abnormality of fibrocytes in the spiral ligament would be expected to disrupt K^+ transport, leading to depression of the EP. A reduced EP would explain the elevation of ABR thresholds in mutants as the receptor potential of hair cells depends on the magnitude of the EP (*15*). The idea that fibrocytes contribute to the generation or maintenance of the EP is thus strongly supported by our present finding.

In the past 5 years, 13 human genes have been identified that are responsible for hereditary nonsyndromic deafness (*16*). Mouse models harboring mutations in the homologous genes are available for *Brn 3.1*/*Brn-3c* and for the myosin VIIA (shaker 1) and myosin XV (shaker 2) genes. In all three of these models, the mice suffer sensorineural deafness because of defects in sensory hair cells of the inner ear. Our analysis of Brn-4-deficient mice has indicated that cochlear fibrocytes, which are nonsensory mesenchymal cells specific to the cochlear duct, may also play an important role in auditory function. Given the high level of *Brn-4* expression in fibrocytes, the pathological changes may be a cell autonomous consequence of Brn-4 deficiency, as is the case for other members of the POU transcription factor family (*17*). Because the number of fibrocytes in Brn-4-deficient mice is similar to that in wild-type mice (*9*), Brn-4 may be essential for the differentiation or function of fibrocytes but not for their survival. The fibrocytes are rich in Na^+,K^+-ATPase and the gap junction protein connexin 26 (*11*), which are thought to be essential for K^+ transport, and mutations in the *GJB2* gene encoding connexin 26 have been shown to be responsible for DFNB1, another nonsyndromic deafness (*18*). Neither *GJB2* nor the Na^+,K^+-ATPase gene, however, appears to be a target of Brn-4-mediated regulation, because Brn-4-deficient fibrocytes showed no dramatic changes in the expression of either gene. Identification of *Brn-4* target genes in cochlear fibrocytes may help to elucidate the role of these cells in auditory function.

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5. A 1.3-kb Acc I–Bam HI fragment and a 6-kb Eco RI fragment isolated from a J1 genomic DNA library were used as short and long homologous sequences of a targeting vector, respectively. The pgk-neo cassette flanked by a pair of loxP sequences was used for positive selections, and the diphtheria toxin A-chain gene cassette without a polyadenylation site was used for negative selection. The Not I-linearized targeting vector was introduced into J1 ES cells derived from 129/sv mice by electroporation and cells were selected with G418 (*17*).
6. An OCTA26 probe (GATCAGTACTAATTAGCATTATAAAG) was used.
7. Mice were anesthetized with sodium pentobarbital (70 mg/kg) and maintained in a headholder within an acoustically and electrically insulated test room. Needle electrodes were placed on the tympanic bulla (positive lead) and scalp vertex (negative lead). Acoustic stimuli evoked by a click were delivered and the ABRs were measured with an evoked potential recording system (NEC, Tokyo). The peak amplitude was measured peak to trough and the threshold was defined as 1 μV.
8. Horizontal sections of the temporal bone showed no gross difference between wild-type and mutant mice in the thickness of the stapes footplate or in the size or shape of the bony labyrinth, including the internal auditory meatus.
9. Under deep anesthesia, cochleas from 11-week-old mice were fixed with 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (pH 7.4) overnight at 4°C. The specimens were decalcified with 0.12 M EDTA, dehydrated, and embedded in paraffin. Serial sections (6 μm) were stained with hematoxylin and eosin. Five sections containing the suprastrial zone of the midbasal turn were selected at random and the number of fibrocytes (mean ± SD) was counted in sections from wild-type (590 ± 25) (n = 4) and mutant (605 ± 50) (n = 4) male mice.
10. Mice at postnatal day 0 were anesthetized and perfused with ice-cold 4% PFA and immersed in 4% PFA at 4°C overnight. Heads were embedded in OCT compound (Sakura) and cryosectioned (20 μm). Digoxigenin-labeled RNA probes were synthesized by using a 390-base-pair fragment of the 3′ noncoding region of mouse *Brn-4* as a template (Boehringer). Tissue sections were treated with 4% PFA, washed in phosphate-buffered saline, and then hybridized with the probes at 72°C for 24 hours. Sections were washed under stringent conditions and hybridization signals were detected by immunohistochemical analysis.
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12. Mice were anesthetized and perfused with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). Cochleas were fixed by perilymphatic perfusion with 2.5% glutaraldehyde overnight at 4°C and immersed in the same fixative for 2 more hours at room temperature. Electron microscopy was performed as in (*19*).
13. In both wild-type and mutant mice, all three cell types of the stria vascularis (marginal, intermediate, and basal cells) were identified and presented a normal appearance. There was no observable difference in the structure of intrastrial capillaries between wild-type and mutant mice. Myelinated axons from spiral ganglion cells to sensory hair cells were well preserved. At the bases of inner hair cells, the fine structure of the afferent nerve terminal synapses appeared to be normal in mutant mice.
14. For EP measurement, each mouse was artificially ventilated with a respirator through a tracheal cannula after deep anesthesia and muscular relaxation. Rectal temperature was maintained at 37°C and an electrocardiometer was monitored. A glass microelectrode filled with 150 mM KCl was inserted into the scala media of the basal turn through the lateral wall of the cochlea and the output was recorded with a high-impedance dual electrometer (WPI), New Haven, CT.
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Long-Term Depression in Hippocampal Interneurons: Joint Requirement for Pre- and Postsynaptic Events

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Long-term depression (LTD) is a well-known form of synaptic plasticity of principal neurons in the mammalian brain. Whether such changes occur in interneurons is still controversial. CA3 hippocampal interneurons expressing Ca^{2+}-permeable AMPA receptors exhibited LTD after tetanic stimulation of CA3 excitatory inputs. LTD was independent of NMDA receptors and required both Ca^{2+} influx through postsynaptic AMPA receptors and activation of presynaptic mGluR7-like receptors. These results point to the capability of interneurons to undergo plastic changes of synaptic strength through joint activation of pre- and postsynaptic glutamate receptors.

LTD and long-term potentiation (LTP) are activity-dependent forms of synaptic plasticity that have been extensively studied in the hippocampus, neocortex, and cerebellum (*1*, *2*). Most studies related to LTD and LTP have examined excitatory synapses onto principal neurons. However, it is important to know whether the same types of long-term plasticity occur at excitatory synapses onto interneurons. It is clear that long-term plasticity at certain hippocampal interneurons is passive, resulting from plasticity of excitatory inputs

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to the principal cells that synapse onto the interneurons (*3*, *4*); however, other evidence points toward direct forms of plasticity (*5*, *6*). Recently, Ca^{2+}-permeable AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate) receptors were shown to mediate synaptic potentiation (*7*, *8*). In some interneurons Ca^{2+}-permeable and Ca^{2+}-impermeable AMPA receptors are localized to different synapses (*9*, *10*), whereas in other interneurons AMPA receptor subtypes appear to intermix in different proportions at the same synapses (*11*, *12*).

We performed whole-cell patch-clamp recordings from visually identified CA3 stratum (st.) radiatum interneurons in thin hippocampal slices prepared from young rats (*13*). Low-intensity electrical stimulation of the CA3 pyramidal cell layer evoked excitatory inputs onto the nearby interneurons (Fig. 1A). Interneurons filled with biocytin revealed that their axons mainly target st. radiatum (Fig. 1B). In these interneurons, excitatory postsynaptic currents (EPSCs) can be classified into two broad groups. Type I EPSCs have an outwardly rectifying or linear current-voltage (*I-V*) relation, are insensitive to polyamines, and are mediated by AMPA receptors with low Ca^{2+} permeability, whereas type II EPSCs are inwardly rectifying, are blocked by polyamines, and show a range of Ca^{2+} permeability that approaches that of NMDA (*N*-methyl-D-aspartate) receptors (*10*–*12*, *14*, *15*). All evoked EPSCs were abolished by 50 μM LY303070, a specific AMPA receptor antagonist (*16*).

High-frequency stimulation of CA3 pyramidal cells induces LTP in both CA1 and CA3 pyramidal cells (*2*, *17*). On the basis of the finding that Ca^{2+}-permeable AMPA receptors may be capable of supporting synaptic potentiation (*7*, *8*), we examined whether type II EPSCs could mediate any form of synaptic plasticity. With NMDA receptors blocked (*13*), we applied three trains of high-frequency stimulation (30 pulses; 100 Hz; one train every 10 s) to the CA3 cell body layer. All type II EPSCs tested (n = 12 interneurons) showed LTD of the mean EPSC amplitude [Fig. 1C; analysis of variance (ANOVA), $P < 0.001$]. LTD started immediately after tetanic stimulation. Within 5 min the EPSC amplitude fell to an average steady-state level of 53 ± 7% of pretetanus control, which persisted up to 60 min after tetanus.

In contrast, none of the type I EPSCs (n = 10 interneurons) showed LTD after tetanus (Fig. 1C), but transient potentiation was observed in some cases (*18*). Two cells with EPSCs of intermediate rectification showed either LTD or no change in response to the tetanus (Fig. 1D, triangles) and were excluded from further analysis. A 50-μM concentration of LY303070 abolished EPSCs occurring within the tetanic trains (n = 3), ruling out the possibility of a substantial contribution of kainate receptor activation to LTD.

The finding that LTD occurred only in interneurons with EPSCs that had low rectification ratios (r.r. < 0.5; Fig. 1D) (*15*) suggests that Ca^{2+} entry through AMPA receptors may be required for LTD. If so, LTD should be prevented by (i) polyamine antagonists that selectively block GluR2-lacking AMPA receptors, (ii) depolarization of the interneuron during the tetanus to reduce Ca^{2+} influx, and (iii) perfusion of the interneuron with BAPTA (to chelate tetanus-induced Ca^{2+} influx).

First, we tested whether type II EPSCs could be selectively blocked by polyamines (*11*, *19*). An inwardly rectifying EPSC was largely blocked by *N*-(4-hydroxyphenylpropanyl)-spermine (NHPP-SP) (Fig. 2, A and C). In contrast, an EPSC with a linear *I-V* relation was unaffected by the same drug (Fig. 2, B and D). The effectiveness of NHPP-SP and of Joro spider toxin (JSTX) was inversely correlated to the rectification ratio (*15*), showing a maximal block of EPSCs having a very low rectification ratio (Fig. 2E) (*11*, *19*). To establish whether inwardly rectifying AMPA receptors were necessary for LTD induction, we delivered trains of stimuli that elicited type II EPSCs in the presence of 10 or 30 μM NHPP-SP. Both concentrations of the polyamine completely blocked LTD (Fig. 2F; ANOVA, $P < 0.05$, n = 5).

LTD requires an inward flux of ions, be-

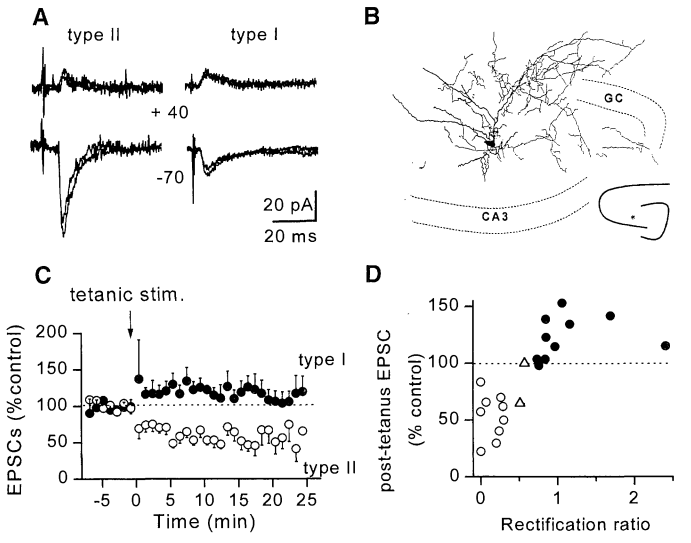

Fig. 1. LTD occurs exclusively at type II EPSCs. (**A**) Type I and type II EPSCs evoked at +40 mV (top) and −70 mV (bottom) in two different interneurons. (**B**) Morphological reconstruction of a typical biocytin-filled CA3 interneuron. The axon is displayed as a thinner line compared with the dendrite. The inset shows the position of the cell body within the st. radiatum (GC, granule cell layer). In all 42 st. radiatum interneurons examined, axons were distributed mainly within st. radiatum but could also extend into the hilus or dentate gyrus as in this cell. Two or three thick primary dendrites arose from a multipolar, bipolar, or fusiform cell body; dendrites branched proximally and ran primarily radially. These cells correspond most closely to Schaffer collateral-associated interneurons (*33*). (**C**) Tetanic stimulation was delivered to CA3 synaptic inputs at the arrow while type II (open circles) and type I EPSCs (solid circles) were recorded. Each point is the mean ± SEM of evoked EPSCs from 12 type II or 10 type I synapses. (**D**) Posttetanic EPSC amplitude measured 10 min after tetanus is expressed as a function of the rectification ratio (r.r.) of the interneuron (*15*). Type II EPSCs are inwardly rectifying with r.r. ≤ 0.5 (open circles) and depress after tetanic stimulation. Type I EPSCs have linear or outwardly rectifying *I-V* relations and show either transient potentiation (*18*) or no effect (solid circles). Two cells with intermediate r.r. are shown as open triangles.

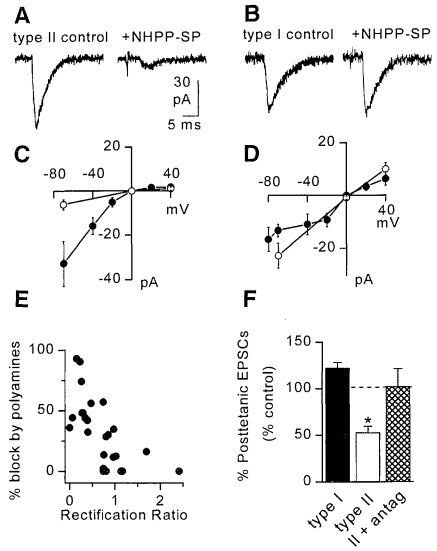

Fig. 2. Polyamine sensitivity of CA3 inputs and LTD. (**A**) CA3-evoked type II EPSCs at −70 mV are reduced by 10 μM NHPP-SP. (**B**) CA3-evoked type I EPSCs in a different cell are unchanged at −70 mV in the control (left) and in the presence of 10 μM NHPP-SP. (**C** and **D**) *I-V* relations of EPSCs from the same interneurons as (A) and (B) before (solid circles) and after perfusion with 10 μM NHPP-SP (open circles). (**E**) Percent block of EPSC by polyamines (8 to 10 μM JSTX or 1 to 10 μM NHPP-SP; holding potential = −70 mV) is inversely related to the rectification ratio. (**F**) Type II EPSCs recorded after tetanic stimulation in the presence of NHPP-SP (10 to 30 μM) (hatched bar) are displayed as percentage of pretetanic control. For comparison, posttetanic type I EPSCs (black bar) and type II (gray bar) EPSCs are also shown (*$P < 0.001$, by analysis of variance post hoc Bonferroni).

cause clamping at +40 mV during the tetanic train prevented LTD induction (Fig. 3A). A subsequent tetanic stimulation delivered at −70 mV, as for control conditions, produced LTD in the same cells (second arrow in Fig. 3A). This result suggests that a postsynaptic event must be involved in the LTD mechanism, but additional presynaptic contributions are not ruled out.

We used the intracellular Ca^{2+} chelator BAPTA [1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid] to determine whether postsynaptic Ca^{2+} influx through inwardly rectifying AMPA receptors is necessary for LTD induction. Inclusion of 10 mM BAPTA in the recording solution prevented LTD in three of six neurons expressing type II synapses and delayed LTD onset in the other three cells. A 30-mM concentration of BAPTA abolished LTD (Fig. 3B). After tetanic stimulation, EPSCs were 98 ± 5% of control with 30 mM BAPTA in the pipette (n = 5), compared with 57 ± 4% of control in the absence of BAPTA (n = 12) ($P < 0.05$). Although 10 mM BAPTA often is sufficient to block Ca^{2+}-mediated plasticity, 30 to 50 mM BAPTA is needed to block plasticity at some synapses (*20*). A high concentration of BAPTA might be required, for example, if the site of action of the intracellular Ca^{2+} is near the mouth of the AMPA receptor channel, or if a low concentration of internal Ca^{2+} is sufficient to trigger LTD (*21*).

The synaptic failure rate increased after tetanus in type II EPSCs (28% in control compared with 51% after tetanus, $P < 0.001$, paired t test, n = 5; Fig. 3E), but the mean amplitude of the EPSCs in successful stimulus trials remained constant after tetanus (102 ± 2% of control, n = 3, Fig. 3C, D). The (coefficient of variation)$^{-2}$ of evoked EPSCs (including failures) was reduced to 63 ± 11% of control in LTD (n = 12), indicative of reduced quantal content. These findings are consistent with, but do not prove (*22*), a reduction in the probability of transmitter release from presynaptic terminals in LTD.

We next investigated the role of presynaptic mGluR receptors in LTD. CA3 inputs to st. radiatum interneurons were not significantly reduced by a high concentration (30 μM) of the selective agonist for group II mGluRs, 2*R*,4*R*-4-aminopyrrolidine-2,4-dicarboxylate (APDC) (Fig. 4A, n = 10), indicating that group II receptors (mGluR2, mGluR3) are absent or ineffective at these terminals. This finding also confirms that the inputs studied are not from mossy fibers (*5*, *9*).

Among the group III mGluRs, mGluR6 appears to be absent in the hippocampus and mGluR4 is expressed mainly in CA2 (*23*), whereas mGluR7 and mGluR8 are heavily expressed by CA3 pyramidal neurons (*24*, *25*). The mGluR7 receptors appear to be exclusively localized to presynaptic terminals in the hippocampus, predominantly at synapses onto interneurons (*24*, *26*). We thus hypothesized that either mGluR7, mGluR8, or both mediate presynaptic inhibition at both type I and type II synapses. The group III–selective agonist L-(+)-2-amino-4-phosphonobutyric acid (L-AP4) has a median effective concentration of 0.4 to 1.2 μM for mGluR8 and 160 to 500 μM for mGluR7 (*27*). A 3-μM concentration of L-AP4 did not depress synaptic transmission at either type I or type II synapses, suggesting that mGluR8 receptors do not influence transmitter release at these synapses. However, both type I and type II EPSCs were depressed by 1 mM L-AP4, consistent with activation of mGluR7 at these synapses (Fig. 4A). L-AP4 at 1 mM increased the incidence of synaptic failures (20% in control compared with 48% in L-AP4, $P <$ 0.001, paired t test, n = 6), consistent with a presynaptic localization of mGluR7 (*24*–*26*).

We then investigated whether activation of group III mGluRs was necessary to produce LTD. The group II and group III antagonist LY341495 [2*S*-2-amino-2-(1*S*,2*S*-2-carboxycyclo-pro-1-yl)-3-(xanth-9-yl) propanoic acid] is active at submicromolar concentrations against recombinant mGluR7 receptors (*28*) and blocks the effect of group III–selective mGluR ago-

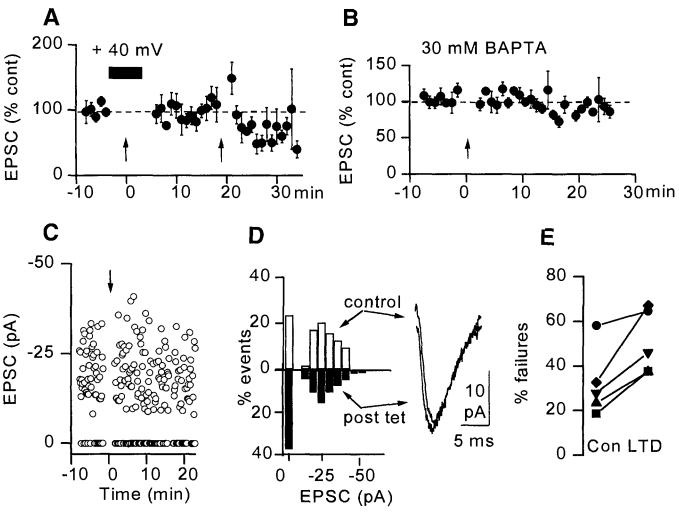

Fig. 3. Postsynaptic Ca^{2+} influx and failure rate in LTD. (**A**) Depolarization blocks LTD. Type II EPSCs normalized to pretetanic control period are shown. At the first arrow, tetanic stimulation was delivered while interneurons were clamped at +40 mV (n = 4). At the second arrow, tetanic stimulation was delivered in standard conditions while the cells were clamped at −70 mV (n = 3). (**B**) Type II EPSCs of interneurons loaded with 30 mM BAPTA do not express LTD (n = 5). (**C**) Type II EPSC amplitude before and after tetanic stimulation (arrow). After the stimulus trains, EPSC amplitude was unchanged but failure rate increased from 23% to 37%. (**D**) Amplitude histogram of EPSCs [from cell of (C)] expressed as percentage of events in control (gray) and after tetanus (black). To the right, averages of 20 EPSCs (nonfailures aligned on rising phase) before and after tetanus show that EPSC kinetics and amplitude were similar after LTD was established. Mean EPSC amplitude (nonfailures) was 20.8 ± 0.9 pA before and 19.7 ± 0.8 pA after the tetanus. EPSCs are offset vertically for clarity. (**E**) LTD was associated with an increase in percentage of failures of type II EPSCs. Each cell is represented by a different symbol (con, control).

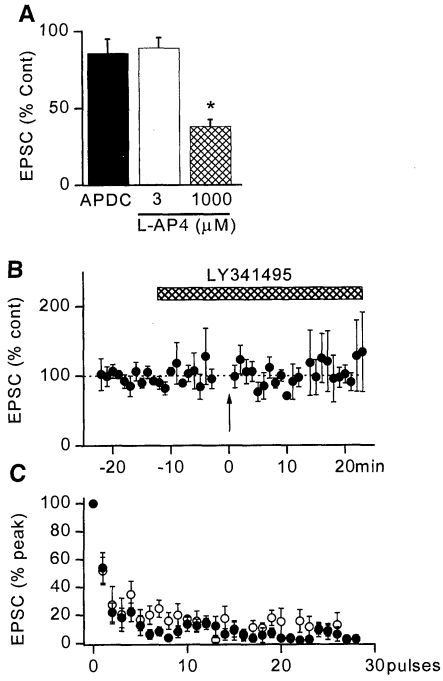

Fig. 4. Metabotropic glutamate receptor involvement in LTD. (**A**) Effect of 30 μM APDC (n = 10, black bar) or L-AP4 (3 μM or 1 mM, respectively, gray bar, n = 6, or hatched bar, n = 10) on type I and type II EPSCs, expressed as a percentage of EPSC amplitude in the control period before exposure. Data on types I and II EPSCs were pooled because drug effects were similar (*$P < 0.001$). (**B**) The group II/III–selective antagonist LY341495 (500 nM) blocked LTD without affecting transmission at low stimulus frequency (n = 3 interneurons with mean r.r. = 0.3 ± 0.1). EPSC amplitude is expressed as a percentage of pretetanic control. At the arrow, tetanic stimulation was delivered. (**C**) EPSCs normalized to the highest amplitude EPSC occurring during 100-Hz trains are plotted in relation to the number of pulses during the train in the absence (solid circles, n = 8) or presence (open circles, n = 5) of 500 nM LY341495. Data are pooled from type I and type II EPSCs.

nists in substantia nigra slices at a concentration of 500 nM (*29*). LY341495 at 500 nM prevented LTD but had no effect on synaptic transmission in the pretetanic control period (Fig. 4B), suggesting a role for presynaptic mGluR7 in LTD. To test whether mGluR7 receptors modulate the probability of release during the stimulus train, we examined the effect of LY341495 on EPSCs elicited by each stimulus during the 100-Hz train. The normal rapid decay of EPSC amplitude observed during 100-Hz trains was not changed by LY341495 (Fig. 4C). Because LTD was blocked by 500 nM LY341495, a presynaptic signaling pathway, likely to involve mGluR7 receptors, appears necessary for LTD to occur. Group II mGluRs appear necessary for LTD at other hippocampal synapses involving principal neurons (*30*). Taken together, these results point to a major role for presynaptic metabotropic receptors in long-term synaptic plasticity, with mGluR2 and mGluR7 regulating excitatory inputs onto principal neurons and hippocampal interneurons, respectively.

A subset of hippocampal inhibitory interneurons is thus capable of undergoing LTD of excitatory inputs that requires activation of both postsynaptic AMPA receptors and presynaptic mGluR7-like receptors. Both types I and II EPSCs are depressed by 1 mM L-AP4, although LTD occurs only at type II synapses, reinforcing the need for joint pre- and postsynaptic receptor activation. Entry of Ca^{2+} through AMPA receptors might trigger a synaptic shape change that enables access of released glutamate to presynaptic mGluR7 receptors, or cause release of a retrograde message that cooperates with presynaptic mGluR7 activation to suppress transmitter release. High-frequency stimulation of CA3 pyramidal cells can induce either LTP, LTD, or no changes in synaptic strength, depending on the postsynaptic target (*2*, *3*, *6*, *17*). Heterogeneity of postsynaptic receptors as well as presynaptic terminal properties are thus key for the sign and magnitude of synaptic plasticity at different targets.

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