Bidirectional Associative Plasticity of Unitary CA3-CA1 EPSPs in the Rat Hippocampus In Vitro

DOMINIQUE DEBANNE, BEAT H. GÄHWILER, AND SCOTT M. THOMPSON

Brain Research Institute, University of Zurich, CH-8029 Zurich, Switzerland

Debanne, Dominique, Beat H. Gähwiler, and Scott M. Thompson. Bidirectional associative plasticity of unitary CA3-CA1 EPSPs in the rat hippocampus in vitro. *J. Neurophysiol.* 77: 2851–2855, 1997. Associative long-term potentiation (LTP) and depression of compound and unitary CA3-CA1 excitatory postsynaptic potentials (EPSPs) were investigated in rat hippocampal slice cultures. The induction of LTP with synchronous pairing of synaptic activation and postsynaptic depolarization resulted in an increase in the amplitude of EPSPs to the same absolute level, regardless of whether the input was naive or had been previously depressed by asynchronous pairing of pre- and postsynaptic activity. Saturated LTP of compound and unitary EPSPs was reversed by asynchronous pairing and could be reinduced by synchronous pairing. The likelihood that an action potential in a presynaptic CA3 cell failed to trigger an unitary EPSP in a postsynaptic CA1 cell decreased after induction of associative potentiation and increased after induction of associative depotentiation. These changes in the rate of transmission failures were accompanied by large changes in the amplitude of nonfailure EPSPs. We conclude that the same CA3-CA1 synapses can alternatively undergo associative potentiation and depression, perhaps through opposite changes in a single expression mechanism.

INTRODUCTION

Long-term potentiation (LTP) in the CA1 region can be reversed with stimulation protocols that induce *homosynaptic* long-term depression (LTD) (Barrionuevo et al. 1980; Dudek and Bear 1993; Heynen et al. 1996; Huerta and Lisman 1995; Mulkey and Malenka 1992). It is not known, however, whether previously potentiated synapses can be depotentiated with stimulation patterns that induce *associative* LTD (Debanne et al. 1994). This issue is particularly important because temporal contiguity of neuronal activity is likely to be an important determinant of the sign of the changes in synaptic efficacy (Debanne et al. 1994; Huerta and Lisman 1995). In addition, although it was previously inferred that depotentiation reflects a true decrease in the strength of previously potentiated synapses, there has been no evidence of bidirectional changes in the strength of unitary excitatory postsynaptic potentials (EPSPs), produced by an action potential in a single presynaptic neuron. Paired recordings from monosynaptically coupled cell pairs are particularly useful for addressing this question because stimulation of only a very small number of synapses is assured.

METHODS

Hippocampal slice cultures were prepared and maintained as described previously (Gähwiler 1981). Hippocampi slices from 5- to 7-day-old rat pups were dissected at 400 μm and attached to glass coverslips in clotted chicken plasma. The coverslips were placed in individual sealed test tubes containing semisynthetic medium and maintained on a roller drum in an incubator for 2–4 wk. For electrophysiological recordings, cultures were transferred to a recording chamber mounted on an inverted microscope and continuously superfused with a warmed (32°C) saline containing (in mM): 149 Na^+, 149 Cl^-, 2.7 K^+, 2.8 Ca^{2+}, 2.0 Mg^{2+}, 11.6 HCO_3^-, 0.4 $H_2PO_4^-$, 5.6 glucose, and also 10 mg/l phenol red, at pH = 7.4. Pyramidal neurons were impaled by using sharp microelectrodes filled with 1 M potassium methylsulphate. EPSPs were recorded from postsynaptic CA1 cells. Compound EPSPs were evoked by field stimulation within area CA3; unitary EPSPs were evoked by generation of single presynaptic action potentials in intracellularly impaled CA3 pyramidal cells. The monosynaptic nature of unitary EPSPs was assessed as described previously (Debanne et al. 1995).

The naive amplitude is the EPSP amplitude before induction of either LTP or LTD in a given culture. Values of potentiation and depression were derived from averages of EPSPs over 2–3-min periods, taken 10 min after the pairing procedure unless otherwise noted (Debanne et al. 1994). The Mann-Whitney U-test was used for all statistical comparisons.

RESULTS

Potentiation of naive and depressed synapses to the same level

Potentiation was induced in area CA1 when extracellularly elicited EPSPs were repeatedly (60–100 times) paired with synchronous postsynaptic depolarizing pulses (240 ms, 0.5–2.5 nA). No further LTP could be induced by repeating the synchronous pairing (SP) procedure 15 min after such potentiation [amplitude after second SP = 95 ± 7% (mean ± SE) of the potentiated amplitude, $n = 3$ cells; not significantly different, $P > 0.5$; Fig. 1*A*]. We conclude that this procedure is sufficient to saturate the ability of synapses to express LTP, and we will assume that the procedure also results in saturated LTP of unitary EPSPs.

The absolute EPSP amplitudes obtained after inducing saturating LTP with the SP protocol were similar for naive EPSPs (152 ± 11% of the naive amplitude, $n = 14$ cells) and for EPSPs recorded from cells in other cultures that had previously been depressed with the use of an asynchronous pairing (AP) protocol (156 ± 28% of the naive amplitude, $n = 6$ cells, $P > 0.5$; Fig. 1*B*). The AP protocol consisted of 80–100 repetitions of a postsynaptic depolarizing pulse (240 ms, 0.5–2.5 nA) followed by a single presynaptic stimulus (delay = 800 ms) (Debanne et al.

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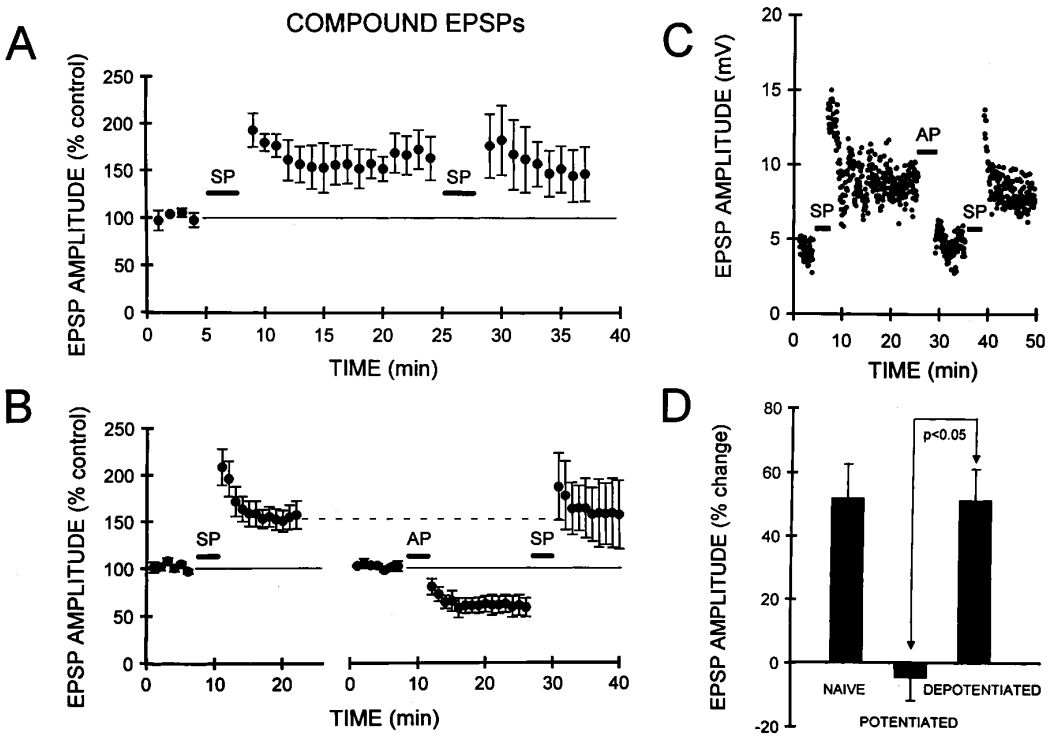

FIG. 1. Bidirectional changes in compound excitatory postsynaptic potential (EPSP) amplitude. *A*: induction of long-term potentiation (LTP) with repeated synchronous pairings (SP) of presynaptic activity and postsynaptic depolarization precludes further potentiation with the SP protocol and is thus saturating. *B*: induction of saturating LTP of naive synapses produces an increase in EPSP amplitude to 152 ± 11% of control ($n = 14$). At other naive synapses, long-term depression (LTD) was first induced with an asynchronous pairing (AP) protocol (see text). Subsequent induction of LTP with the SP protocol then produced an increase in EPSP amplitude to a level not different from that achieved by naive synapses after SP (156 ± 28% of naive amplitude, $n = 6$, $P > 0.5$). *C*: potentiation of a compound EPSP was 1st induced with the SP protocol, followed by induction of depotentiation with the AP protocol. Significant potentiation of the depotentiated EPSP could then be induced by repeating the SP. *D*: percent change in the amplitude of naive ($n = 14$), previously potentiated ($n = 3$), and previously depotentiated ($n = 4$) compound EPSPs produced by the SP protocol. Difference between previously potentiated and depotentiated EPSPs was statistically significant ($P < 0.05$).

1994). Assuming that the naive pathways were equivalent in the two sets of experiments, these results suggest that all synapses that were depressed by AP have been potentiated by SP.

Reversal of potentiation by associative depression

If bidirectional plasticity can occur at a single synapse, then synapses at which saturating potentiation had previously been induced should be able to become depressed and then repotentiated (Dudek and Bear 1993). Indeed, significant potentiation of compound EPSPs could be induced when saturating potentiation had previously been reversed with the AP protocol (Fig. 1*C*). The increase in EPSP amplitude produced by the SP after prior depotentiation was significantly different from the effects of SP on EPSPs that had only been potentiated previously (Fig. 1*D*).

Similar experiments were also performed with simultaneous recordings from monosynaptically coupled cell pairs ($n = 3$; Fig. 2). Saturating potentiation was first induced with the SP protocol. Five minutes later, significant depotentiation (19% decrease, $P < 0.0001$) was induced with asynchronous pairing of postsynaptic depolarizing pulses (240 ms duration, 100 repetitions) and single presynaptic action potentials (800-ms delay). Finally, significant potentiation (22% increase, $P < 0.0001$) of the depotentiated EPSP was induced five minutes later with the SP procedure. These results thus provide unambiguous evidence that the same synapses can undergo bidirectional changes in strength.

Bidirectional changes in synaptic reliability

LTP is associated with a decrease in the rate of synaptic failures (Malinow 1991). Conversely, the failure rate is increased after induction of homosynaptic depression of naive synapses (Stevens and Wang 1994). Because depotentiation affects previously potentiated synapses, opposite changes in the failure rate should occur after potentiation and depotentiation of unitary EPSPs.

Associative potentiation and depotentiation were induced in four cell pairs that exhibited failures in the naive state. To accurately evaluate the rate of synaptic failures, the EPSP and the noise areas were measured within two equivalent time windows (Fig. 3*A*). This pro-

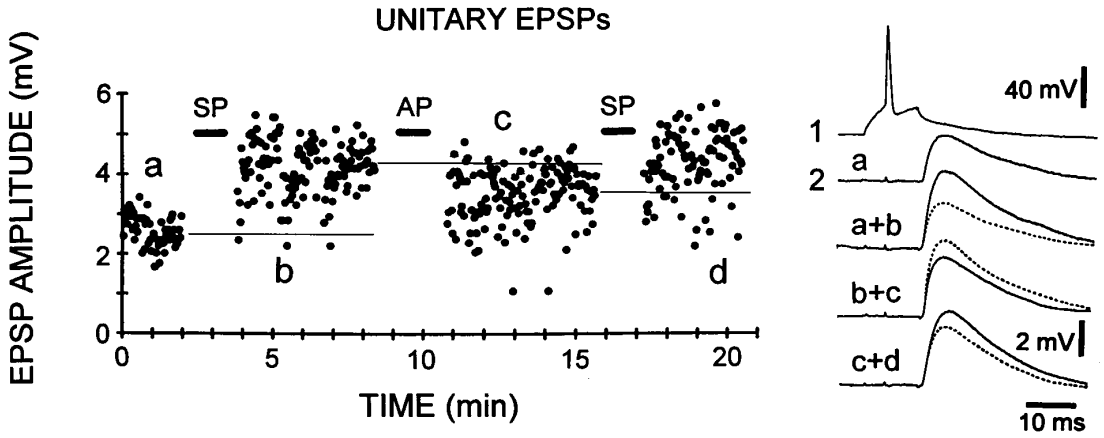

FIG. 2. Bidirectional changes in unitary EPSP amplitude. Saturating potentiation of a CA3-CA1 unitary EPSP was 1st induced with SP (a + b). After stabilization of the potentiation, AP induced significant depotentiation (b + c). A final SP restored the potentiated level (c + d).

cedure was more reliable at extracting small signals from the noise than simply measuring peak EPSP amplitudes (see Jack et al. 1981). To confirm that the failure rate was not over estimated, failures were averaged. As shown in Fig. 3, *A–C*, the average of all sweeps designated as failures was clearly flat, indicating that small EPSPs were not incorrectly designated as failures.

The unitary EPSP failure rate decreased by 84 ± 6% ($n = 4$) after induction of associative potentiation, coincident with a 34 ± 7% increase in the nonfailure EPSP amplitude (Fig. 3, *B* and *D*). In the same cell pairs the failure rate increased by 246 ± 152% ($n = 4$) after depotentiation, coincident with a 7 ± 3% decrease in the nonfailure EPSP amplitude (Fig. 3, *C* and *D*). These changes persisted for >15 min after the pairing procedure in two cell pairs.

DISCUSSION

Previous studies of compound synaptic responses, elicited with extracellular stimulation, have suggested that homosynaptic potentiation and depotentiation can be alternatively induced with a single set of fibers (Dudek and Bear 1993; Heynen et al. 1996; Mulkey and Malenka 1992). We report here similar observations for associative potentiation and depression of compound and unitary EPSPs, induced with synchronous and asynchronous pairing procedures, respectively.

Two lines of evidence suggest that synapses that had previously undergone depression with the AP protocol are the same as those that can be subsequently potentiated by the SP protocol in our experiments. First, compound EPSPs were potentiated to the same final amplitude, regardless of whether the potentiation was induced from a naive or previously depressed state, consistent with previous experiments using the homosynaptic LTD induction protocols (Dudek and Bear 1993). It may also be inferred from this observation that the AP protocol induced a true depression of naive synapses rather than a depotentiation of synapses that had become potentiated before the beginning of the recording. Second, after induction of saturating LTP and subsequent depotentiation with the AP protocol, both compound and unitary EPSPs could be potentiated by the SP procedure. Taken alone, the decrease in EPSP amplitude observed after AP in these experiments could result from depression of nonpotentiatable synapses or true depotentiation of potentiated synapses. Because the EPSP decrease could be reversed by a second SP, the participation of only nonpotentiatable synapses in the decrease in EPSP amplitude can be excluded.

Bidirectional changes in the strength of a given synapse can be most parsimoniously explained if a common expression mechanism accounts for the change in synaptic strength underlying LTP and depotentiation, e.g., increases or decreases in the phosphorylation of the same phosphoprotein(s). This hypothesis received initial support from the recently reported observation of bidirectional changes in quantal size (Oliet et al. 1996). We now report that the failure rate of unitary connections decreases upon induction of associative LTP and increases upon induction of associative depotentiation. Because this observation was made with the use of paired recordings, these events represent true failures of the presynaptic action potential to trigger release, rather than failures of action potentials to propagate to the nerve terminal. Changes in the failure rate might be produced by either the alternative expression and suppression of clusters of AMPA receptors at postsynaptic sites (Liao et al. 1995; Oliet et al. 1996) or by opposite changes in the probability of presynaptic glutamate release (Bolshakov and Siegelbaum 1995; Stevens and Wang 1994). Although decreases in failure rates have been observed to occur in conjunction with LTP induction in many studies, it should be noted that we have also observed an accompanying increase in the amplitude of nonfailure EPSPs, unlike some previous studies in juvenile hippocampal slices (e.g., Bolshakov and Siegelbaum 1995; Stevens and Wang 1994), but consistent with observations in more mature tissue (Malinow 1991).

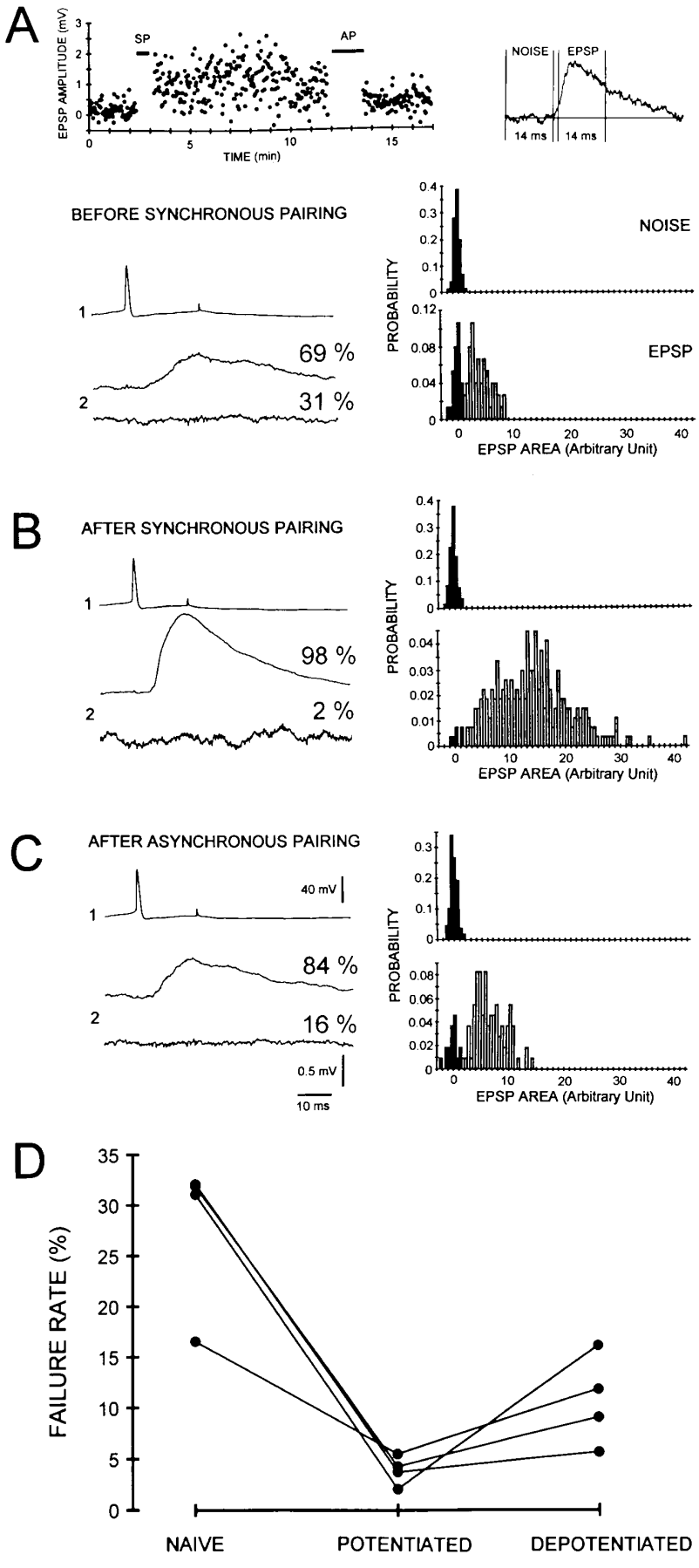

FIG. 3. Bidirectional changes in synaptic reliability. *A*, *top left*: associative LTP of a CA3-CA1 unitary EPSP induced by synchronous pairing and its subsequent depotentiation with asynchronous pairing. *Top right*: for each response to a presynaptic action potential, the trace was integrated, relative to a horizontal baseline cursor, for 2 intervals of 14 ms each, providing a measure of the noise and the EPSP. *Bottom left*: averaged EPSP in *cell 2* before synchronous pairing, detected in response to 69% of action potentials in *cell 1*, and the average failure of transmission in 31% of the responses. *Bottom right*: distribution of noise integrals (*upper graph*) and responses designated as transmission failures (*lower graph*, black bars) were similar. EPSP area given as arbitrary units (=14 mV∗ms). *B*: after induction of LTP with synchronous pairing, the failure rate decreased to 2% and the distribution of nonfailure EPSPs was shifted to the right (same cell as *A*). *C*: after induction of depotentiation with asynchronous pairing, the failure rate increased to 16% and the distribution of nonfailure EPSPs was shifted to the left (same cell as *A*). *D*: changes in the failure rate for 4 cell pairs after induction of potentiation and depotentiation. Some values were determined using data <10 min after the end of the pairing procedure.

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Present address of D. Debanne: Unité de Neurocybernétique Cellulaire, UPR 9041, 280 Bld. Sainte Marguerite, 13009 Marseille, France.

Address for reprint requests: S. M. Thompson, Brain Research Institute, August Forel-Strasse 1, CH-8029 Zurich, Switzerland.

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REFERENCES

BARRIONUEVO, G., SCHOTTLER, F., AND LYNCH, G. The effects of repetitive low frequency stimulation on control and ''potentiated'' synaptic responses in the hippocampus. *Life Sci.* 27: 2385–2391, 1980.

BOLSHAKOV, V. Y. AND SIEGELBAUM, S. A. Regulation of hippocampal transmitter release during development and long-term potentiation. *Science Wash. DC* 269: 1730–1734, 1995.

DEBANNE, D., GÄHWILER, B. H., AND THOMPSON, S. M. Asynchronous pre- and postsynaptic activity induces associative long-term depression in area CA1 of the rat hippocampus *in vitro*. *Proc. Natl. Acad. Sci. USA* 91: 1148–1152, 1994.

DEBANNE, D., GUÉRINEAU, N. C., GÄHWILER, B. H., AND THOMPSON, S. M. Physiology and pharmacology of unitary synaptic connections between pairs of cells in areas CA3 and CA1 of rat hippocampal slice cultures. *J. Neurophysiol.* 73: 1282–1294, 1995.

DUDEK, S. M. AND BEAR, M. F. Bidirectional long-term modification of synaptic effectiveness in the adult and immature hippocampus. *J. Neurosci.* 13: 2910–2918, 1993.

GÄHWILER, B. H. Organotypic monolayer cultures of nervous tissue. *J. Neurosci. Methods* 4: 329–342, 1981.

HEYNEN, A. J., ABRAHAM, W. C., AND BEAR, M. F. Bidirectional modification of CA1 synapses in the adult hippocampus *in vivo*. *Nature Lond.* 381: 163–166, 1996.

HUERTA, P. T. AND LISMAN, J. E. Bidirectional synaptic plasticity induced by a single burst during cholinergic theta oscillation in CA1 in vitro. *Neuron* 15: 1053–1063, 1995.

JACK, J.J.B., REDMAN S. J., AND WONG, K. The components of synaptic potentials evoked in cat spinal motoneurones by impulses in single group Ia afferents. *J. Physiol. Lond.* 321: 65–96, 1981.

LIAO, D., HESSLER, N. A., AND MALINOW, R. Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. *Nature Lond.* 375: 400–404, 1995.

MALINOW, R. Transmission between pairs of hippocampal slice neurons: quantal levels, oscillations, and LTP. *Science Wash. DC* 252: 722–724, 1991.

MULKEY, R. M. AND MALENKA, R. C. Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. *Neuron* 9: 967–975, 1993.

OLIET, S.H.R., MALENKA, R. C., AND NICOLL, R. A. Bidirectional control of quantal size by synaptic activity in the hippocampus. *Science Wash. DC* 271: 1294–1297, 1996.

STEVENS, C. F. AND WANG, Y. Changes in reliability of synaptic function as a mechanism for plasticity. *Nature Lond.* 371: 704–707, 1994.