conotoxin GVIA \( n = 6 \) obtained with 0.1 mM fura-2 and 10 µM nifedipine \( n = 5 \); L-type blocker) obtained with 0.1 mM Oregon Green (see Materials and Methods). Dendritic ROIs were located in the stratum radiatum at a distance of \(0–40\) µm or \(40–100\) µm from the soma. C, Application of 0.5 µM \(\omega\)-conotoxin GVIA \( n = 9 \) reduced basal synaptic transmission and prevented the induction of LTD by APS. D, A 10 µM concentration of nifedipine did not affect basal transmission but similarly inhibited LTD induction \( n = 7 \). Voltage traces in C and D are EPSPs from a single representative experiment, respectively, at the times indicated by the asterisks.
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\(^{2+}\) buffers (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 µM 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 µm of the apical dendrite. In contrast, higher concentrations (30 µM) 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). Hippocampal oriens-alevus 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 Reece, 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.

REFERENCES


