Neuron, Vol. 44, 917–923, December 16, 2004, Copyright ©2004 by Cell Press

How Monocular Deprivation Shifts Ocular Dominance in Visual Cortex of Young Mice

Report

Mikhail Y. Frenkel and Mark F. Bear*
Howard Hughes Medical Institute
The Picower Center for Learning and Memory
Department of Brain and Cognitive Sciences
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Summary

We used a chronic recording method to document the kinetics of ocular dominance (OD) plasticity induced by temporary lid closure in young mice. We find that monocular deprivation (MD) induces two separate modifications: (1) rapid, deprivation-induced response depression and (2) delayed, deprivation-enabled, experience-dependent response potentiation. To gain insight into how altering retinal activity triggers these cortical responses, we compared the effects of MD by lid closure with monocular inactivation (MI) by intravitreal injection of tetrodotoxin. We find that MI fails to induce deprived-eye response depression but promotes potentiation of responses driven by the normal eye. These effects of MI in juvenile mice closely resemble the effects of MD in adult mice. Understanding how MI and MD differentially affect activity in the visual system of young mice may provide key insight into how the critical period ends.

Introduction

A classic example of experience-dependent cortical plasticity is the shift in ocular dominance that occurs in visual cortex when one eyelid is temporarily closed during a postnatal critical period (Wiesel and Hubel, 1963). OD plasticity is usually detected as a persistent shift in the relative responsiveness of the visual cortex to stimulation of the two eyes. The ocular response ratio obviously can be altered by modification of the numerator (deprived-eye response), the denominator (nondeprived-eye response), or both; yet little is known about the absolute changes in visual response that underlie the ocular dominance shift—information that is vital to understanding the mechanism(s).

To address this question, we established a method of recording visual responses chronically from mouse visual cortex. Although the pioneering studies of visual cortical plasticity were performed using kittens and primates, mice have proven to be valuable models for mechanistic investigations. Mice exhibit robust OD plasticity (Drager, 1978; Gordon and Stryker, 1996) and offer several advantages over traditional species. First, it is feasible in mice to perform coordinated molecular, biochemical, electrophysiological, and behavioral analyses. Second, mice can be manipulated genetically to test mechanistic hypotheses in vivo. Third, mouse visual cortex is relatively undifferentiated (e.g., compared to mouse somatosensory cortex or monkey visual cortex), suggesting that findings here may reflect general principles of cortical plasticity that might apply across species and cortical areas. The relative simplicity of visual cortical organization of mice (lacking, for example, ocular dominance columns and other anisotropy) also makes it well-suited for chronic recording methods.

In a previous study using this method, we made the unexpected discoveries that (1) OD can be shifted by monocular deprivation in adult mice and (2) that this shift can occur solely by potentiation of responses to the nondeprived eye (Sawtell et al., 2003). These results suggest that the classical "critical period" for OD plasticity (that ends around adolescence) might be more precisely defined as the period of development when MD triggers a rapid loss of cortical responsiveness to the deprived eye (and amblyopia). The first goal of the present study was to understand the relative timing and contributions of deprived-eye depression and open-eye potentiation to the OD shift in young mice. This information is basic for the interpretation and design of experiments to dissect the mechanisms of OD plasticity during the critical period.

A second goal of the present study was to understand more precisely how closing the eyelid triggers the two responses to monocular deprivation. We reasoned that MD has at least two effects. First, by degrading image formation on the retina, it replaces well-correlated ganglion cell responses with poorly correlated spontaneous activity. Second, it reduces visually evoked postsynaptic activity in the cortex. A way to determine the relative contribution of these effects to the OD shift is to inactivate one eye with tetrodotoxin (TTX), which should reduce cortical activity like MD, but eliminate all residual retinal activity. Previous investigations of the effects of monocular inactivation (MI), which relied on shifts in the OD ratio, have yielded variable results, however. In kittens, 2 days of MI has much less effect on cortical OD than 2 days of lid closure (Rittenhouse et al., 1999); whereas longer periods ($\geq$7 days) of MI reportedly are as effective as MD in shifting OD, both in kittens (Chapman et al., 1986; Greuel et al., 1987) and mice (Hensch et al., 1998). We thought that the chronic recording method could provide answers to the questions of when and how MI shifts OD; answers that are fundamental to understanding how activity triggers synaptic modifications in visual cortex.

Results

To monitor changes in cortical responsiveness to stimulation of the two eyes, we recorded visually evoked potentials (VEPs; Figure 1A). Electrodes were implanted in primary visual cortex at a site yielding the maximal binocular response and at a depth yielding the maximum negative-going VEP (Huang et al., 1999; Sawtell et al., 2003). The ratio of VEP amplitudes evoked by alternating patterned visual stimulation of the two eyes provides a reliable index of ocular dominance, in close agreement

*Correspondence: mbear@mit.edu

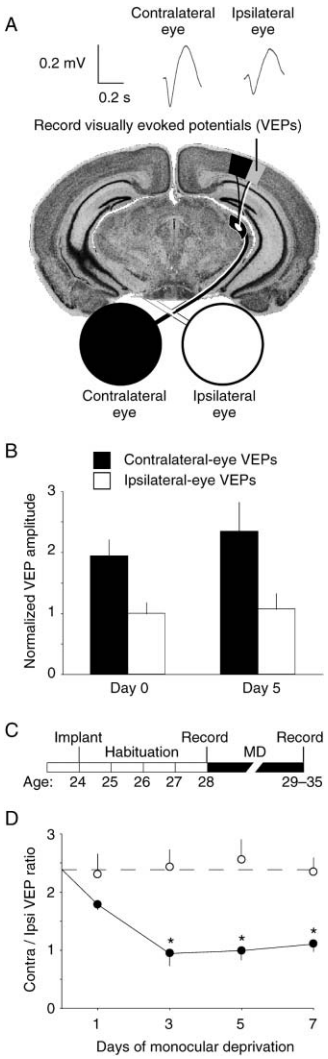

Figure 1. Assessment of Ocular Dominance Plasticity in Mice Using VEPs

(A) VEPs are recorded from the binocular region of primary visual cortex (shaded) at a depth that yields the maximum negative-going potential. Representative VEPs in response to contralateral and ipsilateral eye stimulation are shown (averages of 200 trials). Note the innate contralateral-eye dominance.

(B) VEPs recorded from awake mice are stable over days. Displayed are the average magnitude (±SEM; n = 6 mice) of VEPs in response to contralateral eye (filled bars) and ipsilateral eye (open bars) stimulation during a baseline (day 0 = P28) and after 5 days of normal visual experience. For display purposes, responses are normalized to the average value of the day 0 ipsilateral-eye VEPs. No significant change in VEP amplitude is observed over the course of 5 days (day 0: contra, 148 ± 18 μV; ipsi, 74 ± 10 μV; day 5: contra, 157 ± 24 μV; ipsi, 77 ± 14 μV).

(C) Experimental design used to study ocular dominance plasticity in juvenile mice.

(D) Kinetics of the shift in the ratio of contralateral-eye to ipsilateral-eye VEPs following periods of MD. Data at each time point are from a separate group of animals (n = 5–7 per group); open symbols are mean (±SEM) values during baseline recordings, and filled symbols are recordings from the same animals after the designated period of MD. Dashed horizontal line represents the mean baseline value. A significant ocular dominance shift is observed after 3 days of MD (asterisks).

with ratios derived from single-unit recordings (Hanover et al., 1999; Huang et al., 1999). VEPs have the advantages that they are less subject to sampling bias, the data are quantitative (absolute levels of response, in addition to ratios), and they can be recorded chronically to yield information about the kinetics of synaptic plasticity (Sawtell et al., 2003). In a normal visual environment, we find that the amplitude of the responses evoked by the two eyes and their ratio remain stable for many days (Figure 1B).

Time Course of the OD Shift after MD

The experimental design, illustrated in Figure 1C, was to implant young mice with VEP recording electrodes, collect baseline recordings, and monocularly deprive by lid suture for various durations (1, 3, 5, or 7 days); then open the eyelid and record the consequences of the deprivation. During baseline recordings at postnatal day (P) 28, we consistently observed that the ratio of contralateral eye VEP amplitude to ipsilateral eye VEP amplitude (the C/I ratio) was approximately 2.4, reflecting the normal contralateral eye dominance in mice (Figure 1D, open symbols). This ratio was rapidly shifted as a consequence of contralateral eyelid closure (Figure 1D, filled symbols). After 3 days of MD, the C/I ratios were significantly decreased (2.43 ± 0.29 pre- versus 0.94 ± 0.20 post-MD; $n = 6$, $p < 0.03$, Wilcoxon signed rank test) and remained shifted after 5 (2.56 ± 0.34 pre- versus 0.99 ± 0.16 post-MD, $p < 0.02$) and 7 days (2.35 ± 0.25 pre- versus 1.11 ± 0.13 post-MD, $p < 0.03$) of deprivation. These experiments confirm earlier studies showing that a robust ocular dominance shift can occur in mice with ≤4 days of MD (Gordon and Stryker, 1996).

Two Responses to MD in Young Mice

The central goal of our study was to monitor absolute changes in the cortical responses to deprived-eye and nondeprived-eye stimulation. It is possible to achieve this goal despite interanimal variations in absolute VEP amplitude by using the same animals as their own controls, comparing pre- and post-MD values (Table 1). This analysis revealed two temporally distinct responses to monocular deprivation: (1) rapid, deprivation-induced depression of contralateral (deprived) eye responses, occurring over the first 3 days of MD, and (2) delayed potentiation of ipsilateral (nondeprived) eye responses, emerging after 5 days of MD (Figures 2A and 2B).

The depression of deprived-eye VEP amplitude was apparent after 1 day and statistically significant after ≥3 days of monocular deprivation (Table 1). Contralateral-eye responses remained significantly depressed after 5 and 7 days of MD, although there was significant relaxation of responses toward baseline with time [ANOVA $F_{(3,20)} = 3.686$; $p < 0.03$]. Potentiation of the ipsilateral-eye VEPs was apparent after 5 days of contralateral eyelid closure and was highly statistically significant after 7 days (Table 1). The gradual potentiation of responses failed to occur in a parallel study where mice underwent binocular deprivation for 1, 3, or 7 days. Binocular lid suture produced no significant changes in either absolute VEP amplitude or C/I ratios (Table 1). Taken together, the data support the conclusion reached in a previous study of OD plasticity in adult

Table 1. Effects of Temporary Lid Closure on Ocular Dominance and Visual Responsiveness

Contralateral Eye VEPs									
		1		3		5		7	
	Days Post Pre	Pre	Post	Pre	Post	Pre	Post	Pre	Post
MD	Mean ± SEM	191 ± 30	151 ± 36	188 ± 43	80 ± 23*	131 ± 13	85 ± 12*	213 ± 19	157 ± 15*
	n	5	5	6	6	7	7	6	6
BD	Mean ± SEM	223 ± 29	246 ± 44	222 ± 31	227 ± 35			274 ± 21	211 ± 27
	n	6	6	6	6			7	7
Ipsilateral Eye VEPs									
		1		3		5		7	
	Days Post Pre	Pre	Post	Pre	Post	Pre	Post	Pre	Post
MD	Mean ± SEM	91 ± 21	84 ± 18	85 ± 26	85 ± 10	56 ± 10	99 ± 18	92 ± 6	144 ± 11*
	n	5	5	6	6	7	7	6	6
BD	Mean ± SEM	97 ± 11	126 ± 24	87 ± 11	92 ± 11			138 ± 15	113 ± 21
	n	6	6	6	6			7	7
C/I Ratios									
		1		3		5		7	
	Days Post Pre	Pre	Post	Pre	Post	Pre	Post	Pre	Post
MD	Mean ± SEM	2.3 ± 0.3	1.8 ± 0.1	2.4 ± 0.3	0.9 ± 0.2*	2.6 ± 0.3	1.0 ± 0.2*	2.3 ± 0.2	1.1 ± 0.1*
	n	5	5	6	6	7	7	6	6
BD	Mean ± SEM	2.3 ± 0.1	2.2 ± 0.4	2.6 ± 0.3	2.5 ± 0.3			2.1 + 0.2	2.2 + 0.4
	n	6	6	6	6			7	7

VEP amplitudes (in μV) and the C/I ratios before (pre) and after (post) various forms of visual experience. MD, monocular deprivation by lid closure of the contralateral eye; BD, binocular deprivation by lid closure of both eyes. C/I ratios of individual animals are first calculated and then averaged across the experimental group.

*denotes statistically significant from "pre" condition at $p < 0.05$ using two-tailed paired t test.

mice (Sawtell et al., 2003) that the delayed ipsilateral-eye potentiation is (1) *enabled* by reducing activity in the competing contralateral eye but is (2) *dependent* on visual experience in the ipsilateral eye.

Monocular TTX Abolishes the First and Promotes the Second Response to MD

The most obvious change in cortex that results from closing the contralateral eye is a reduction in retinally driven activity. To investigate if quieting activity in the dominant eye is indeed an enabling factor for ipsilateral-eye potentiation, we inactivated the contralateral eye with TTX for 3 days. Unlike 3 days of lid closure, monocular inactivation (MI) clearly allowed robust potentiation of ipsilateral-eye responses (pre-TTX 131 ± 20 μV versus post-TTX 192 ± 25 μV, $p < 0.04$, paired t test, $n = 6$; Figure 2B). We therefore conclude that experience-dependent potentiation of the nondominant input is facilitated by reducing cortical activity driven by the dominant input.

To investigate the consequences of MI on deprivation-induced response depression, young mice (P28) received daily intravitreal injections of TTX ($n = 5$) for 3 days, followed by lid closure of the injected eye to limit visual experience for an additional 2 days while the TTX wore off (Figure 3A). In each animal, daily pupil inspections and VEP recordings through the injected eye confirmed that the retinal activity blockade was complete and spanned the time between injections. This monocular inactivation group was compared with a second group of animals that were monocularly deprived by lid suture for 5 days (and underwent the same regimen of anesthesia as animals receiving eye injections; Figure 3C).

The effects of MI were dramatic. As expected from our previous experiments, we found that inactivating the contralateral eye enhanced potentiation of the ipsilateral-eye responses (101 ± 19 μV pre versus 161 ± 33 μV post, $p < 0.05$). However, there was no depression of the responses to the deprived eye (Figure 3B). Contralateral-eye responses before MI (209 ± 30 μV) were not different from those after (239 ± 46 μV) despite 5 days of monocular activity blockade. This finding contrasted with the effect of monocular lid closure. The animals lid sutured for 5 days beginning at P28 showed the ocular dominance shift we expected from our previous experiments (Figure 3D). The deprived-eye responses were significantly decreased (224 ± 30 μV pre-MD versus 157 ± 30 μV post-MD, $p < 0.04$, paired t test), and the nondeprived-eye responses showed a trend toward potentiation that did not reach statistical significance (114 ± 23 μV pre-MD versus 143 ± 29 μV post-MD). Neither contralateral-eye nor ipsilateral-eye responses showed any significant changes in the saline-injected control group (Figure 3F). The lid closure had no effect on deprived-eye responses in this group, presumably because the very brief deprivation (2 days) was initiated at the end of the critical period for response depression, which is thought to end at P32 (Gordon and Stryker, 1996).

Discussion

Our experiments show that MD in mice during the preadolescent critical period causes (1) rapid, deprivation-

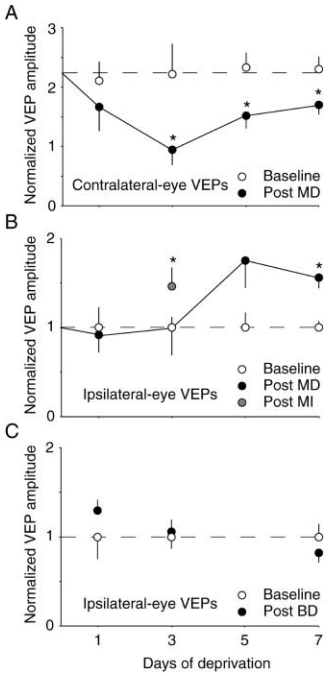

Figure 2. Two Responses to Monocular Deprivation

Displayed are the absolute VEP amplitudes (normalized to average baseline ipsilateral-eye VEP) before (open symbols) and after (filled symbols) various periods of visual deprivation. (A) Depriving the contralateral eye of vision by lid closure causes a rapid depression of contralateral-eye VEP amplitude that reaches statistical significance after 3 days of MD and remains significant at 5 and 7 days, despite a trend to relax toward baseline (same animals displayed in Fig 1D). (B) Depriving the contralateral eye of vision by lid closure leads to a delayed potentiation of the ipsilateral-eye VEPs, significant after 7 days. If the contralateral eye is inactivated with TTX, ipsilateral-eye VEP potentiation is significant after only 3 days (shaded symbol). (C) In contrast to the effects of MD, binocular deprivation (BD) has no effect on VEP amplitude.

induced response depression and (2) delayed, deprivation-enabled response potentiation. These two responses to MD are mechanistically distinct because they have different developmental regulation and are affected differently by reducing activity in the deprived eye.

Depression of responses in visual cortex during MD is abolished by intraocular TTX, suggesting that it is triggered by activity originating in the deprived retina. Studies in vitro have revealed a number of mechanisms by which untimely presynaptic activity can induce long-term synaptic depression (LTD) in visual cortex (Bear, 2003; Sjostrom et al., 2003); one of these is the loss of postsynaptic glutamate receptors (Dodt et al., 1999). It is of interest to note that a recent study showed in rats that monocular lid closure significantly reduced surface expression of glutamate receptors, whereas monocular TTX had no effect (Heynen et al., 2003). Our new findings are entirely consistent with the conclusion of that study. Together, the data suggest that the loss of postsynaptic glutamate receptors, triggered by the activity in the deprived-eye cortical afferents, is a mechanism for deprivation-induced response depression.

The delayed potentiation of the nondeprived-eye response resembles what we described previously in adults (Sawtell et al., 2003). In that study, we showed that closing the contralateral eyelid for several days leads to a progressive NMDAR-dependent increase in ipsilateral-eye responses. We show here that response potentiation emerges faster and more reliably when all activity is blocked in the competing eye. Thus, reducing the activity in one eye appears to be permissive for the strengthening of responses to the other eye. Response potentiation does not appear to be merely a passive homeostatic adaptation to reducing cortical activity (Turrigiano and Nelson, 2004), however, since it fails to occur following binocular deprivation. Rather, our data suggest that the deprivation-enabled potentiation is experience dependent.

Our interpretation of the effects of intraocular TTX is based on the assumption that it reduces activity relayed to the visual cortex by the lateral geniculate nucleus (LGN). In anesthetized kittens, it has been shown that TTX injection in the eye produces an immediate (Rittenhouse et al., 1999) and sustained (Stryker and Harris, 1986) reduction in the "spontaneous" activity of LGN neurons postsynaptic to the injected eye. Furthermore, it has been shown that intraocular TTX greatly reduces constituitive expression of activity-regulated immediate-early genes in the visual cortex of behaving rodents (Worley et al., 1991). However, studies in immature ferrets have shown that LGN activity, driven by cortical feedback connections, can recover following transection of the optic nerves (Weliky and Katz, 1999). Moreover, a recent study in head-restrained awake ferrets reports surprisingly modest differences in visual cortical activity between the conditions of natural vision and complete darkness (Fiser et al., 2004). Regardless of how subtle the activity distinctions prove to be, however, our findings demonstrate profoundly different functional consequences of monocular TTX, lid closure, and normal visual experience. Considerable insight into how patterns of activity trigger synaptic changes in vivo could be gained by chronic recording from the LGN in awake, behaving animals over the course of the several days required to elicit cortical modifications.

Our findings may help to resolve apparent discrepancies in the literature regarding the effects of monocular TTX. In kittens, Rittenhouse et al. (1999) found that the OD shift after 2 days of monocular lid closure was dramatically reduced if TTX was injected in the deprived eye. In contrast, Chapman et al. (1986) and Greuel et al. (1987) reported comparable OD shifts after $\geq$7 days of deprivation. Here we find that MI prevents depression of deprived eye responses and promotes potentiation of the open eye responses. Thus, TTX impairs the early OD shift that is accounted for solely by deprived-eye depression ($<$3 days) without significantly reducing the late OD shift which is accounted for mainly by open-eye potentiation ($>$5 days).

The finding of rapid, deprivation-induced response depression followed by a gradual potentiation of nondeprived-eye responses in mice is reminiscent of results obtained in kitten visual cortex after the manipulation called "reverse suture." As the name implies, the deprived eyelid is opened after a period of MD, and the initially open eye is closed. The state of the kitten visual system after the initial MD is similar to that in the normally reared mouse, because the cortex is dominated

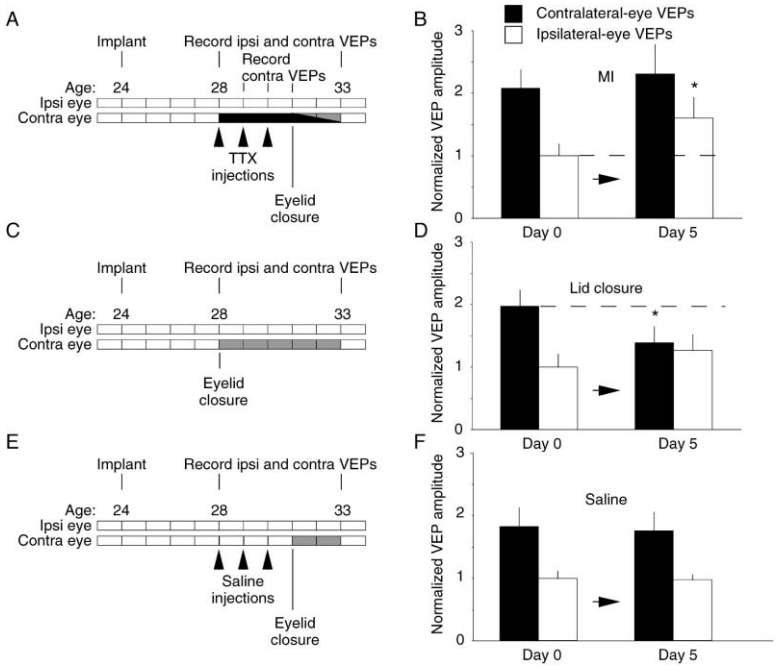

Figure 3. Monocular Lid Closure and Inactivation Shift Ocular Dominance in Different Ways

(A) Design of MI experiment. Retinal blockade spanned the 3 TTX injections as confirmed by the absence VEPs from the injected eye. During the washout period, the lid was closed to limit visual experience. Complete recovery from TTX was confirmed by return of the pupillary light reflex.

(B) Inactivation of the contralateral eye for 5 days produced no depression of deprived-eye responses but did promote significant potentiation of the ipsilateral- (nondeprived-) eye responses.

(C) Design of control experiments in which the eyelid was closed for a comparable period.

(D) MD by lid closure significantly depresses contralateral- (deprived-) eye responses. The trend toward potentiation of ipsilateral-eye responses does not reach statistical significance.

(E) Design of control experiments in which the eye was injected with saline rather than TTX.

(F) Saline injections do not alter responses of the injected eye. The brief MD by lid closure produced no effect because it was initiated at the end of the critical period for response depression.

by inputs from one eye. Like what we observe in the mouse, the dominant response is rapidly depressed by deprivation, followed by a gradual potentiation of responses to the initially weak eye (Mioche and Singer, 1989). The kinetics of the ocular dominance shift during reverse suture has been modeled using the BCM theory (Bienenstock et al., 1982; Blais et al., 1999; Clothiaux et al., 1991). According to this theory, closing the dominant eye has two effects. First, patterned retinal activity is replaced by "noise," which drives mechanisms of homosynaptic LTD in visual cortex. Second, because the dominant input is deprived, cortical activity is sharply reduced. This leads to an adjustment in the properties of synaptic plasticity (metaplasticity) that facilitates the mechanisms of activity-dependent response potentiation. The temporal delay between the initial deprived-eye response depression and the open-eye response potentiation depends on the amount of noise in the deprived eye and the rate of the metaplasticity. Thus, the BCM theory seems to provide a useful description of ocular dominance plasticity in the mouse.

We have not systematically compared the different consequences of MD in the two hemispheres in young mice, although our previous experiments in adults suggest that open-eye response potentiation may be less prominent in the visual cortex ipsilateral to the deprived eye (Sawtell et al., 2003). The BCM theory suggests that the rate of response potentiation depends on how much cortical activity is reduced by closing one eye, but it can still occur even if the competing input is not initially dominant (Blais et al., 1999). The situation in the hemisphere ipsilateral to the deprived eye may be analogous to what happens in the somatosensory "barrel" cortex when all but one vibrissa is trimmed during development. Similar to what we report here, there is a rapid depression of responses to the deprived whiskers. However, by the third week of deprivation there is also significant potentiation of responses to the nondeprived whisker (Glazewski and Fox, 1996). We note that the BCM theory also has been invoked to account for these findings (Benuskova et al., 1999). Regardless, the close similarity in the kinetics and outcome of sensory deprivation in these two sensory systems suggests that our findings may be broadly applicable to understanding the mechanisms of cortical plasticity.

Deprivation-induced response depression appears to occur most readily during a critical period that normally ends at approximately 5 weeks of age and correlates with maturation of cortical GABAergic inhibition (Fagiolini and Hensch, 2000; Huang et al., 1999; Iwai et al., 2003; Kirkwood et al., 1995; Rozas et al., 2001). Our MI experiments suggest a simple explanation for how inhibition can shape the critical period. In adult mice (P80), depriving the contralateral eye fails to cause response depression but still enables potentiation of ipsilateral-eye responses (significant after only 5 days MD) (Sawtell et al., 2003). It is remarkable that eliminating activity in the deprived eye of juvenile mice with TTX is sufficient to mimic the effect of adult MD. Thus, the critical period for deprivation-induced response depression could end simply by a reduction in the amount of poorly correlated afferent activity reaching modifiable synapses in visual cortex. Developmental noise reduction conceivably could occur in the retina or lateral geniculate nucleus. However, there is already considerable evidence suggesting that the late maturation of inhibition in the cortex, particularly in layer 4, filters the type of activity relayed to the superficial layers (Kirkwood and Bear, 1994; Rozas et al., 2001). Inhibitory circuits may therefore contribute to a "plasticity gate" that controls the presynaptic activity that is required to induce deprivation-induced synaptic depression in the visual cortex.

Experimental Procedures

Electrode Implantation

Mice were anesthetized with 50 mg/kg ketamine and 10 mg/kg xylazine i.p., and a local anesthetic of 1% lidocaine hydrochloride was injected over the scalp. For purposes of head fixation, a post was fixed to the skull just anterior to bregma using cyanoacrylate and a further application of dental cement. Two small ($<$0.5 mm) burr holes were made in the skull overlying the binocular visual cortex (3 mm lateral of lambda), and tungsten microelectrodes (FHC, Bowdoinham, ME) were inserted 450 μm below the cortical surface. Reference electrodes were placed bilaterally in prefrontal cortex. Electrodes were secured in place using cyanoacrylate, and the entire exposure was covered with dental cement. Animals were monitored postoperatively for signs of infection or discomfort and were allowed at least 24 hr recovery before habituation to the restraint apparatus.

Eyelid Suture

Mice were anesthetized by inhalation of isoflurane (IsoFlo 2%–3%) and placed under a surgical microscope. Lid margins were trimmed and antibiotic ophthalmic ointment (Vetropolycin, Pharmaderm) was applied to the eye. Three mattress stitches were placed using 6-0 vicryl, opposing the full extent of the trimmed lids. Mice were recovered by breathing room air and were monitored daily to be sure that the sutured eye remained shut and uninfected. Animals whose eyelids did not fully seal shut were excluded from further experiments. At the end of the deprivation period, mice were reanesthetized, stitches were removed, and lid margins were separated. Eyes were then flushed with sterile saline and checked for clarity under a microscope. Mice with corneal opacities or signs of infection were excluded from further study.

Eyeball Injections

Mice were anesthetized by inhalation of isoflurane (2%–3%) and placed under a surgical microscope. The superior part of the conjuctiva was exposed, and the globe was stabilized with 7-0 silk suture through the conjuctiva. Ophthalmic ointment was applied to keep the eye moist. After conjuctival dissection, a small puncture was made at conreoscleral junction into the vitreous chamber with a fine needle. A glass micropipette attached to microinjection apparatus (MMP, World Precision Instruments) was inserted to a depth of approximately 0.7 mm. TTX solution (1 μl) (Sigma, 1 mM) or saline was injected into vitreous chamber. After the injection, the micropipette was held for 2 min before being withdrawn. The eye was rinsed several times with sterile eye drops. The pupil was dilated in the TTX-treated eyes within 5 min, and the pupilary light reflex was abolished; this was not the case for the saline-injected eyes. Total retinal activity blockade following each injection lasted $>$24 hr, as confirmed by daily VEP recordings. After 3 daily injections, pupils of TTX-treated eyes stayed dilated for at least 36 hr.

Visual Stimuli

Stimuli consisted of full-field sine-wave gratings of 0% and 100% contrast, square reversing at 1 Hz, and presented at 0.05 cycles/degree. Stimuli were generated by a VSG2/2 card (Cambridge Research System, Cheshire, UK) and presented on a computer monitor suitably linearized by γ correction. VEPs were elicited by either horizontal or vertical bars. The display was positioned 20 cm in front of the mouse and centered on the midline, thereby occupying 92° $\times$ 66° of the visual field. Mean luminance, determined by a photodiode placed in front of the computer screen, was 27 cd/m^2.

Recording Procedure

VEP recordings were conducted in awake mice. The animals were alert and still during recording. Visual stimuli were presented to left and right eyes randomly. A total of 100 to 200 stimuli were presented per each condition. VEP amplitude was quantified by measuring peak-peak response amplitude, as described previously (Huang et al., 1999; Sawtell et al., 2003). Responses to 0% contrast were also collected to measure activity not evoked by patterned visual stimuli.

Acknowledgments

We thank Nathaniel Sawtell, Carlos Lois, Michelle Adams, Arnold Heynen, Robert Crozier, Erik Sklar, and Suzanne Meagher for assistance and advice. Research partially funded by grants from the National Eye Institute and National Institute for Mental Health.

Received: July 13, 2004
Revised: November 3, 2004
Accepted: November 24, 2004
Published: December 15, 2004

References

Bear, M.F. (2003). Bidirectional synaptic plasticity: from theory to reality. Philos. Trans. R. Soc. Lond. B Biol. Sci. *358*, 649–655.

Benuskova, L., Ebner, F.F., Diamond, M.E., and Armstrong-James, M. (1999). Computational study of experience-dependent plasticity in adult rat cortical barrel-column. Network *10*, 303–323.

Bienenstock, E.L., Cooper, L.N., and Munro, P.W. (1982). Theory for the development of neuron selectivity: Orientation specificity and binocular interaction in visual cortex. J. Neurosci. *2*, 32–48.

Blais, B.S., Shouval, H.Z., and Cooper, L.N. (1999). The role of presynaptic activity in monocular deprivation: comparison of homosynaptic and heterosynaptic mechanisms. Proc. Natl. Acad. Sci. USA *96*, 1083–1087.

Chapman, B., Jacobson, M.D., Reiter, H.O., and Stryker, M.P. (1986). Ocular dominance shift in kitten visual cortex caused by imbalance in retinal electrical activity. Nature *324*, 154–156.

Clothiaux, E.E., Bear, M.F., and Cooper, L.N. (1991). Synaptic plasticity in visual cortex: comparison of theory with experiment. J. Neurophysiol. *66*, 1785–1804.

Dodt, H., Eder, M., Frick, A., and Zieglgansberger, W. (1999). Precisely localized LTD in the neocortex revealed by infrared-guided laser stimulation. Science *286*, 110–113.

Drager, U.C. (1978). Observations on monocular deprivation in mice. J. Neurophysiol. *41*, 28–42.

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

Fiser, J., Chiu, C., and Weliky, M. (2004). Small modulation of ongoing cortical dynamics by sensory input during natural vision. Nature *431*, 573–578.

Glazewski, S., and Fox, K. (1996). Time course of experience-dependent synaptic potentiation and depression in barrel cortex of adolescent rats. J. Neurophysiol. *75*, 1714–1729.

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.

Greuel, J.M., Luhmann, H.J., and Singer, W. (1987). Evidence for a threshold in experience-dependent long-term changes of kitten visual cortex. Brain Res. *431*, 141–149.

Hanover, J.L., Huang, Z.J., Tonegawa, S., and Stryker, M.P. (1999). Brain-derived neurotrophic factor overexpression induces precocious critical period in mouse visual cortex. J. Neurosci. *19*, RC40.

Hensch, T.K., Fagiolini, M., Mataga, N., Stryker, M.P., Baekkeskov, S., and Kash, S.F. (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.

Huang, Z.J., Kirkwood, A., Pizzorusso, T., Porciatti, V., Morales, B., Bear, M.F., Maffei, L., and Tonegawa, S. (1999). BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. Cell *98*, 739–755.

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.

Kirkwood, A., and Bear, M.F. (1994). Hebbian synapses in visual cortex. J. Neurosci. *14*, 1634–1645.

Kirkwood, A., Lee, H.-K., and Bear, M.F. (1995). Co-regulation of long-term potentiation and experience-dependent plasticity in visual cortex by age and experience. Nature *375*, 328–331.

Mioche, L., and Singer, W. (1989). Chronic recordings from single sites of kitten striate cortex during experience-dependent modifications of receptive-field properties. J. Neurophysiol. *62*, 185–197.

Rittenhouse, C.D., Shouval, H.Z., Paradiso, M.A., and Bear, M.F. (1999). Monocular deprivation induces homosynaptic long-term depression in visual cortex. Nature *397*, 347–350.

Rozas, C., Frank, H., Heynen, A.J., Morales, B., Bear, M.F., and Kirkwood, A. (2001). Developmental inhibitory gate controls the relay of activity to the superficial layers of the visual cortex. J. Neurosci. *21*, 6791–6801.

Sawtell, N.B., Frenkel, M.Y., Philpot, B.D., Nakazawa, K., Tonegawa, S., and Bear, M.F. (2003). NMDA receptor-dependent ocular dominance plasticity in adult visual cortex. Neuron *38*, 977–985.

Sjostrom, P.J., Turrigiano, G.G., and Nelson, S.B. (2003). Neocortical LTD via coincident activation of presynaptic NMDA and cannabinoid receptors. Neuron *39*, 641–654.

Stryker, M.P., and Harris, W.A. (1986). Binocular impulse blockade prevents the formation of ocular dominance columns in cat visual cortex. J. Neurosci. *6*, 2117–2133.

Turrigiano, G.G., and Nelson, S.B. (2004). Homeostatic plasticity in the developing nervous system. Nat. Rev. Neurosci. *5*, 97–107.

Weliky, M., and Katz, L.C. (1999). Correlational structure of spontaneous neuronal activity in the developing lateral geniculate nucleus in vivo. Science *285*, 599–604.

Wiesel, T.N., and Hubel, D.H. (1963). Single cell responses in striate cortex of kittens deprived of vision in one eye. J. Neurophysiol. *26*, 1003–1017.

Worley, P.F., Christy, B.A., Nakabeppu, Y., Bhat, R.V., Cole, A.J., and Baraban, J.M. (1991). Constitutive expression of zif268 in neocortex is regulated by synaptic activity. Proc. Natl. Acad. Sci. USA *88*, 5106–5110.