injected into BALB/c mice to generate antibodies. Injections were repeated as necessary. Titers of antibodies were estimated by enzyme-linked immunosorbent assay. The antisera were used to screen a human cortex cDNA library (Clontech, Palo Alto, CA). Out of 1 million recombinants, one positive clone that contained a cDNA insert of 480 bp was found, purified, and used to screen a rat striatum cDNA library generated in the laboratory in a lambda ZAP vector (Stratagene). One million clones were screened at high stringency with the human insert. One full-length cDNA clone (GR33) was obtained and sequenced in both strands.

8. M. K. Bennett, N. Calakos, R. H. Scheller, *Science* **257**, 255 (1992).
9. A. Inoue, K. Obada, K. Akagawa, *J. Biol. Chem.* **267**, 10613 (1992).
10. R. Miledi and K. Sumikawa, *Biomed. Res.* **3**, 390 (1982).
11. T. A. Verdoorn and R. Dingledine, *Mol. Pharmacol.* **34**, 298 (1988).
12. L. Nowak, P. Bregestovski, P. Ascher, A. Herbert, A. Prochiantz, *Nature* **307**, 462 (1984); K. Moriyoshi *et al.*, *ibid.* **354**, 31 (1991).
13. C. Pfeiffer-Linn and R. M. Glantz, *Synapse* **9**, 35 (1991).
14. S. Nakanishi, *Science* **258**, 597 (1992).
15. H. Monyer *et al.*, *ibid.* **256**, 1217 (1992).
16. F. Revah *et al.*, *Nature* **353**, 846 (1991).
17. K. Folander *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 2975 (1990); S. A. N. Goldstein and C. Miller, *Neuron* **7**, 403 (1991).
18. A. Inoue and K. Akagawa, *Biochem. Biophys. Res. Commun.* **187**, 1144 (1992).
19. Y. Shoji-Kasai *et al.*, *Science* **256**, 1820 (1992).
20. R. A. Gross, R. L. Macdonald, T. Ryan-Jastrow, *J. Physiol. (London)* **411**, 585 (1989).
21. N. I. Chernevskaya, A. G. Obukhov, O. A. Krishtal, *Nature* **349**, 418 (1991).
22. The electrophysiology of oocytes was studied with a two-electrode voltage clamp at room temperature in a Mg^{2+}-free OR2 solution [82.5 mM NaCl, 2.5 mM KCl, 1 mM $CaCl_2$, and 10 mM Hepes buffer (pH 7.4) containing 10 μM glycine] 3 to 4 days after injection of cRNA (5 ng). Mutation L278T was generated with a polymerase chain reaction (PCR)–based technique and was verified by sequence analysis. The PCR product was subcloned under the T3-RNA polymerase promoter introduced in the pUC19 plasmid and subjected thereafter to in vitro RNA synthesis.
23. The dissociated cells from the cortex of fetal Wistar rats (E17) were cultured during 4 to 5 days in the absence of serum as described [S. Kure *et al.*, *Biochem. Biophys. Res. Commun.* **179**, 39 (1991)]. Non-neuronal cells were found to represent less than 0.5% of the population. Neurons were incubated with the primary GR33-specific antibodies (1:100) in the presence of 10% fetal calf serum during 2 hours at 37°C. After six washes with phosphate-buffered saline (PBS), the cells were exposed to secondary antibodies (1:50 dilution of rhodamine-conjugated goat antibody to rabbit immunoglobulins in PBS) for 1 hour at 37°C. Cells were washed twice with PBS and were observed with a fluorescence Nikon Diaphot TMD microscope.
24. A fragment of the GR33 cDNA that carried the coding region was generated by PCR amplification with GR33-specific oligonucleotides as primers. The product was subcloned into an *Escherichia coli* pGEX-2 expression vector [D. B. Smith and K. S. Johnston, *Gene* **67**, 31 (1988)] so that a fusion protein [glutathione-S-transferase–GR33] was obtained. The fusion protein was purified and used to produce rabbit polyclonal antibodies.
25. In situ hybridization was performed on 15-μm brain slices, fixed in 4% paraformaldehyde, and dried through ascending ethanol baths, with a 45-mer antisense oligonucleotide (specific to GR33 and labeled with ^{35}S-labeled adenosine triphosphate) at 42°C overnight in 4× saline sodium citrate (SSC) (1× SSC: 0.15 M NaCl and 0.015 M sodium citrate) solution containing 50% formamide, 10% dextran sulfate, 10 mM dithiothreitol (DTT), 1× Denhardt's solution, and 250 μM transfer RNA. After a final washing at 55°C in 0.5× SSC and 10 mM DTT, the slices were dried and exposed to βmax film (Amersham) for 3 to 4 days.
26. We thank R. Bibilashvilly for supporting the initiation of this work and J. Rossier for help during this work, S. Nakanishi for providing us with the NMDAR1 clone, S. Dumas for the help with in situ hybridization experiments, P. Vernier for providing us with the cDNA library, N. Daniel for help in immunological experiments, P. Ascher, F. Revah, A. Hicks, and J. J. Robert for helpful discussions, and C. Freed for critically reading the manuscript. Supported by Foundation S. & C. Del Duca, Centre National de la Recherche Scientifique, Institut de la Santé et de la Recherche Médicale, Ministère de la Recherche et de la Technologie, Association de la Recherche contre le Cancer, and the Bioavenir Program (Rhône-Poulenc Rorer, Ministère de la Recherche et de l'Espace, Ministère de l'Industrie et du Commerce Extérieur).

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Transsynaptic Expression of a Presynaptic Glutamate Receptor During Hippocampal Long-Term Potentiation

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Repetitive activation of excitatory synapses in the hippocampus produces a persistent enhancement of synaptic efficiency known as long-term potentiation (LTP). In anesthetized and in freely moving rats, the induction of LTP in the perforant path led to a transient increase in the amount of messenger RNA (mRNA) coding for a presynaptic glutamate receptor (GR33) in dentate granule cells. The amount of GR33 mRNA was increased for at least 5 hours after the induction of LTP but was indistinguishable from control values 1 day after induction. The *N*-methyl-D-aspartate receptor antagonist 2-aminophosphonovalerate prevented the induction of both LTP and the increase in GR33 mRNA. The amount of GR33 protein was increased in the mossy fiber terminal zone of dentate granule cells 5 hours after the induction of LTP. These results suggest that the induction of LTP in synapses at one stage in a neural network may lead to modification in synaptic function at the next stage in the network.

Long-term potentiation (LTP) in the dentate gyrus and hippocampus is an extensively studied form of activity-dependent synaptic plasticity in the vertebrate nervous system (*1*) and is considered to be a cellular model for the changes that underlie learning and memory (*2*). Induction of LTP in the CA1 region of the hippocampus and in the dentate gyrus requires simultaneous presynaptic release of neurotransmitter and sufficient postsynaptic depolarization to cause activation of the *N*-methyl-D-aspartate (NMDA) receptor channel complex, which results in an increased calcium influx into the postsynaptic cell. This initial triggering event activates both pre- and postsynaptic mechanisms to generate a persistent increase in synaptic strength (*3*). In the CA3 region, however, one form of LTP, mossy fiber LTP, is independent of NMDA receptor activation (*4*). Mossy fibers are granule cell axons that make synaptic connections with spines located on the proximal dendrites of CA3 pyramidal cells (*5*). Although the mechanism of induction of LTP at the mossy fiber–CA3 synapse remains controversial (*6*), it is possible that maintenance of LTP is entirely presynaptically mediated (*4*) and involves presynaptic receptors that modulate the release of excitatory neurotransmitters (*7*). Recent studies that demonstrate that the presynaptic glutamate autoreceptor of the metabotropic (mGluR) type regulates transmitter release (*8*) suggest that presynaptic glutamate receptors have an important role in the maintenance of LTP.

One of the candidates for such a role is GR33, a presynaptic glutamate receptor with a pharmacological profile similar to that of the postsynaptic NMDA receptor (*9*). We studied the effect of LTP, which was induced in the perforant path–dentate gyrus synapses, on the expression of the GR33 receptor gene in the rat hippocampus. In situ hybridization (*10*) was performed on brain slices taken 2 or 5 hours after induction of LTP in anesthetized rats (*11*) ($n = 2$ for each time point) and 2 hours, 1 day, 2 days, or 5 days after induction of LTP in freely moving rats (*12*) ($n = 3$ for each time point). Freely

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moving and anesthetized controls (one rat for each time point) were subjected to low-frequency stimulation that did not induce LTP and were matched for analysis at similar time points after stimulation (*11, 12*).

High-frequency stimulation of the perforant path induced reliable LTP both of the evoked field excitatory postsynaptic potential (EPSP) (Fig. 1) and of the synchronous discharges of dentate granule cells (*13*). There was no change of either measure in stimulated controls (Fig. 1). The amount of GR33 mRNA in the dentate gyrus increased 2 hours after the induction of LTP in both unanesthetized and anesthetized animals in which LTP had been established (Fig. 2, A and B). The increase was restricted to the granule cells of the entire dorsal region of the dentate gyrus and was not seen in the contralateral hippocampus. Control stimulation had no effect on the expression of GR33 (Fig. 2, A and B). The relative amount of GR33 mRNA (d_{dg}/d_{CA1}) (*14*) in the dentate gyrus of control rats was compared with that of rats that received tetanic stimulation (Table 1). There was a threefold increase (two rats of two analyzed) and 1.7-fold increase (three rats of three analyzed) in the amount of GR33 mRNA in anesthetized and freely moving rats, respectively.

The amount of GR33 mRNA in the dentate gyrus of anesthetized rats was increased 5 hours after the induction of LTP on the ipsilateral (tetanized) side and also on the contralateral side, which had not been stimulated (Fig. 2B). At this time point, the increase in the amounts of GR33 mRNA was identical on both sides (LTP side: $d_{dg}/d_{CA1} = 4.15$, SEM = 0.31, $n = 3$ sections; contralateral side: $d_{dg}/d_{CA1} = 3.76$, SEM = 0.34, $n = 3$ sections) and was similar to the unilaterally restricted increase at 2 hours. Amounts of GR33 mRNA in freely moving rats killed 1, 2, and 5 days after the induction of LTP were indistinguishable from those in controls (Fig. 2A). Tetanic stimulation of the perforant path had no significant effect on the amounts of GR33 mRNA in the CA1 and CA3 pyramidal cell layers at any time after the induction of LTP.

In further experiments, we established that both LTP and the increased amount of GR33 mRNA after 2 hours and 5 hours (Fig. 2C) were blocked by infusion of the competitive NMDA receptor antagonist 2-aminophosphonovalerate (APV) (*15*). This suggests that alteration in the amount of GR33 mRNA requires the activation of NMDA receptors on granule cells. We next examined the possibility that the expression of the postsynaptic NMDA receptor was regulated in LTP. In rat brain, mRNA for GR33 is colocalized with mRNA for a major component of the NMDA receptor complex, a subunit NMDAR1 (*9, 16*). The amount of NMDAR1 mRNA did not change during the 5 days after the induction of LTP (Fig. 2A).

We then studied the amount of GR33 protein in the hippocampus of potentiated and control anesthetized rats (*17*). In the hippocampus, GR33 antibodies labeled the mossy fiber terminal zone of the dentate granule cells (Fig. 3). No changes in the amount of GR33 protein were detected in mossy fibers 2 hours after induction of LTP, nor in the perforant path terminal zone in the dentate gyrus, nor in any other region of the hippocampus. However, the intensity of labeling of the dentate granule cells in the mossy fiber terminal zone was increased 5 hours after the induction of LTP when compared with the intensity in the stimulated controls (Fig. 3).

These results show that LTP leads to transient induction of GR33 gene expression in dentate granule cells and to a subsequent increase in protein amounts in the mossy fiber terminal zone. These findings have important implications for the mechanisms that underlie long-lasting increases in synaptic strength in hippocampal circuits and for the function of the GR33 glutamate receptor. Our results suggest that NMDA-dependent postsynaptic mechanisms responsible for the induction of LTP in the dentate gyrus induce not only presynaptic changes in the perforant path–dentate gyrus synapses (*3, 18*) but also postsynaptic changes in GR33 gene expression, which results in presynaptic modifications downstream in the hippocampal circuitry at the level of mossy fiber–CA3 synapses. Moreover, the bilateral increase

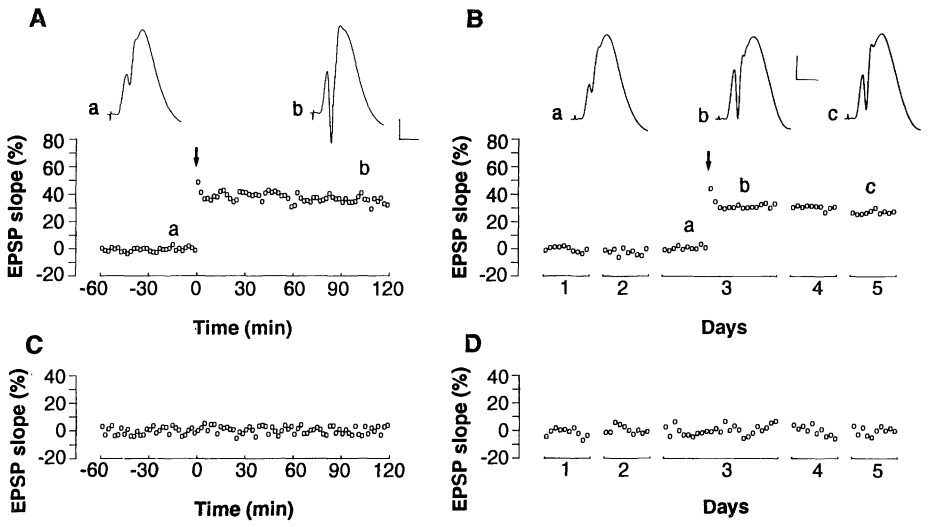

Fig. 1. LTP-inducing (**A** and **B**) and control (**C** and **D**) stimulation in the dentate gyrus in anesthetized (A and C) and freely moving (B and D) rats. The slope of the EPSP is plotted as a function of time and as a percentage change from the mean value obtained before high-frequency [1 hour in (A); 1 to 3 days in (B)] or control stimulation [identical periods in (C) and (D)]. Tetanic stimulation is indicated by arrows in (A) and (B); control stimulation was given at corresponding times in (C) and (D). Each point on the graphs represents the average of four consecutive evoked responses. In (A) and (B), tetanic stimulation induced a pronounced and long-lasting increase in the slope of the EPSP in both groups of rats. In freely moving rats, potentiation of the EPSP was stable with almost no decrement 2 days (B) and 5 days (data points not shown) after induction. In (C) and (D), no change was seen in stimulated controls. Sample waveforms are averages of 40 evoked potentials obtained at the times indicated by the lowercase letters. Calibration bars are 4 mV and 4 ms.

Table 1. Increase of GR33 mRNA in the dentate gyrus of rats 2 hours after the induction of LTP. The relative amount of GR33 mRNA ipsilaterally and contralaterally to tetanic or control stimulation was calculated for each rat as described (*14*). The first number in each pair is the number of brain sections analyzed; the second is the number of animals.

Rat model	LTP-inducing stimulation		Control stimulation	
	Ipsilateral	Contralateral	Ipsilateral	Contralateral
Freely moving	4.00 ± 0.65 $n = 4, 3$	2.35 ± 0.33 $n = 4, 3$	2.12 ± 0.18 $n = 3, 1$	2.15 ± 0.47 $n = 3, 1$
Anesthetized	3.70 ± 0.55 $n = 3, 2$	1.45 ± 0.10 $n = 3, 2$	1.28 ± 0.06 $n = 3, 1$	ND*

*Not determined, because one of two anesthetized rats was stimulated on both sides.

in the expression of GR33 after LTP suggests that similar mechanisms may contribute to the bilateral modulation of downstream synaptic function in hippocampal networks. In summary, our data highlight the potential importance of transsynaptically regulated gene expression for information processing in the brain and suggest that the glutamate receptor GR33, which is located presynaptically, functions in this process.

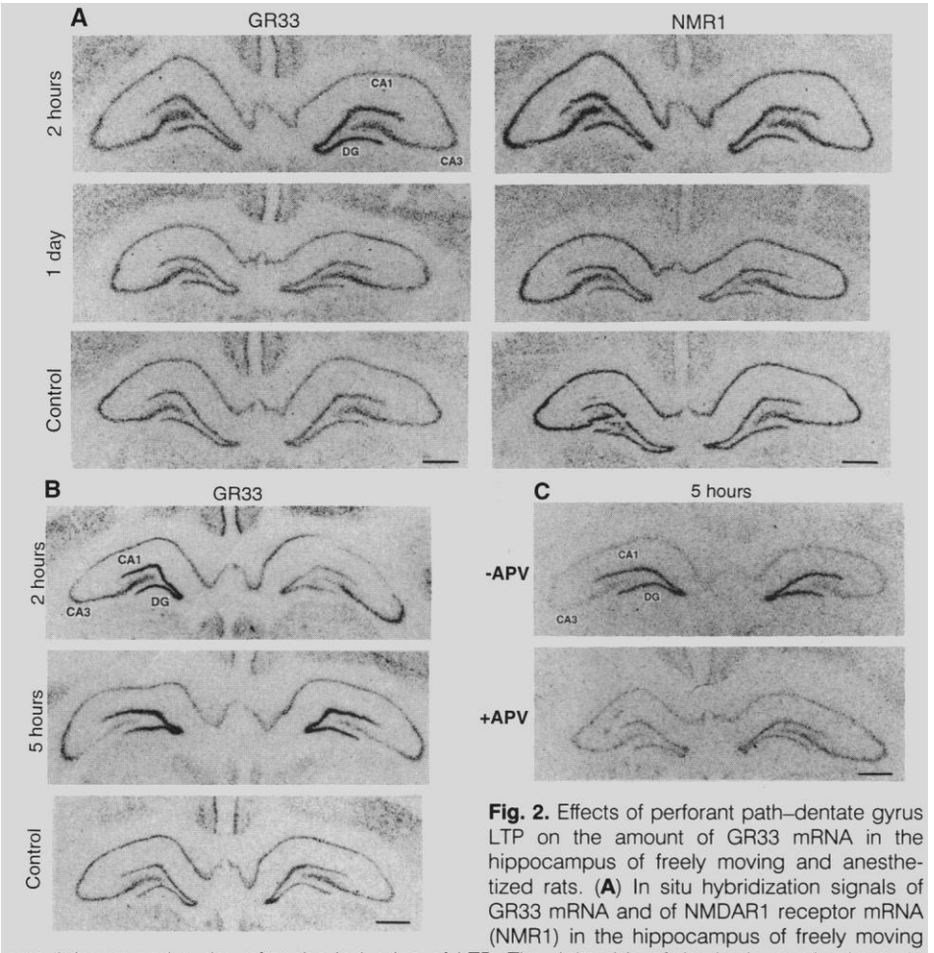

Fig. 2. Effects of perforant path–dentate gyrus LTP on the amount of GR33 mRNA in the hippocampus of freely moving and anesthetized rats. (**A**) In situ hybridization signals of GR33 mRNA and of NMDAR1 receptor mRNA (NMR1) in the hippocampus of freely moving rats 2 hours and 1 day after the induction of LTP. The right side of the brain received tetanic stimulation. The hybridization signal obtained in a rat killed 2 hours after control stimulation is defined as the control (Fig. 1). (**B**) GR33 mRNA hybridization signals in the hippocampus of anesthetized rats killed 2 hours and 5 hours after LTP induction and control stimulation. The left side of the brain was stimulated in each case. (**C**) Effect of APV infusion (30 min before tetanus) on the amount of GR33 mRNA in the hippocampus of a rat killed 5 hours after LTP induction. The right side of the brain received LTP-inducing stimulation. Control stimulation was applied to the left side. The CA1 and CA3 regions of the hippocampus and the dentate gyrus (DG) are indicated. Scale bars: 850 μm (A and B) and 770 μm (C).

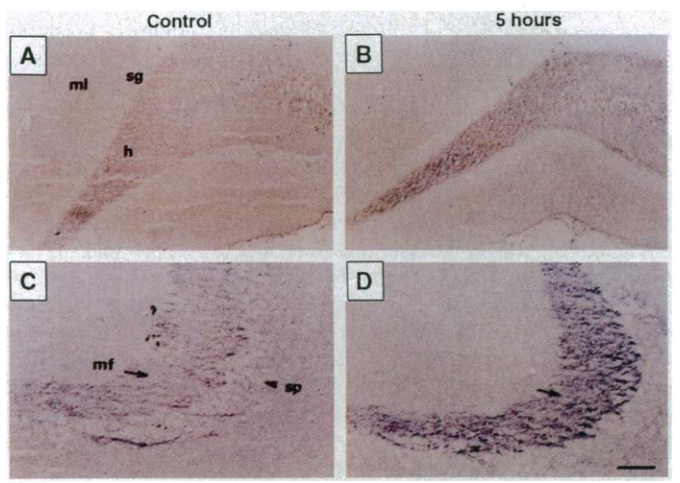

Fig. 3. Immunohistochemical detection of GR33 protein in the dentate gyrus of anesthetized rats. Immunodetection was done with GR33 antibodies 5 hours after the induction of LTP (**B** and **D**) or with control stimulation (**A** and **C**) (Fig. 1); ml, molecular layer; mf, mossy fiber terminal zone; sg, stratum granulosum; sp, stratum pyramidale; and h, hilus. Scale bar: 40 μm for (A) and (B) and 20 μm for (C) and (D).

REFERENCES AND NOTES

1. G. L. Collingridge and T. V. P. Bliss, *Trends Neurosci.* **10**, 288 (1987); R. A. Nicoll, J. A. Kauer, R. C. Malenka, *Neuron* **1**, 97 (1988).
2. R. G. M. Morris, S. Davis, S. P. Butcher, *Philos. Trans. R. Soc. London Ser. B* **329**, 187 (1990); V. Doyère and S. Laroche, *Hippocampus* **2**, 39 (1992).
3. T. V. P. Bliss and G. L. Collingridge, *Nature* **361**, 31 (1993).
4. E. W. Harris and C. W. Cotman, *Neurosci. Lett.* **70**, 132 (1986); R. A. Zalutsky and R. A. Nicoll, *Science* **248**, 1619 (1990).
5. B. J. Claiborne, D. G. Amaral, W. N. Cowan, *J. Comp. Neurol.* **246**, 435 (1986).
6. D. Jaffe and D. Johnston, *J. Neurophysiol.* **64**, 948 (1990).
7. C. Alzheimer, J. Rohrenbeck, G. ten Bruggencate, *Brain Res.* **543**, 163 (1991); R. L. Gannon and D. M. Terrian, *Neuroscience* **41**, 401 (1991).
8. I. Herrero, M. T. Miras-Portugal, J. Sánchez-Prieto, *Nature* **360**, 163 (1992).
9. T. Smirnova, J. Stinnakre, J. Mallet, *Science* **262**, 430 (1993).
10. Brains were cut into 15-μm sections mounted on coated slides, postfixed in 4% paraformaldehyde, and dried. In situ hybridization was performed with a 45-mer oligonucleotide (specific to GR33 and labeled with ^{35}S-labeled adenosine triphosphate) at 42°C overnight in 4× saline sodium citrate (SSC) solution containing 50% formamide, 10% dextran sulfate, 10 mM dithiothreitol (DTT), 1× Denhardt's, and 250 μM transfer RNA. After a final washing at 55°C in 0.5× SSC and 10 mM DTT, the slices were exposed to βmax film (Amersham) for 3 to 4 days.
11. The methods and procedures described below (*12*) were used in urethane-anesthetized rats (1.5 g per kilogram of body weight, given intraperitoneally). Evoked potentials were monitored every 30 s for 1 hour before, and for 2 hours after, the induction of LTP or control stimulation.
12. Adult male Sprague-Dawley rats were anesthetized with sodium pentobarbitone (60 mg/kg, given intraperitoneally) and prepared for chronic recording as described [S. Laroche *et al.*, *Neuroscience* **28**, 375 (1989)]. Pairs of recording electrodes made of nickel-chromium alloy (nichrome) wire were implanted in the hilus of the dentate gyrus, and concentric bipolar stimulating electrodes were positioned in the ipsilateral angular bundle. Rats were allowed to recover for at least 10 days and were habituated to the recording chamber for 2 days before the experiment. Forty perforant path test shocks (100 μs) were then delivered each day at a rate of one per 30 s. The test intensity was adjusted to give a population spike of approximately 1 mV before the experiment (234.2 ± 29.5 μA). Evoked potentials were amplified, digitized, averaged by groups of four, and stored for off-line analysis with the use of conventional measures of EPSP slope and spike height. On day 3, the session was lengthened and LTP was induced after testing by delivery of six series (at 2-min intervals) of six high-frequency trains (400 Hz for 20 ms) at 0.1 Hz. To increase the number of potentiated synapses, we increased the intensity of stimulation during the tetanus to a value (537.5 ± 16.5 μA) that gave a population spike of near maximal amplitude. Testing was then resumed for 30 min and then on days 1, 2, and 5 after the induction of LTP. The same protocol was applied to stimulated controls, except that each high-frequency train on day 3 was replaced by single shocks at high intensity.
13. High-frequency stimulation of the perforant path also caused a stable and long-lasting increase in

the amplitude of the population spike, which attained average increases of 259% and 237% in freely moving and in anesthetized rats, respectively. The population spike height in stimulated controls was unchanged.

14. After in situ hybridization, quantification of the amount of GR33 mRNA in the dentate gyrus of rat brain sections was performed with the BIOCOM computer program (System Photometrique d'Analyse des Autoradiogrammes RAG 200, France). The densities of silver grains in the test areas were calculated separately for the dentate gyrus (d_{dg}) and the CA1 region (d_{CA1}) such that

$$d = \log[ng_c/ng_t] \times A_t$$

where ng_c is the number of grains in the control area, ng_t is the number of grains in the area of the test, and A_t is the test area. The relative amount of GR33 mRNA (d_{dg}/d_{CA1}) on the potentiated side of the hippocampus was compared with that on the contralateral side or that from comparable sections from animals that received unilateral control stimulation.

15. Two additional adult male Sprague-Dawley rats were anesthetized with urethane (1.5 g/kg, given intraperitoneally), and electrodes were positioned bilaterally to stimulate the perforant path. Glass microelectrodes were advanced bilaterally into the dorsal granule cell layer of the dentate gyrus. Test shocks of 50-μs monopolar pulses were given at 30-s intervals throughout the experiment. Three sets of trains (250 Hz for 200 ms) at 1-min intervals were used to induce LTP. The stimulus intensity was doubled during the tetanus on the conditioned side (a total of 150 strong shocks). On the control side, the same number of strong shocks was given by interpolating a single strong shock between weak test shocks for a period of 75 min. Thus, the control side received a substantial number of strong shocks, which produced population spikes that were as large as the potentiated responses on the tetanized side. The APV (Sigma) was injected intraventricularly (20 μl of 200 μM solution) 30 min before the tetanus. After either 2 or 5 hours, the animals were perfused intracardially with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), postfixed for 2 hours at 4°C, and saturated with 30% sucrose phosphate buffer before being stored at −70°C.

16. K. Moriyoshi *et al.*, *Nature* **354**, 31 (1991).

17. Immunohistochemistry was performed with GR33-specific antibodies (1:400) on 15-μm fixed brain slices after pretreatment with 0.2% Triton X-100 in phosphate-buffered saline solution. Antibody binding was revealed with DAB (3,3′-diaminobenzidine) in the presence of nickel with the Vectastain kit (Vector) following the manufacturer's instructions.

18. T. V. P. Bliss *et al.*, *J. Physiol. (London)* **377**, 391 (1986); J. H. Williams, M. L. Errington, M. A. Lynch, T. V. P. Bliss, *Nature* **341**, 739 (1989); D. M. Lovinger, P. A. Colley, R. F. Akers, R. B. Nelson, A. Routtenberg, *Brain Res.* **399**, 205 (1986).

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Binding of L-Selectin to the Vascular Sialomucin CD34

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The adhesive interactions between leukocyte L-selectin and the endothelium are involved in the migration of lymphocytes through peripheral lymph nodes and of neutrophils to sites of inflammation. A recombinant L-selectin stains high endothelial venules (HEVs) in lymph nodes and recognizes sulfated carbohydrates found on two endothelial glycoproteins, Sgp50 and Sgp90. Amino acid sequencing of purified Sgp90 revealed a protein core identical to that of CD34, a sialomucin expressed on hematopoietic stem cells and endothelium. A polyclonal antiserum to recombinant murine CD34 stains peripheral lymph node endothelium and recognizes Sgp90 that is functionally bound by L-selectin. Thus, an HEV glycoform of CD34 can function as a ligand for L-selectin.

The ability of leukocytes to adhere efficiently to the endothelium under conditions of vascular flow is a key event in the inflammatory response (*1–4*). Leukocyte adhesion and extravasation appear to constitute a multistep phenomenon in which the initial, relatively low-affinity binding event (leukocyte rolling) is mediated by the selectin family of adhesion molecules (*3, 4*). Chemotactically activated leukocytes then induce a higher avidity binding that is mediated by the leukocyte integrins and their cognate endothelial ligands, the immunoglobulin (Ig) superfamily glycoproteins including the intercellular adhesion molecules (ICAMs) and vascular cell adhesion molecule–1 (VCAM-1) (*1–4*). Although the adhesion that is mediated by the leukocyte integrins is due to protein-dependent binding (*2*), the selectins bind through the calcium-dependent lectin recognition of carbohydrate ligands on specific endothelial or leukocyte glycoproteins (*4*). Two such endothelial ligands that are specifically recognized by leukocyte L-selectin are sulfated glycoproteins of 50 and 90 kD that have been termed Sgp50 and Sgp90 (*5–9*). The interactions between recombinant L-selectin and Sgp50 and Sgp90 appear to exactly mimic the L-selectin–mediated binding of lymphocytes to the HEVs of peripheral lymph nodes (PLNs) (*5–9*). The nature of Sgp50 was determined by the cloning of a complementary DNA (cDNA) encoding the protein backbone of this glycoprotein (*10*). The results revealed a potentially soluble mucin-like molecule that could function as a scaffold for the high-density presentation of the appropriate, sulfated carbohydrates to cell surface L-selectin on lymphocytes. The clustering of carbohydrate ligands in a mucin organization was an obvious mechanism to enhance the avidity of this ligand for L-selectin. Previous analyses did not define the molecular nature of Sgp90.

The earlier biochemical characterization of the Sgp50 and Sgp90 L-selectin ligands was done with a chimeric molecule containing the extracellular domain of L-selectin and human IgG1 (L-selectin–IgG) (*5–9*). Isolated Sgp50 interacts with L-selectin–IgG in a calcium-dependent, carbohydrate-mediated manner, as is the case for the binding of lymphocytes to HEVs (*5–7*). To prove that Sgp90 also has independent ligand activity that is carbohydrate-dependent, and is not merely coprecipitated with Sgp50, we electroeluted Sgp90 from an SDS gel and examined its ability to interact directly with L-selectin–IgG. As shown in Fig. 1, purified Sgp90 was quantitatively reprecipitated by L-selectin–IgG only in the presence of calcium, demonstrating the involvement of the calcium-dependent lectin domain of L-selectin (*4*). A CD4-IgG chimera did not react with the 90-kD component. We used affinity chromatography on wheat germ agglutinin and L-selectin–IgG columns to purify Sgp90 from detergent lysates of mouse mesenteric lymph nodes (*7, 10*). The final EDTA-released fraction from the L-selectin affinity column was electrophoresed on an SDS gel, and the region of the gel containing Sgp90 was isolated. Electroblotted material was subjected to amino acid sequence analysis.

A weak (~5 pmol) 12-residue NH_2-terminal sequence was determined that contained a number of gaps ("X"). Comparison of the NH_2-terminal sequence of the purified ligand with the deduced NH_2-terminus of the murine sialomucin CD34 (mCD34) (*11*) revealed an exact match at 7 out of 12 positions (Sgp90 sequence: XXETSXQGIXPT, CD34 sequence: TTE-

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