Neuron, Vol. 44, 1031–1041, December 16, 2004, Copyright ©2004 by Cell Press

Experience-Dependent Pruning of Dendritic Spines in Visual Cortex by Tissue Plasminogen Activator

Nobuko Mataga, Yoko Mizuguchi, and Takao K. Hensch*
Laboratory for Neuronal Circuit Development
Institute of Physical and Chemical Research (RIKEN)
Brain Science Institute
2-1 Hirosawa, Wako-shi
Saitama 351-0198
Japan

Summary

Sensory experience physically rewires the brain in early postnatal life through unknown processes. Here, we identify a robust anatomical consequence of monocular deprivation (MD) in layer II/III of visual cortex that corresponds to the rapid, functional loss of responsiveness preceding any changes in axonal input. Protrusions on pyramidal cell apical dendrites increased steadily after eye opening, but were transiently lost through competitive mechanisms after brief MD only during the physiological critical period. Proteolysis by tissue-type plasminogen activator (tPA) conversely declined with age and increased with MD only in young mice. Targeted disruption of tPA release or its upstream regulation by glutamic acid decarboxylase (GAD65) prevented MD-induced spine loss that was pharmacologically rescued concomitant with critical period plasticity. An extracellular mechanism for structural remodeling that is limited to the binocular zone upon proper detection of competing inputs thus links early sensory experience to visual function.

Introduction

Occluding one eye during development ultimately trims its input to the neocortex, while thalamic axons serving the open eye progressively expand (Antonini and Stryker, 1993; Antonini et al., 1999). Yet, the most immediate and potent cortical plasticity occurs beyond thalamo-recipient layer IV (Gordon and Stryker, 1996; Shatz and Stryker, 1978; Trachtenberg et al., 2000), for which the structural basis is obscured by the complexity of the cortical microcircuit. Synapse remodeling is one fundamental process that may reflect dramatic changes in neuronal activity (reviewed in Nimchinsky et al., 2002; Segal, 2001; Yuste and Bonhoeffer, 2001). In visual cortex, altered spine number has been observed on apical dendrites of layer V or basal dendrites of layer III pyramidal neurons following prolonged manipulation of sensory input, such as dark rearing or continuous illumination from birth (Parnavelas et al., 1973; Riccio and Matthews, 1985; Valverde, 1967, 1971; Wallace and Bear, 2004). However, the rapid morphological consequence of competition resulting in loss of visual acuity during the critical period (CP) remains unknown (Hensch, 2004; Prusky and Douglas, 2003).

More recently, the motility of living spines has been monitored using two-photon imaging techniques in transgenic mice expressing yellow fluorescent protein in a subset of pyramidal cells. While basal spine dynamics generally decline with age, the specific correspondence to the CP for MD effects remains controversial (Grutzendler et al., 2002; Konur and Yuste, 2004; Majewska and Sur, 2003). Interestingly, spines on layer V pyramidal neurons may increase their motility in response to sensory deprivation only during the CP (Majewska and Sur, 2003; Oray et al., 2004 [this issue of *Neuron*]). It remains unclear whether these rapid events ultimately produce a structural redistribution of connectivity on a time scale consistent with the full shift of ocular dominance requiring more than 2 days of MD (Gordon and Stryker, 1996). This saturated functional plasticity, in turn, occurs days to weeks ahead of the classical sculpting of thalamic afferent axons and boutons (Antonini and Stryker, 1993; Antonini et al., 1999; Silver and Stryker, 1999).

Extracellular proteolysis by serine proteases offers a candidate molecular basis for linking neuronal activity with morphological change (reviewed in Liu et al., 1994; Mataga and Hensch, 2004; Shiosaka and Yoshida, 2000). The tissue plasminogen activator (tPA)-plasmin system in particular is expressed in various regions of the brain, including the cerebral cortex (Basham and Seeds, 2001), where it participates in both normal and pathological events (Benchenane et al., 2004; Mataga and Hensch, 2004). Our previous findings indicate that tPA has a permissive role for functional ocular dominance plasticity (Mataga et al., 2002) downstream of the excitatory-inhibitory balance that triggers it (Fagiolini and Hensch, 2000; Fagiolini et al., 2004; Hensch et al., 1998; Hensch, 2004; Iwai et al., 2003). Here, we show that rapid and saturating experience-dependent plasticity is accompanied by a transient loss of spines on supragranular apical dendrites only during a CP in the binocular zone of mouse visual cortex through the action of tPA downstream of an optimal excitatory-inhibitory balance.

Results

Development and Experience-Dependent Pruning of Dendritic Protrusions

Dendritic spine formation is the characteristic morphological feature of mature excitatory synapses (reviewed in Nimchinsky et al., 2002; Segal, 2001; Yuste and Bonhoeffer, 2001). To establish the developmental profile of dendritic protrusions in upper cortical layers of visual cortex, neurons were visualized by particle-mediated transfer of lipophilic dye-coated beads (DiOlistic labeling; Gan et al., 2000). The number of protrusions (filopodia and spines) was counted from randomly labeled apical dendrites of typical layer II/III pyramidal neurons in the binocular zone of mouse visual cortex. Neonatal

*Correspondence: hensch@riken.jp

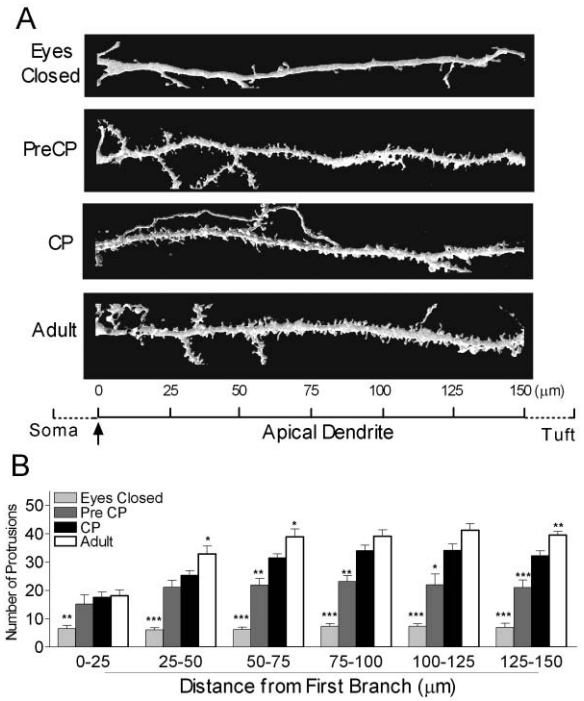

Figure 1. Emergence of Dendritic Protrusions on Apical Dendrites in Developing Mouse Visual Cortex

(A) Complete three-dimensional confocal microscope reconstructions of apical dendrites from typical layer II/III pyramidal neurons in the binocular zone of wt mouse visual cortex. Top, before eye opening (eyes closed, postnatal days P9–10); second, precritical period (Pre-CP, P15–17); third, during the critical period (CP, P24–31); bottom, adulthood (adult, P57–66). Arrow indicates first dendritic branch point. (B) Number of protrusions per 25 μm segment between the first dendritic branch point and the apical tuft. Eyes closed (8 mice, 12 cells), Pre-CP (6 mice, 9 cells), CP (6 mice, 18 cells), and adult (11 mice, 17 cells). Mean ± SEM; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus CP value for each segment. Bonferroni's multiple comparison test for four groups.

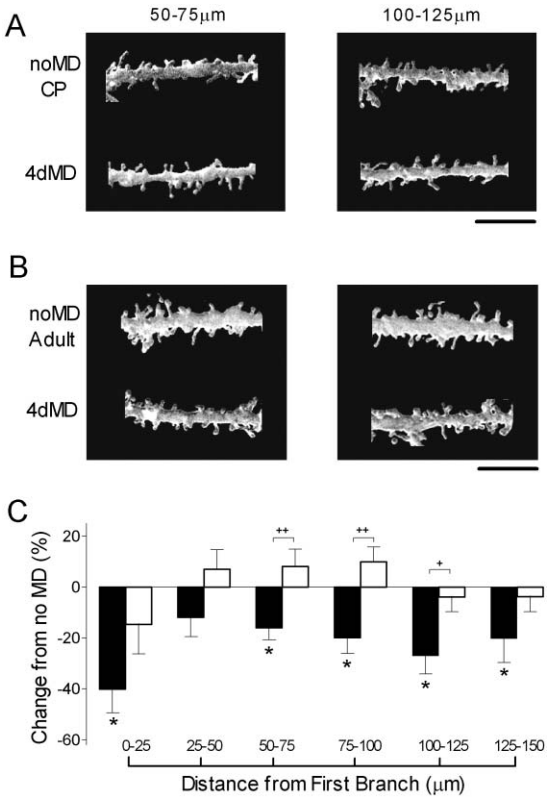

Figure 2. Age-Limited Pruning of Dendritic Protrusions by Monocular Deprivation

Representative three-dimensional confocal reconstructions of apical dendrites (third and fifth segments) after short-term MD (4 days MD) (A) during the CP or (B) in adulthood. Scale bars, 10 μm. (C) Spine loss is restricted to 4 days MD in the CP (filled columns, 5 mice, 10 cells) and is absent in adults (open columns, 6 mice, 10 cells). *$p < 0.05$ versus nondeprived mean at each age (Figure 1B); $^{+}p < 0.05$, $^{++}p < 0.01$, CP versus adult; Student's t test.

pyramidal cell dendrites were relatively bare (Figure 1, eyes closed), as reported previously (reviewed in Nimchinsky et al., 2002; Segal, 2001; Yuste and Bonhoeffer, 2001).

After eye opening, the density of dendritic protrusions dramatically increased at each segment along the apical dendrite (Figure 1, Pre-CP) and continued to proliferate throughout the CP for ocular dominance plasticity (Figure 1, CP and Adult). Only the first apical segment (0–25 μm from the first dendritic branch point) rapidly reached a plateau level by the third week of life, while the number of protrusions in other segments increased significantly into adulthood (2 months old). Similarly, it is reported that matching growth and elaboration of geniculocortical axon arbors continues beyond the peak of the CP (Antonini et al., 1999).

Although there was no simple relationship between CP plasticity levels and the development of spines (Konur and Yuste, 2004), their emergence is reported to reflect neuronal activity (Parnavelas et al., 1973; Riccio and Matthews, 1985; Valverde, 1967, 1971). We therefore directly assessed whether morphological refinement is dependent upon visual experience and age. The number of dendritic protrusions was compared before and after short-term MD (4 days MD) either during the CP (from postnatal days P25–26, Figures 2A and 2C, closed columns) or in adulthood (from P60, Figures 2B and 2C, open columns).

Robust pruning of protrusions was observed only in young mice in tight correlation with the minimum time course (4 days) for a full physiological shift of ocular dominance by MD (Gordon and Stryker, 1996; Fagiolini and Hensch, 2000). The number of protrusions tended to decrease in all apical segments after 4 days MD (*$p < 0.05$, Student's t test versus nondeprived mice, no MD; Figure 1B, CP), but was unchanged in adult animals, confirming the distinctive nature of the earlier developmental stage in mice (Antonini et al., 1999; Gordon and Stryker, 1996). These results are further consistent with an age-limited increase in spine motility induced by an even shorter (2 day) deprivation visualized by two-photon laser microscopy (Majewska and Sur, 2003; Oray et al., 2004).

Increased tPA Activity Permits Spine Loss by Sensory Deprivation

Two-day MD during the CP leads to an increase in proteolytic activity in visual cortex that precedes and persists during the rapid loss of deprived-eye responsiveness.

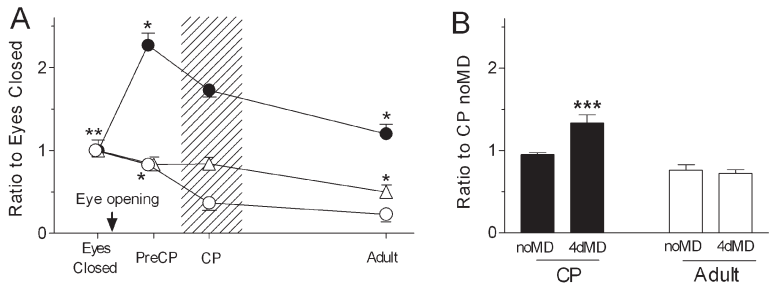

Figure 3. Elevation of tPA Proteolytic Activity by Monocular Deprivation Only during the Critical Period

(A) Developmental profile of tPA mRNA (open circles), tPA protein (open triangles), and tPA activity (filled circles) in wild-type mouse visual cortex. Shaded region represents peak of the CP. Arrow indicates eye opening (approximately P14 in mice). *$p < 0.05$, **$p < 0.01$ versus CP. Bonferroni's multiple comparison test for four groups. (B) Increased tPA activity induced by 4 days MD is seen only in the CP mouse binocular zone. ***$p < 0.001$ versus nondeprived mean (no MD); Student's t test.

Both proteolytic activity and loss of responsiveness are disrupted in the absence of tPA (Mataga et al., 2002). To explore whether tPA levels might reflect dynamics of protrusions during development and visual deprivation, we analyzed endogenous tPA expression in visual cortex. Inverse to the development of dendritic protrusions (Figure 1), the basal expression of tPA mRNA, tPA protein, and its proteolytic activity in visual cortex declined steadily throughout the CP with no particular relation to the expected level of plasticity (Figure 3A). Instead, tPA activity in the binocular zone was induced by 4 days MD in an age-restricted manner only during the CP and not in adult mice (Figure 3B).

Based on the hypothesis that tPA may then contribute to the pruning of dendritic spines accompanying ocular dominance plasticity, we examined the number of protrusions in three different groups of tPA knockout (KO) mice, which showed no gross abnormality of layer II/III pyramidal cell dendrites (Figure 4A). First, the total number of filopodia and spines was similar between nondeprived wild-type (wt) and tPA KO mice during the CP (Figure 1B versus Figure 4D, gray open triangles). Second, a dramatic difference appeared between wt and tPA KO mice after 4 days MD (Figure 4D, red filled circles versus red filled triangles, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). There was no evidence of pruning by deprivation in the tPA KO mice, but rather a trend toward increased spine number at some sites, perhaps indicating uninhibited growth mechanisms in the absence of tPA.

Finally, we combined eyelid suture of tPA KO mice for 4 days together with exogeneous recombinant tPA injection (Figure 4D, 4 days MD + tPA) (E6010, Eisai Co. Ltd., 160 IU/μl, i.c.v., starting 3 days before MD), which largely rescues functional ocular dominance plasticity in this mutant (Mataga et al., 2002). Curiously, the loss of protrusions by experience was partially restored, starting from dendritic segments nearest the cell body (Figures 4B and 4D; black filled rectangles, $^{++}p < 0.01$ versus 4 days MD and $p < 0.05$ versus no MD KO). Such a pattern of dendritic spine elimination radiating from the soma outward has also been observed for the intracellular action of serum-inducible kinase (SNK) in vitro (Pak and Sheng, 2003).

Excitatory-Inhibitory Balance Triggers Experience-Dependent Spine Loss

We further explored whether loss of protrusions by sensory deprivation through tPA specifically reveals CP plasticity based on competition. For this purpose, we analyzed spines in mice lacking the synaptic isoform of GABA-synthetic enzyme glutamic acid decarboxylase. GAD65 KO mice preserve a latent, pre-CP cortex throughout life until they are treated with benzodiazepine agonists, such as diazepam or zolpidem (Fagiolini and Hensch, 2000; Fagiolini et al., 2004; Hensch et al., 1998; Iwai et al., 2003). Several findings indicate that tPA acts downstream of an optimal excitatory-inhibitory balance during functional ocular dominance plasticity. Proteolytic activity is not elevated by 2 day MD in the binocular zone of GAD65 KO mice (Mataga et al., 2002). Ocular dominance shifts are restored to GAD65 KO mice by a minimum 2 day infusion of diazepam (Iwai et al., 2003) or, in part, by longer exogenous tPA injections (1 week, i.c.v.). In contrast, plasticity remains low in tPA KO mice, even after full diazepam treatment (4 days, i.c.v.) throughout MD.

Loss of dendritic protrusions was not observed in GAD65 KO mice after 4 days MD from P25–26 (Figures 5B and 5D; red filled rectangles), despite normal gross morphology of layer II/III pyramidal neurons (Figure 5A). Instead, as expected, the number of filopodia and spines on apical dendrites was decreased by 4 days MD during this CP in the binocular zone of wt mice (Figures 5B and 5D, red filled circles, $^{+}p < 0.05$, $^{+++}p < 0.001$ versus no MD; data not shown). A tendency for more protrusions than in wt mice was even observed at some apical segments in nondeprived GAD65 KO mice, consistent with reports indicating that reduced GABAergic transmission increases basal dendritic spine density (Murphy et al., 1998a, 1998b).

Importantly, the number of protrusions after 4 days MD was significantly higher along the entire apical dendrite of GAD65 KO mice than in wt mice (Figures 5B and 5D; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus wt after 4 days MD). Pruning of spines was fully restored by diazepam injections concomitant with the MD (Figures 5B and 5D; black filled rectangles). In this animal model, total recovery of spine loss mirrors the complete rescue of CP plasticity by our drug protocol that facilitates GABAergic transmission (Fagiolini and Hensch, 2000; Hensch et al., 1998; Iwai et al., 2003). Thus, the loss of dendritic protrusions is triggered, and the refinement of experience-dependent circuits proceeds when competing inputs can be properly detected by an optimal excitatory-inhibitory balance.

Spine Loss Reflects Competition and Is Transient

In mouse visual cortex, competitive interactions occur only within a narrow binocular zone in which the two eyes' inputs first converge to represent the central 30°

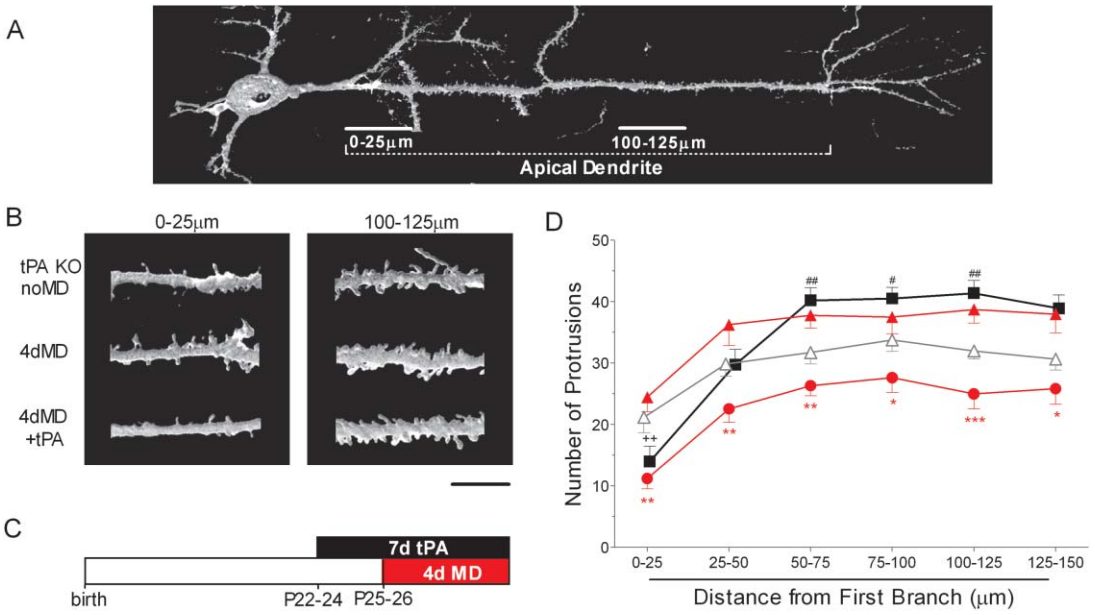

Figure 4. Pruning of Dendritic Spines Is Reversibly Impaired in tPA KO Mouse Visual Cortex

(A) Layer II/III pyramidal neuron dendritic arbors develop normally in the binocular zone of tPA KO mice. Scale bars, 25 μm. (B) Typical confocal images of apical dendrites (first and fifth segments) in nondeprived (no MD), 4 days MD from P25–26, and 4 days MD of tPA KO concomitant with exogenous tPA injection (4 days MD + tPA). Scale bar, 10 μm.

(C) Schedule of exogenous tPA injection (7 d) and MD (4 d).

(D) Summary of total number of protrusions per apical dendritic segment in tPA KO mice. Nondeprived tPA KO (gray open triangles; N = 8 mice, n = 16 cells). Spine loss due to 4 days MD fails to occur in the tPA KO (red filled triangles; N = 5, n = 11), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus 4 days MD wt (red filled circles; N = 5, n = 10, CP in Figure 2C). Dendritic spine loss by 4 days MD is partially rescued nearest the soma in the KO by concomitant exogenous tPA injection (black filled rectangles; N = 5, n = 16), $^{++}p < 0.01$ versus 4 days MD, and $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus nondeprived tPA KO. Bonferroni's multiple comparison test for four groups.

of visual space (Dräger, 1975; Wagor et al., 1980; Gordon and Stryker, 1996). The larger monocular region receives exclusively contralateral eye input serving the lateral visual fields. We took advantage of the laterally displaced rodent visual system to determine whether spine loss requires competition between inputs or simply reflects a lack of sensory input. The number of dendritic filopodia and spines on typical layer II/III pyramidal neurons in the monocular zone of wt mice remained unchanged after the contralateral eye was sutured for 4 days during the CP (Figure 6A, closed columns). Similar results were obtained ipsilateral to the closed eye (data not shown, 6 animals, 14 cells). Uniform (noncompetitive) deprivation of both eyes, examined previously, also does not affect average spine length, class distribution, or density in the binocular zone (Majewska and Sur, 2003).

These results indicate that competition between both eyes is essential for the induction of rapid dendritic spine refinements on excitatory pyramidal neurons in visual cortex. On the presynaptic side, the shrinkage of geniculocortical arbors serving the deprived eye occurs prior to the expansion of open-eye arbors (Antonini and Stryker, 1993; Antonini et al., 1999). We therefore examined whether reduction of dendritic protrusions is transient or sustained throughout long-term sensory deprivation (>30 days MD). One eyelid of wt mice was trimmed and sutured at P18, and protrusions were counted from the binocular zone at the end of the CP (P58–64; Gordon and Stryker, 1996; Fagiolini and Hensch, 2000). The number of filopodia and spines largely recovered along the apical dendrite, except nearest the cell body (Figure 6B, closed columns). These results suggest that rapid spine loss may signal the earlier retraction of deprived-eye inputs, followed by new spine formation to meet the growing axons serving the open eye in a scenario very much like that in the developing neuromuscular junction (Figure 6C; Hensch, 2004; Sanes and Lichtman, 1999).

Discussion

Experience-dependent plasticity translates learning into synaptic remodeling. Direct modification of dendritic spine structure may be a common intermediary for rapid, activity-dependent rewiring across brain systems (Liu et al., 1994; Matsuzaki et al., 2004; Okamoto et al., 2004; Yuste and Bonhoeffer, 2001). Several reports implicate tPA not only in ocular dominance plasticity (Mataga et al., 1996, 2002; Müller and Griesinger, 1998) but also in learning and memory in the cerebellum (Seeds et al., 2003), striatum, and hippocampus (Calabresi et al., 2000; Huang et al., 1996; Madani et al., 1999; Pawlak et al., 2002). In hippocampal cultures, the role of tPA lies in the creation or growth of new presynaptic varicosities (Baranes et al., 1998) or in the formation of perforated shaft synapses after induction of long-term potentiation (LTP) through NMDA receptors in vitro (Neuhoff et al., 1999). Strikingly, we found that in the visual cortex in vivo, tPA initially acts to eliminate protrusions upon sensory deprivation.

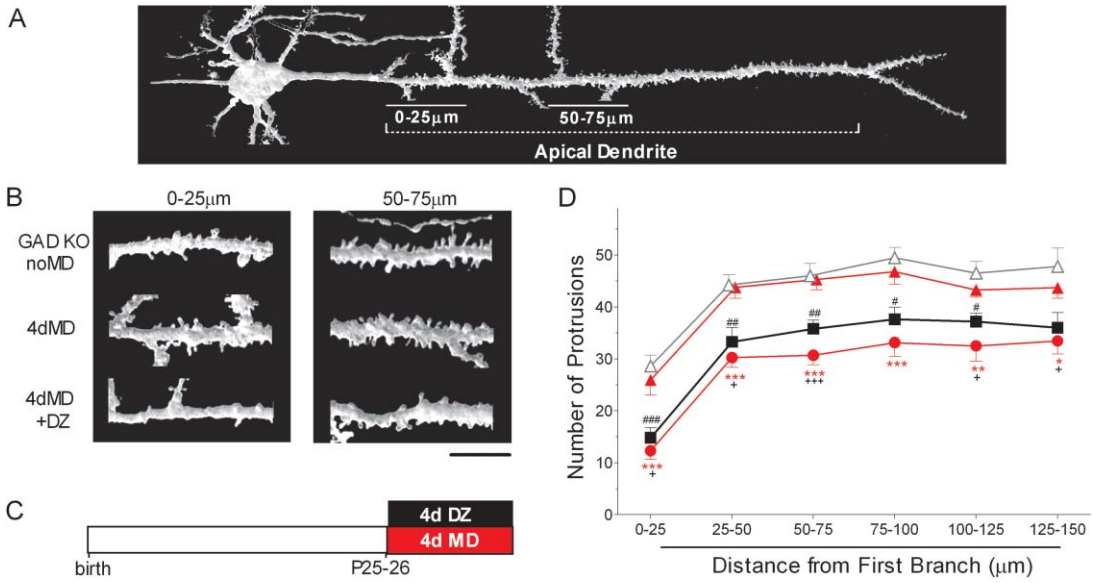

Figure 5. Pruning of Dendritic Spines Is Reversibly Impaired in GAD65 KO Mouse Visual Cortex

(A) Gross morphology of typical layer II/III pyramidal neurons is normal in the binocular zone of GAD65 KO mice.

(B) Typical confocal images of apical dendrites (first and third segments) in nondeprived (no MD), 4 days MD (initiated from P25–26), and 4 days MD of GAD65 KO concomitant with exogenous diazepam injection (4 days MD + DZ). Scale bar, 10 μm.

(C) Schedule of MD concomitant with diazepam (DZ) injection (4 days).

(D) Summary of total number of protrusions per apical dendritic segment in GAD65 KO mice. Rapid reduction of spines in wt mice by 4 days MD during the CP (red filled circles; N = 6, n = 16), $^{+}p < 0.05$, $^{+++}p < 0.001$ versus nondeprived wt (data not shown; N = 4, n = 14). Spine loss due to 4 days MD fails to occur at all apical segments of GAD65 KO mice (red filled triangles; N = 5, n = 11), no significant differences versus nondeprived KO (gray open triangles; N = 5, n = 14); $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus 4 days MD in wt (red filled circles). Dendritic spine loss by 4 days MD is fully restored along the entire apical dendrite by DZ injection (black filled rectangles; N = 7, n = 10). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ versus nondeprived GAD65 KO (gray open triangles). Bonferroni's multiple comparison test for five groups.

tPA-Mediated Spine Elimination in Visual Cortex

We propose that there are three steps to complete morphological rearrangement during ocular dominance plasticity (Figure 6C). First, spines become motile due to increased tPA proteolytic activity within 2 days of sensory deprivation (Figure 6C, left). Very brief (2 day) deprivation is indeed sufficient to increase spine dynamics outside layer IV in an age- and protease-dependent manner before any changes in functional responsiveness occur (Gordon and Stryker, 1996; Oray et al., 2004). Second, by 4 days MD, spines are eliminated prior to axons serving the deprived eye (Figure 6C, center). Finally, as deprivation proceeds, axonal arbors relaying strong neuronal activity from the open eye sprout to freed spaces (Figure 6C, asterisk) along the apical dendrite (Figure 6C, right). Thus, physiological plasticity, triggered by visual experience and an intracortical excitatory-inhibitory balance (Hensch et al., 1998), may gradually induce morphological refinements through a multistep proteolytic action of tPA.

A full shift in visual responses is recorded by single-unit electrodes within a few days of MD that requires cortical (but not thalamic) protein synthesis consistent with intracortical growth/retraction (Taha and Stryker, 2002). Whole-scale dendritic field realignment is limited to a subset of layer IV stellate cells at the borders of ocular dominance columns in cats (Kossel et al., 1995). Moreover, after MD, structural plasticity of geniculocortical axon terminals and boutons follows only slowly, by weeks to months, the initial physiological shift in ocular dominance (Antonini and Stryker, 1993; Antonini et al, 1999; Silver and Stryker, 1999). Previous work focused on basal dendrites receiving direct thalamic input in layer IV and may, thus, have missed the transient and robust spine phenotype we describe here upon apical dendrites (Konur and Yuste, 2004).

Our findings establish a crucial proteolytic link in the temporal profile and molecular cascade that converts early functional regulation to lasting morphological disconnection in the visual cortex (Hensch, 2004). Outside the central nervous system, tPA is found predominantly in the blood, where it functions primarily as a proteolytic enzyme whose principal substrate is the zymogen plasminogen. Although present in the cortex and hippocampus of both neonatal and adult mice (Basham and Seeds, 2001; Tsirka et al., 1997), the natural substrate for tPA within the brain could, in principle, be distinct from plasminogen (Benchenane et al., 2004). Enhanced NMDA receptor function upon cleavage of the NR1 subunit by tPA is both pathological and controversial (Horwood et al., 2004; Matys and Strickland, 2003; Nicole et al., 2001). While prolonged NMDA currents would reduce ocular dominance plasticity (Fagiolini et al., 2003), tPA clearly acts differently as it restores plasticity to tPA KO mice (Mataga et al., 2002).

Alternatively, tPA in association with low-density lipoprotein receptor-related protein (LRP) may be involved in such cellular events as LTP in the hippocampus (Zhuo et al., 2000) or in the regulation of the blood-brain barrier (Yepes et al., 2003). Overall, while some findings support

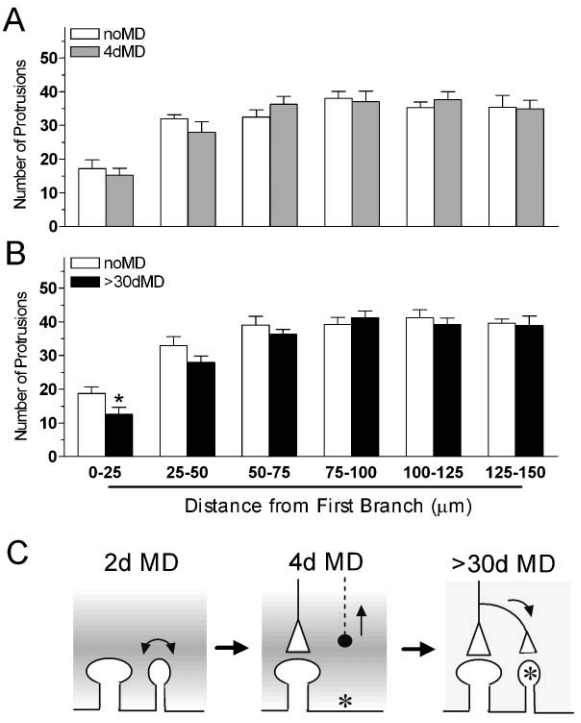

Figure 6. Experience-Dependent Pruning of Protrusions Reflects Competition and Is Transient

(A) Sensory deprivation does not induce spine loss in the monocular zone of wt mice during the CP. There was no significant difference in the total number of protrusions between nondeprived (open columns, N = 9, n = 14) and 4 days MD of the contralateral eye (filled columns; N = 6, n = 14). (B) Loss of protrusions is transient at all apical segments, except nearest the cell body. Spines largely recovered in the binocular zone of adult wt mice after prolonged MD (>30 days MD, P58–64) (filled columns; N = 11, n = 17). *$p < 0.05$, Student's t test versus nondeprived adult (open columns; Figure 1, Adult). (C) Model of morphological changes by sensory deprivation during the critical period. (Left, step 1) At MD onset during the CP, motility of spines is increased on apical dendrites of excitatory pyramidal neurons within 2 days by elevated tPA-plasmin proteolytic activity (gray background; Figure 3B; Mataga et al, 2002; Oray et al., 2004). (Center, step 2) Concurrent with the first physiologically detectable plasticity (4 days MD; Gordon and Stryker, 1996), the total number of spines is transiently and significantly reduced (asterisks) to signal the early retraction of deprived-eye axons (Antonini and Stryker, 1993). Extracellular tPA-plasmin activity remains high (Mataga et al., 2002). (Right, step 3) Upon prolonged MD (>30 days), new spines emerge to receive synaptic input from sprouting open-eye axons (Antonini and Stryker, 1993; Antonini et al., 1999) as tPA activity returns to basal levels despite continued deprivation (Mataga et al., 2002).

a role for the tPA-plasmin axis in neural plasticity, others suggest that tPA acts independently of plasminogen (Nagai et al., 2004; Pawlak et al., 2003; Zhuo et al., 2000). Even at the classical neuromuscular junction, the role of proteases in synapse elimination remains controversial (Bidoia et al., 2004; Quattrocchi et al., 2003). We therefore directly confirmed that ocular dominance plasticity is impaired in plasminogen KO mice following 4 days MD during the CP (unpublished data; contralateral bias index [CBI] = 0.66 ± 0.02, 11 mice versus wt, CBI = 0.49 ± 0.02, 10 mice; $p < 0.001$, Student's t test).

Proteases such as plasmin, tPA, and urokinase plasminogen activator (uPA) can activate secreted growth factors by cleaving their immature proforms. For instance, hepatocyte growth factor is processed by tPA or uPA and is required for the migration of parvalbumin-positive interneurons from the ganglionic eminence to appropriate cortical loci during embryonic development (Levitt et al., 2004; Mars et al., 1993; Powell et al., 2001; Thewke and Seeds, 1996). Weakened GABA circuits would be expected to impair visual cortical plasticity and spine elimination by 4 days MD (Figure 5; Hensch et al., 1998). The total number of parvalbumin-positive cells in the binocular zone of tPA KO mice was no different from that in wt (72.5 ± 5.8 and 64.4 ± 3.0 cells/field, 8 and 9 mice, respectively; p = 0.22, Student's t test) and GABA content was normal as well (wt, 1.87 ± 0.26; KO, 1.67 ± 0.12 nmol/mg; Mataga and Hensch, 2004). Moreover, while acute tPA directly rescues plasticity in these mutants (Figure 4), diazepam fails to do so (Mataga et al., 2002), indicating that tPA lies downstream rather than upstream of inhibitory function.

Mature forms of brain-derived neurotrophic factor (BDNF) are also produced by the tPA-plasmin cascade acting on secreted pro-BDNF (Lee et al., 2001). This contributes to long-lasting forms of LTP and spine/axon growth on hippocampal pyramidal cells (Baranes et al., 1998; Pang et al., 2004; Tyler and Pozzo-Miller, 2003). In contrast, BDNF blocks synaptic depression (Jiang et al., 2003) and is unlikely to mediate the rapid, experience-dependent pruning of spines found in the visual cortex. In principle, fewer spines could simply reflect retarded growth upon sensory deprivation, but this is unlikely, as acute application of tPA or diazepam during MD rapidly leads to spine loss from a normal (or elevated) basal level in tPA or GAD65 KO mice. Instead, protease-activated BDNF could contribute to later regrowth of spines and sprouting of thalamocortical axons as deprivation continues and initial tPA activity levels subside (Figure 6C, right).

Competition through Extracellular Mechanisms in Visual Cortex

Rapid spine elimination in visual cortex was observed only under conditions of competition between the two eyes and was limited to the binocular zone during the CP. Unlike homosynaptic depression that saturates within minutes to hours independent of competition (Heynen et al., 2003), ocular dominance plasticity in vivo follows days of protein synthesis (Taha and Stryker, 2002) and physical spine loss through a gradual build-up of proteolytic activity after MD regulated by an optimal excitatory-inhibitory balance (Figures 3 and 5; Mataga et al., 2002). It is widely accepted that the synaptic cleft material consists of extracellular matrix (ECM) components and cell adhesion molecules (Dityatev and Schachner, 2003). Figure 7 shows our hypothesis for spine dynamics before (Figure 7A) and after brief MD (Figures 7B and 7C). Spines become mobile due to the degradation of ECM components by increased extracellular proteolysis (Figure 3B; Mataga et al., 2002; Oray et al., 2004) and may eventually be removed by continued lack of input from the deprived eye (Figure 7C; Contra).

For instance, the neuronal cell adhesion molecule (NCAM), an Ig superfamily protein that has a site of cleavage by tPA and plasmin (Figure 7; yellow bars) (Sumi et al., 1992), is well characterized as a substrate

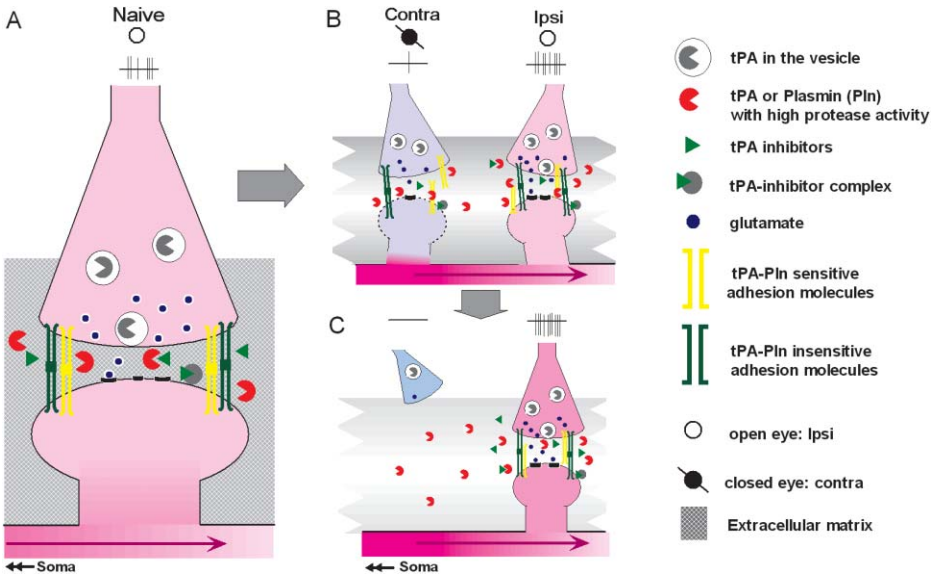

Figure 7. Model of Molecular Machinery for Experience-Dependent Synapse Refinement

(A) Normally active synapses are stabilized through a combination of cell adhesion molecules, ECM factors, and delicately balanced proteases and their inhibitors.

(B) Gross imbalance of input following sensory deprivation (MD), initially detected by local circuit excitation-inhibition (Fagiolini and Hensch, 2000; Hensch et al., 1998), leads to a downstream release of tPA (Figure 3B; Mataga et al., 2002). Cleavage of protease-sensitive adhesion molecules and ECM components creates a permissive milieu for increased spine motility within 2 days of MD (Oray et al., 2004).

(C) Competitive loss of inactive inputs may eventually result from unbalanced extracellular proteolytic activity, a further decline in protease-insensitive adhesion, and/or an intracellular gradient of spine removal signals (pink arrow).

for this system in the brain (Endo et al., 1999; Hoffman et al., 1998). In addition, ECM proteins laminin and phosphacan are also reported as targets of tPA-plasmin (Figure 7; gray background) (Wu et al., 2000; Chen and Strickland, 1997). Interestingly, NCAM expression was slightly but significantly decreased in the binocular zone by brief MD only in wt, not in tPA KO mice (unpublished data, $p < 0.05$ versus nondeprived wt).

Proteolytic removal of chondroitin sulfate proteoglycans (CSPGs) in the ECM by infusion of other proteases (chondroitinase) restores visual plasticity to adult animals (Pizzorusso et al., 2002). Curiously, this treatment eliminates so-called perineuronal nets (Pizzorusso et al., 2002), which preferentially surround large parvalbumin-positive interneurons and may buffer cations to control their fast-spiking behavior (Härtig et al., 1999). The reactivation of ocular dominance shifts in adult animals by ablating these nets may thus reflect a resetting of the initial GABAergic trigger for the CP, as defined previously (Fagiolini and Hensch, 2000). Instead, our work here identifies the first rapid anatomical consequence of CP plasticity.

The competitive mechanism whereby active spines might be preferentially retained is provocative. We propose two potential scenarios based on tPA-plasmin availability in the extracellular milieu. Further study will require more specific reagents (verified in plasminogen or tPA KO mice) to visualize cellular localization, release, and regulation by activity. First, the balance between proteases and their endogenous inhibitors is important for synapse stabilization (Figure 7; tPA-plasmin, red circle; inhibitors, green triangle). tPA can be stored in presynaptic vesicles with low protease activity (Figure 7; gray circle) to be released upon stimulation in a Ca^{2+}-dependent manner (Figure 7; red circle) (Gualandris et al., 1996; Lochner et al., 1998; Müller and Griesinger, 1998; Parmer et al., 1997) where it is rapidly inactivated by coreleased tPA inhibitors (Figure 7; gray circle + green triangle) (Fiumelli et al., 1999).

At least two endogenous inhibitors, neuroserpin (for tPA) and nexin-1 (for tPA and plasmin), are regulated by activity in the brain (Shiosaka and Yoshida, 2000). Expression of neuroserpin mRNA, in particular, is decreased in the hemisphere contralateral to the closed eye (Figure 7; green triangle) (Wannier-Morino et al., 2003), while tPA activity is increased under the same conditions (Figure 3B; Mataga et al., 2002). These findings indicate that proteolytic activity may dominate around synapses receiving poor input (Figure 7B; Contra).

Second, there are also protease-insensitive cell adhesion molecules in the synaptic glue. For instance, N-cadherin becomes resistant to proteases on the spine, and β-catenin is inserted into spines by activity (Figure 7; green bars) (Tanaka et al., 2000; Murase et al., 2002). Thus, active synapses could be retained despite increased extracellular proteolysis (Figures 7B and 7C; Ipsi). In contrast, deprived spines may finally be pruned when protease-insensitive molecules eventually disappear from these inactive synapses while the surrounding ECM continues to be degraded by tPA-plasmin (Figures 7B and 7C; Contra). Alternatively, intracellular signals for spine removal, such as SNK emanating from the soma (Pak and Sheng, 2003), may underlie the proximal spine loss once extracellular proteases permit motility by loosening up the ECM.

In this context, it is interesting that the endogenous control of visual cortical plasticity is likely driven by inhibitory contacts impinging upon the soma (Fagiolini

et al., 2004; Hensch, 2004). Such GABA circuits may eventually exert their influence on slowly shifting thalamocortical axons (Hensch and Stryker, 2004), but also initiate early physiological changes (Fagiolini and Hensch, 2000; Hensch et al., 1998; Iwai et al., 2003) and rapid pruning of spines (Figure 5). The morphological refinement identified here offers a novel perspective on the relevant functional time scale, as well as specific molecular targets, at the dendritic spine level for understanding experience-dependent brain development.

Experimental Procedures

Animals

Mice carrying a targeted disruption (knockout [KO]) of the tPA (Carmeliet et al., 1994; Mataga et al., 2002) or the GAD65 gene (Asada et al., 1996) and respective wild-type (wt) mice were used for gene gun experiments (N = 108), Northern blot analysis (N = 16), ELISA (N = 16), and tPA proteolytic activity (N = 40). No abnormalities of growth or survival were observed in either tPA or GAD65 KO mice. Animals were maintained on a 12 hr light/dark cycle with access to food and water ad libitum. All experimental groups were sacrificed at a similar time (afternoon) to avoid possible circadian effects.

DiI Labeling

Mice were anesthetized with halothane and nembutal (50 mg/kg). Animals were perfused transcardially with 0.9% saline and 4% paraformaldehyde in PBS. Brains were trimmed and cut into coronal sections at 200 μm thickness on a vibratome. Lipophilic dye (DiI, Molecular Probes) was coated onto tungsten or gold particles (0.6–1.7 μm diameter, Bio-Rad, UK), according to Gan et al. (2000) with minor modifications. DiI-coated particles were delivered to the slices using a Helios Gene Gun system (Bio-Rad). A polycarbonate filter with a 2.0 or 3.0 μm pore size and 1.6×10^6 pores/cm^2 density (Neuro Probe, MD, or BD) was inserted between the gun and the preparation to remove clusters of large particles. Density of labeling was controlled by gas pressure (95–105 psi helium). A confocal microscope (40× oil immersion objective and 4× zoom, μRadiance, Bio-Rad) was used to image the labeled structures. Randomly labeled typical pyramidal neurons were selected from deep layer II/III in the binocular zone of visual cortex. In the case of MD animals, we obtained DiI-positive neurons from hemispheres contralateral to the closed eye. Images at 0.29 μm steps (corrected for the refractive index) were acquired and stacked for 3D reconstruction using VG Studio Max software (Volume Graphics GmbH, Heidelberg, Germany; NVS, Japan). All protrusions along each apical dendrite were counted from the first dendritic branch toward the apical tuft at 25 μm intervals, using Image Pro Plus software (Media Cybernetics, Inc., MD). Dendritic protrusions were not divided by morphology (filopodia, spines) due to inherent classification ambiguities in stacked 3D images.

Northern Blot Analysis

Mice were anesthetized with halothane and sacrificed by cervical dislocation. Each visual cortex was extracted using acid guanidine isothiocyanate-phenol chloroform (AGPC) (Mataga et al., 2001). Total RNA (20 μg) was subjected to electrophoresis through a 1% agarose/formaldehyde (0.44 M) gel and transferred to a nylon filter (Hybond N, Amersham Biosciences) by electroblotting. Complementary DNA probes (tPA) were radiolabeled with [α-^{32}P] dCTP by the random priming method. Hybridization was carried out at 42°C in prehybridization buffer (50% formamide, 5× SSC, 50 mg/ml denatured herring sperm DNA, and 5× Denhardt's solution) with radiolabeled probes. Filters were washed and exposed to X-ray films at −80°C using intensifying screens. Autoradiographic signals corresponding to tPA mRNA-specific hybridization were analyzed using NIH Image.

Measurement of tPA Protein

The AngioMax human tPA ELISA kit (ET1001-1, AngioPharma, MO) was used for detecting mouse tPA protein in the binocular zone of visual cortex. This assay employs a quantitative sandwich enzyme immunoassay technique: murine antibody specific for tPA is precoated onto a microplate. Mice were anesthetized with halothane and sacrificed by cervical dislocation. Each binocular zone of visual cortex was homogenized with 100 μl of Tris buffer and mixed with the same volume of dilution buffer (50 mM Tris buffer containing 1% Triton X-100). tPA in standards and diluted homogenates (50 μl) was added to microtest plate wells and incubated overnight in the refrigerator. The sample was sandwiched by the immobilized antibody and 50 μl of biotinylated polyclonal antibody specific to tPA for 1 hr. Afterward, tPA complex was recognized by a streptavidin-peroxidase conjugate (50 μl for 30 min). All unbound material was then washed away, and a perioxidase enzyme substrate (chromogen) was added. Color development was stopped by 0.5 N hydrochloric acid (a color change from blue to yellow within 10 min), and intensity was immediately measured at a wavelength of 450 nm.

tPA Clot Lysis Analysis

The tPA activity in the binocular zone was determined by a chromogenic assay kit (Spectrolyse/fibrin, Biopool, Umea, Sweden). Briefly, homogenates were mixed with 20 volumes of Tris buffer. As standard samples, several different concentrations of human melanoma cell line-derived tPA were also mixed with Tris buffer. Twenty microliters of diluted homogenates or standard solutions were applied to each well of a microtiter plate. Each sample was mixed with 200 μl of chromogenic plasmin substrate (human Glu-plasminogen with D-Bit-CHT-Lsy-pNA) and incubated in the refrigerator for 15 min. Then, a tPA substrate (soluble *des*AA fibrinogen) was added to each well and agitated for 10 s. After 3 hr incubation at 37°C, each well was treated with 25 μl of stop solution and left for 15 min, before measuring the absorbance at 405 nm and 492 nm (as background) for each well. Specificity of the tPA ELISA and clot lysis assay was confirmed using tPA KO mice.

Drug Treatment

Recombinant tPA (E6010), also termed Cleactor and Monteplace (with EGF domain Cys84 modified to Ser to prolong half-life; Eisai, Ibaraki, Japan), was dissolved in vehicle solution (containing 4.4 mM arginine, 53 mM aspartate, 168 mM mannitol, and 0.9% saline) and stored at −80°C. Diazepam (2 mg/ml, Wako Pure Chemical, Osaka, Japan) was dissolved in propylene glycol and diluted with 0.9% saline. Solutions of tPA (160 IU/μl) or diazepam (2 mg/ml) were injected daily into the third ventricles (1.5 μl per hemisphere) according to indicated protocols (Figures 4 and 5C).

Statistical Analysis

All population data were expressed as the mean ± SEM. The data were evaluated by Student's t test (for two groups) or ANOVA with Barlett's test for equal variances, followed by Bonferroni's multiple comparison test (t test for 3–5 groups) using Prism software (GraphPad, San Diego, CA).

Acknowledgments

We thank Drs. N. Nagai, P. Carmeliet, and D. Collen (Leuven University, Belgium) for providing tPA KO mice; Dr. K. Obata (BSI, RIKEN) for providing GAD65 KO mice; Ms. Y. Tsuchimoto, M.H. Chung, S. Ohashi, A. Oba, and Mr. T. Kuji for technical assistance, genotyping, and maintenance of mouse colonies; Eisai Co., Ltd for recombinant tPA (E6010); and R.O.L. Wong (Washington University, St. Louis, MO) for kind instruction on lipophilic dye-coating methods. This work was supported by the RIKEN Brain Science Institute and Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science, and Technology (N.M.).

Received: May 7, 2004
Revised: September 13, 2004
Accepted: October 19, 2004
Published: December 15, 2004

References

Antonini, A., and Stryker, M.P. (1993). Rapid remodeling of axonal arbors in the visual cortex. Science *260*, 1819–1821.

Antonini, A., Fagiolini, M., and Stryker, M.P. (1999). Anatomical correlates of functional plasticity in mouse visual cortex. J. Neurosci. *19*, 4388–4406.

Asada, H., Kawamura, Y., Maruyama, K., Kume, H., Ding, R.-G., Ji, F.Y., Kanbara, N., Kuzume, H., Sanbo, M., Yagi, T., and Obata, K. (1996). Mice lacking the 65 kDa isoform of glutamic acid decarboxylase (GAD65) maintain normal levels of GAD67 and GABA in their brains but are susceptible to seizures. Biochem. Biophys. Res. Commun. *229*, 891–895.

Baranes, D., Lederfein, D., Huang, Y.-Y., Chen, M., Bailey, C.H., and Kandel, E.R. (1998). Tissue plasminogen activator contributes to the late phase of LTP and to synaptic growth in the hippocampal mossy fiber pathway. Neuron *21*, 813–825.

Basham, M.E., and Seeds, N.W. (2001). Plasminogen expression in the neonatal and adult mouse brain. J. Neurochem. *77*, 318–325.

Benchenane, K., López-Atalaya, J.P., Fernández-Monreal, M., Touzani, O., and Vivien, D. (2004). Equivocal roles of tissue-type plasminogen activator in stroke-induced injury. Trends Neurosci. *27*, 155–160.

Bidoia, C., Misgeld, T., Weinzierl, E., Buffelli, M., Feng, G., Cangiano, A., Lichtman, J.W., and Sanes, J.R. (2004). Comment on "Reelin promotes peripheral synapse elimination and maturation." Science *303*, 1977.

Calabresi, P., Napolitano, M., Centonze, D., Marfia, G.A., Gubellini, P., Teule, M.A., Berretta, N., Bernardi, G., Frati, L., Tolu, M., and Gulino, A. (2000). Tissue plasminogen activator controls multiple forms of synaptic plasticity and memory. Eur. J. Neurosci. *12*, 1002–1012.

Carmeliet, P., Schoonjans, L., Kieckens, L., Ream, B., Degen, J., Bronson, R., De Vos, R., van den Oord, J.J., Collen, D., and Mulligan, R.C. (1994). Physiological consequences of loss of plasminogen activator gene function in mice. Nature *368*, 419–424.

Chen, Z.-L., and Strickland, S. (1997). Neuronal death in the hippocampus is promoted by plasmin-catalyzed degradation of laminin. Cell *91*, 917–925.

Dräger, U.C. (1975). Receptive fields of single cells and topography in mouse visual cortex. J. Comp. Neurol. *160*, 269–290.

Dityatev, A., and Schachner, M. (2003). Extracellular matrix molecules and synaptic plasticity. Nat. Rev. Neurosci. *4*, 456–468.

Endo, A., Nagai, N., Urano, T., Takada, Y., Hashimoto, K., and Takada, A. (1999). Proteolysis of neuronal cell adhesion molecule by the tissue plasminogen activator-plasmin system after kainate injection in the mouse hippocampus. Neurosci. Res. *33*, 1–8.

Fagiolini, M., and Hensch, T.K. (2000). Inhibitory threshold for critical-period activation in primary visual cortex. Nature *404*, 183–186.

Fagiolini, M., Katagiri, H., Miyamoto, H., Mori, H., Grant, S.G.N., Mishina, M., and Hensch, T.K. (2003). Separable features of visual cortical plasticity revealed by N-methyl-D-aspartate receptor 2A signaling. Proc. Natl. Acad. Sci. USA *100*, 2854–2859.

Fagiolini, M., Fritschy, J.-M., Löw, K., Möhler, H., Rudolph, U., and Hensch, T.K. (2004). Specific $GABA_A$ circuits for visual cortical plasticity. Science *303*, 1681–1683.

Fiumelli, H., Jabaudon, D., Magistretti, P.J., and Martin, J.-L. (1999). BDNF stimulates expression, activity and release of tissue-type plasminogen activator in mouse cortical neurons. Eur. J. Neurosci. *11*, 1639–1646.

Gan, W.-B., Grutzendler, J., Wong, W.T., Wong, R.O.L., and Lichtman, J.W. (2000). Multicolor 'DiOlistic' labeling of the nervous system using lipophilic dye combinations. Neuron *27*, 219–225.

Gordon, J.A., and Stryker, M.P. (1996). Experience-dependent plasticity of binocular responses in the primary visual cortex of the mouse. J. Neurosci. *16*, 3274–3286.

Grutzendler, J., Kasthuri, N., and Gan, W.-B. (2002). Long-term dendritic spine stability in the adult cortex. Nature *420*, 812–816.

Gualandris, A., Jones, T.E., Strickland, S., and Tsirka, S.E. (1996). Membrane depolarization induces calcium-dependent secretion of tissue plasminogen activator. J. Neurosci. *16*, 2220–2225.

Härtig, W., Derouiche, A., Welt, K., Brauer, K., Grosche, J., Mäder, M., Reichenbach, A., and Brückner, G. (1999). Cortical neurons immunoreactive for the potassium channel $K_v3.1b$ subunit are predominantly surrounded by perineuronal nets presumed as a buffering system for cations. Brain Res. *842*, 15–29.

Hensch, T.K. (2004). Critical period regulation. Annu. Rev. Neurosci. *27*, 549–579.

Hensch, T.K., and Stryker, M.P. (2004). Columnar architecture sculpted by GABA circuits in developing cat visual cortex. Science *303*, 1678–1681.

Hensch, T.K., Fagiolini, M., Mataga, N., Stryker, M.P., Baekkeskov, S., and Kash, S. (1998). Local GABA circuit control of experience-dependent plasticity in developing visual cortex. Science *282*, 1504–1508.

Heynen, A.J., Yoon, B.J., Liu, C.H., Chung, H.J., Huganir, R.L., and Bear, M.F. (2003). Molecular mechanism for loss of visual cortical responsiveness following brief monocular deprivation. Nat. Neurosci. *6*, 854–862.

Hoffman, K.B., Martinez, J., and Lynch, G. (1998). Proteolysis of cell adhesion molecules by serine proteases: a role in long term potentiation? Brain Res. *811*, 29–33.

Horwood, J.M., Ripley, T.L., and Stephens, D.N. (2004). Evidence for disrupted NMDA receptor function in tissue plasminogen activator knockout mice. Behav. Brain Res. *150*, 127–138.

Huang, Y.-Y., Bach, M.E., Lipp, H.-P., Zhuo, M., Wolfer, D.P., Hawkins, R.D., Schoonjans, L., Kandel, E.R., Godfraind, J.-M., Mulligan, R., et al. (1996). Mice lacking the gene encoding tissue-type plasminogen activator show a selective interference with late-phase long-term potentiation in both schaffer collateral and mossy fiber pathways. Proc. Natl. Acad. Sci. USA *93*, 8699–8704.

Iwai, Y., Fagiolini, M., Obata, K., and Hensch, T.K. (2003). Rapid critical period induction by tonic inhibition in visual cortex. J. Neurosci. *23*, 6695–6702.

Jiang, B., Akaneya, Y., Hata, Y., and Tsumoto, T. (2003). Long-term depression is not induced by low-frequency stimulation in rat visual cortex in vivo: a possible preventing role of endogenous brain-derived neurotrophic factor. J. Neurosci. *23*, 3761–3770.

Konur, S., and Yuste, R. (2004). Developmental regulation of spine and filopodial motility in primary visual cortex: reduced effects of activity and sensory deprivation. J. Neurobiol. *59*, 236–246.

Kossel, A., Löwel, S., and Bolz, J. (1995). Relationships between dendritic fields and functional architecture in striate cortex of normal and visually deprived cats. J. Neurosci. *15*, 3913–3926.

Lee, R., Kermani, P., Teng, K.K., and Hempstead, B.L. (2001). Regulation of cell survival by secreted proneurotrophins. Science *294*, 1945–1948.

Levitt, P., Eagleson, K.L., and Powell, E.M. (2004). Regulation of neocortical interneuron development and the implications for neurodevelopmental disorders. Trends Neurosci. *27*, 400–406.

Liu, Y., Fields, R.D., Fitzgerald, S., Festoff, B.W., and Nelson, P.G. (1994). Proteolytic activity, synapse elimination, and the Hebb synapse. J. Neurobiol. *25*, 325–335.

Lochner, J.E., Kingma, M., Kuhn, S., Meliza, C.D., Cutler, B., and Scalettar, B.A. (1998). Real-time imaging of the axonal transport of granules containing a tissue plasminogen activator/green fluorescent protein hybrid. Mol. Biol. Cell *9*, 2463–2476.

Madani, R., Hulo, S., Toni, N., Madani, H., Steimer, T., Muller, D., and Vassalli, J.-D. (1999). Enhanced hippocampal long-term potentiation and learning by increased neuronal expression of tissue-type plasminogen activator in transgenic mice. EMBO J. *18*, 3007–3012.

Majewska, A., and Sur, M. (2003). Motility of dendritic spines in visual cortex in vivo: Changes during the critical period and effects of visual deprivation. Proc. Natl. Acad. Sci. USA *100*, 16024–16029.

Mars, W.M., Zarnegar, R., and Michalopoulos, G.K. (1993). Activation of hepatocyte growth factor by the plasminogen activators uPA and tPA. Am. J. Pathol. *143*, 949–958.

Mataga, N., and Hensch, T.K. (2005). Serine proteases and neuronal plasticity in vivo. In Proteases in the Brain (Proteases in Biology and Disease, volume 3), U. Lendeckel, N. Hooper, and M. Nigel, eds. (New York: Springer), in press.

Mataga, N., Imamura, K., Shiomitsu, T., Yoshimura, Y., Fukamauchi,

F., and Watanabe, Y. (1996). Enhancement of mRNA expression of tissue-type plasminogen activator by L-threo-3,4-dihydrophenylserine in association with ocular dominance plasticity. Neurosci. Lett. *218*, 149–152.

Mataga, N., Fujishima, S., Condie, B.G., and Hensch, T.K. (2001). Experience-dependent plasticity of mouse visual cortex in the absence of the neuronal activity-dependent marker egr1/zif268. J. Neurosci. *21*, 9724–9732.

Mataga, N., Nagai, N., and Hensch, T.K. (2002). Permissive proteolytic activity for visual cortical plasticity. Proc. Natl. Acad. Sci. USA *99*, 7717–7721.

Matsuzaki, M., Honkura, N., Ellis-Davies, G.C., and Kasai, H. (2004). Structural basis of long-term potentiation in single dendritic spines. Nature *429*, 761–766.

Matys, T., and Strickland, S. (2003). Tissue plasminogen activator and NMDA receptor cleavage. Nat. Med. *9*, 371–372.

Müller, C.M., and Griesinger, C.B. (1998). Tissue plasminogen activator mediates reverse occlusion plasticity in visual cortex. Nat. Neurosci. *1*, 47–53.

Murase, S., Mosser, E., and Schuman, E.M. (2002). Depolarization drives β-catenin into neuronal spines promoting changes in synaptic structure and function. Neuron *35*, 91–105.

Murphy, D.D., Cole, N.B., Greenberger, V., and Segal, M. (1998a). Estradiol increases dendritic spine density by reducing GABA neurotransmission in hippocampal neurons. J. Neurosci. *18*, 2550–2559.

Murphy, D.D., Cole, N.B., and Segal, M. (1998b). Brain-derived neurotrophic factor mediates estradiol-induced dendritic spine formation in hippocampal neurons. Proc. Natl. Acad. Sci. USA *95*, 11412–11417.

Nagai, T., Yamada, K., Yoshimura, M., Ishikawa, K., Miyamoto, Y., Hashimoto, K., Noda, Y., Nitta, A., and Nabeshima, T. (2004). The tissue plasminogen activator-plasmin system participates in the rewarding effect of morphine by regulating dopamine release. Proc. Natl. Acad. Sci. USA *101*, 3650–3655.

Neuhoff, H., Roeper, J., and Schweizer, M. (1999). Activity-dependent formation of perforated synapses in cultured hippocampal neurons. Eur. J. Neurosci. *11*, 4241–4250.

Nicole, O., Docagne, F., Ali, C., Margaill, I., Carmeliet, P., MacKenzie, E.T., Vivien, D., and Buisson, A. (2001). The proteolytic activity of tissue-plasminogen activator enhances NMDA receptor-mediated signaling. Nat. Med. *7*, 59–64.

Nimchinsky, E.A., Sabatini, B.L., and Svoboda, K. (2002). Structure and function of dendritic spines. Annu. Rev. Physiol. *64*, 313–353.

Okamoto, K.-I., Nagai, T., Miyawaki, A., and Hayashi, Y. (2004). Rapid and persistent modulation of actin dynamics regulates postsynaptic reorganization underlying bidirectional plasticity. Nat. Neurosci. *7*, 1104–1112.

Oray, S., Majewska, A., and Sur, M. (2004). Dendritic spine dynamics are regulated by monocular deprivation and extracellular matrix degradation. Neuron *44*, 1021–1030.

Pak, D.T.S., and Sheng, M. (2003). Targeted protein degradation and synapse remodeling by an inducible protein kinase. Science *302*, 1368–1373.

Pang, P., Teng, H.K., Zaitsev, E., Woo, N., Sakata, K., Zhen, S., Teng, K.K., Yung, W.-H., Hempstead, B.L., and Lu, B. (2004). Cleavage of ProBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. Science *306*, 487–491.

Parmer, R.J., Mahata, M., Mahata, S., Sebald, M.T., O'Connor, D.T., and Miles, L.A. (1997). Tissue plasminogen activator (tPA) is targeted to the regulated secretory pathway. J. Biol. Chem. *272*, 1976–1982.

Parnavelas, J.G., Globus, A., and Kaups, P. (1973). Continuous illumination from birth affects spine density of neurons in the visual cortex of the rat. Exp. Neurol. *40*, 742–747.

Pawlak, R., Nagai, N., Urano, T., Napiorkowska-Pawlak, D., Ihara, H., Takada, Y., Collen, D., and Takada, A. (2002). Rapid, specific and active site-catalyzed effect of tissue-plasminogen activator on hippocampus-dependent learning in mice. Neuroscience *113*, 995–1001.

Pawlak, R., Magarinos, A.M., Melchor, J., McEwen, B., and Strickland, S. (2003). Tissue plasminogen activator in the amygdala is critical for stress-induced anxiety-like behavior. Nat. Neurosci. *6*, 168–174.

Pizzorusso, T., Medini, P., Berardi, N., Chierzi, S., Fawcett, J.W., and Maffei, L. (2002). Reactivation of ocular dominance plasticity in the adult visual cortex. Science *298*, 1248–1251.

Powell, E.M., Mars, W.M., and Levitt, P. (2001). Hepatocyte growth factor/scatter factor is a motogen for interneurons migrating from the ventral to dorsal telencephalon. Neuron *30*, 79–89.

Prusky, G.T., and Douglas, R.M. (2003). Developmental plasticity of mouse visual acuity. Eur. J. Neurosci. *17*, 167–173.

Quattrocchi, C.C., Huang, C., Niu, S., Sheldon, M., Benhayon, D., Cartwright, J.J., Mosier, D.R., Keller, F., and D'Arcangelo, G. (2003). Reelin promotes peripheral synapse elimination and maturation. Science *301*, 649–653. Erratum: Science *301*, 1849.

Riccio, R.V., and Matthews, M.A. (1985). Effects of intraocular tetrodotoxin on dendritic spines in the developing rat visual cortex: a Golgi analysis. Brain Res. *351*, 173–182.

Seeds, N.W., Basham, M.E., and Ferguson, J.E. (2003). Absence of tissue plasminogen activator gene or activity impairs mouse cerebellar motor learning. J. Neurosci. *23*, 7368–7375.

Segal, M. (2001). Rapid plasticity of dendritic spines: hints to possible functions? Prog. Neurobiol. *63*, 61–70.

Sanes, J.R., and Lichtman, J.W. (1999). Development of the vertebrate neuromuscular junction. Annu. Rev. Neurosci. *22*, 389–442.

Shatz, C.J., and Stryker, M.P. (1978). Ocular dominance in layer IV of the cat's visual cortex and the effects of monocular deprivation. J. Physiol. *281*, 267–283.

Shiosaka, S., and Yoshida, S. (2000). Synaptic microenvironments-structural plasticity, adhesion molecules, proteases and their inhibitors. Neurosci. Res. *37*, 85–89.

Silver, M.A., and Stryker, M.P. (1999). Synaptic density in geniculocortical afferents remains constant after monocular deprivation in the cat. J. Neurosci. *19*, 10829–10842.

Sumi, Y., Dent, M.A.R., Owen, D.E., Seeley, P.J., and Morris, R.J. (1992). The expression of tissue and urokinase-type plasminogen activators in neuronal development suggests different modes of proteolytic involvement in neuronal growth. Development *116*, 625–637.

Taha, S., and Stryker, M.P. (2002). Rapid ocular dominance plasticity requires cortical but not geniculate protein synthesis. Neuron *34*, 425–436.

Tanaka, H., Shan, W., Phillips, G.R., Arndt, K., Bozdagi, O., Shapiro, L., Huntley, G.W., Benson, D.L., and Colman, D.R. (2000). Molecular modification of N-cadherin in response to synaptic activity. Neuron *25*, 93–107.

Thewke, D.P., and Seeds, N.W. (1996). Expression of hepatocyte growth factor/scatter factor, its receptor, c-met and tissue-type plasminogen activator during development of the murine olfactory system. J. Neurosci. *16*, 6933–6944.

Trachtenberg, J.T., Trepel, C., and Stryker, M.P. (2000). Rapid extragranular plasticity in the absence of thalamocortical plasticity in the developing primary visual cortex. Science *287*, 2029–2032.

Tsirka, S.E., Rogove, A.D., Bugge, T.H., Degen, J.L., and Strickland, S. (1997). An extracellular proteolytic cascade promotes neuronal degeneration in the mouse hippocampus. J. Neurosci. *17*, 543–552.

Tyler, W.J., and Pozzo-Miller, L. (2003). Miniature synaptic transmission and BDNF modulates dendritic spine growth and form in rat CA1 neurons. J. Physiol. *553*, 497–509.

Valverde, F. (1967). Apical dendritic spines of the visual cortex and light deprivation in the mouse. Exp. Brain Res. *3*, 337–352.

Valverde, F. (1971). Rate and extent of recovery from dark rearing in the visual cortex of the mouse. Brain Res. *33*, 1–11.

Wagor, E., Mangini, N.J., and Pearlman, A.L. (1980). Retinotopic organization of striate and extrastriate visual cortex in the mouse. J. Comp. Neurol. *193*, 187–202.

Wallace, W., and Bear, M.F. (2004). A morphological correlate of synaptic scaling in visual cortex. J. Neurosci. *24*, 6928–6938.

Wannier-Morino, P., Rager, G., Sonderegger, P., and Grabs, D. (2003). Expression of neuroserpin in the visual cortex of the mouse during the developmental critical period. Eur. J. Neurosci. *17*, 1853–1860.

Wu, Y.P., Siao, C.-J., Lu, W., Sung, T.-C., Frohman, M.A., Milev, P., Bugge, T.H., Degen, J.L., Levine, J.M., Margolis, R.U., and Tsirka, S.E. (2000). The tissue plasminogen activator (tPA)/plasmin extracellular proteolytic system regulates seizure-induced hippocampal mossy fiber outgrowth through a proteoglycan substrate. J. Cell Biol. *148*, 1295–1304.

Yepes, M., Sandkvist, M., Moore, E.G., Bugge, T.H., Strickland, D.K., and Lawrence, D.A. (2003). Tissue-type plasminogen activator induces opening of the blood-brain barrier via the LDL receptor-related protein. J. Clin. Invest. *112*, 1533–1540.

Yuste, R., and Bonhoeffer, T. (2001). Morphological changes in dendritic spines associated with long-term synaptic plasticity. Annu. Rev. Neurosci. *24*, 1071–1089.

Zhuo, M., Holtzman, D.M., Li, Y., Osaka, H., DeMaro, J., Jacquin, M., and Bu, G. (2000). Role of tissue plasminogen activator receptor LRP in hippocampal long-term potentiation. J. Neurosci. *20*, 542–549.