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Dendritic Spine Dynamics Are Regulated by Monocular Deprivation and Extracellular Matrix Degradation

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Summary

The mammalian primary visual cortex (V1) is especially susceptible to changes in visual input over a well-defined critical period, during which closing one eye leads to a loss of responsiveness of neurons to the deprived eye and a shift in response toward the open eye. This functional plasticity can occur rapidly, following even a single day of eye closure, although the structural bases of these changes are unknown. Here, we show that rapid structural changes at the level of dendritic spines occur following brief monocular deprivation. These changes are evident in the supra- and infragranular layers of the binocular zone and can be mimicked by degradation of the extracellular matrix with the tPA/plasmin proteolytic cascade. Further, monocular deprivation occludes a subsequent effect of matrix degradation, suggesting that this mechanism is active in vivo to permit structural remodeling during ocular dominance plasticity.

Introduction

Ocular dominance plasticity in the visual cortex occurs during a well-defined critical period, first observed in cats (Hubel and Wiesel, 1970; Olson and Freeman, 1975; Wiesel and Hubel, 1963) and recently described in mice (Gordon and Stryker, 1996), and has served as a model for experience-dependent plasticity throughout the central nervous system. In mice, the critical period for ocular dominance plasticity in V1 occurs just as the processes of excitatory and inhibitory synaptogenesis are coming to a close (Blue and Parnavelas, 1983; De Felipe et al., 1997) and is likely to be controlled by the functional maturation of inhibitory connections (Fagiolini et al., 2004; Fagiolini and Hensch, 2000; Hensch et al., 1998; Huang et al., 1999). Monocular deprivation at the peak of the critical period, for even 1 or 2 days, shifts the responses of cells toward the open eye (Hubel and Wiesel, 1970; Olson and Freeman, 1975; Trachtenberg et al., 2000). This rapid change in the functional properties of cortical neurons is accompanied by long-term depression (LTD) of synapses in the deprived cortex (Heynen et al., 2003). In contrast, the reduction of cortical area driven by the closed eye and concomitant expansion of that driven by the open eye, as examined by the extent and complexity of thalamocortical axonal arbors, proceeds on the timescale of weeks to months (Antonini et al., 1999; Antonini and Stryker, 1993; Shatz and Stryker, 1978). This indicates that following monocular deprivation, processes at the level of synapses may guide large-scale reorganization of anatomical connectivity. Further, recent work has found that changes in both functional properties and anatomical connectivity may occur more rapidly in the horizontal connections of the superficial and deep cortical layers before being relayed to layer IV (Trachtenberg and Stryker, 2001; Trachtenberg et al., 2000), suggesting that there might be a top-down reorganization following ocular dominance plasticity. However, the mechanisms that intervene between the rapid loss of physiological responses driven by the deprived eye and the anatomical reorganization of dendritic and axonal arborizations remain unknown.

One possible locus of both functional and anatomical change is at the level of dendritic spines, which are structural specializations that contain the postsynaptic elements of excitatory synapses (Hering and Sheng, 2001). Spines receive the majority of excitatory input in the mammalian CNS (Gray, 1959) and are known to be important for compartmentalizing synaptic signals (Emptage et al., 1999; Koester and Sakmann, 1998; Majewska et al., 2000a; Yuste and Denk, 1995). Additionally, spines are motile structures (Dunaevsky et al., 1999; Fischer et al., 1998) whose dynamics are likely to play a role in their functional properties (Majewska et al., 2000b). These dynamics may be further regulated by glutamatergic activity (Fischer et al., 2000; but see Dunaevsky et al., 1999). Spine dynamics decrease over development (Lendvai et al., 2000), and by the critical period for ocular dominance plasticity in mice, spines have achieved a relatively stable state (Konur and Yuste, 2004; Majewska and Sur, 2003). Binocular deprivation, by reducing visual cortical activity over long periods of time, increases spine motility (Majewska and Sur, 2003). This increase in dynamics may reflect a process whereby spines destabilize and increase their synaptic drive.

Here we examine rapid structural changes at the level of synapses by imaging dendritic spines following brief monocular deprivation. Our findings provide clear evidence that brief monocular deprivation initiates a rapid upregulation in structural dynamics in the binocular segment of V1 and that this effect is initially restricted to the superficial and deep layers of the cortex. We also find that spine motility is elevated by proteolysis of the extracellular matrix (ECM) with the tissue-type plasminogen activator (tPA)/plasmin cascade, which has previously been implicated in ocular dominance plasticity (Mataga et al., 2002; Müller and Griesinger, 1998). Further, monocular deprivation occludes a subsequent effect of ECM degradation in a lamina-specific manner, strongly suggesting that this mechanism may be active in vivo to permit structural remodeling during experience-dependent plasticity in the visual system.

Results

MD Alters Spine Dynamics In Vivo

In order to examine whether monocular deprivation rapidly alters dendritic structure in a manner consistent

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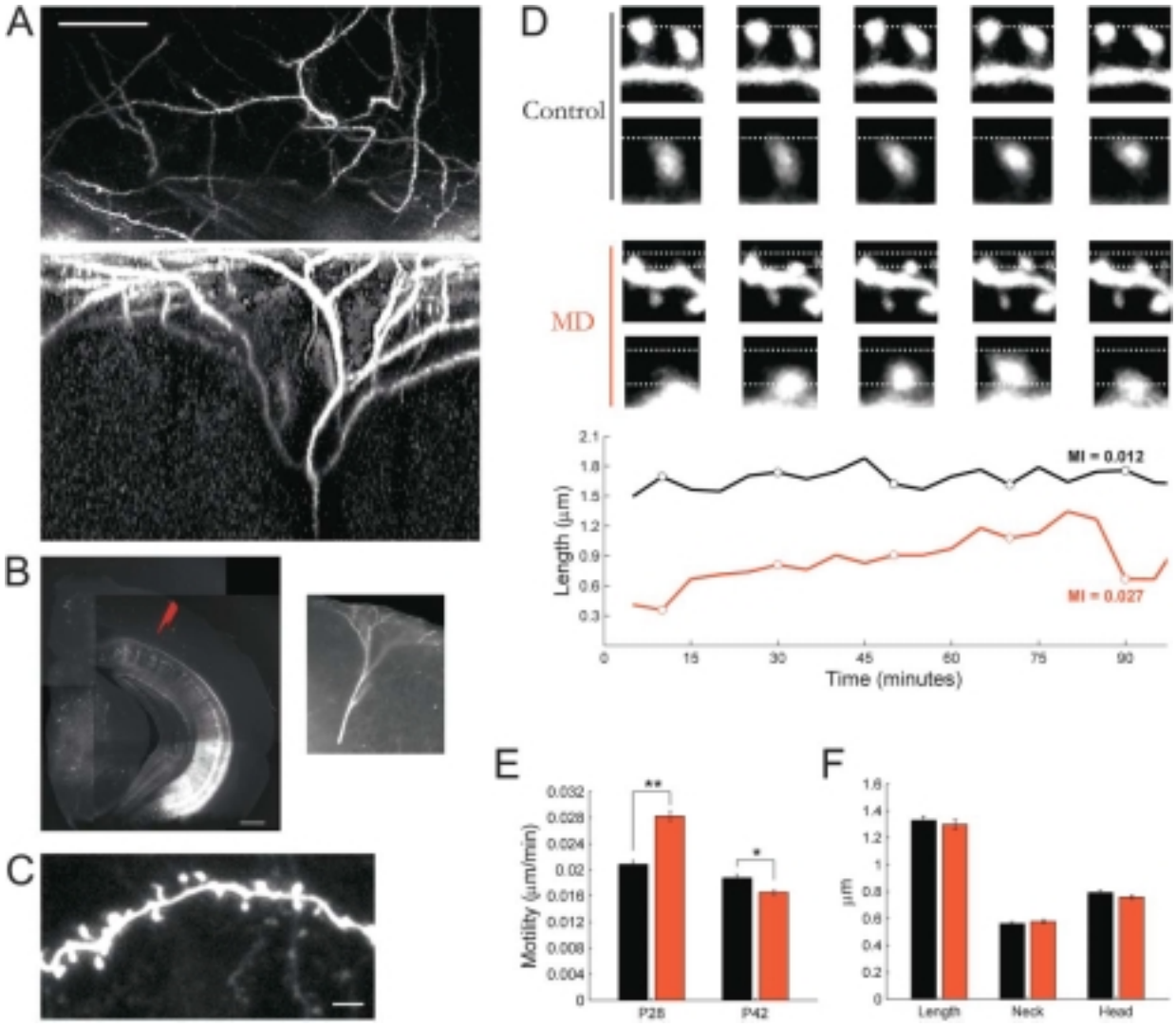

Figure 1. Spine Motility Is Elevated In Vivo Contralateral to the Deprived Eye following Short Monocular Deprivation during the Critical Period

(A) Apical dendritic arbors from mice expressing GFP in a subset of their layer V pyramidal neurons are visualized with two-photon microscopy. The top panel shows a collapsed z stack of the arbor from a top-down view, and the lower panel shows a side view of the same arbor after a volumetric projection in the x-z axis. Scale bar, 50 μm in the x and y axes; 100 μm in the z axis.

(B) The location of in vivo imaging is marked with an injection of Alexa Fluor 594, which is then used to verify that the imaged neuron was in the binocular region of V1. The panel on the right is a blow-up of the neuron immediately adjacent to the Alexa injection. Scale bar, 500 μm.

(C) A sample image (the first image of a time series) indicating that spines are readily identifiable in vivo with a sufficient signal-to-noise ratio that they can be tracked reliably over several hours. Scale bar, 5 μm.

(D) Two spines typifying the mean motility from control (black) and monocularly deprived (red) cortex are tracked over time. Each spine is shown at low magnification with the local dendrite and nearby spines and at higher magnification for a closer examination of morphology. Dotted lines are included to facilitate the comparison between time points. The images in the top panels correspond to spine length measurements at the open circles in the bottom panel. The motility index for each spine is listed next to the relevant trace.

(E) Spine motility is significantly elevated following brief deprivation during the critical period.

(F) There is no apparent change in average spine length, neck diameter, or head diameter after brief deprivation. Black bars, spines from nondeprived cortex; red bars, spines from cortex contralateral to the deprived eye. **$p < 0.0001$; *$p < 0.005$.

with physiological changes, we visualized the dynamic structural properties of synapses by imaging dendritic spines in vivo. Spines from the apical arbor of layer V pyramidal neurons were imaged using two-photon microscopy (Figures 1A–1D) at the height of the critical period (P28-29) (Gordon and Stryker, 1996) either with or without short-term monocular deprivation (2–3 days, starting on P26). Spine motility in the binocular region of V1, contralateral to the deprived eye, was 35% higher than motility in control, nondeprived animals (Figure 1E; control, 147 spines, 8 cells, 6 animals; deprived, 221 spines, 4 cells, 4 animals; $p < 0.0001$). This indicates that sensory deprivation in a fully innervated, yet plastic, cortex was able to initiate a rapid sequence of events leading to increased structural dynamics at the level of individual spines. Such an increase in spine dynamics may reflect structural destabilization of a population of spines whose function is affected by visual deprivation.

This, in turn, could precede a change in spine density (Mataga et al., 2004 [this issue of *Neuron*]). Interestingly, the upregulation of spine motility is restricted to the critical period, as monocular deprivation at later ages (P42) had a modest effect of reducing spine motility (Figure 1E; control, 112 spines, 4 cells, 4 animals; deprived, 153 spines, 4 cells, 4 animals; $p < 0.005$), suggesting that in older animals, deprivation may selectively stabilize spines. This may reflect alternate processes that regulate ocular dominance plasticity in the adult cortex (Sawtell et al., 2003).

In addition to spine dynamics, it is possible that spine morphology might also be altered by monocular deprivation. Although initial reports using serial electron microscopy suggested that spine morphology was not altered by long-term potentiation (LTP) in the hippocampus (Sorra and Harris, 1998), more recent evidence suggests that spine size is increased by LTP (Harris et al., 2003; Matsuzaki et al., 2004) and that spine length is decreased by dark rearing (Wallace and Bear, 2004). However, analysis of spine morphology in vivo following brief monocular deprivation revealed no significant differences in average spine dimensions (Figure 1F) between control and deprived spines. This indicates that, unlike spine motility, it may be difficult to detect subtle changes in spine morphology following brief periods of deprivation. This further suggests that an analysis of spine morphology in a fixed preparation could overlook an early indicator of potential structural remodeling that is only observed by examining the dynamic properties of spines.

MD Alters Spine Dynamics In Vitro

In vivo imaging with two-photon microscopy has the advantage of visualizing small structures in the living animal, though the signal-to-noise ratio becomes limiting as one images deeper into the tissue. To examine spines situated throughout the cortical layers, we took coronal slices of visual cortex from deprived and nondeprived animals (Figure 2A). Spine motility in this preparation also decreases as development proceeds (S.O., submitted) and reaches a stable level by the critical period. As with in vivo imaging, the motility of spines contralateral to the deprived eye was elevated compared with that in nondeprived cortex (9%; control, 502 spines, 14 cells, 13 animals; deprived, 581 spines, 9 cells, 8 animals; $p < 0.0001$) following brief monocular deprivation (Figure 2D). This suggests that the mechanism which induces structural rearrangement at the level of dendritic spines after monocular deprivation is preserved in the acute slice preparation.

As with the in vivo analysis, the elevation of spine dynamics was not accompanied by a change in average spine length, neck diameter, or head diameter (Figure 2F). Interestingly, a direct comparison between spine morphologies in vivo and in the acute slice preparation showed significant differences; average spine length was shorter ($p < 0.01$) and head diameters were larger ($p < 0.001$) in vivo. Also, the prevalence of thin spines was nearly twice as high in the acute slice (11.5% of total in vivo, 21.6% of total in acute slice), and there was a dramatic increase in the number of filopodia (0.5% of total in vivo, 7.7% of total in acute slice). These results are consistent with the finding that spines are longer (Kirov et al., 2004) and that new spines (particularly thin spines) are created after slice preparation in the hippocampus (Kirov et al., 1999). Therefore, the population of spines imaged in the slice is likely to be slightly different from what is observed in vivo. However, since average morphological dimensions are not altered by brief deprivation in vivo or in the acute slice, the mechanisms that are activated by slice preparation are not likely to be masking a significant effect of deprivation on spine morphology. This finding again highlights the importance of examining the dynamic properties of dendritic spines in order to detect the earliest structural effects of monocular deprivation.

MD Alters Spine Dynamics in a Laminar Fashion in the Binocular Zone

Motivated by the finding that electrophysiological changes in supra- and infragranular layers precede those in layer IV (Trachtenberg et al., 2000), we further divided our data set into three separate compartments along the apical dendritic arbor (Figures 3A and 3B). These compartments were defined either as a distance from the soma or as a distance from the pial surface, though both methods gave similar groupings and identical results. With this compartmental analysis, the population of spines closest to the cortical surface, which are closest in laminar location to the population visualized in vivo, increased their motility $\sim$20% following deprivation as compared to nondeprived spines (control, 297 spines, 9 cells, 8 animals; deprived, 231 spines, 6 cells, 5 animals; $p < 0.0001$). Furthermore, an inspection of spines in other parts of the arbor revealed clear laminar differences in spine motility. Those spines located in the middle of the dendritic arbor, in layer IV, were not influenced by monocular deprivation (control, 42 spines, 2 cells, 2 animals; deprived, 237 spines, 5 cells, 5 animals; $p > 0.5$), while those spines in the deep infragranular region showed an elevation of motility following deprivation (control, 164 spines, 5 cells, 5 animals; deprived, 113 spines, 3 cells, 3 animals; $p < 0.001$). These results indicate that the distribution of synaptic contacts across the apical dendritic arbor is not homogenous and that spines on a single neuron, separated only by several hundred micrometers, can experience differential environments for structural plasticity. Further, since the altered dynamics are present in the extragranular layers, they are likely to contribute to the remodeling of horizontal connections in these regions (Callaway and Katz, 1990, 1991).

The mouse primary visual cortex is dominated by contralateral eye inputs and consists of a medial monocular zone, which receives exclusive input from the contralateral eye, and a lateral binocular zone, which receives inputs from both eyes (Antonini et al., 1999). All of the previously described changes in spine motility were obtained from spines in the binocular region. However, in order to clarify whether the laminar effects of monocular deprivation were due to reduced synaptic activity or were induced by competition between deprived and nondeprived inputs, we further examined spine motility in the monocular segment of V1 following short-term deprivation. Spine motility in the superficial part of the dendritic arbor, while elevated in the binocular zone,

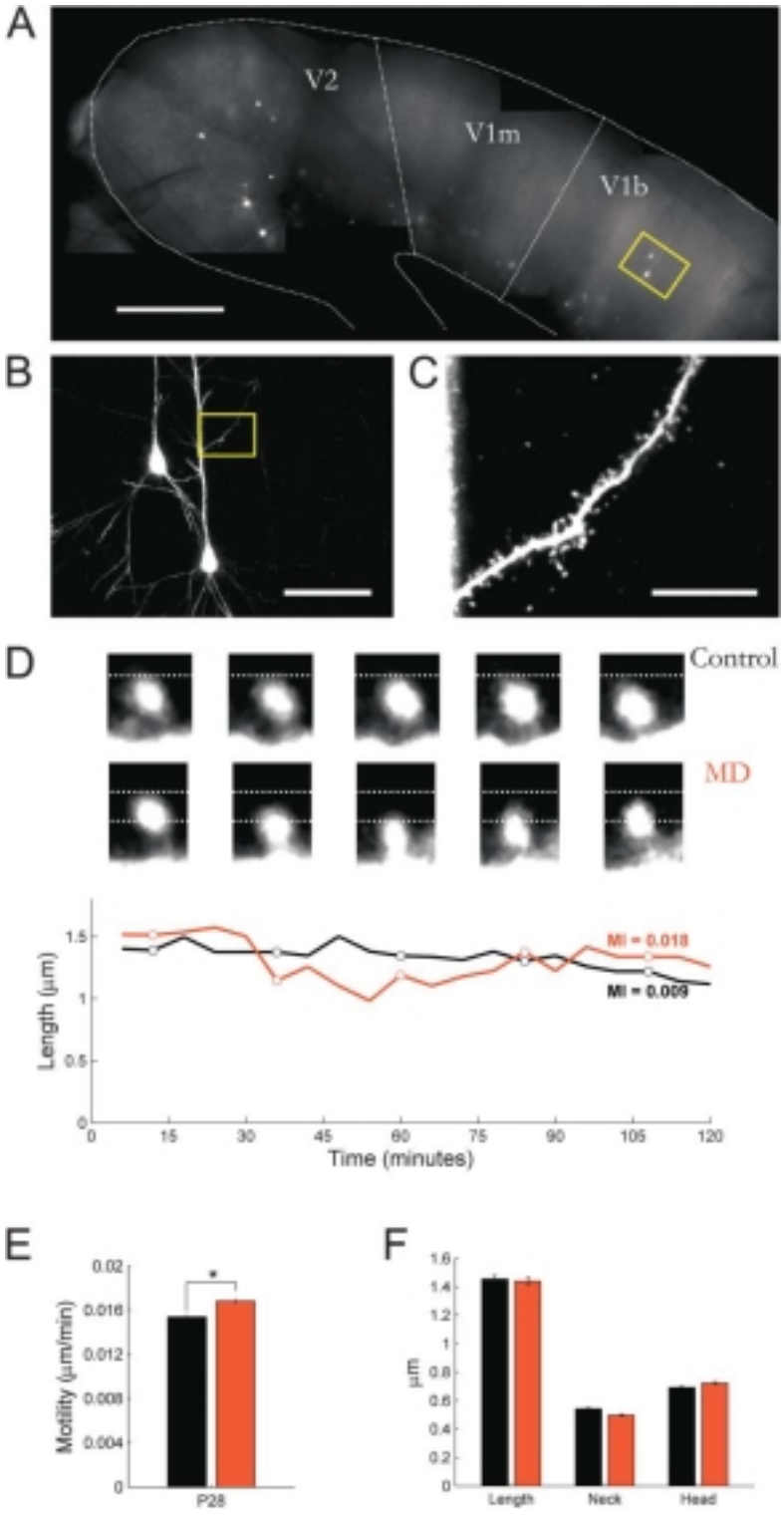

Figure 2. Spine Motility Is Elevated in Acute Slice following Short Monocular Deprivation

(A) GFP-expressing neurons are identified in the binocular portion of V1. Scale bar, 500 μm.

(B) The two neurons shown boxed in (A) are imaged at 60× with two-photon microscopy. Scale bar, 50 μm.

(C) A population of spines, proximal to the cell body, from the region shown in (B) are imaged every 6 min for 120 min. Scale bar, 10 μm.

(D) Two representative spines from control (black) and monocularly deprived (red) cortex are tracked over time. The images in the top panels correspond to the length measurements at the open circles in the bottom panel. The motility index for each spine is listed next to the relevant trace. In this example, the deprived spine is much more motile than the control spine.

(E) Considering the entire population of spines from across the dendritic arbor, spine motility is elevated following deprivation during the critical period in acute slice.

(F) There is no apparent change in average spine length, neck diameter, or head diameter. Black bars, spines from nondeprived cortex; red bars, spines from cortex contralateral to the deprived eye. *$p < 0.0001$.

was unaffected by deprivation in the monocular zone (Figure 3C, right; 97 spines, 3 cells, 3 animals; $p > 0.1$ in comparison to superficial binocular zone control). This finding provides evidence that the competition between deprived and nondeprived inputs, which is only present in the binocular segment of V1, is critical in creating a permissive local environment for structural reorganization.

ECM Degradation by tPA or Plasmin Alters Spine Dynamics

One way in which structural plasticity might be differentially regulated in different anatomical regions is by localized remodeling of the extracellular matrix (Pizzorusso et al., 2002). The extracellular matrix is composed of a multitude of molecules that directly interact with cell surface proteins and provide structural support (Dit-

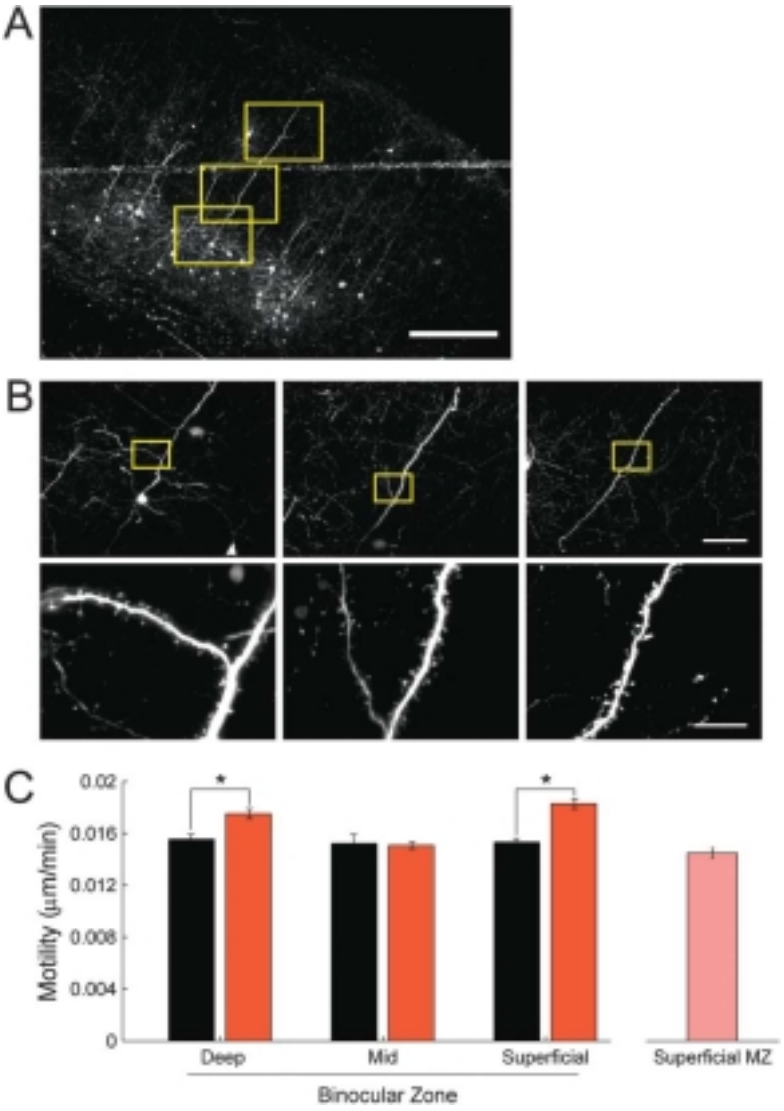

Figure 3. The Upregulation of Spine Motility after Monocular Deprivation Follows a Laminar Pattern

(A) A large population of cells expressing GFP can be seen at 10× magnification, many of them extending long apical dendritic arbors. Scale bar, 300 μm.

(B) The three boxed regions shown in (A) at 60× magnification, representing multiple dendritic compartments on a single layer V pyramidal neuron, progressing from regions close to the cell body (left panel), midway up the apical arbor (middle panel), and distal from the cell body (right panel). Below each 60× image is a high-magnification image of the population of spines from the boxed region in the upper panels. Scale bars, 50 μm for top panels and 10 μm for lower panels.

(C) Spine motility is elevated in the binocular region of V1 in superficial and deep compartments and is unaltered in the middle of the apical dendrite. Additionally, this effect is specific to the binocular zone, as spine motility in the superficial compartment in the monocular segment of V1 is unaltered by deprivation. Black bars, spines from nondeprived cortex; red or pink bars, spines from cortex contralateral to the deprived eye. *$p < 0.005$.

yatev and Schachner, 2003). tPA, which acts by cleaving extracellular plasminogen and converting it to enzymatically active plasmin, has been implicated in structural remodeling in peripheral nerve regeneration (Siconolfi and Seeds, 2001) and synaptic remodeling in LTP (Baranes et al., 1998), as well as fibrinolytic activity around sensory neuron growth cones (Krystosek and Seeds, 1981). tPA transcription (Qian et al., 1993) and secretion (Gualandris et al., 1996) occur in an activity-dependent manner through BDNF exposure (Fiumelli et al., 1999), and although protein levels do not appear to change during ocular dominance plasticity (Müller and Griesinger, 1998), enzyme activity is significantly increased with monocular deprivation (Mataga et al., 2002). Given that tPA may be important in both ocular dominance plasticity and tissue remodeling, we examined the effect of tPA and plasmin on spine motility. Spines from P28 animals were imaged in visual cortex slices before and after a 45 min period of enzyme application. Treatment with either exogenous plasmin or exogenous tPA (without exogenous plasminogen) significantly increased spine motility (Figure 4A; plasmin, 21% increase, 191 spines, 5 cells, 5 animals; $p < 0.0001$; Figure 4B, tPA, 17% increase, 94 spines, 3 cells, 3 animals, $p < 0.0001$). There was no apparent laminar specificity to this effect, as spines situated through all layers of the cortex were equally affected by tPA and plasmin. These results indicate that proteolysis through the tPA/plasmin pathway can either induce structural plasticity or provide a permissive environment in which spine dynamics can be altered.

MD Occludes Subsequent Effects of ECM Degradation in a Laminar Fashion

During ocular dominance plasticity, tPA exerts a critical role, as tPA knockout animals fail to enter a critical period (Mataga et al., 2002). To determine whether tPA/plasmin might be involved in the structural plasticity of dendritic spines during the critical period, we examined whether monocular deprivation would occlude a subsequent effect of exogenous tPA/plasmin. If a selective local secretion of tPA is responsible for the laminar upregulation of spine motility, then those spines in the middle parts of the apical arbor, corresponding to layer

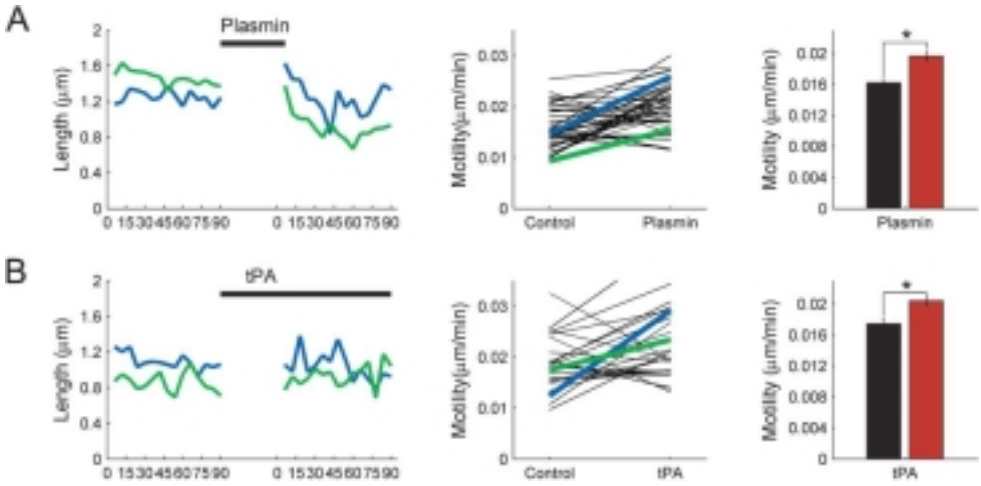

Figure 4. Spine Motility Is Upregulated by Degradation of the Extracellular Matrix

For each experimental condition, the blue and green traces in the left panels describe the change in length over time for two example spines before and after enzymatic degradation. Likewise, the middle panels show the motility index of a population of spines from a single experiment, including the spines from the left panel. The final column of panels shows the pooled effect of enzymatic degradation from all experiments. (A) Spine motility is significantly elevated after proteolytic degradation with plasmin. (B) Similarly, spine motility is significantly elevated after application of tPA with no exogenous plasminogen. Black bars, spines from nondeprived cortex; dark red bars, spines from nondeprived cortex following enzyme degradation. *$p < 0.0001$.

IV, would be predicted to receive the least endogenous tPA, while extragranular spines should receive the most endogenous tPA. Consistent with this hypothesis, those regions where spine motility is upregulated by monocular deprivation (e.g., superficial layers) were unaffected by additional plasmin application (Figure 5B; 88 spines, 2 cells, 2 animals; $p > 0.5$), suggesting that monocular deprivation occluded a further increase in spine dynamics via proteolysis of the extracellular matrix. However, in middle regions where spine motility is unchanged following deprivation, spine motility was significantly increased by enzymatic treatment with plasmin (Figure 5A; 60 spines, 2 cells, 2 animals, $p < 0.0001$). These results strongly suggest that the tPA/plasmin proteolytic cascade is active in vivo following brief monocular deprivation in the infra- and supragranular layers and provides a permissive extracellular environment in which spines are free to move, potentially as a prelude to changing their synaptic connectivity.

Discussion

Remodeling of the primary visual cortex during the critical period for ocular dominance plasticity is thought

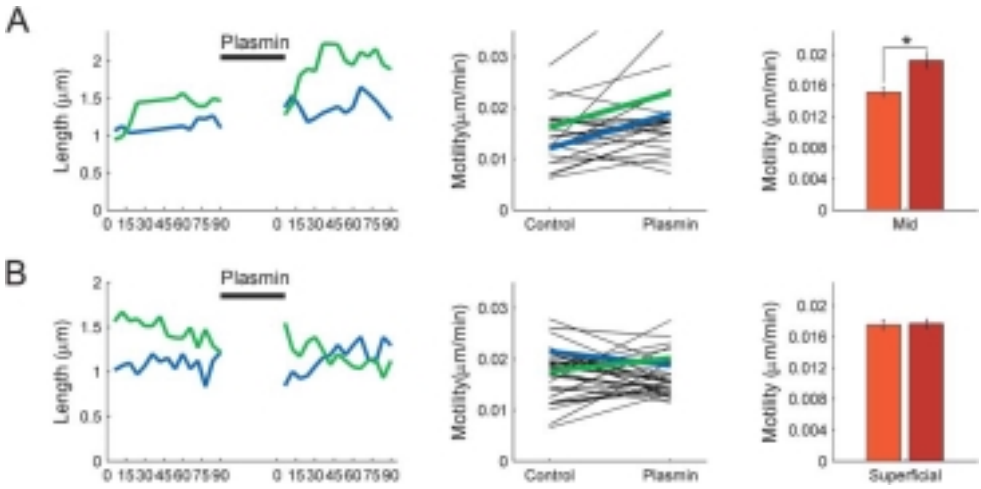

Figure 5. Monocular Deprivation Occludes Extracellular Matrix Degradation in a Laminar Fashion

The change in length of example spines, motility before and after extracellular matrix degradation, and pooled population results are as in Figure 4. (A) After monocular deprivation, plasmin significantly upregulates spine motility in the middle of the dendritic arbor. (B) However, in the superficial part of the dendritic arbor, where spine motility is already upregulated by monocular deprivation, further upregulation by plasmin is occluded. Red bars, spines from cortex contralateral to the deprived eye; dark red bars, spines from cortex contralateral to the deprived eye following enzyme application. *$p < 0.0001$.

to progress from functional alterations in the response properties of single neurons (Gordon and Stryker, 1996; Hubel and Wiesel, 1970; Trachtenberg et al., 2000) to large anatomical shifts in axonal arborization (Antonini et al., 1999; Antonini and Stryker, 1993; Shatz and Stryker, 1978). This idea is based on the relative timing of functional and anatomical events and presupposes that the thalamocortical projection to layer IV is the principal indicator of altered connectivity following ocular dominance plasticity. However, recent evidence suggests that both functional (Trachtenberg et al., 2000) and anatomical (Trachtenberg and Stryker, 2001) changes in extragranular layers may precede, and subsequently inform, the altered connectivity in layer IV. Consistent with this hypothesis, we find that the dynamic properties of dendritic spines are substantially altered in laminar regions outside layer IV following 2–3 days of monocular deprivation during the peak of the critical period. Spines imaged both in vivo and in vitro showed elevated dynamics during this period, potentially reflecting a series of events that destabilized both synapses and spine structure. Longer periods of deprivation, greater than 3 days, may be required to alter both the properties of spines in the middle region of the cortex and the pattern of thalamocortical connectivity (Antonini and Stryker, 1993).

Spines in Cortical Plasticity

Previous studies in other sensory regions as well as the visual cortex have found differing effects on spine dynamics. For example, in the somatosensory cortex, early sensory deprivation results in a reduction of protrusive motility (Lendvai et al., 2000). However, since this effect was seen at relatively early ages, it may be more closely related to inhibition of normal synaptogenesis than to critical period plasticity. Notably, a recent study of the visual cortex failed to observe any significant effect on spine motility with dark rearing or eye enucleation during the critical period for ocular dominance plasticity (Konur and Yuste, 2004). This study focused on the basal dendrites of layer II/III pyramidal neurons in order to examine spines postsynaptic to thalamic afferents. Based on the laminar effects of monocular deprivation reported here, motility would not be expected to be altered in a population of spines residing in the granular layer. However, it should be noted that the focus of our data has been on layer V pyramidal neurons, and the laminar effects that we have observed may not generalize to all cell types that have dendritic arborizations within layer IV.

Another discrepancy between our findings and the previous literature highlights the paucity of information regarding the specific mechanisms underlying different forms of plasticity in the visual system. For example, neither dark rearing nor binocular deprivation involves competition between deprived and nondeprived eye inputs, which is the hallmark of monocular deprivation. Previous data from our own laboratory suggest that several weeks of binocular deprivation have a strong effect on spine motility in superficial regions of the visual cortex (Majewska and Sur, 2003). A priori, this finding would predict that short-term monocular deprivation should affect both the monocular and binocular segments of V1 equally, as spine motility would be elevated regardless of competition. Instead, we find that brief periods of monocular deprivation are insufficient to change spine dynamics in the monocular segment, suggesting that competition is indeed critical for this structural plasticity. It is plausible that two competing sets of inputs can be functionally and structurally remodeled very quickly through mechanisms that prefer active inputs, while complete deprivation of all inputs might require longer timescales to activate the same mechanisms.

Synaptic Influences on Spines

Processes such as long-term synaptic depression, which can account for the rapid functional changes following monocular deprivation (Heynen et al., 2003), may also induce the translation from synaptic and functional modification to increased structural dynamics. Spines are likely to be influenced by persistent changes in synaptic efficacy as prolonged activation can induce the formation of new protrusions (Engert and Bonhoeffer, 1999; Maletic-Savatic et al., 1999) and accumulation of actin within dendritic spines (Fukazawa et al., 2003). Further, spines are stabilized by synaptic input (Fischer et al., 2000; McKinney et al., 1999), and significant loss of synaptic drive or long-lasting alteration of synaptic strength could lead to increased structural dynamics (Baranes et al., 1998; Majewska and Sur, 2003). Monocular deprivation is likely to destabilize spines that are initially part of the neural circuitry driven by inputs from the deprived eye as synapses attempt to maintain homeostatic levels of activity by reoptimizing their connection to presynaptic partners. This may also serve as a prelude to eventual synaptic loss and spine withdrawal (Figure 6) on a time course consistent with functional shifts in ocular dominance in the mouse visual cortex (Gordon and Stryker, 1996). The relationship between spine stability and spine loss has not been directly proven, though with monocular deprivation, significant spine loss occurs within 4 days (Mataga et al., 2004), suggesting that synapses that had initially served the deprived eye have likely either been lost or have altered their connections within this short period of time.

Mechanisms of Structural Remodeling at the Synapse

The mechanisms by which structural dynamics are controlled at the level of spines remain unknown. However, as spines are enriched in actin (Matus et al., 1982) and spine dynamics are regulated by the rate of actin polymerization (Fischer et al., 1998; Star et al., 2002), potential mechanisms for altering spine dynamics should modify the network of filamentous actin. Molecules that might directly fill this role are the Rho family of small GTPases (Bonhoeffer and Yuste, 2002; Hall, 1998), which can either promote or reduce spine number while altering spine morphology (Tashiro et al., 2000; Tashiro and Yuste, 2004). However, as important as intracellular cues may be for altering the properties of actin dynamics, properties of the extracellular space may also exert an influence on the structure of spines, either by binding to proteins expressed at the postsynaptic membrane or by forming physical barriers. Degradation of supportive molecules like fibrin, fibronectin, tenascin, and laminin by enzymes such as plasmin (Werb, 1997) may provide

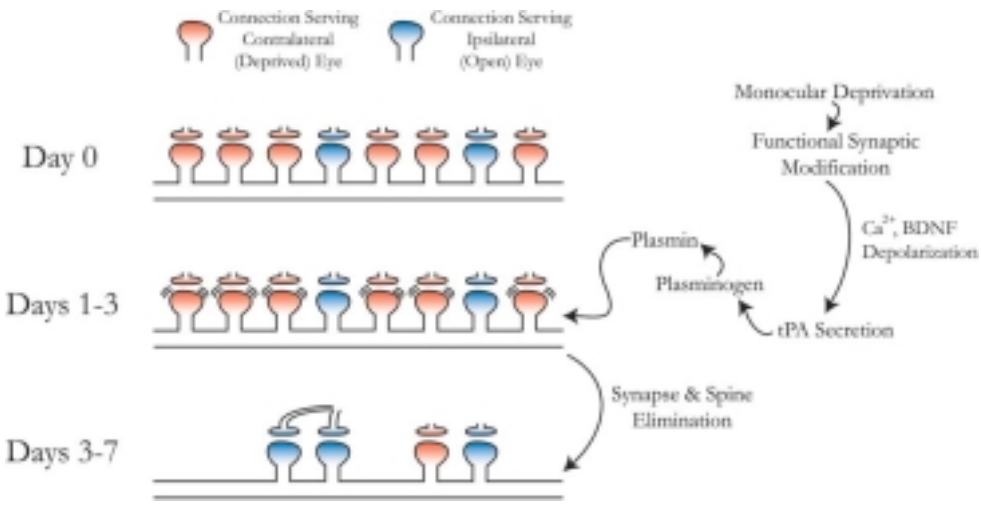

Figure 6. The Proposed Time Course of Functional and Structural Changes following Monocular Deprivation and Key Elements of Extracellular Matrix Remodeling

Monocular deprivation during the critical period induces tPA secretion and the conversion of extracellular plasminogen into plasmin. Plasmin then acts on a number of molecules in the extracellular matrix, allowing increased structural dynamics (schematically depicted with wavy lines around spines). This increase in structural dynamics then presumably facilitates a change in synaptic connectivity, such that spines receiving input from the deprived eye (shown in red) are either lost or converted, while those spines receiving input from the open eye (shown in blue) are maintained.

the physical flexibility required to alter existing neural structures. This has been dramatically demonstrated recently by the reactivation of ocular dominance plasticity in adult animals following enzymatic treatment to degrade extracellular chondroitin sulfate proteoglycans (Pizzorusso et al., 2002). This degradation, either by altering the inhibitory properties of perineuronal nets or by removing physical barriers, induced new functional and structural plasticity.

Similarly, we find that proteolysis of proteins in the extracellular matrix by tPA or plasmin can mimic the effect of ocular dominance plasticity by upregulating spine motility. This effect is also occluded by prior monocular deprivation as a function of the laminar location of the spines. tPA release into the extracellular space has previously been demonstrated in culture following depolarization (Gualandris et al., 1996) or exposure to BDNF (Fiumelli et al., 1999), and tPA mRNA is upregulated as an immediate early gene after seizure (Qian et al., 1993) or by induction of long-lasting potentiation (Baranes et al., 1998). This implies that the tPA/plasmin cascade, which is known to be involved in ocular dominance plasticity (Mataga et al., 2002; Müller and Griesinger, 1998), can respond to activity-dependent signals and may act on the extracellular matrix to provide a flexible and permissive environment for competitive structural plasticity during the critical period (Figure 6). Evidence from the lateral geniculate nucleus also suggests that the expression of extracellular matrix proteins is sensitive to binocular competition (Sur et al., 1988). In this way, activity of proteolytic enzymes is likely to lie downstream of the initial induction of plasticity and serve as an effector of structural rearrangement to reshape the connectivity of the primary visual cortex.

In conclusion, we have identified rapid structural plasticity at the level of individual synaptic connections following monocular deprivation. This plasticity occurs in a lamina-specific manner, proceeding in step with functional changes that are known to occur rapidly in the horizontal connections present in the superficial and deep layers of visual cortex. Additionally, deprivation-induced structural plasticity is limited to the binocular zone where competition between inputs from the two eyes occurs. We show that one component of this structural plasticity is likely to be mediated by enzymes such as tPA and plasmin, which locally degrade the extracellular matrix and generate a flexible and permissive environment for structural rearrangement.

Experimental Procedures

Monocular Deprivation

Mice (C57/Bl6) expressing GFP (strain GFP-M) or YFP (strain YFP-H) in a subset of their cortical neurons (principally layer V pyramidal neurons) (Feng et al., 2000) were anesthetized at postnatal days 26 or 40 and maintained in deep anesthesia with isoflurane. Monocular deprivation was performed by scoring the eyelids and then sealing them shut with tissue adhesive (Vetbond, 3M, St. Paul, MN). Mice were checked over the next 2–3 days to ensure that the eye remained closed. A total of 18 mice were used in the in vivo experiments (6 control, 4 deprived at P26; 4 control, 4 deprived at P40), and 27 mice were used in the slice experiments (15 control, 12 deprived).

Two-Photon Imaging

Mice were prepared for in vivo imaging and imaged as described previously (Majewska and Sur, 2003). Briefly, primary visual cortex was identified using stereotaxic coordinates, and either a small craniotomy was drilled in this area, or the skull was thinned without making a craniotomy. Imaging was performed with a custom-made two-photon microscope consisting of a Ti:S laser (Tsunami, Spectra-Physics, Mountain View, CA) pumped by a 10W solid state source (Millennia, Spectra-Physics) coupled to a modified Fluoview confocal scanhead (Olympus, Melville, NY). Superficial dendrites were initially identified with wide-field epifluorescence illumination and were subsequently imaged using a 60x N.A. (0.9) lens with digital zoom (5–10×). Volumetric z stacks were typically collected with an 800 × 600 acquisition window with 0.5–1 μm z steps every 5 min over 2 hr. In some animals, at the end of the imaging session,

injections of cholera toxin subunit B (CTB, List Biologic, Campbell, CA) coupled to Alexa Fluor 594 (Molecular Probes, Eugene, OR) were made adjacent to imaged areas in order to facilitate identification of imaged cells after fixation. Mice were transcardially perfused and fixed with paraformaldehyde, and coronal sections were cut to verify the location of imaged cells in layer V1.

Slice Preparation and Enzyme Application

Acute slices were prepared from P28–29 mice after deep anesthesia with sodium pentobarbitol (35 mg/kg, i.p.; Henry Schein Inc., Indianapolis, IN). The brain was removed and sectioned in cold (4°C) solution containing 1 mM NaH_2PO_4, 25 mM $NaHCO_3$, 3 mM KCl, 2 mM $MgS0_4$, 10 mM dextrose, 252 mM sucrose, 2.5 mM $CaCl_2$, and 5 mM kynurenic acid in a coronal plane with a thickness of 300 μm. After sectioning, slices were transferred to a holding chamber containing room temperature artificial cerebrospinal fluid (ACSF) containing 1 mM NaH_2PO_4, 25 mM $NaHCO_3$, 3 mM KCl, 2 mM $MgS0_4$, 10 mM dextrose, 126 mM NaCl, and 2.5 mM $CaCl_2$. Slices were allowed to equilibrate for 1 hr before transferring to the microscopy submersion chamber, which was continuously perfused with warm (35°C) ACSF. For enzymatic reactions, slices were either perfused with 0.5 μg/mL tPA (American Diagnostica, Stamford, CT) for 45 min or were removed from the submersion chamber and incubated with 0.2 U/mL plasmin (Sigma, St. Louis, MO) in a small volume of ACSF for 45 min before being returned to the microscopy submersion chamber. Images were collected with two-photon microscopy every 6 min for 1.5 hr before and 1.5 hr after enzyme treatment.

Image Analysis

Z stack images were exported to MATLAB (Mathworks, Natick, MA) and processed using custom algorithms. Spine motility was analyzed on two-dimensional projections in ImageJ (NIH, Bethesda, MD), where motility was defined as the average change in spine length per unit time (Lendvai et al., 2000; Majewska and Sur, 2003). Filopodia, distinguished by their morphology and dynamic properties, were excluded from all analyses. The minimum detectable motility was measured for both in vivo and acute slice preparations by making repeated length measurements from the same image or by making repeated length measurements from a series of interleaved images and then calculating the average change in spine length from these "artificial time series." From these measurements, the minimum detectable motility was 0.005 μm/min in vivo and 0.007 μm/min in slices in vitro. Although spines were motile in both preparations, spine turnover was rarely observed in single imaging sessions. All values are presented as the mean ± standard error of the mean. The number of observations for each experimental manipulation is listed as the number of spines, the number of cells, and the number of animals for each condition. All statistical analyses were performed using either a parametric t test for populations of monocularly deprived and control spines or a parametric paired t test for enzymatic treatments.

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