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Molecular mechanism for loss of visual cortical responsiveness following brief monocular deprivation

Arnold J Heynen[1,3], Bong-June Yoon[1,3], Cheng-Hang Liu[1], Hee J Chung[2], Richard L Huganir[2] & Mark F Bear[1]

A dramatic form of experience-dependent synaptic plasticity is revealed in visual cortex when one eye is temporarily deprived of vision during early postnatal life. Monocular deprivation (MD) alters synaptic transmission such that cortical neurons cease to respond to stimulation of the deprived eye, but how this occurs is poorly understood. Here we show in rat visual cortex that brief MD sets in motion the same molecular and functional changes as the experimental model of homosynaptic long-term depression (LTD), and that prior synaptic depression by MD occludes subsequent induction of LTD. The mechanisms of LTD, about which there is now a detailed understanding, therefore contribute to visual cortical plasticity.

For decades it has been appreciated that monocular deprivation during early postnatal life sets in motion a cascade of events in the primary visual cortex that culminates in severe visual impairment[1]. At the peak of the sensitive period, even 24 h of MD is sufficient to cause neurons in visual cortex to lose responsiveness to stimulation of the deprived eye[2]. This effect of MD is not merely a passive consequence of reduced neural activity. Rather, the residual 'noise' in the visually deprived retina actively stimulates the loss of synaptic efficacy[3]. The synaptic modifications induced by MD reflect processes that normally refine cortical receptive fields and visual capabilities during postnatal development. A major challenge has been to identify the mechanisms responsible for this type of visual cortical plasticity.

Inspired originally by a theory of visual cortical plasticity[4], research over the past 15 years has also established that in visual cortex and the CA1 region of hippocampus, long-term synaptic depression can be induced by activation of postsynaptic NMDA receptors (NMDARs)[5] and a protein phosphatase cascade[6,7]. This synaptic activity leads to a very specific pattern of altered AMPA receptor (AMPAR) phosphorylation, reduced cell-surface receptor expression, and depressed AMPAR-mediated transmission[8,9]. Precisely how these mechanisms contribute to experience-dependent synaptic modifications *in vivo*, however, has remained uncertain.

One way to connect the effects of MD and the mechanisms of LTD is to correlate deficits after genetic or pharmacological manipulations. However, correlation does not establish causality, and the absence of correlation can reflect degeneracy—the ability of elements that are structurally different to perform the same function or yield the same output—that is ubiquitous in biological systems[10]. Showing that the effects of MD survive deletion of one mechanism for LTD is obviously not grounds for rejecting the hypothesis that this mechanism normally provides a substrate for the effects of MD. An alternative approach is to ask the simple question: Does MD induce LTD in visual cortex? The aim of the current study was to exploit knowledge of the molecular mechanisms of LTD to address this question, focusing on alterations in AMPAR phosphorylation and surface expression.

The AMPAR phosphorylation sites that have been monitored during LTD are serine residues 831 and 845 on the GluR1 subunit, and Ser880 on the GluR2 subunit. Although Ser831 is selectively phosphorylated by calcium/calmodulin-dependent protein kinase II (CaMKII) during long-term synaptic potentiation (LTP)[11], it is unaltered during LTD[12,13]. In contrast, Ser845 and Ser880 are unaltered by LTP, but are selectively and persistently dephosphorylated and phosphorylated, respectively, during LTD[12–14]. Dephosphorylation of Ser845 can lead to synaptic depression, in part, by decreasing AMPAR channel open probability[15]. However, changes in phosphorylation also reflect a loss of AMPARs at the synapse. It has been established that AMPARs are rapidly internalized in response to LTD-inducing stimulation[16] and that internalized receptors are dephosphorylated at GluR1 Ser845[17]. Moreover, phosphorylation of GluR2 at Ser880 alters the relative affinity of the receptor for intracellular anchoring proteins[18], and current models suggest that this change either stimulates internalization or causes internalized AMPARs to be retained inside the neuron. Regardless of their specific roles, however, these unique changes in AMPAR phosphorylation and the concomitant changes in cell-surface expression constitute a molecular 'fingerprint' for the occurrence of NMDAR-dependent homosynaptic LTD.

Here we report that 24 h of MD during the sensitive period precisely mimics LTD with respect to AMPAR phosphorylation, and also significantly reduces the expression of AMPARs on the surface of visual cortical neurons. Moreover, the changes induced by MD occlude the subsequent expression of LTD at synapses *ex vivo*. Therefore, we conclude that MD induces LTD in visual cortex. A subset of these results was presented at the 2002 Society for Neuroscience meeting, and published in abstract form.

[1]Howard Hughes Medical Institute, The Picower Center for Learning and Memory, and Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA. [2]Howard Hughes Medical Institute and Department of Neuroscience, Johns Hopkins University Medical School, Baltimore, Maryland 21205, USA. [3]These authors contributed equally to this work. Correspondence should be addressed to M.F.B. (mbear@mit.edu).

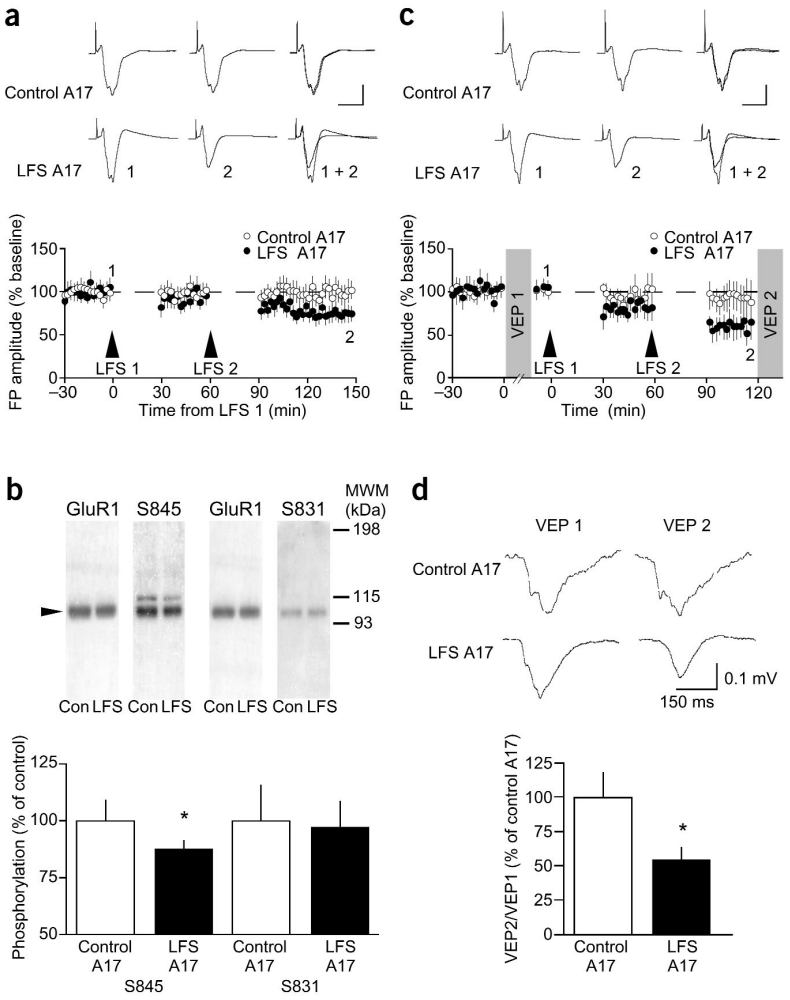

Figure 1 LTD *in vivo* is accompanied by a dephosphorylation of GluR1 at Ser845 and a decrease in visually evoked potentials. (**a**) Application of LFS to the LGN elicited LTD of field potentials in A17 *in vivo*. After a 30-min baseline recording period, application of two episodes of LFS (1,800 pulses, 1 Hz; arrows LFS1 and LFS2) to the LGN of one hemisphere produced LTD of the negative-going component of LGN-evoked field potentials (FP) recorded in A17 ($n = 10$, 77 ± 8% of baseline; $P < 0.01$, paired *t*-test). No change in FP amplitude was observed in contralateral control A17 that received only baseline stimulation (101 ± 10% of baseline; $P > 0.7$). Traces here and in **c** are taken from one representative animal in each group and are averages of ten consecutive FPs obtained at times indicated by numerals. Scale bars (**a**,**c**): 0.5 mV, 10 ms. (**b**) Immunoblots were made of membrane fractions from A17 ipsilateral and contralateral (Con) to the LGN receiving LFS using antibodies against phosphorylated Ser845 (S845) or Ser831 (S831) on GluR1. Blots were stripped and reprobed with an antibody recognizing the C-terminal end of GluR1. Extraneous bands on the S845 immunoblot are not related to GluR1[50]. The bar graph summarizes the immunoblot analysis: LFS-induced LTD was accompanied by a significant decrease in Ser845 phosphorylation in A17. Ser-845 phosphorylation in A17 undergoing LTD was 87 ± 4% of contralateral control A17 ($n = 10$; $P < 0.05$ versus 100 ± 9%), whereas no change in Ser831 phosphorylation was observed ($P > 0.6$, 97 ± 11% versus 100 ± 15%). (**c**,**d**) LTD induction *in vivo* depresses VEP amplitude. (**c**) LGN-evoked FPs are reduced in A17 ipsilateral to application of LFS to the LGN ($n = 5$, closed circles, 61 ± 18% of baseline; $P < 0.01$), whereas no change in FP amplitude is observed in contralateral control A17 (open circles; 95 ± 16% of baseline; $P > 0.6$). Shaded areas indicate periods when VEPs in response to grating stimuli were collected prior to (VEP 1) and following (VEP 2) induction of LTD. (**d**) Summary of VEP amplitude data for animals undergoing LTD (same animals as in **c**). In A17 in which LTD was induced, binocular VEPs were significantly depressed relative to contralateral control A17. Representative VEPs were obtained from one animal before (VEP1) and following (VEP2) induction of LTD.

RESULTS

LTD *in vivo* alters AMPAR phosphorylation and visual responses

NMDAR-dependent homosynaptic LTD was first described in hippocampal area CA1, where it can be induced *in vitro*[5] and *in vivo*[19] by prolonged presynaptic stimulation at 1 Hz. A biochemical requirement for LTD expression at CA1 synapses is dephosphorylation of postsynaptic protein kinase A (PKA) substrates[20]. Inhibition of postsynaptic PKA both mimics and occludes LTD, demonstrating that dephosphorylation of postsynaptic PKA substrates initially mediates LTD expression[21]. One PKA substrate is GluR1 Ser845. This site is highly phosphorylated under basal conditions, and undergoes rapid, specific and persistent dephosphorylation in response to LTD-inducing stimulation in the hippocampus *in vitro*[12,13]. Thus, dephosphorylation of Ser845 of GluR1 serves as a molecular marker for the occurrence of NMDAR-dependent LTD by reporting the status of postsynaptic PKA-dependent phosphorylation.

We felt it was important to confirm that LTD has the same biochemical signature in visual cortex *in vivo*. To this end, we established a preparation in which LTD could be elicited by 1-Hz stimulation of the dorsal lateral geniculate nucleus (LGN) of anesthetized rats at postnatal day (P) 21–25, an age when monocular deprivation also causes synaptic depression[22]. Concentric bipolar stimulating electrodes were placed bilaterally in the LGN, and recording electrodes were inserted into the visual cortex (Brodmann's area 17, A17) of each hemisphere at a depth yielding the maximum negative-going field potential in response to LGN stimulation, as described previously for adult rats[23].

After collecting stable baseline responses, the LGN of one hemisphere received 1-Hz stimulation; the other hemisphere served as a within-animal control. We found that reliable LTD in this preparation required two 30-min episodes of 1-Hz stimulation (**Fig. 1a**). One hour later, the animals were killed with an overdose of anesthetic, and the brains were removed. The visual cortex of each hemisphere was then probed for changes in GluR1 phosphorylation, using quantitative immunoblot analysis as previously described[12,13]. Important features of this and all other biochemical assays in this study were (i) that the experimenters performing the analyses were always blind to the stimulation history of the tissue, and (ii) that there was always a yoked, within-animal control sample run at the same time under identical conditions, allowing for pair-wise statistical comparisons. We found that 1-Hz stimulation of the LGN

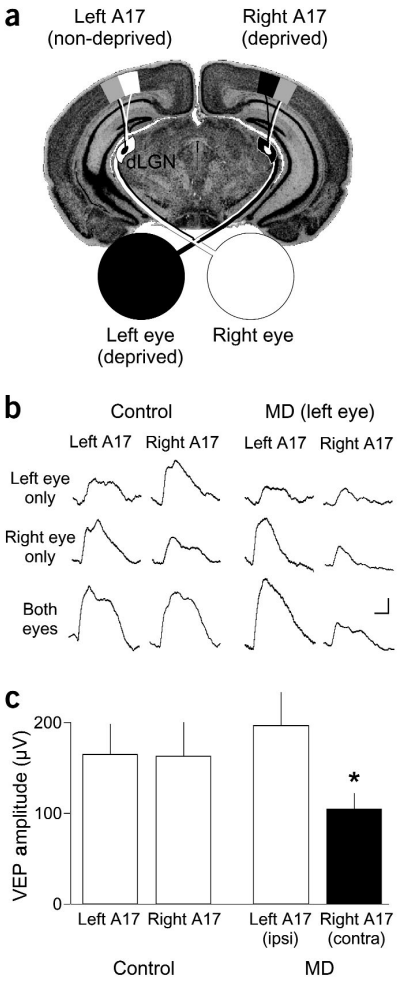

Figure 2 Brief MD leads to depression of synaptic transmission in A17 contralateral to the deprived eye (DE). (**a**) Schematic diagram of rat visual pathways illustrating the predominance of contralateral retinal input to A17. (**b**) Representative visually evoked potentials (VEPs) recorded simultaneously from recording electrodes on the dural surfaces of left and right A17 from control and MD animals (P21–25). Traces are averages of 100 consecutive stimulus presentations (scale bar: 50 μV, 100 ms). (**c**) Summary of VEP amplitude data for control and MD animals ($n = 7$, respectively). In control animals, there was no difference in VEP amplitude between the hemispheres (left A17, 165 ± 34 μV; right A17, 164 ± 37 μV; $P > 0.60$, Wilcoxon signed-ranks test). In rats that had been monocularly deprived for 24 h before recording, binocular VEPs contralateral to the deprived eye were significantly depressed (106 ± 17 μV, ∗) relative to control (198 ± 36 μV; $P < 0.02$, Wilcoxon signed-ranks test).

produces a modest but significant dephosphorylation of Ser845 (normalized to total GluR1) in the ipsilateral A17 compared to contralateral control A17 (**Fig. 1b**). In contrast, there was no significant change in phosphorylation of Ser831. Neither LTD nor dephosphorylation of Ser845 occurred after 1-Hz stimulation in animals treated with an NMDAR antagonist (data not shown).

The finding of modest but selective dephosphorylation of Ser845 in A17 *in vivo* mimics what has previously been reported for hippocampal slices following induction of LTD. To understand the meaning of a change of this magnitude, it is helpful to consider two points. First, bath application of NMDA to hippocampal slices, which induces LTD in the entire population (100%) of modifiable synapses, produces an approximately 50% dephosphorylation of GluR1 Ser845. Second, LTD induced in the same slice preparation by electrical stimulation of the Schaffer collaterals with a microelectrode produces a phosphorylation change of about 10%[12,13]. Therefore, it can be estimated that the electrical stimulation induces LTD in ~20% of the modifiable synapses in the hippocampal slice. Applying the same logic to A17, our data suggest that approximately 25% of the modifiable synapses may have undergone LTD as a consequence of 1-Hz stimulation of the LGN.

To gain some insight into the functional consequences of inducing LTD in the geniculocortical pathway, we performed an additional series of experiments in which field potentials evoked by high-contrast visual grating stimuli were monitored before and after conditioning stimulation of the LGN. This study revealed that the amplitude of the maximum negative-going visually evoked potential (VEP), which reflects a synaptic current sink in deep layer 3 of visual cortex[23,24], is also significantly depressed by induction of LTD (**Fig. 1c,d**).

Brief MD depresses VEPs contralateral to the deprived eye

Because of the significant decussation of the retinofugal projection in rodents, even the binocular regions of rat visual cortex are dominated by input from the contralateral eye[25] (**Fig. 2a**). Thus, in principle, MD should depress transmission in a substantially larger fraction of synapses in A17 contralateral to the deprived eye as compared with the ipsilateral cortex, which would enable within-animal comparisons of AMPAR changes associated with naturally occurring synaptic modifications.

To investigate the possibility of an interhemispheric asymmetry in the effects of MD in rats, we recorded VEPs from the surface of primary visual cortex in precisely matched locations in the two hemispheres. Recording positions were chosen to maximize the response from the ipsilateral eye, thus ensuring that we were sampling from the binocular region of A17. In control urethane-anesthetized rats (P21–25), we confirmed that the responses evoked by full-field pattern-reversing sinusoidal gratings are symmetrical in the two hemispheres when both eyes are open (**Fig. 2b**). When each eye was tested individually, the VEP through the contralateral eye was dominant, as expected (contralateral/ipsilateral (C/I) VEP ratio, 2.4 ± 0.3; $n = 5$).

Because of our interest in studying the early molecular events responsible for rapid ocular dominance (OD) plasticity, we chose to investigate the minimal period of MD that produces reliable changes in A17. We found that 24 h of MD in P21 rats was sufficient to produce a substantial OD shift (C/I VEP ratio contralateral to the deprived eye = 0.63 ± 0.04, $n = 6$; $P < 0.01$ compared to non-deprived controls, Mann-Whitney U-test). More importantly, we found that this period of MD was sufficient to depress responses to binocular stimulation in the hemisphere contralateral to the deprived eye as compared with the ipsilateral A17 (**Fig. 2c**). Although the binocular VEP ipsilateral to the deprived eye was comparable in magnitude to the VEP evoked in control animals, the response contralateral to the deprived eye was significantly reduced. Thus, 24 h of MD is sufficient to produce a net depression of synaptic transmission in A17 contralateral to the deprived eye. These data are also consistent with the conclusion that depression of deprived-eye responses occurs very rapidly following MD and precedes increases in open-eye responses[2,26].

Brief MD alters AMPAR phosphorylation in visual cortex

We took advantage of this interhemispheric asymmetry in the effect of MD to test the hypothesis that deprivation-induced synaptic depression, like LTD, is associated with specific changes in AMPAR phosphorylation. After 24 h of MD, the visual cortex of both hemi-

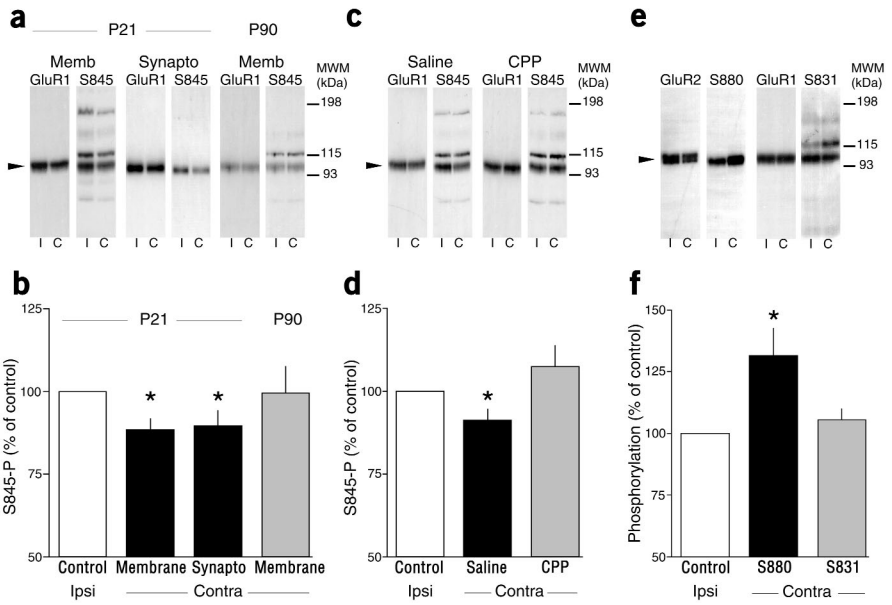

Figure 3 Brief MD alters AMPAR phosphorylation in visual cortex. (**a**) Representative immunoblots of membrane (Memb) and synaptoneurosome (Synapto) fractions from A17 contralateral (C) and ipsilateral (I) to the deprived eye using an antibody against phosphorylated Ser845 (S845; PKA site) on GluR1. Immunoblots were stripped and reprobed with an antibody recognizing the C-terminal end of GluR1. Extraneous bands on the S845 immunoblot are other PKA substrates not related to GluR1[50]. (**b**) Summary of immunoblot analysis showing that 24 h of MD produced a significant decrease in Ser845 phosphorylation in A17 contralateral to the deprived eye in juvenile (P21) but not adult (P90) animals. Asterisk in this and subsequent figures indicates significant ($P < 0.05$) decrease/increase as compared to A17 ipsilateral to the deprived eye (paired *t*-tests). Ser845 phosphorylation contralateral to the deprived eye was 88 ± 3% ($n = 18$; $P < 0.02$ versus 100 ± 15) and 90 ± 5% ($n = 18$; $P < 0.03$ versus 100 ± 7) of the ipsilateral A17 for Memb and Synapto preparations, respectively. (**c**) Representative immunoblots of membrane fractions from A17 of saline- and CPP-treated animals. (**d**) Summary of immunoblot analysis showing absence of an MD-associated decrease in Ser845 phosphorylation (107 ± 6% of ipsilateral (100 ± 7%) A17; $n = 8$) in animals treated during the time of deprivation with the NMDA receptor antagonist CPP. Saline-injected animals again showed a significant decrease (91 ± 3% of ipsilateral (100 ± 5%) A17; $n = 8$; $P < 0.05$). (**e**) Representative immunoblots of membrane fractions using antibodies against phosphorylated Ser880 on GluR2 and phosphorylated Ser831 on GluR1. Immunoblots were stripped and reprobed with an antibody recognizing the N terminus of GluR2 and C terminus of GluR1. Extraneous bands on the S831 immunoblot are not related to GluR1[50] and do not show consistent differences following MD. (**f**) Summary of immunoblot analysis: 24 h of MD produced a significant increase in Ser880 phosphorylation (132 ± 11% of ipsilateral (100 ± 7%) A17; $n = 30$; $P < 0.02$), whereas no change in Ser831 phosphorylation was observed (106 ± 4% of ipsilateral (100 ± 9%) A17; $n = 19$).

spheres was removed and prepared for quantitative immunoblot analysis. Using phosphorylation site-specific antibodies, the phosphorylation state of GluR1 and GluR2 in the cortex contralateral to the deprived eye was compared with the ipsilateral cortex.

We found that MD reliably induces dephosphorylation of Ser845 (normalized to total GluR1) in the visual cortex contralateral to the deprived eye (**Fig. 3a**,**b**). The magnitude of this change (10–12%) is comparable to that observed after induction of LTD in A17 and hippocampus with 1-Hz synaptic stimulation, suggesting that approximately 20% of the modifiable synapses may undergo LTD as a consequence of 24 h of MD. The observed decrease in VEP amplitude could be fully accounted for by this change, especially if the affected synapses are concentrated in the superficial layers (1–4), as is suggested by additional experiments presented below.

If the change in AMPAR phosphorylation contributes to the synaptic depression following MD, we expect this response to be absent under conditions where MD is without effect. Therefore, we investigated whether similar changes occur when MD is initiated in mature animals aged beyond the critical period for OD plasticity. Unlike the effect observed in young animals, MD did not induce any changes in Ser845 phosphorylation in P90 rats (**Fig. 3a**,**b**). Another condition in which MD does not trigger OD plasticity is when cortical NMDARs are blocked[27]. Therefore, we treated P21 animals with the NMDAR antagonist CPP (5.0 mg/kg) or with saline at 5-h intervals during the period of MD. Although saline-injected animals still showed significant dephosphorylation of Ser845 contralateral to the deprived eye after 24 h of MD, CPP-injected animals did not (**Fig. 3c**,**d**). Thus, the deprivation-induced dephosphorylation of GluR1, like the deprivation-induced synaptic depression, is restricted to a critical period of postnatal development and is dependent on activation of NMDARs.

As mentioned above, not all phosphorylation sites on AMPARs respond the same way during LTD. We therefore used antibodies against GluR1 phospho-Ser831 and GluR2 phospho-Ser880 to examine the selectivity of the change in AMPAR phosphorylation in visual cortex after 1 d of MD. This analysis showed a variable but statistically significant increase in Ser880 phosphorylation, and no change in Ser831 phosphorylation, following MD (**Fig. 3e**,**f**), similar to what occurs following induction of LTD.

Because immunoblot analyses of all three phosphorylation sites were typically performed on tissue from the same animals, the entire A17 of each hemisphere was used to obtain enough protein. However, this procedure left us vulnerable to the criticism that the true modifications in AMPARs might actually occur ipsilateral to the deprived eye, where they would be the opposite of what occurs during LTD. Although we considered this possibility to be exceedingly unlikely based on the interhemispheric asymmetry in VEPs after MD, we were nevertheless compelled to rule it out. The ideal within-animal control tissue is the monocular segment of visual cortex ipsilateral to the deprived eye, because it is still area 17, but MD has no effect on activity in this region of cortex (**Fig. 2a**). Therefore, we repeated the experiment, performing an analysis of GluR1 Ser845 phosphorylation in monocular and binocular segments, ipsilateral and contralateral to the deprived eye.

This analysis confirmed that there is no significant change in Ser845 phosphorylation in the binocular segment of A17 ipsilateral to the deprived eye and that Ser845 is significantly dephosphorylated in the binocular segment contralateral to the deprived eye (**Fig. 4a**,**b**). However, we also observed dephosphorylation of Ser845 in the monocular segment contralateral to the deprived eye. This finding suggests that brief deprivation induces synaptic depression in both monocular and binocular segments contralateral to the deprived eye. To address this possibility, we repeated the analysis of VEPs and discovered that significant deprived-eye response depres-

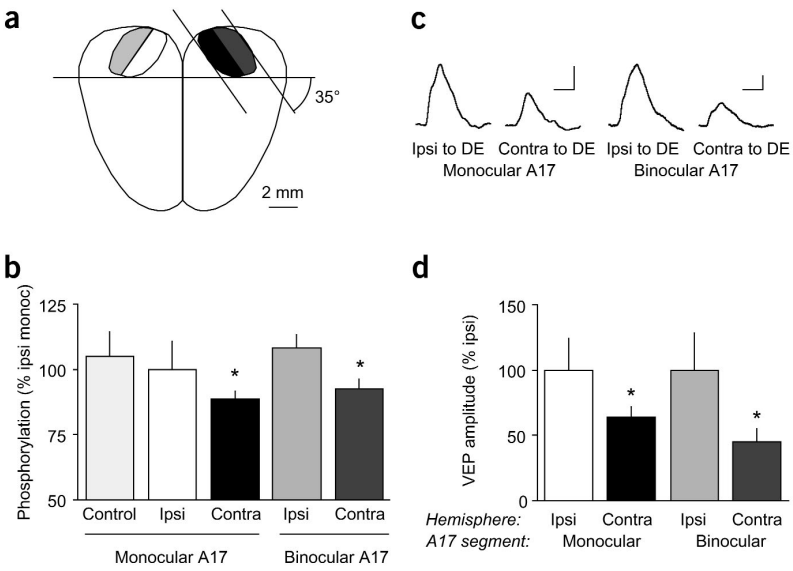

Figure 4 Brief MD produces synaptic depression and dephosphorylation of GluR1 at Ser845 in monocular and binocular segments of A17 contralateral to the deprived eye. (**a**) Dorsal view of a rat brain showing the monocular and binocular segments of A17 dissected for biochemical analysis. (**b**) Summary of immunoblot analysis showing that 24 h of MD produced a significant decrease in Ser845 phosphorylation in monocular and binocular regions of A17 contralateral to the deprived eye (Contra). Data are expressed as a percentage of monocular segment ipsilateral to the deprived eye (Ipsi), in which visual experience is unaffected by MD. Asterisks indicate significant decrease as compared to corresponding region of A17 ipsilateral to the deprived eye (paired *t*-test). Ser845 phosphorylation in A17 contralateral to the deprived eye was 89 ± 3% ($n = 28$; $P < 0.001$ versus 100 ± 11%) and 92 ± 4% ($n = 28$; $P < 0.002$ versus 100 ± 11%) of the ipsilateral A17 for monocular and binocular segments, respectively. Note that Ser845 phosphorylation in the monocular region ipsilateral to the deprived eye of MD animals did not differ from monocular segment of nondeprived control animals (105 ± 9%; $P > 0.6$, unpaired *t*-test). (**c**) Representative binocular VEPs recorded from electrodes on the dural surfaces of the monocular and binocular segments of A17 in the hemispheres ipsilateral and contralateral to the deprived eye of one animal. Traces are averages of 100 consecutive stimulus presentations (scale bars: monocular A17, 25 μV, 100 ms; binocular A17, 50 μV, 100 ms). (**d**) Summary of binocular VEP amplitude data for monocular and binocular segments of A17 (expressed as a percentage of values ipsilateral to the deprived eye, $n = 8$). In rats that had been monocularly deprived for 24 h before recording, VEPs contralateral to the deprived eye were significantly depressed in both monocular and binocular segments relative to the corresponding regions of A17 ipsilateral to the deprived eye (monocular A17: 29 ± 7 versus 46 ± 11 μV, $P < 0.02$; binocular A17: 67 ± 12 versus 191 ± 54 μV, $P < 0.02$; Wilcoxon signed-ranks tests).

sion also occurs in the monocular segment (**Fig. 4c,d**). This finding replicates with brief MD what has been observed previously in rats after longer periods of deprivation[25].

Brief MD alters AMPAR surface expression in visual cortex

There is considerable evidence that an expression mechanism for NMDAR-dependent LTD is the internalization of synaptic AMPARs, and internalized receptors show changes in phosphorylation that are similar to those observed following MD. We therefore hypothesized that MD triggers the loss of surface-expressed AMPARs in visual cortex. To test this idea, young rats were monocularly deprived for 1 d, and surface receptors were labeled with biotin in slices of visual cortex prepared from the hemispheres ipsilateral and contralateral to the deprived eye. To enrich for modified synapses in the superficial layers, the slices were bisected and the deep layers (5 and 6) were discarded. Biotinylated receptors were then precipitated and the ratio of surface to total AMPAR protein was determined by quantitative western blotting in each hemisphere. Control experiments showed that only surface proteins are biotinylated in the slice preparation (**Fig. 5a**).

This biochemical analysis (again performed 'blind') showed, remarkably, that the number of AMPARs on the surface of visual cortical neurons is significantly reduced, by approximately 20%, in A17 contralateral to the deprived eye in P21 rats (**Fig. 5b**). The magnitude of this change is comparable to that observed following induction of LTD in hippocampus *in vivo*[28]. Both the GluR1 and GluR2 subunits showed similar reductions (80.7 ± 6.4% and 81.5 ± 4.1% of ipsilateral controls; $n = 7$ rats), but the same analysis of $GABA_A$ receptor surface-expression revealed no significant difference. MD in adult (P90) rats failed to alter AMPAR surface expression (97.7 ± 5.8% and 93.1 ± 6.2% of ipsilateral controls for GluR1 and GluR2, respectively; $n = 7$ rats), showing that this response to MD, like synaptic depression, is restricted to a sensitive period of postnatal development (**Fig. 5c**).

Homosynaptic LTD, by definition, requires presynaptic activity (*i.e.*, the release of glutamate) for induction. Presynaptic activity is also known to be important for deprivation-induced synaptic depression in A17, as the consequences of brief monocular inactivation with tetrodotoxin (TTX) are much less severe than those of MD by lid closure[3]. We were therefore interested to compare the consequences of MD and monocular inactivation on AMPAR surface expression in rat visual cortex. Under anesthesia, animals received unilateral vitreal injections of saline (2 μl; $n = 7$) or TTX (0.4 mM, 2 μl; $n = 7$) and the lid over this eye was sutured closed. Twenty-four hours later, slices of A17 were prepared as described above. These experiments replicated the finding that MD induces a significant ~20% reduction in AMPAR surface expression (saline; **Fig. 5d**). However, in striking contrast, depriving the contralateral cortex of all retinally driven activity had no effect (TTX; **Fig. 5e**). Therefore, the decrease in AMPAR surface expression caused by MD is not merely a passive consequence of reduced input activity; rather it is caused by the uncorrelated noise that drives deprivation-induced synaptic depression.

Changes induced by brief MD occlude synaptic expression of LTD

Altered AMPAR phosphorylation and/or surface expression could contribute substantially to the reduced responsiveness of visual cortical neurons to deprived eye stimulation following MD, if these changes actually occur at synapses. The fact that phosphorylation changes are observed in synaptoneurosomes (**Fig. 3a,b**) provides some support for the conclusion that synaptic AMPARs are modified. Another way to address this question is to exploit the fact that synaptic expression of NMDAR-dependent LTD uses the same, saturable modifications in AMPARs. Thus, if MD partially exhausts the same expression mechanism as LTD, then we predict that LTD magnitude at saturation would be reduced in visual cortex that has already undergone deprivation-induced synaptic depression.

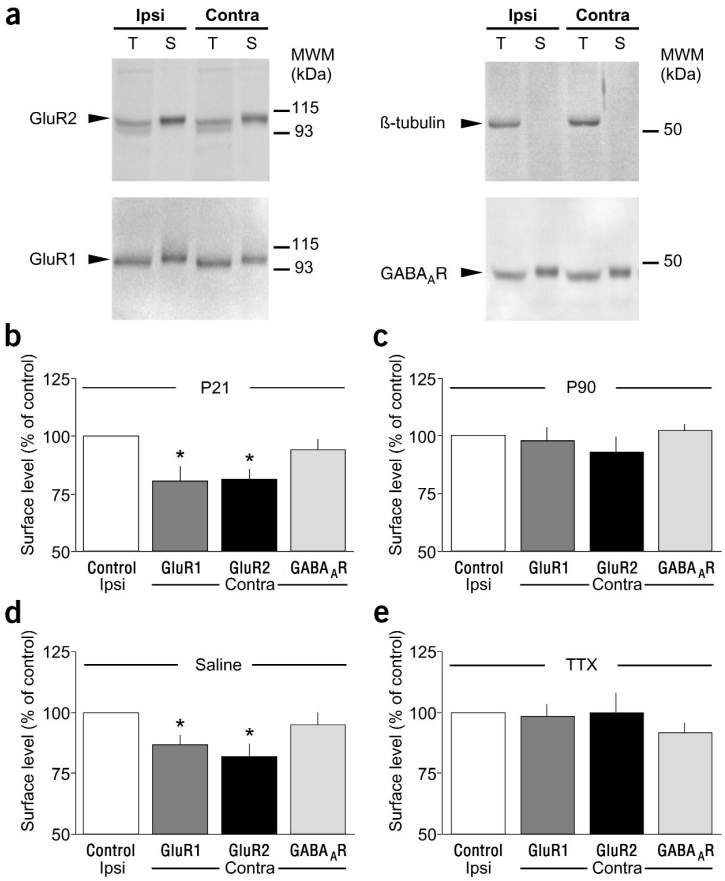

Figure 5 Brief MD is accompanied by a loss of surface-expressed AMPARs in juvenile animals. (**a**) Representative immunoblots of the biotinylated surface protein of A17 from P21 MD animals. Blots were probed with an antibody, stripped and reprobed with additional antibodies. Immunoblot with anti β-tubulin antibody shows that biotin specifically labeled cell surface proteins (Ipsi, ipsilateral hemisphere to the deprived eye; Contra, contralateral hemisphere to the deprived eye; T, total cell homogenate; S, biotinylated surface protein). (**b**) Summary of surface protein biotinylation assays of P21 MD animals ($n = 7$). Surface levels of both GluR1 ($80.7 \pm 6.4\%$, $P < 0.05$, *t*-test) and GluR2 ($81.5 \pm 4.1\%$, $P < 0.01$, *t*-test) were significantly lower in the visual cortex contralateral to the deprived eye. However, there was no significant difference in the surface level of $GABA_A$ receptor α1 subunit. (**c**) Summary of surface protein biotinylation assays of P90 MD animals ($n = 7$). In contrast to juvenile animals, no significant change in surface levels of both GluR1 ($97.7 \pm 5.8\%$, $P > 0.90$, *t*-test) and GluR2 ($93.1 \pm 6.2\%$, $P > 0.30$, *t*-test) were observed in the visual cortex contralateral to the deprived eye of adult animals. Similarly, no significant difference in the surface level of $GABA_A$ receptor α1 subunit was observed. (**d**) Summary of surface protein biotinylation assays of animals in which the deprived eye was injected with saline prior to lid closure ($n = 7$). Both GluR1 ($86.6 \pm 4.1\%$, $P < 0.035$, *t*-test) and GluR2 ($81.8 \pm 5.3\%$, $P < 0.019$, *t*-test) showed the similar loss of surface level as shown in **c**. (**e**) Summary of surface protein biotinylation assays of animals in which the deprived eye was injected with TTX prior to lid closure ($n = 7$). TTX injection prevented the loss of surface protein of both GluR1 ($98.4 \pm 4.8\%$) and GluR2 ($99.8 \pm 8.4\%$) normally caused by MD from lid closure.

We tested this prediction by preparing slices of A17 after 1 d of MD and comparing LTD ipsilateral and contralateral to the deprived eye. Synaptic field potentials were recorded from layer 3 in response to layer-4 stimulation, as described previously[7]. Three episodes of LFS (1 Hz, 900 pulses) spaced at 25-min intervals were used to saturate LTD. There was a striking difference in LTD magnitude at saturation in the two hemispheres (**Fig. 6**). Consistent with our prediction, LTD was diminished in the cortex contralateral to the deprived eye (ipsilateral A17, $66.4 \pm 5.0\%$ of baseline; contralateral A17, $81.4 \pm 4.0\%$ of baseline). Repeating the experiment in non-deprived animals confirmed that there is normally no interhemispheric asymmetry in LTD, and further, that the magnitude of LTD ipsilateral to the deprived eye is not different from controls (left A17, $68.8 \pm 10.3\%$ of baseline; right A17, $67.3 \pm 7.4\%$ of baseline). Together, the data strongly support the conclusion that prior MD, by triggering internalization of AMPARs, partially occluded LTD in A17 contralateral to the deprived eye. We note that this effect of MD on LTD is observed in layer 3, supporting the notion that the superficial layers are a site for early OD plasticity[29,30].

Time course of MD-induced changes in AMPAR phosphorylation

In a final series of experiments, we tracked the time course of changes in AMPAR phosphorylation following MD. The trends of decreased GluR1 phosphorylation at Ser845 and increased GluR2 phosphorylation at Ser880 were already apparent after 6 h of MD, but disappeared after 2 d of MD (**Fig. 7a,b**). This transient change in receptor phosphorylation following MD was expected, as receptors dephosphorylated at GluR1 Ser845 are degraded[17]. It was also expected that the markers for net LTD in A17 contralateral to the deprived eye would disappear with time as the ipsilateral inputs grow in strength. Nonetheless, at a minimum the data suggest that the mechanisms of LTD contribute to the rapid loss of visual responsiveness that occurs during the first 2 d of MD.

With long periods of MD, there are well-documented changes in axonal arbors in visual cortex[1], but these changes may lack the speed necessary to account for rapid OD plasticity[31]. Therefore, the mechanisms of LTD can bridge the early functional consequences of MD with the later anatomical modifications. However, we further suggest that the mechanisms of LTD—particularly the loss of postsynaptic glutamate receptors—actually serve to trigger the anatomical loss of visually deprived connections[32]. This hypothesis is supported by work performed on synapse elimination at the neuromuscular junction, where it has been shown that the loss of receptors postsynaptically precedes and triggers the withdrawal of the presynaptic axon[33].

DISCUSSION

There are well established criteria that must be met to conclude that two manipulations induce plasticity by the same mechanism[21]. The first is mimicry. Here we show that the effects of MD in the contralateral visual cortex (relative to the ipsilateral cortex) mimic LTD with respect to (i) depressed synaptic transmission, (ii) decreased phosphorylation of GluR1 Ser845, (iii) increased phosphorylation of GluR2

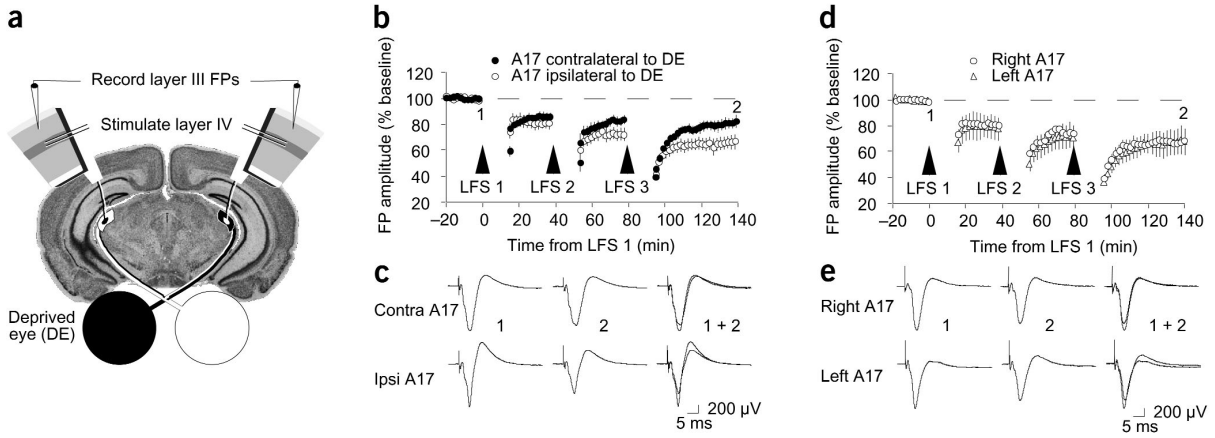

Figure 6 Prior monocular deprivation occludes LFS-induced decreases in synaptic strength. (**a**) Slices of A17 were prepared following 24 h of MD *in vivo*. Layer-3 field potentials were recorded in response to layer-4 stimulation, and LTD was induced with multiple episodes of LFS. (**b**) Summary of the effects of MD on the saturation level of LTD in A17 contralateral ($n = 7$ slices from 6 rats) and ipsilateral ($n = 7$ slices from 5 rats) to the deprived eye. For statistical analysis, the magnitudes of the field potentials obtained during the last 5 min of the baseline recording period and the final 5 min following each LFS period were compared. A two-way repeated measures ANOVA showed a significant difference between hemispheres ($F_{1,36} = 3.29$; $P < 0.03$) of MD animals. Subsequent Student-Newman-Keuls *post hoc* tests showed that the magnitude of LTD contralateral to the deprived eye was saturated after the first LFS episode, as no significant increase in LTD magnitude was observed after the second and third LFS periods. In contrast, in the ipsilateral A17, a significant increase in LTD magnitude was still observed following the third LFS episode ($P < 0.05$). (**c**) Representative layer 2/3 field potentials obtained from A17 contralateral (Contra A17) and ipsilateral (Ipsi A17) to the eye of deprivation. Traces are averages of ten consecutive field potentials obtained at times indicated by numerals in **a**. (**d**) Summary of LTD saturation levels in A17 of the right ($n = 4$ slices from 4 rats) and left ($n = 4$ slices from 4 rats) hemispheres of control animals. No significant difference in the magnitude of LTD was observed between left and right A17 (ANOVA; $P > 0.9$). (**e**) Representative layer 2/3 field potentials obtained from A17 of the right and left hemispheres of control animals.

Ser880, (iv) unaltered phosphorylation of GluR1 Ser831, (v) decreased surface expression of GluR1, (vi) decreased surface expression of GluR2, (vii) unaltered surface expression of $GABA_A$ receptor protein, (viii) sensitivity to NMDAR blockade and (ix) dependence on presynaptic activity. The second criterion is occlusion. Here we show that synaptic depression induced by MD *in vivo* reduces the level of synaptic depression that can be attained by the mechanism of LTD *in vitro*. We therefore conclude that MD induces LTD in visual cortex. We hasten to add that we are not suggesting that LTD is the *only* mechanism for initiating OD plasticity. For example, there is evidence for NMDAR-independent mechanisms of response depression[27], and responses driven by the non-deprived eye eventually potentiate[2,26]. However, a possible contribution of other mechanisms does not diminish the significance of the present findings. There is now a very detailed understanding of the molecular basis for NMDAR-dependent LTD. We can therefore reconstruct, for the first time, a molecular chain of events that is set in motion by MD in visual cortex.

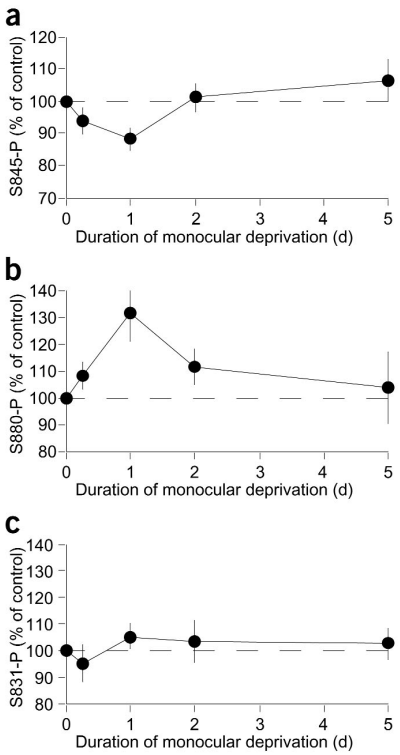

Figure 7 Time course of changes in AMPAR phosphorylation following monocular deprivation. Changes in phosphorylation at Ser845 of GluR1 (**a**) and Ser880 (**b**) of GluR2 were apparent after 6 h of MD, but disappeared after two days of MD. No significant change in phosphorylation of Ser831 on GluR1 was observed at any time point (**c**).

The data support a model in which the activity in the deprived retina, relayed to the visual cortex by the lateral geniculate nucleus, weakly activates postsynaptic NMDARs. The activation of NMDARs is 'weak' because it rarely correlates with responses evoked by visual stimulation of the open eye. Activated NMDARs admit Ca^{2+} ions into the postsynaptic neuron that, in turn, regulate a network of protein phosphatases and kinases. Among the consequences of the modest rise in intracellular calcium is dephosphorylation of postsynaptic PKA substrates, including Ser845 of the AMPAR GluR1 subunit, and the phosphorylation of Ser880 on the GluR2 subunit. These changes in phosphorylation alter the binding of intracellular scaffolding proteins, leading to net endocytosis, retention, and eventual degradation of synaptic glutamate receptors. Consequently, the deprived eye no longer effectively drives synaptic excitation in the visual cortex.

The biochemical and functional changes caused by brief MD are homosynaptic—they are driven by activity generated in the deprived retina—and are expressed initially in both the monocular and binocular segments of visual cortex. It should be noted that these findings are entirely compatible with the notion of 'binocular competition'[34] if it is assumed that the activity of the non-deprived eye influences the potential for subsequent recovery of deprived-eye responses via regulation of the LTD/LTP modification threshold[35–37], for which there is now ample evidence[38–40]. Regardless, even under conditions where cortical neurons are not under the influence of a competing input (*e.g.*, binocular lid closure), it is well established that deprivation induces a rapid and profound visual impairment in both cats and rodents[2,41,42].

The homosynaptic model for deprivation-induced synaptic depression provides a new framework to address several fundamental unanswered questions about experience-dependent cortical development; for example, how OD plasticity is regulated by age. We show here that MD is no longer sufficient to dephosphorylate or internalize AMPARs in adults. One possibility is that the expression of key signaling molecules is altered during development, rendering synapses incapable of supporting LTD[43]. A second possibility is that the retinal activity required to drive the NMDAR-dependent process of LTD fails to reach the modifiable synapses in A17 as a consequence of the late maturation of cortical inhibition[44]. We note in this context that the developmental decline in OD plasticity is accelerated in genetically modified mice displaying a precocious maturation of inhibition[45]. The model also accounts for the seemingly contradictory finding that global reductions in cortical inhibition can reduce OD plasticity[46,47]. Under conditions of heightened cortical excitability, activity from the deprived eye is more likely to correlate with evoked postsynaptic responses, and thus fails to satisfy the conditions necessary to trigger LTD (*i.e.*, 'weak' NMDAR activation). The model also suggests novel explanations for how OD plasticity is modulated by behavioral state (LTD is powerfully modulated by acetylcholine and norepinephrine[48]).

Studies of LTP and LTD have revealed much about the mechanisms of bidirectional synaptic plasticity; yet the questions of how, when, and where these mechanisms contribute to brain function remain largely unanswered. We have now shown that brief MD sets in motion the same molecular and electrophysiological changes as LTD, and that prior synaptic depression by MD occludes subsequent induction of LTD in visual cortex. Thus, the mechanisms of LTD contribute to the rapid loss of visual responsiveness after brief MD. LTD also appears to participate in the loss of responses in somatosensory cortex following whisker deprivation[49]. It therefore seems likely that these same mechanisms also contribute to the processes that refine cortical connections during early postnatal development.

METHODS

Animals. Juvenile (P21–25) and adult (P90 or older) Long Evans black-hooded rats (Charles River Labs) were used. Animals were group-housed, with food and water available *ad libitum* and maintained on a 12:12 h light:dark cycle. All protocols were approved by the Brown University Institutional Animal Care and Use Committee.

LTD of geniculocortical transmission *in vivo*. Electrophysiological recordings of LGN-evoked field potentials were performed in urethane-anesthetized animals. Briefly, monopolar recording electrodes were positioned bilaterally in the supragranular cell layers of A17, and concentric bipolar stimulating electrodes were positioned bilaterally in the LGN. Final positions of the stimulating and recording electrodes were adjusted to maximize the amplitude of the field potential recorded in A17 in response to pulsed electrical stimulation of the LGN. Field potentials were elicited using square-wave pulses of 0.2 ms duration and 0.2–0.5 mA intensity. Evoked responses were amplified, filtered (0.1 kHz and 3.0 kHz), digitized (160 kHz), then stored and analyzed using Experimenter's Workbench. Before each experiment, a full input-output series was performed and a stimulation intensity yielding a field potential amplitude 50–60% of maximum was used for the remainder of the experiment. Baseline responses were collected every 30 s for 30 min before application of low-frequency stimulation (LFS; 1,800 pulses at 1 Hz). Electrophysiological data are expressed as a percentage of the mean response magnitude recorded during the baseline recording period.

Monocular deprivation and VEP recordings. MD was performed by eyelid suture between the hours of 9:00 and 11:00 in the morning. For VEP recordings, rats were anesthetized with urethane (2 g/kg, i.p.) and positioned in a modified stereotaxic frame which allowed for unobstructed viewing of visual stimuli. Monopolar recordings from the dural surface of A17 were made relative to a ground screw inserted into the bone overlying the frontal cortex. Maximum amplitude VEPs were obtained with the recording electrodes positioned >7.0 mm posterior to Bregma and ≥4.0 mm lateral to the midline, corresponding to the binocular region of A17 in the rat. Visual stimuli consisted of full-field, horizontally oriented sinusoidal gratings (0.05 c.p.d.) presented on a computer monitor placed 16 cm from the eyes, in a darkened room. Mean luminance of the monitor was 28 cd/m^2. VEPs were amplified, filtered (0.1 kHz and 1.0 kHz), digitized at 20 kHz, and averaged (>500 events per block) in synchrony with stimulus contrast reversal using a PC. Transient VEPs in response to abrupt contrast reversing stimuli (1 Hz) were analyzed by measuring the amplitude of the positive peak of the major response component.

Immunoblot analyses. Membrane fractions (or, in some cases, synaptoneurosomes) of A17 were prepared and immunoblotted as previously described[12,13,28]. The signals were quantified using NIH Image 1.62 software or ImageQuant software (Molecular Dynamics). Relative amounts of AMPAR phosphorylation were ascertained by determining the ratio (P/G) of the signal detected using the phosphorylation-specific antibody (P) and the phosphorylation-independent antibody (G). All analyses were performed blind. The P/G ratios from the experimental hemispheres (ipsilateral to LFS of the LGN, or contralateral to the deprived eye) were compared with the values in the yoked control hemispheres using paired two-tailed *t*-tests. For display purposes, changes in AMPAR phosphorylation levels for experimental A17 are expressed as a percentage of control A17, according to previously established conventions[13]. We confirmed that absolute GluR1 levels do not differ across hemispheres after 24 h MD (GluR1 normalized to total protein contralateral to the deprived eye, 103 ± 7% of ipsilateral value; $n = 7$).

Cortical slice biotinylation. With the experimenter blind to the deprivation history of the rat, A17 from both hemispheres were dissected into ice-cold dissection buffer containing 2 μM jasplakinolide (Molecular Probes) and sectioned simultaneously into 400-μm-thick slices using a vibratome. Slices were incubated in ice-cold, oxygenated artificial cerebrospinal fluid (ACSF) containing 2 μM jasplakinolide for 30–60 min, and then incubated in 1 mg/ml biotin (EZ-link, sulfo-NHS-SS-Biotin, Pierce) solution in ACSF on ice for 20 min. After three washes with ACSF, the superficial layers were microdissected, homogenized in RIPA buffer containing 0.5% SDS, and the protein concentration of the RIPA soluble fraction determined. Samples were mixed with NeutrAvidin (Pierce) and incubated at 4 °C overnight. Biotinylated protein-avidin complex was then spun down and washed 3× with RIPA before resuspension in SDS sample buffer. Samples of total protein and the pulled-down fraction were loaded side by side on 7.5% SDS gels. The average values from triplicate runs of total protein and surface protein were used to determine the relative level of surface protein. The surface/total ratio of the hemisphere contralateral to the deprived eye was then normalized to the ratio of the ipsilateral hemisphere to obtain a percentage of control value. It should be noted that it is not necessary for this analysis to assume that biotin fully penetrates the slice. Broken membranes do not contribute significantly to the signal because the intracellular protein β-tubulin was not biotinylated. We established the validity of this approach by showing that induction of LTD by brief bath application of NMDA[13] in visual cortical slices decreases surface expres-

sion of GluR1 to approximately 56.5 ± 14.3% of control (n = 5), consistent with the well-established finding that AMPARs are internalized during LTD.

Cortical slice recordings. Animals that had been monocularly deprived for 24 h were anesthetized with Beuthanasia-D (Schering-Plough), and their brains were removed and dissected in ice-cold dissection buffer containing 212.7 mM sucrose, 2.6 mM KCl, 1.23 mM NaH_2PO_4, 26 mM $NaHCO_3$, 3 mM $MgCl_2$, 1 mM $CaCl_2$ and 10 mM dextrose. Slices of 400 μm thickness containing the primary visual cortices were isolated and allowed to recover for 1–2 h at 31 °C in ACSF containing 124 mM NaCl, 5 mM KCl, 1.23 mM NaH_2PO_4, 26 mM $NaHCO_3$, 1 mM $MgCl_2$, 2 mM $CaCl_2$ and 10 mM dextrose. ACSF and dissection buffer were saturated with 95% O_2/5% CO_2. Recordings were performed in a submersion chamber with the experimenter blind to the hemisphere from which slices were obtained. Extracellular recording electrodes were placed in layer 2/3 of the binocular region of A17 to monitor field potentials evoked by stimulating electrodes placed at the border of layer 4 and upper layer 5, as described previously[7]. In every experiment, a full input-output curve was generated, and baseline responses were obtained every 30 s with a stimulation intensity that yielded a half-maximal response. To induce LTD, three episodes of low-frequency stimulation (LFS) consisting of 900 pulses at 1 Hz were applied, each separated by a 25-min interval. Baseline recordings continued for ≥45 min after the conclusion of the final LFS period.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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