the patient's age at each testing point. Patients (and their age-matched controls) were included only if they completed both the immediate and 1-hour tests for both eyes. For the comparison to the patient's immediate and 1-hour tests, the control infant was tested upon arrival at the lab and again after 1 hour of additional visual experience. Later measurements were available for most patients (and their controls) and occurred 1 week (mean, 1 week; range, 1 to 2 weeks) and 1 month (mean, 5 weeks; range, 3 to 8 weeks) after the immediate test.

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14. Although both bilateral and unilateral cases improved significantly between the 1-hour and 1-month tests (all P's < 0.02), bilateral cases showed an improvement in each eye between 1 hour and 1 week (both P's < 0.05) but unilateral cases did not ($P > 0.10$) (Fig. 3).
15. Across patients, the mean number of hours per day that the nondeprived eye was patched during the first month ranged from 0 to 7.7 hours/day with an overall group mean of 4.6 hours/day.
16. The fact that the nondeprived eye of children treated for unilateral congenital cataract improved on average 0.26 octaves between the immediate and 1-hour tests raises the possibility that some of the improvement in the treated eyes might have arisen from nonvisual factors such as recovery from the eye exam. However, the improvement in the nondeprived eyes was not significant ($P > 0.20$, two-tailed t test), and the treated eyes of unilateral cases tended to improve more than the fellow nondeprived eyes, on average 0.32 octaves more ($P = 0.07$, one-tailed paired t test). There was no such improvement in 17 additional infants in a control experiment in whom one treated eye (eight from bilateral cases and nine from unilateral cases) was patched between the two tests instead of receiving 1 hour of patterned visual input, with a mean decline of 0.19 octaves. Between the immediate and 1-hour tests, acuity improved more in eyes from the main experiment that had received visual input during the hour than in eyes from the control experiment that had not and this was true whether the initial deprivation had been binocular or monocular (all P's ≤ 0.02, one-tailed unpaired t tests). In the six bilateral cases included in the control experiment for which we were able to measure changes in acuity both for the eye that had been patched and for the fellow eye that had received 1 hour of visual input, the experienced eye improved by a mean of 0.50 octaves, whereas the patched eyes declined by a mean of 0.24 octaves ($P = 0.04$, one-tailed paired t test). Although the significant difference results from both a significant decline in patched eyes and a nonsignificant increase in experienced eyes, it nevertheless indicates that the nervous system is sensitive to short-term changes in visual input and that recovery from the eye exam and contact lens insertion (which should have been equal in patched and experienced eyes) does not account for any improvement in acuity. The mean increase of 0.50 octaves in the experienced eyes is similar in magnitude to the mean from the main experiment (Fig. 3) and almost significant despite the small n (paired t test comparing immediate and 1-hour acuity for six experienced eyes, $P = 0.08$, one-tailed). The robustness of the improvement observed at 1 hour in the experienced eyes in the main and control experiments was confirmed by the finding that their acuity remained significantly better than the initial value when they were retested at 1 week, whereas there was no change in the nondeprived eyes in unilateral cases nor in the age-matched groups (unilateral cases in the main experiment: $P < 0.05$; right eye of bilateral cases in the main experiment: $P < 0.002$; left eye of bilateral cases in the main experiment: $P < 0.0001$; six experienced eyes of bilateral cases in the control experiment: $P < 0.002$; nondeprived eyes of unilateral cases in the main and control experiments, both P's > 0.10; age-matched groups, all P's > 0.10). The pattern of improvement after the onset of patterned visual input was also confirmed in eyes from the control experiment that were patched during the first hour after treatment; they improved significantly between the immediate and 1-week tests, with average improvements similar to those shown in Fig. 3 for the main experiment: 0.58 octaves in unilateral cases ($n = 9$; $P = 0.03$, one-tailed paired t test) and 0.88 to 1.24 octaves in bilateral cases (nine right eyes, $P < 0.004$; seven left eyes, $P < 0.02$).
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11 February 1999; accepted 19 August 1999

Precisely Localized LTD in the Neocortex Revealed by Infrared-Guided Laser Stimulation

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In a direct approach to elucidate the origin of long-term depression (LTD), glutamate was applied onto dendrites of neurons in rat neocortical slices. An infrared-guided laser stimulation was used to release glutamate from caged glutamate in the focal spot of an ultraviolet laser. A burst of light flashes caused an LTD-like depression of glutamate receptor responses, which was highly confined to the region of "tetanic" stimulation (<10 micrometers). A similar depression of glutamate receptor responses was observed during LTD of synaptic transmission. A spatially highly specific postsynaptic mechanism can account for the LTD induced by glutamate release.

The locus of long-term potentiation (LTP) and LTD is still a matter of debate. Mainly indirect methods have been used to separate contributions of pre- and postsynaptic factors. As these analyses are based on assumptions concerning neurotransmitter release, the results have been controversial (*1*). More direct approaches, probing glutamate sensitivity during LTP by microiontophoresis, also yielded conflicting results (*2*). Therefore, techniques for applying exogenous receptor agonists over a time course and volume, which approximate the release of transmitter at a single synapse, are required (*3*). Such brief and localized releases of neurotransmitter can be achieved by focusing ultraviolet (UV) light on the slice to release glutamate from its caged form, which has been added to the slice superfusate. LTD can be induced in hippocampal neurons by direct glutamate application (*4*). We used the method of glutamate application by photolysis in combination with infrared videomicroscopy to address these questions.

The soma and dendrites of pyramidal neurons of layer V were visualized in neocortical slices with infrared videomicroscopy, with the use of a gradient contrast system (*5*). Neurons ($n = 55$) were recorded with patch-clamp pipettes in whole-cell mode (*6*). Caged glutamate was added to the superfusion medium, and presynaptic input was eliminated by the addition of tetrodotoxin (TTX) (1 μM) in all experiments without synaptic stimulation. With a setup developed especially for infrared-guided laser stimulation [Fig. 1A and supplementary figure (*7*)], we were able to direct a 1-μm UV spot under visual control on the surface of the neuron being studied. The UV spot was positioned on the dendrite at distances of 100 to 150 μm from the soma.

As glutamate is released from its caged form by constant UV flashes in constant quantities (*8*) and presynaptic input was blocked by TTX, any changes in neuronal responses had to be of postsynaptic origin. After establishing a baseline of responses by releasing glutamate every 20 s for 10 min, a train of 5-Hz light flashes for 1 min was used

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to induce LTD (Fig. 1B). This tetanuslike stimulation paradigm reliably elicited LTD of more than 30% on average (33 ± 2%, $n = 9$) for up to 1 hour of recording.

To exclude the possibility that neurons were damaged by the UV laser radiation itself, in control experiments, we also applied the 5-Hz "light tetanus" to neurons without caged glutamate in the perfusion medium. Neuronal responses were recorded in the presence of caged glutamate, caged glutamate was washed out of the slice, and then the 5-Hz train was applied to the neuron (Fig. 1C). When caged glutamate was washed in again, no reduction of the amplitude of the membrane depolarization could be detected (3 ± 4%, $n = 3$). After applying the 5-Hz train in caged glutamate solution, robust LTD was elicited (Fig. 1C). Additional depolarization of the neuron by current injection (*4*) was not necessary to elicit LTD. This could be due to the depolarizing summation of the glutamate responses elicited by photostimulation at 5 Hz. Another factor could be the very efficient photolytic release of glutamate by the focused light of the UV laser.

To elucidate whether the LTD induced by photolytic glutamate release shares common features with synaptic LTD, we investigated whether the photolytically induced LTD was *N*-methyl-D-aspartate (NMDA) receptor–mediated and Ca^{2+}-dependent. The NMDA receptor antagonist MK801 (20 μM) was added to the superfusion medium (*9*), and the Mg^{2+} concentration was increased from 1 to 4 mM. Under these conditions, only a statistically insignificant reduction of 7 ± 2% ($n = 6$) of the response remained (Fig. 1D), suggesting that the LTD observed is NMDA receptor–dependent. Addition of the Ca^{2+} chelator 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) (10 mM) to the recording pipette solution (*10*) left only an insignificant reduction of 9 ± 2% ($n = 6$) of the membrane depolarizations (Fig. 1E).

The spatial spread of LTD is of great importance (*11*) to the selective modulation of neurotransmission at single synapses. We exploited the high spatial resolution of the infrared-guided laser stimulation to investigate this question. We determined the accuracy of the laser stimulation by laterally offsetting the laser point and measuring the induced neuronal depolarizations (Fig. 2A). The accuracy obtained was 4 ± 1 μm laterally [full width half maximum (FWHM), $n =$ 6 neurons] (Fig. 2, A and B) and 18 ± 2 μm axially ($n = 4$ neurons). Thus, the effective "glutamate release site" can be regarded roughly as a spot of 10-μm diameter. To test for spatial specificity, we stimulated sites along the neuronal dendrite spaced 10 μm apart (Fig. 2C). During the control and follow-up period, seven points along the dendrite were stimulated consecutively at low frequency. Five-hertz tetanic stimulation was applied to only one point. Figure 2C shows that only at this point was a significant LTD of 29 ± 4% ($n = 9$ neurons) induced. The reductions measured 10 μm to the left and right of this point were already statistically insignificant ($P > 0.05$). Thus, the LTD observed in our experiments had a spatial specificity of at least 10 μm. The actual extension of LTD may be even smaller, as the accuracy of the method is limited to the 10-μm range.

One could argue that such experiments only describe a reduction of glutamate sensitivity and not the mechanisms of synaptic LTD. Therefore, we also probed glutamate sensitivity before and after induction of synaptic LTD by electrical stimulation. By positioning the laser stimulation on the dendrite at 100 to 150 μm distance from the soma, glutamate receptor sensitivity was tested in a dendritic region where a high number of spines and glutamatergic inputs are found (*12*). Electrical stimulation of synaptic input was performed with a standard tungsten stimulation electrode in white matter below the recording site. To activate a large number of glutamatergic synapses, we used a higher stimulation intensity (two times the threshold of action potential generation) for the 5-Hz tetanus than for eliciting the control excitatory postsynaptic potentials (EPSPs). This synaptic stimulation (*13*) reliably evoked LTD of the EPSPs of 32 ± 6% [$n = 14$, measured at 48 min (minutes 46 to 50 averaged) after the tetanus] (Fig. 3). The glutamate sensitivity showed the same amount of LTD 48 min after the tetanus (31 ± 3%, $n = 8$). Some difference between the time course of glutamate sensitivity and the time course of EPSP amplitudes directly after the tetanus is apparent in Fig. 3C. LTD of glutamate sensitivity developed more slowly than LTD of EPSPs. Thirty-five minutes after LTD induction, this difference was statistically insignificant ($P > 0.5$). Thus, a reduction of postsynaptic glutamate sensitivity is sufficient to explain synaptic LTD after half an hour, the time point usually taken as the beginning of "long-term" depression.

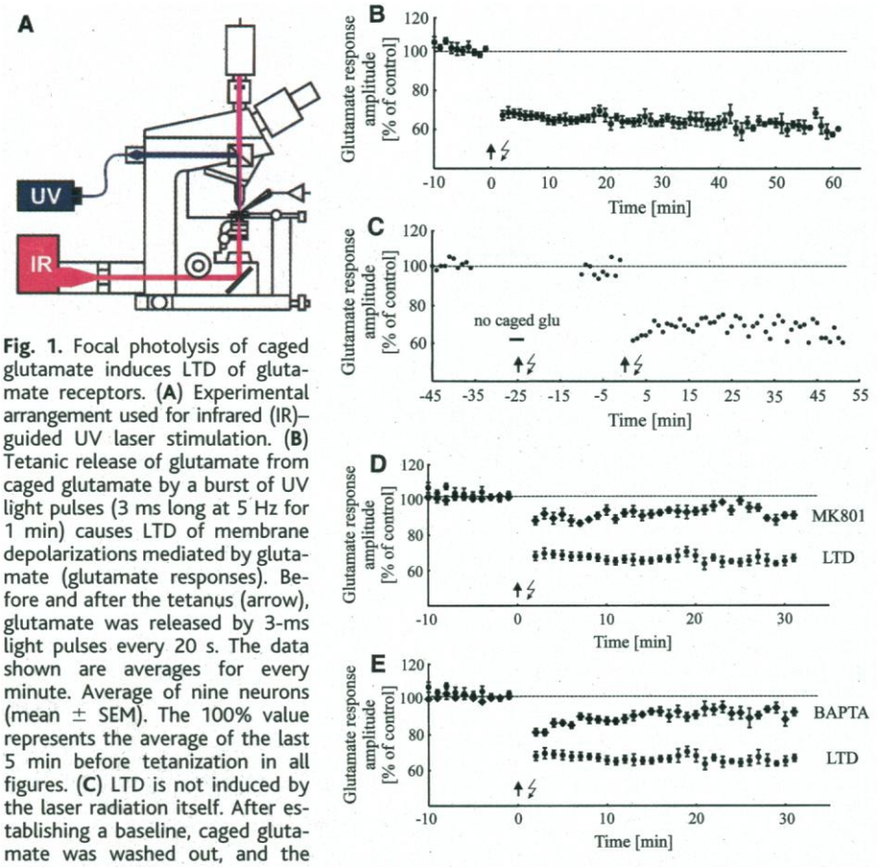

Fig. 1. Focal photolysis of caged glutamate induces LTD of glutamate receptors. (**A**) Experimental arrangement used for infrared (IR)–guided UV laser stimulation. (**B**) Tetanic release of glutamate from caged glutamate by a burst of UV light pulses (3 ms long at 5 Hz for 1 min) causes LTD of membrane depolarizations mediated by glutamate (glutamate responses). Before and after the tetanus (arrow), glutamate was released by 3-ms light pulses every 20 s. The data shown are averages for every minute. Average of nine neurons (mean ± SEM). The 100% value represents the average of the last 5 min before tetanization in all figures. (**C**) LTD is not induced by the laser radiation itself. After establishing a baseline, caged glutamate was washed out, and the 5-Hz light tetanus was applied. Afterward, caged glutamate was washed in again. Glutamate responses were not affected by the light tetanus (single experiment shown). Reapplication of the light tetanus in the presence of caged glutamate induced robust LTD. (**D**) LTD of glutamate responses requires activation of NMDA receptors. Application of the NMDA receptor antagonist MK801 (20 μM) strongly reduced the depression of glutamate responses after the 5-Hz stimulation to a statistically insignificant 7 ± 2% ($n = 6$, data of 16 to 20 min after the light tetanus pooled). (**E**) LTD is Ca^{2+}-dependent. Addition of the Ca^{2+} chelator BAPTA (10 mM) to the recording pipette solution left only an insignificant reduction of 9 ± 2% ($n = 6$) of the membrane depolarizations.

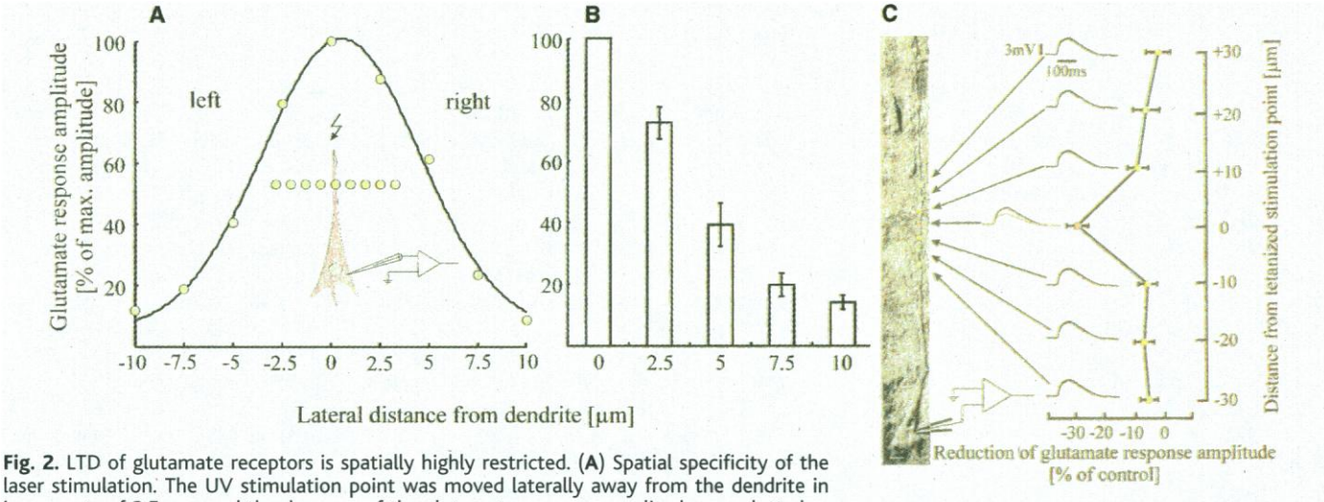

Fig. 2. LTD of glutamate receptors is spatially highly restricted. (**A**) Spatial specificity of the laser stimulation. The UV stimulation point was moved laterally away from the dendrite in increments of 2.5 μm, and the decrease of the glutamate response amplitude was plotted as a function of this distance (single experiment shown). (**B**) Average of results of six neurons; only the measurements of one side were considered, as the gaussian shape of the other side was often distorted, probably because of an invisible secondary dendritic branch (*22*). FWHM = 4 ± 1 μm. (**C**) Example of a pyramidal neuron of neocortical layer V overlaid with seven UV stimulation points on the dendrite (scale bar, 10 μm). The reduction of the glutamate responses after 5-Hz stimulation to the central point only is indicated in red. Only at this stimulation site was LTD (29 ± 4%) induced, illustrated in the figure by the glutamate responses, scaled according to the reduction. At all other stimulation sites, reductions were not significant compared with values before tetanic stimulation ($P > 0.05$, $n = 9$ neurons). Values were obtained by averaging five stimulations for each stimulation site on each neuron before and 10 to 20 min after the 5-Hz stimulation and calculating the relative reduction.

To explain the slow development of LTD of glutamate sensitivity after synaptic tetanization, it is important to realize that glutamate is released by synaptic stimulation mainly onto the postsynaptic density on the spine head. Photostimulation releases glutamate onto a bigger membrane area of about 10-μm diameter including extrasynaptic receptors. The response of these extrasynaptic receptors will not be depressed at first hand by synaptic tetanization. However, photostimulation measures the response of all glutamate receptors. Thus, the slow development of the LTD of glutamate sensitivity may indicate some kind of slow recruitment of glutamate receptors for LTD, which lie adjacent to postsynaptic densities. Such neighboring extrasynaptic glutamate receptors have been found, and rapid redistribution phenomena of glutamate receptors have been described during LTD (*14*). An alternative explanation for the different time course in Fig. 3C would be a contribution of presynaptic mechanisms to the depression of EPSPs in the first 15 min after the tetanus.

Further evidence for common mechanisms of LTD induction by synaptic stimulation and by photostimulation was provided by occlusion experiments (Fig. 3, B and C). Forty minutes after synaptic tetanic stimulation, the 5-Hz train of light flashes for LTD induction was applied for 1 min to 6 out of 14 neurons (yellow diamonds). No additional LTD could be induced in these neurons, whereas in the experi-

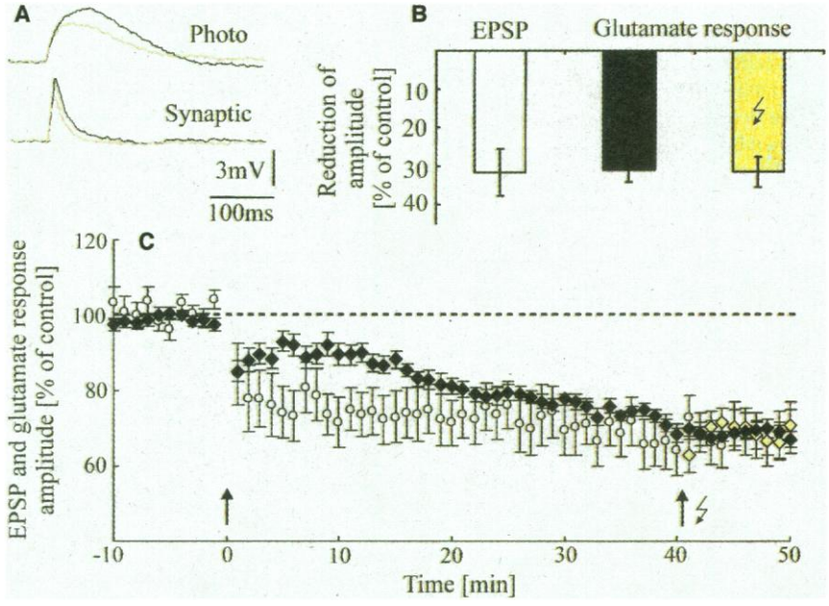

Fig. 3. LTD of EPSPs is associated with a reduction of glutamate receptor sensitivity. EPSPs were elicited by electrical stimulation with a standard stimulation electrode placed in white matter below the recording electrode. During the control and follow-up period, electrical stimulation and photostimulation were performed every 20 s with an interval of 10 s between the two kinds of stimulation. LTD was induced by a tetanus of electrical stimulation of 5 Hz for 1 min with two times the threshold voltage for action potential generation while photostimulation was stopped. (**A**) Traces of photostimulation and synaptic potentials before (black) and 35 min after (red) electrical 5-Hz stimulation. Potentials evoked by photostimulation had a time to peak (62 ± 3 ms) that was three times as long as potentials evoked by electrical stimulation (20 ± 2 ms) ($n = 7$ neurons). (**B**) Comparison of LTD of synaptic potentials (32 ± 6%, $n = 14$ neurons) and photostimulation potentials (31 ± 3%, $n = 8$ neurons) 48 min after electrical LTD induction (data of minutes 46 to 50 averaged). LTD of both potentials showed no difference ($P > 0.5$) at this time. The yellow column indicates the average of photostimulation potentials of 6 out of the 14 neurons at 48 min after additional glutamate release by a 5-Hz light tetanus (31 ± 2%). (**C**) Time course of LTD of membrane potentials evoked by electrical stimulation (○) or photostimulation (◆). Half an hour after the 5-Hz stimulation, LTD of both kinds of potentials was nearly equal. Additional glutamate release by a 5-Hz light tetanus (indicated by the flash symbol) was without any effect (yellow diamonds), demonstrating complete occlusion of synaptic and photostimulation LTD.

ments without prior synaptic tetanization (Fig. 1B), robust LTD of about 30% was always apparent 1 min after the light tetanus. Thus, synaptically induced LTD occludes photostimulation LTD, suggesting similar mechanisms of expression of both kinds of LTD (*15*).

The mechanism of LTD induction is very quick. The reduction of the neuronal depolarizations was always apparent at the first stimulation, 1 min after the tetanus. It should therefore be mediated by a local, membrane-bound process. One possibility could be changed ion channel kinetics, but we obtained no evidence for this explanation. Another explanation could be a modulation of glutamate channel conductance (*16*).

The method of infrared-guided laser stimulation allows the stimulation of selected target points on neurons with a spatial precision of 10 μm. Neurons can be depolarized by light flashes with a duration of a few milliseconds for at least 1 hour without any signs of photodamage. The use of infrared videomicroscopy for the visualization of neurons obviates the necessity of neuronal staining by fluorescent dyes (*17*) or biocytin (*18*), which have inherent drawbacks.

LTD is spatially restricted. Tetanic activation of postsynaptic glutamate receptors by photolysis alone can induce LTD. This postsynaptic LTD of glutamate receptors is sufficient to account for the LTD of EPSPs seen 30 min after electrical stimulation of afferent fibers. The LTD of a small membrane patch (Fig. 2C) suggests that, during synaptic transmission, a few or even single synapses may undergo LTD in isolation. These results are compatible with observations that single spines show transient $[Ca^{2+}]$ increases after synaptic stimulation (*19*).

In conclusion, our experiments indicate the importance of the postsynaptic site for the generation of LTD. At this site, LTD can be induced very quickly and with high precision. Our finding of high spatial specificity would allow for a model of fine tuning of the transmission strength of each individual synapse, depending on its input. Such a specific mechanism would be very attractive for "high-density storage" of memory traces.

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5. The gradient contrast system (GC) we developed gives images of neurons similar to images obtained with differential interference contrast but with superior contrast and light intensity. As in the GC system (Luigs and Neumann), no optical elements for contrast generation had to be placed behind the objective (60×, 0.9 numerical aperture, Olympus); the full power of the UV laser light can reach the neuron. The light of the UV laser was focused through the microscope objective to an optical spot of 1-μm diameter. The power of the laser (Enterprise II, Coherent) was adjusted with its remote control to 5 to 10 mW to evoke a depolarizing response of about 3 to 5 mV at the soma in response to 3-ms shuttered light pulses (Uniblitz shutter; Vincent Associates). Glutamate was applied by photolysis (wavelength = 351 to 364 nm) of γ-(α-carboxy-2-nitrobenzyl) (CNB)–caged glutamate (Molecular Probes) added at a concentration of 0.25 to 0.5 mM to the superfusion solution, which was oxygenated in a recirculation system. No indications for effects of spontaneously hydrolyzed γ-CNB–caged glutamate were found. All photostimulation experiments were started at least 15 min after the addition of caged glutamate and TTX.
6. Parasaggital slices of the parietal neocortex (300 μm thick) from 14- to 21-day-old Sprague-Dawley rats were prepared according to standard procedures (*20*). The brain slices were placed in the recording chamber of the "infrapatch" setup (Luigs and Neumann) and superfused with solutions containing the following: 125 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 25 mM $NaHCO_3$, and 25 mM glucose (pH 7.38; 20° to 22°C). Whole-cell recordings from visually identified somata of layer V pyramidal neurons were made with a standard intracellular amplifier (npi) in bridge mode. Patch-clamp electrodes with open-tip resistances of 4 to 7 megaohms were used that contained the following: 130 mM K-gluconate, 5 mM KCl, 0.5 mM EGTA, 2 mM Mg-adenosine triphosphate, 10 mM Hepes, and 5 mM glucose (pH 7.2) (*21*). Data were stored and analyzed with a Macintosh-based recording system and standard software (Pulse, HEKA).
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23. We thank K. Lee for comments on this manuscript. Supported by a grant from the Sonderforschungsbereich 391.

22 June 1999; accepted 27 August 1999

Regulation of Intestinal α-Defensin Activation by the Metalloproteinase Matrilysin in Innate Host Defense

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Precursors of α-defensin peptides require activation for bactericidal activity. In mouse small intestine, matrilysin colocalized with α-defensins (cryptdins) in Paneth cell granules, and in vitro it cleaved the pro segment from cryptdin precursors. Matrilysin-deficient ($MAT^{-/-}$) mice lacked mature cryptdins and accumulated precursor molecules. Intestinal peptide preparations from $MAT^{-/-}$ mice had decreased antimicrobial activity. Orally administered bacteria survived in greater numbers and were more virulent in $MAT^{-/-}$ mice than in $MAT^{+/+}$ mice. Thus, matrilysin functions in intestinal mucosal defense by regulating the activity of defensins, which may be a common role for this metalloproteinase in its numerous epithelial sites of expression.

One role of the mucosal epithelium is to function as an active barrier against the external environment. Secretion of antibiotic peptides by epithelial cells appears to be an important component of innate immunity (*1*). The α- and β-defensins comprise a family of cationic peptides that kill bacteria by membrane disruption (*2*, *3*). Granulocytes and several epithelial tissues (*4*), including the Paneth cells of the small intestine of most mammals (*3*, *5*, *6*), produce α-defensins as prepropeptides. In mice, Paneth cell α-defensins are termed cryptdins (crypt defensins) (*5*–*7*), of which six distinct peptides have been iso-