Rapid recovery from the effects of early monocular deprivation is enabled by temporary inactivation of the retinas

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A half-century of research on the consequences of monocular deprivation (MD) in animals has revealed a great deal about the pathophysiology of amblyopia. MD initiates synaptic changes in the visual cortex that reduce acuity and binocular vision by causing neurons to lose responsiveness to the deprived eye. However, much less is known about how deprivation-induced synaptic modifications can be reversed to restore normal visual function. One theoretically motivated hypothesis is that a period of inactivity can reduce the threshold for synaptic potentiation such that subsequent visual experience promotes synaptic strengthening and increased responsiveness in the visual cortex. Here we have reduced this idea to practice in two species. In young mice, we show that the otherwise stable loss of cortical responsiveness caused by MD is reversed when binocular visual experience follows temporary anesthetic inactivation of the retinas. In 3-mo-old kittens, we show that a severe impairment of visual acuity is also fully reversed by binocular experience following treatment and, further, that prolonged retinal inactivation alone can erase anatomical consequences of MD. We conclude that temporary retinal inactivation represents a highly efficacious means to promote recovery of function.

ocular dominance plasticity | metaplasticity | amblyopia | visual cortex | lateral geniculate nucleus

Amblyopia is a widespread form of human visual disability that arises from imbalanced visual experience in the two eyes during infancy or early childhood. The core pathophysiological process underlying amblyopia is ocular dominance plasticity in the primary visual cortex (V1). Ocular dominance plasticity, evolutionarily conserved in mammals with binocular vision, has become a classic paradigm for studying how brain development is influenced by experience and deprivation. A brief period of monocular deprivation (MD) during early postnatal life causes functional depression of synapses in V1 that serve the deprived eye (1–7). Consequently, early-life MD severely and persistently degrades visual acuity through the deprived eye, which typically fails to recover spontaneously when binocular vision is restored (8–10). A critical step in developing targeted interventions for treating amblyopia is to identify strategies for reversing deprivation-driven synaptic modifications in V1.

The traditional approach to promote recovery following MD has been to occlude the strong eye to force vision through the weak amblyopic eye. This "reverse occlusion" approach has been validated in animals (11, 12) and represents the cornerstone of current treatment (patching therapy) of human amblyopia (13–16). However, this treatment has well-known limitations that include poor compliance, potential loss of vision through the newly patched eye, failure to recover binocular vision, and a declining treatment efficacy with age (15, 17–21). Nevertheless, the success of reverse occlusion strategies demonstrates that severely weakened synaptic inputs in the brain can be rejuvenated under appropriate circumstances.

An insight into how reverse occlusion might promote recovery came from a study in cat V1, which showed that neurons lose responsiveness to the newly occluded eye days before gaining responses to the newly open (amblyopic) eye (22). It is now understood that the qualities of cortical synaptic plasticity depend on the recent history of cortical activity, a property called metaplasticity (23). After a period of attenuated cortical activity, the threshold for synaptic potentiation is reduced (24–26). Thus, the period of quiescence that immediately follows initiation of reverse occlusion lowers the threshold for synaptic potentiation, enabling subsequent visual experience to increase synaptic effectiveness when it would have otherwise been without effect (27). An intriguing possibility is that imposition of a period of binocular inactivity following early MD could be sufficient to promote visual recovery without forcing the eyes to compete.

In support of this concept, studies have shown that exposure of adult rats and kittens to a period of continuous darkness can prime the visual cortex for recovery from the effect of MD when vision is restored to the deprived eye (28–30). However, available data suggest that this priming effect requires ≥10 d of darkness, which cannot be interrupted, even briefly, by light exposure and cannot be substituted with binocular lid closure (31, 32). Further, work in kittens has shown that when initiated before 10 wk of age, exposure to total darkness itself can cause temporary visual impairment and even blindness before 7 wk of age (33). Thus, although these results show the potential of using insights gained from the study of synaptic plasticity to promote recovery of function (34), application of this approach to clinical practice presents many challenges.

Significance

Normal development of the visual cortex depends critically on early life visual experience. In humans, a disparity in the quality of vision between two eyes during infancy or early childhood leads to a visual impairment called amblyopia. In animal models, amblyopia can be induced early in life using a brief period of monocular deprivation via eyelid closure. Here we show in two evolutionarily divergent species that experimental amblyopia can be rapidly corrected if binocular visual experience is restored after temporarily silencing the retinas with a local anesthetic. These findings point the way to an approach for clinical management of amblyopia with advantages over the current standard of care.

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In the current study, we set out to examine an alternative method involving temporary anesthetic inactivation of the retinas. For decades, researchers of visual system development have used tetrodotoxin (TTX) to investigate the consequences of blocking action potentials in retinal ganglion cells. A single dose, administered by microinjection into the vitreous humor, can locally block all impulse activity in the optic nerve for a day or two. Of significance, inactivation of one eye with TTX fails to trigger depression of deprived-eye responses in V1 that is observed after comparable periods of monocular deprivation by lid closure or image blurring (5, 7, 35–37). Thus, brief inactivation of both eyes with TTX could potentially augment recovery of function by lowering the plasticity threshold without the liability associated with other forms of deprivation. Furthermore, because the retinas are silent until the drug wears off, compliance is assured.

To test this hypothesis, we performed parallel studies in mice and kittens. Our interest in mice was driven by the utility of this species for mechanistic studies. Although the immediate effects of MD in mice have been well documented, less was known about the stability of these effects. Therefore, we first conducted a longitudinal electrophysiological study to track V1 responsiveness to stimulation of the two eyes in awake mice for several weeks following early-life MD. We found that 7 d of MD drove a profound reduction in visual cortical responsiveness to deprived eye vision that persisted through adulthood, validating this species as a model to study the pathophysiology of amblyopia. We then investigated the effect of bilateral retinal inactivation and observed a complete recovery from the effect of MD once binocular visual experience was restored. We also studied treatment effectiveness in the cat, which for decades has been the preferred species to study the effects of MD on visual system physiology, anatomy, and behavior. We found that bilateral retinal inactivation followed by a short period of binocular visual experience promoted fast recovery of the visual acuity of the deprived eye to normal levels. Further, we discovered that even in the absence of subsequent visual experience, prolonged TTX treatment led to recovery from the anatomical consequences of MD on the visual thalamus of kittens. Our experiments demonstrate, in two species, that temporary retinal inactivation is an effective strategy for promoting rapid recovery from MD-driven visual impairments.

Results

Monocular Deprivation Drives Lasting Visual Impairment in Mice.
Mice are commonly used for mechanistic studies of ocular dominance plasticity in response to early-life MD (3, 5, 38). Recent investigations have focused on the mechanisms responsible for the loss of responsiveness to the deprived eye (39, 40), but very little is known about the stability of these changes or whether they can be reversed. To address these questions, we chronically recorded visual evoked potentials (VEPs) in thalamo-recipient layer 4 of binocular V1 over the course of several weeks in three cohorts of mice (Fig. 1). One group had normal visual experience; the second was monocularly deprived by eyelid closure from postnatal day (P) 26 to P33 followed by normal binocular visual experience; and the third group was treated as the second except that the period of MD was followed by a single dose of TTX injected into the vitreous humor of both eyes (Fig. 1 A–C). TTX is a long-lasting voltage-gated sodium channel blocker, and in agreement with previous studies (7, 41), we found that a single dose completely abolished visual responses for 24–48 h, followed by a gradual recovery. In all MD cases, VEPs were recorded from V1 contralateral to the deprived eye (Fig. 1A).

In agreement with previous reports (5, 42), 7-d MD drove a profound reduction in the magnitude of VEPs during presentation of phase-reversing sinusoidal grating stimuli to the deprived eye [Fig. 1D, Center; $P = 0.0006$; 0.2 cycles per degree (cpd)]. Further, we found that deprived-eye VEP magnitude

remained significantly reduced after 1 wk and 1 mo of binocular visual experience following MD, compared with pre-MD baseline values (Fig. 1D, Center; 1 wk: $P = 0.0246$; 1 mo: $P = 0.0023$) and to sham littermate controls (Fig. 1D, Left; 1 wk: $P = 0.0020$; 1 mo: $P = 0.0204$). The sustained depression of deprived-eye responses was also observed at lower and higher spatial frequencies (Fig. S1). These results demonstrate that the visual deficit observed after 7 d of MD is long lasting and further validate the mouse as a useful animal model of amblyopia.

Temporary Retinal Inactivation Promotes Electrophysiological Recovery in Amblyopic Mice.
After confirming depression of deprived-eye VEP magnitude immediately following 7-d MD (Fig. 1D, Right; $P = 0.0099$; 0.2 cpd), a cohort of mice received a binocular intravitreal TTX injection, eliminating retinal activity for ~2 d, followed by normal binocular experience. Subsequent VEP recordings revealed responses to the deprived eye that actually exceeded baseline values 1 wk later ($P = 0.0562$) and were no different from baseline 1 mo later ($P = 0.9454$). Visually evoked responses in animals that experienced binocular inactivation following MD were significantly increased over littermates that experienced MD only (Fig. 1 D and E; 1 wk: $P < 0.0001$; 1 mo: $P = 0.0028$) and were statistically indistinguishable from sham littermate controls that had no MD (1 wk: $P = 0.6442$; 1 mo: $P = 0.7981$). Assessment of VEPs measured with lower and higher spatial frequency stimuli (0.05 and 0.4 cpd, respectively) revealed similar trends, although recovery for the higher spatial frequency was not observed immediately, but instead occurred between 1 wk and 1 mo following the injection (Fig. S1). Meanwhile, binocular inactivation had no lasting impact on nondeprived eye responses (Fig. 1E and Fig. S1). Thus, whereas early-life MD in mice can drive a sustained deficit in V1 responsiveness to vision through the deprived eye, this deficit can be eliminated following binocular retinal inactivation.

Temporary Retinal Inactivation Restores Visual Acuity in Amblyopic Kittens.
Although mice are useful for mechanistic studies of amblyopia, the species with the longest history and richest database is the cat. Advantages of the cat include visual pathways that share a similar organization to those of primates, good visual acuity, stereoscopic vision, and functional and morphological responses to early MD that are very large and similar to those observed in primates. The lineage of modern cats and mice diverged early in mammalian evolution, even before the divarication of rodents and primates (43). A similar response to treatment in cats and mice would thus be consistent with evolutionary conservation of a common mechanism that might apply generally to visual mammals, including humans.

Visual acuity was measured in kittens using a two-alternative forced-choice discrimination task (30, 44) (Fig. 2A). Consistent with previous studies, we found that 7-d MD initiated at P30 induced a long-lasting deficit in deprived-eye visual acuity (Fig. 2 B–D). In our first pilot experiment (animal C349) we injected the eyes with two doses of TTX 2 d apart, starting on P99, ~2 mo after the deprived eye had been opened (Fig. 2B). Remarkably, within 1 wk of the return of normal acuity in the nondeprived eye (reflecting clearance of the TTX), we observed a rapid and complete recovery from amblyopia in the fellow deprived eye. To confirm this encouraging result, we repeated the experiment in three littermates using a similar experimental design (Fig. 2C). Assessed with the pupillary light reflex and visual placement behavior, it appeared that blockade of retinal activity lasted ~5 d, with full restoration of vision through the nondeprived eye apparent 10 d after the first TTX dose. Over the next 10 d, acuity in the deprived (amblyopic) eyes of all three additional kittens increased until it was indistinguishable from that of the nondeprived eye.

In a fifth kitten (C362) we attempted to investigate the effect of a single binocular dose of TTX at P94 (Fig. 2D). Unfortunately, although visuomotor behavior was affected by the
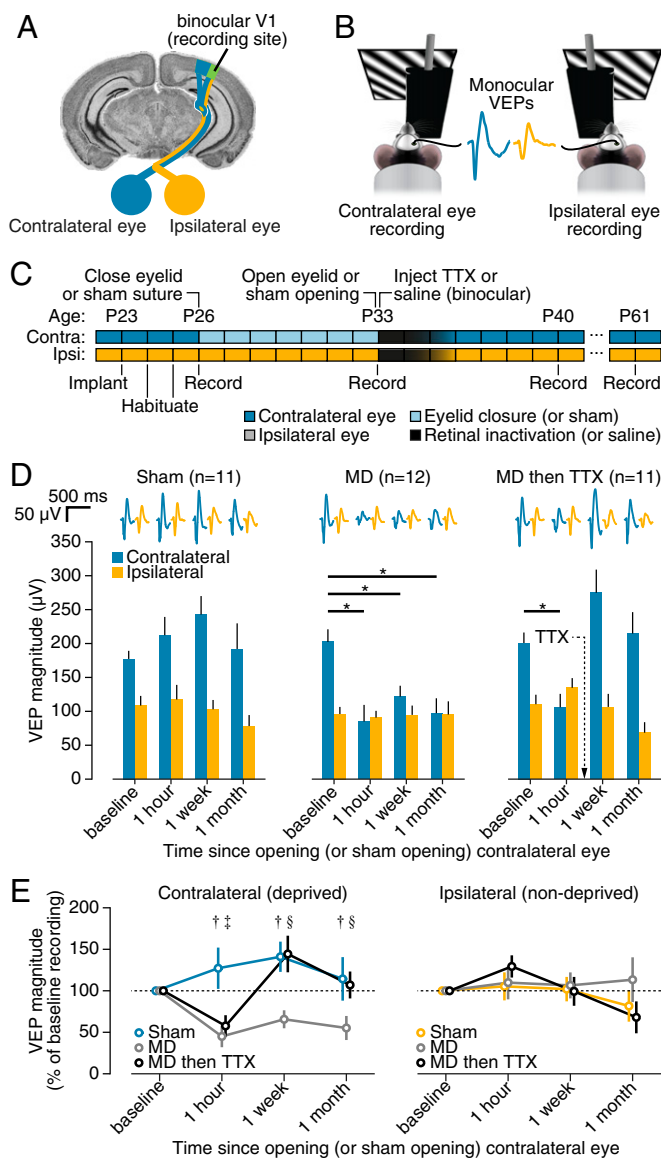
Fig. 1. Binocular retinal inactivation following MD promotes recovery of visually evoked responses in mouse V1. (*A*) Coronal section of mouse brain showing recording site relative to eyes. (*B*) Cartoon showing VEPs measured during visual stimulus presentation to the contralateral or ipsilateral eyes. (*C*) Experimental timeline. Seven days of MD or sham MD of the contralateral eye were followed by intravitreal injections of TTX or saline into both eyes. (*D*) Mean monocular VEP magnitudes for littermates that underwent sham eyelid closure followed by saline injections (sham, *n* = 11), MD followed by saline injections (MD, *n* = 12), or MD followed by TTX injections (MD then TTX, *n* = 11). Deprived eye responses varied for each treatment group over time [time, $F_{(3, 93)}$ = 7.116, $P < 10^{-3}$; treatment, $F_{(2, 31)}$ = 7.963, $P < 10^{-2}$; and interaction, $F_{(6, 93)}$ = 5.166, $P < 10^{-3}$], with MD significantly reducing responses to vision through the deprived, contralateral eye compared with its own baseline (Dunnett: MD, P = 0.0006; MD then TTX, P = 0.0099; and sham, P = 0.5524) and compared with sham controls (Tukey: sham vs. MD, P = 0.0011; sham vs. MD then TTX, P = 0.0093; and MD vs. MD then TTX, P = 0.82). Contralateral responses recovered following binocular retinal inactivation (Dunnett: baseline vs. 1 wk, P = 0.0562 and baseline vs. 1 mo, P = 0.9454) and were statistically indistinguishable from sham controls (Tukey: 1 wk, P = 0.9866 and 1 mo, P = 0.9347). Error bars, SEM. Asterisks denote statistically significant differences from baseline (Dunnett, adjusted $P < 0.05$). Stimulus spatial frequency: 0.2 cpd. (*E*) Mean monocular VEP magnitudes over time normalized to baseline values. Contralateral VEP magnitudes varied for each treatment group over time [time, $F_{(3, 93)}$ = 5.514, $P < 10^{-2}$; treatment, $F_{(2, 31)}$ = 7.481, $P < 10^{-2}$; and interaction, $F_{(6, 93)}$ = 4.225, $P < 10^{-3}$]. Error

treatment, the kitten showed incomplete pupil dilation as well as weak light reflexes and visual placing responses. The animal's impairment on the behavioral task indicated that retinal activity was at least partially disrupted, although the sustained visual reflexes suggested that activity blockade was incomplete. Interestingly, we also failed to observe any recovery of acuity in the deprived eye, suggesting that a partial activity blockade is insufficient for promoting visual recovery. We attempted a second TTX injection, 1.5 mo after the initial injection. This time, the treatment successfully blocked visual reflexes (indicative of a complete retinal activity blockade) in both eyes, and we observed a full recovery of acuity in the deprived eye over the next 2 wk (Fig. 2*D*). Together, these data suggest that complete blockade of retinal spiking is required to promote visual recovery, and that a single administration of an inactivating agent can promote normal visual acuity even several months after MD.

Sustained Retinal Inactivation Drives Anatomical Recovery from Consequences of MD in Kittens. In cats as in monkeys, substantial shrinkage of neurons occurs in the layers of the lateral geniculate nucleus (LGN) that relay information to V1 from the deprived eye. Shrinkage of these neurons correlates well with the loss of their axonal arbors and synaptic influence in V1 (45–48). Thus, assessment of LGN cell-size changes after MD ± TTX has the potential to provide additional insight into how the treatment promotes recovery. We found that 7-d MD produced a clear deprivation effect (expressed as the percent difference in cell size between deprived and nondeprived LGN layers) (48) (Fig. 3 *A* and *D*), which was still apparent in animals receiving 2–3 doses of TTX every 2 d immediately following MD (Fig. 3*D*). This finding indicates that brief binocular activity blockade itself does not restore function to deprived-eye cortical inputs, but possibly primes the cortex for recovery when the TTX wears off and binocular vision is provided. However, we also found that with five binocular doses over 10 d, soma sizes in deprived layers of LGN returned to normal and the deprivation effect was eliminated (Fig. 3 *B*, *D*, and *E*). This striking finding contrasts with the effect of treating only the deprived eye with TTX, which further exacerbated the deprivation effect (Fig. 3 *C* and *E*). Thus, some aspects of recovery are experience independent, as they occur during the period of retinal inactivation and presumably provide a scaffold for visual recovery that ensues with subsequent binocular vision.

Discussion

Our experiments reveal that stable electrophysiological, anatomical, and behavioral consequences of early-life MD can be fully and rapidly reversed in two evolutionarily distant species, kittens and mice, when binocular visual experience is restored following temporary bilateral anesthetic inactivation of the retinas. These findings suggest the possibility of developing a treatment modality for amblyopia with significant advantages over the current standard of care.

Kittens historically have been a preferred animal model to study ocular dominance plasticity, in part because of clear relevance of the findings to human amblyopia. As is the case in humans and nonhuman primates, MD and reverse occlusion are effective in kittens only during a well-defined sensitive period of early postnatal development; the physiological and anatomical changes in LGN and V1 correlate with altered acuity and binocularity assessed behaviorally; and temporary deprivation produces visual deficits that persist even when normal visual experience is restored. However, over the past

bars, SEM. Statistical significance between treatment groups (Tukey, $P < 0.05$) is denoted by † (sham vs. MD), ‡ (sham vs. MD then TTX), or § (MD vs. MD then TTX). Ipsilateral VEP magnitudes were not significantly different across time or between treatment groups [time, $F_{(3, 93)}$ = 1.879, P = 0.1386; treatment, $F_{(2, 31)}$ = 0.3222, P = 0.7270; and interaction, $F_{(6, 93)}$ = 1.020, P = 0.4172].

Fong et al.

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A

C

Close eyelid / Open eyelid — Inject TTX / Inject TTX

DE: / NDE: — P30 P37 — P94 P96 / TTX — P122

■ Deprived eye
■ Non-deprived eye
□ Eyelid closure
■ Retinal inactivation

Range of normal acuity @ P100

Grating acuity (cycles/degree) — 8 7 6 5 4 3 2 1 0 / open door / blind

Days since opening deprived eye — 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85

C363 C364 C365 □○△ DE / □○△ NDE / □○△ BV

B

Close eyelid / Open eyelid — Inject TTX / Inject TTX

DE: / NDE: — P30 P37 P67 — P99 P101 / TTX — P122

Grating acuity (cycles/degree) — 8 7 6 5 4 3 2 1 0 / open door / blind

Days since opening deprived eye — 30 35 40 45 50 55 60 65 70 75 80 85

○ DE / ○ BV

D

Close eyelid / Open eyelid — Inject TTX — Inject TTX

DE: / NDE: — P30 P37 P67 — P94 / TTX (visual reflexes not eliminated) — P135 / TTX — P157

Grating acuity (cycles/degree) — 8 7 6 5 4 3 2 1 0 / open door / blind

Days since opening deprived eye — 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120
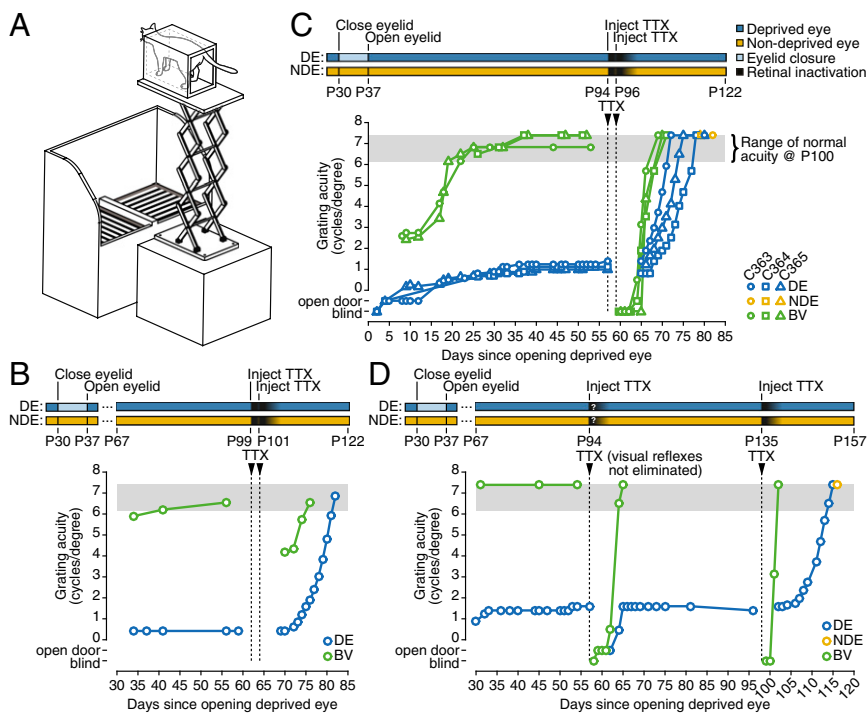
○ DE / ○ NDE / ○ BV

Fig. 2. Binocular retinal inactivation following MD promotes recovery of visual acuity in behaving kittens. (*A*) The jumping stand used to test grating acuity. (*B*) Visual spatial acuity of the deprived eye (DE: blue) and nondeprived eye (NDE), assessed using binocular vision (BV: green) of a kitten (C349) that received two injections of TTX at P99 and P101. The period of complete and waning retinal inactivation that ensued is depicted by completeness of black shading. As with subsequent panels, the rearing history is displayed in schematic form above the graph. Gray shading shows the range of grating acuities measured in normal kittens by use of the same testing procedure. The designations "blind" and "open door" refer, respectively, to an inability or ability to locate a closed door on the jumping stand by visual cues alone. (*C*) The visual acuity of the DE and NDE tested in three littermates (C363, C364, and C365) before and after two binocular intravitreal injections of TTX made at P94 and P96. All animals received a 7-d period of MD at P30 (NDE acuity assessed monocularly: yellow; other conventions as in *A*). (*D*) Results from C362 that received an initial binocular TTX injection at P94 that apparently did not achieve full retinal activity block as the pupils were not dilated fully and visual placing behavior was still evident. Although the animal appeared blind on the jumping stand, the acuity of the NDE recovered to normal in 8 d but the acuity of the DE recovered only to its preinjection level. Remarkably, following a second binocular intravitreal injection at P135 the acuity of this eye recovered to normal levels.

20 y, mice have supplanted other species for the investigation of visual cortical plasticity because of their numerous advantages for interventional mechanistic studies. Mice show many of the expected responses to temporary MD, including robust depression of deprived-eye responses. However, one unusual feature of mouse ocular dominance plasticity is that it persists well into adulthood (42), particularly when visual experience is enriched (49, 50). An additional complication is that the ocular dominance shift initiated in adults reverts spontaneously when binocular vision is restored (51, 52) or if both eyelids are closed (53). Thus, for the mouse to serve as a useful model for amblyopia following early-life MD, it was of particular importance to understand the degree to which the juvenile ocular dominance shift persists when binocular experience is restored. Previous studies in juvenile mice have shown that deprived-eye visual deficits persist through adulthood following 13-d MD ending at P32 (10), but not 4- to 5-d MD ending at P30 (51, 54). In our study, we monitored spontaneous recovery following 7-d MD (P26–P33). We took advantage of the fact that the effects of MD could be followed in the same animals through chronically implanted electrodes in V1. These longitudinal recordings revealed that like cats and primates, 7-d early MD in mice depresses responses to stimulation of the deprived eye, and this response depression persists for weeks. Whereas we did observe some evidence of spontaneous recovery during the first week after MD (Fig. S1*B*), deprived-eye responses in MD animals were still well below sham controls. These findings establish 7 d of early MD in mice as a useful protocol to investigate strategies for reversing ocular dominance plasticity and promoting recovery from amblyopia.

It has been known for many years from work in kittens and monkeys that deprivation-induced synaptic depression can be reversed if normal vision is restored in the weak (amblyopic) eye and the fellow eye is occluded (11, 12). However, gains in the amblyopic eye often come at the expense of vision in the fellow eye, may be temporary, and are rarely accompanied by improvement in binocularity (9, 17–19). Further, as a treatment for human amblyopia, patching is limited by poor compliance, has a variable outcome, and is effective only when initiated in early childhood (16, 19). A better understanding of what allows recovery with patching might suggest improvements

over the current standard of care. One view is that recovery is enabled by a reduction in cortical activity that occurs when the strong eye is occluded (27). Support for this idea comes from findings that exposure of rats (28) and kittens (30, 32) to complete darkness for 10–14 d can promote recovery from the effects of MD when visual experience is restored. The fact that darkness, but not bilateral lid closure (31) is effective suggests that a key variable in changing the threshold for cortical plasticity is the level and/or variance of residual retinal activity. Our current findings using TTX to silence retinal activity directly support this hypothesis.

Dark exposure (to lower the plasticity threshold) followed by repetitive (55) or dichoptic (56) visual training exercises (to use binocular cooperativity to strengthen weak synapses in V1) offers a neurobiologically sound alternative to reverse occlusion (patching) as a treatment for amblyopia. However, previous studies in kittens have found that very brief daily light exposure eliminated the therapeutic effects of an otherwise uninterrupted 10-d period of darkness (32). Further, shortening the duration of absolute darkness to 5 d was insufficient to promote recovery of visual acuity or mobilize neuroplasticity mechanisms (30, 32). Therefore, the infrastructure and strict compliance requirements for effective dark exposure therapy could present a serious obstacle for clinical application of this approach, particularly to medically underserved communities (57, 58). In these respects, complete silencing of the retinas could offer significant advantages over dark exposure. Our data show rapid and complete recovery of visual responsiveness and acuity after binocular TTX treatment can be achieved following a single dose. In addition to shortening the treatment duration, pharmacological inactivation of the retinas represents a portable treatment that can circumvent compliance issues associated with enforcing an extended period of darkness. If the risks of the procedure and the duration of blockade can be minimized, while maintaining efficacy, the brevity of the period of visual incapacitation makes it potentially of greater appeal than the longer period of total darkness required to promote equivalent recovery. We believe the current findings are sufficiently compelling to justify additional preclinical studies to establish safety, minimal treatment duration, the maximal age of
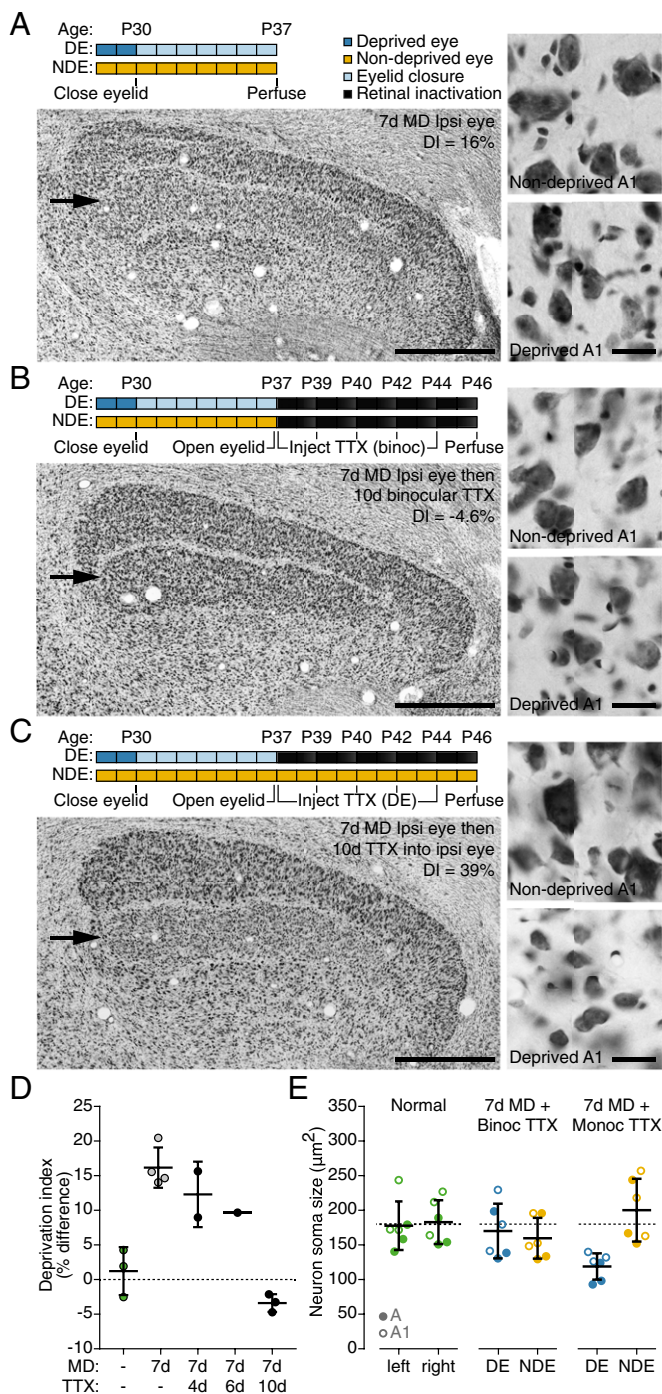
Fig. 3. Recovery of neuron soma size in the kitten LGN following prolonged binocular retinal inactivation. (*A*, *Top*) Schematic depicting rearing history. Kittens received retinal TTX immediately after the period of MD to isolate the effect of inactivation on recovery and in the absence of any visual experience. (*Left*) Low magnification image of the LGN stained for Nissl substance after 7-d MD started at P30. Arrow indicates deprived eye (DE) layer (lamina A1, ipsilateral to the DE). (Scale bar, 1 mm.) (*Right*) High magnification images from NDE (*Top*) and DE (*Bottom*) A1 layers after 7-d MD. (Scale bar, 25 μm.) (*B* and *C*) Same as *A* except that MD was followed by 10 d of binocular retinal silencing (*B*) or monocular retinal silencing of the DE (*C*). (*D*) Stereological quantification of neuron soma size within DE and NDE A and A1 LGN layers (deprivation index, DI) revealed that after MD, DE neurons recovered progressively during the period of binocular inactivation so that DE and NDE neurons were of equal size by 10 d. Dashed line denotes DI observed in normal kittens. Colors denote normal visual experience (green), 7-d MD alone (gray), or 7-d MD followed by binocular TTX (black).

treatment effectiveness, and the maximal duration of MD that can be corrected.

In light of the translational potential, it is important to consider questions and concerns raised by this approach. First, TTX is a highly potent blocker of sodium-dependent action potentials. Although systemic exposure to TTX can be lethal, this risk is minimized by microinjection into the eyes. TTX was chosen for our experiments because a single treatment produces a long-lasting block of sodium channels, but there are many other approaches to achieve the same goal of silencing retinal output and it will be important for future studies to evaluate these alternatives. Second, although intravitreal injections are a routine ophthalmological procedure, they are not entirely without risk to the retina. The benefits of this or related approaches (e.g., retrobulbar block) will need to be weighed carefully against the risk of complications. Finally, there is an obvious requirement for monitoring patient safety during the period of temporary blindness.

Caveats aside, the remarkable effects of temporary inactivation of the visual pathway demonstrate that V1 synapses can be rejuvenated and at least some forms of amblyopia can be reversed. An urgent goal of future studies will be to assess precisely how retinal inactivation creates conditions in the ascending visual pathway that promote recovery. For example, it has been shown in mice that retinal silencing with TTX can promote bursting in the LGN (41) and burst stimulation of the geniculate can potentiate VEPs (59). Homeostatic adaptations that facilitate experience-dependent synaptic potentiation (24, 60, 61), such as reduced cortical inhibition and alterations in the properties of NMDA receptors (62–64), could also contribute to the therapeutic effects of retinal inactivation. We are encouraged to believe that the approach described here, and/or the knowledge gained by studying it, might ultimately have therapeutic potential for the treatment of amblyopia and other types of neurological rehabilitation.

Materials and Methods

All mouse experiments adhered to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (65) and were approved by the Committee on Animal Care at the Massachusetts Institute of Technology (MIT). All cat experiments conformed to guidelines of the Canadian Council on Animal Care and were conducted according to protocols approved by the University Committee on Laboratory Animals at Dalhousie University. Details on experimental procedures are provided in *SI Materials and Methods*.

Center values and error bars, mean ± SD. (*E*) Average left- and right-eye neuron soma size from age-matched normal controls (*Left*), and when MD was followed by binocular (*Center*) or monocular (*Right*) retinal inactivation. The restoration of balanced DE and NDE neuron size induced by binocular inactivation was the product of recovery rather than generalized shrinkage because (*i*) compared with monocular inactivation the size of DE neurons after binocular treatment was significantly larger [$F(2, 15) = 5.79$, $P = 0.013$; Bonferroni, $P < 0.05$], and (*ii*) neuron size after binocular silencing was not different from normal control data (Bonferroni, $P > 0.05$). Dashed line denotes average value observed from normal kittens. Closed and open symbols indicate measurements from A and A1 LGN layers, respectively, and colors denote normal visual experience (green), the DE (blue), or the NDE (yellow). Center values and error bars, mean ± SD.

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