Development/Plasticity/Repair

Visual Acuity Development and Plasticity in the Absence of Sensory Experience

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Visual circuits mature and are refined by sensory experience. However, significant gaps remain in our understanding how deprivation influences the development of visual acuity in mice. Here, we perform a longitudinal study assessing the effects of chronic deprivation on the development of the mouse subcortical and cortical visual circuits using a combination of behavioral optomotor testing, *in vivo* visual evoked responses (VEP) and single-unit cortical recordings. As previously reported, orientation tuning was degraded and onset of ocular dominance plasticity was delayed and remained open in chronically deprived mice. Surprisingly, we found that the development of optomotor threshold and VEP acuity can occur in an experience-independent manner, although at a significantly slower rate. Moreover, monocular deprivation elicited amblyopia only during a discrete period of development in the dark. The rate of recovery of optomotor threshold upon exposure of deprived mice to light confirmed a maturational transition regardless of visual input. Together our results revealed a dissociable developmental trajectory for visual receptive-field properties in dark-reared mice suggesting a differential role for spontaneous activity within thalamocortical and intracortical circuits.

Introduction

Experience shapes brain connectivity and its function during specific developmental time windows called critical periods (CPs). In the visual system, function rapidly matures after eye-opening, as experience sculpts the cortical circuit underlying receptive-field (RF) properties. In humans and other species, disruption of sensory experience during this CP can lead to permanent visual dysfunction (Daw, 2006). Imbalance of visual inputs between the two eyes, such as with monocular deprivation (MD), for example, shifts the spiking response of visual cortical neurons in favor of the open eye and it is accompanied by an enduring loss of visual acuity or amblyopia in the deprived eye (Wiesel and Hubel, 1963; Dews and Wiesel, 1970; Hubel and Wiesel, 1970; Movshon and Dürsteler, 1977; Blakemore et al., 1978; Dräger, 1978; Giffin and Mitchell, 1978; Olson and Freeman, 1980; Fagiolini et al., 1994; Gordon and Stryker, 1996; Daw, 1998; Kiorpes et al., 1998; Issa et al., 1999; Fagiolini and Hensch, 2000; Prusky et al., 2000). Complete removal of sensory experience by dark-rearing from birth (chronic dark rearing; CDR), affects the general maturation of several RF properties and the expression of their respective CP plasticity (Regal et al., 1976; Teller et al., 1978; Cynader and Mitchell, 1980; Mower, 1991; Fagiolini et al., 1994; Crair et al., 1998; Gianfranceschi et al., 2003). To identify the underlying molecular and cellular mechanisms of circuit refinement, the effects of different manipulations of sensory experience have been extensively studied in the rodent visual system due to its rapid postnatal development and the power of genetic manipulations (Valverde, 1971; Fagiolini et al., 1994, 2000; Gordon and Stryker, 1996; Gianfranceschi et al., 2003; Tropea et al., 2006, 2010). Similar to higher mammals, rodent visual system develops in an experience-dependent manner (Regal et al., 1976; Teller et al., 1978; Cynader and Mitchell, 1980; Mower, 1991; Fagiolini et al., 1994; Crair et al., 1998; Gianfranceschi et al., 2003). However, it is still not clear whether this disrupted state represents a circuit permanently fixed in the immature state, a normal circuit that is delayed in maturation, or a circuit that is miswired in a configuration not seen during normal development.

To distinguish between these three possibilities, mice were reared in total darkness from birth and exposed to normal light experience at different developmental ages. We first tested the development of spatial frequency threshold to moving gratings using the behavioral optomotor task (OPT) and visual acuity by *in vivo* extracellular recording of visual evoked potentials (VEP). Then, we evaluated the ability of the CDR visual circuits to respond to MD by recording ocular dominance (OD) plasticity and induction of amblyopia compared with light-reared (LR) mice. Our longitudinal studies demonstrated that CDR slows the maturation of spatial vision rather than indefinitely halting its development. Moreover, they revealed a dissociable developmental trajectory for visual RF properties in complete absence of visual sensory experience, suggesting that spontaneous activity alone is sufficient to establish mature spatial vision.

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Materials and Methods

All procedures were approved by the IACUC of Boston Children's Hospital. Experiments were conducted in C57BL/6J males. CDR mice were placed in a light-tight holding chamber within a darkroom at birth, and LR animals were raised on a normal 12 h light/dark cycle in the standard colony room.

Monocular deprivation. Eyelids were trimmed and sutured under isoflurane anesthesia as previously described (Gordon and Stryker, 1996). The integrity of the suture was checked daily and mice were used only if the eyelids remained closed throughout the duration of the deprivation period. The eyelids were reopened immediately before recording and the pupil was checked for clarity.

Visual behavioral test: OPT. Behavioral threshold acuity was evaluated using standard methods in unrestrained mice (Prusky et al., 2004). The highest spatial frequency tracked for each direction of rotation of the vertical sine wave gratings was recorded as the visual acuity. Mice were tested every 3–5 d from eye opening until P60. Experimenters were blind to rearing condition of the mice and the animal's previously recorded thresholds.

VEPs. VEPs were recorded from anesthetized mice (50 mg/kg Nembutal and 0.12 mg chlorprothixene) using standard techniques in mice as previously described (Porciatti et al., 1999; Durand et al., 2012). A tungsten electrode was inserted into binocular V1 at ~2.8 mm from the midline where the visual receptive field was approximate 20° from the vertical meridian and lowered to 400–600 μm from the surface of the cortex corresponding to bottom of layer 3 or top of layer 4. This position yielded the largest amplitude signal in response to a 0.05 cpd stimulus in V1b. Visual stimulus were generated using a VSG2/2 card (Cambridge Research System) and custom made software (developed by Mr. Orsini, Instituto di Neuroscienze CNR, Pisa Italy) and presented on a CRT monitor suitably linearized by gamma correction. The display (mean luminance 25 cd/m^2) was placed in front of the animal covering the binocular visual field of the mouse. Signals were bandpass-filtered (0.1–100 Hz), amplified, and fed to a computer for analysis. Transient VEPs in response to abrupt contrast reversal (100%, 1 Hz) were evaluated in the time domain by measuring the peak-to-trough amplitude and peak latency of the principal negative component (N1). At least 3–4 trials consisting of 20 events each were averaged in synchrony with the abrupt stimulus contrast reversal. The mean amplitude of the negative peak (N1) was plotted against the log of the spatial frequency, and the threshold of visual acuity was obtained by extrapolation to zero amplitude of the linear regression through the last 4 data points.

Single-unit electrophysiology in vivo. Electrophysiological recordings were made from the binocular visual cortex (V1b) of anesthetized mice (50 mg/kg Nembutal and 0.12 mg chlorprothixene) using standard methods (Gordon and Stryker, 1996; Hensch et al., 1998; Durand et al., 2012).

Orientation selectivity. Orientation selectivity was evaluated by recording single unit activity across all cortical layers I–VI using a 16-channel probe (Neuronexus Technologies, A1x16-3mm50-177) lowered into V1b at 3–4 different locations between 2.6 and 3.0 mm lateral to the midline in each mouse. Signals were filtered from 300 to 5000 Hz and amplified 1000 times, and a threshold was set to separate spikes from noise (SortClient, Plexon Technologies). Visual stimuli were delivered to the contralateral eye and consisted of 3 s long presentations of drifting (2 Hz) black and white bars (100% contrast, 0.025 cpd) at 12 different orientations (0–360°) spaced 30° apart. Each orientation presentation was repeated 8 times in random order. Eight repetitions of a blank stimulus of intermediate luminance were interspersed throughout the session to evaluate spontaneous activity.

Spikes were sorted based on waveform characteristics (Offline Sorter, Plexon Technologies), and a minimum interstimulus interval of 1.5 ms was imposed to ensure single unit isolation. Spiking rates in response to visual stimuli were determined using Matlab via SigTOOL (Lidierth, 2009). Spontaneous activity (SA) was calculated as the mean firing rate in response to blank stimuli. Maximum evoked response (R_{max}) was defined as the maximum firing rate during any stimulus presentation. Signal-to-noise ratio (SNR) = $(R_{max} - SA)/R_{max}$. The orientation selectivity index (OSI) = $(R_{max} - R_{ortho})/(R_{max} + R_{ortho})$, where R_{ortho} is the response to the orientation that is orthogonal to the preferred orientation. Only visually responsive cells were included in the analysis ($R_{max} \geq 1.5 \times SA$; $R_{max} \geq 0.5$ spikes/s, unless OSI ≥ 0.33). Fast-spiking putative inhibitory cells were excluded from analysis based on trough to peak time $\leq$0.37 ms and peak:trough amplitude ratio $\geq$0.43 (Niell and Stryker, 2008).

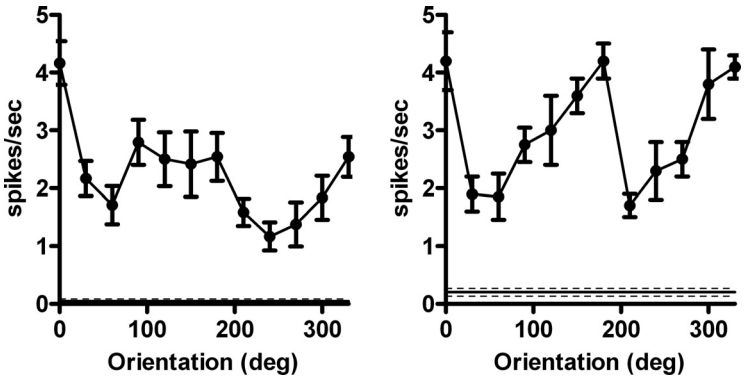


Figure 1. Sample orientation selectivity plots showing the response of a binocular visual cortical neuron to the stimulation of the contralateral (left plot) and ipsilateral (right plot) eye. Twelve different orientations spaced 30 degrees apart were shown. The ocular dominance score for this cell was −0.015 and the cell would be classified as a 4 on the 1–7 ocular dominance scale, meaning that the cell responded equally strong to stimulation of both eyes.

OD. Multiple penetrations (3–4) across the lateral extent of V1b were made with a tungsten electrode (FHC) and 5–8 cells spaced at least 70 μm apart were recorded for each penetration across all cortical layers I–VI. The stimulus was a vertical bar of light (90% luminance), 3° wide, moving horizontally across the screen at a velocity of 19.05 °/s. The stimulus was repeated six times for each cell and for each eye, and the responses were averaged across all repetitions. The signal was filtered from 300 to 5000 Hz and amplified 1000 times. Baseline activity was determined based on spiking during 2.5 s of blank screen between each repetition.

The OD score of each cell was calculated by peristimulus time histogram analysis of spiking in response to stimulation of each eye. OD score = $[(PI - BI) - (PC - BC)]/[(PI - BI) + (PC - BC)]$, where P, peak; B, baseline; C, contralateral eye; I, ipsilateral eye (Hensch et al., 1998; Fig. 1).

Each cell was also assigned a value on the 7-point OD classification scale (Wiesel and Hubel, 1963). A contralateral bias index (CBI) was calculated to represent the weighted average of overall ocular dominance in a population of cells. CBI = $[(n1 - n7) + 2/3(n2 - n6) + 1/3(n3 - n5) + N]/2N$, where N = total number of cells and nx = number of cells with a ranking of x on the 7-point scale (Gordon and Stryker, 1996). The receptive-field location of a subset of cells was determined by the location in degrees from the vertical meridian of the visual field where the maximum firing rate was evoked by the passing bar of light.

Statistical analysis. We first performed the Kolmogorov–Smirnov test to see whether the data were normally distributed. Student's t test was used for datasets that passed the Kolmogorov–Smirnov test, and Mann–Whitney test was used for those that were not normally distributed. Unless otherwise noted, we used a Student's t test for statistical significance and present the data as mean ± SEM.

Results

Delayed maturation of visual acuity in CDR mice

To assess the effects of deprivation on visual development, we first measured spatial frequency threshold using the OPT on

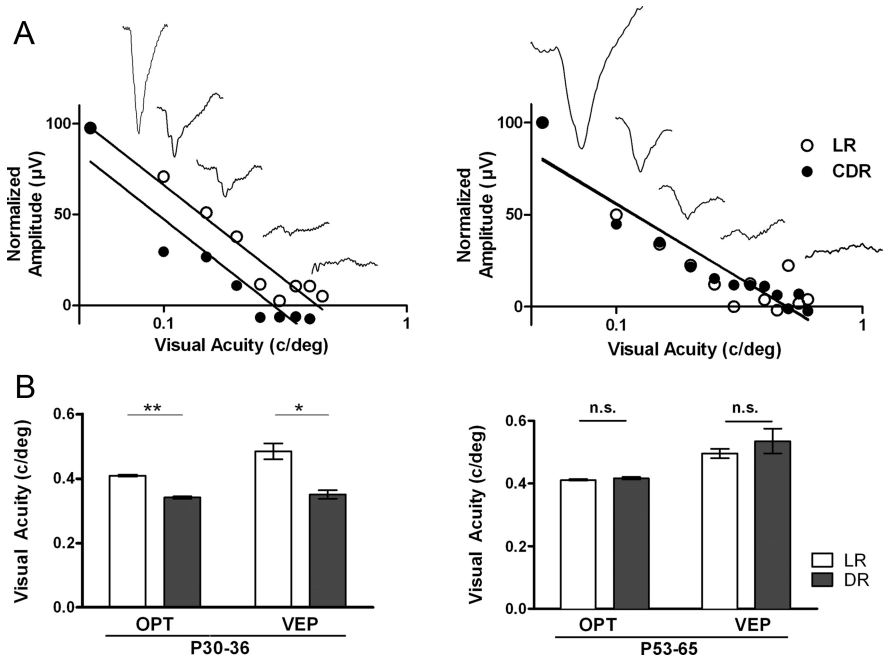


Figure 2. Development of optomotor threshold and visual acuity is delayed by CDR. ***A***, Representative examples of VEPs in response to alternating gratings of different spatial frequencies in a LR and CDR mouse at P34–P35 (left) and P55–P65 (right), respectively. VEP amplitudes decrease with increasing spatial frequency of stimulus and become undistinguishable from response to a blank field (noise). Visual acuity is calculated by linear extrapolation (log coordinates) to 0 μV of the last 4 data points. ***B***, Left, Both optomotor threshold and VEP acuity were significantly reduced in CDR (gray) compared with LR (white) mice at postnatal day P34–P35 (OPT: $n = 7$–12 mice, $^{***}p < 0.001$; VEP: $n = 4$–5 mice, $^{**}p = 0.002$). ***B***, Right, Both optomotor threshold and VEP acuity reached LR levels by in CRD (gray) compared with LR (white) mice at postnatal day P55–P65 (OPT: $n = 10$–12 mice; VEP: $n = 11$ mice, $p = 0.37$).

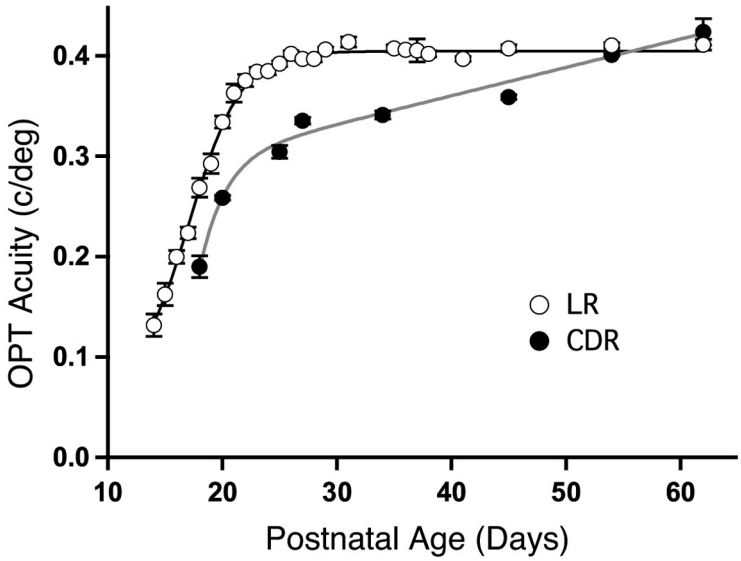


Figure 3. Development of optomotor threshold. The developmental trajectory of optomotor responses is delayed in CDR (black circle) compared with LR mice (open circles) reaching the adult LR level only after P55 (LR vs CDR, $^{***}p < 0.001$ for P18, P25, and P27; $^{**}p < 0.01$ for P34/35, P45). LR and CDR conditions are significantly different by two-way ANOVA [taking a random subset of equal data points at ages P18, P20, P25, P27, P45, P54; the p value for interaction, age and visual condition (LR vs CDR) are all <0.0001].

P34–P35 mice that were dark-reared from birth (CDR). The OPT has been used in mice to document that spatial vision rapidly increases from eye-opening (P12–P14) reaching a stable fully developed level by P30 (Prusky et al., 2004; Durand et al., 2012). Similarly, we found that the spatial frequency threshold of mice raised in a normal 12 h light/dark cycle (LR) was 0.41 ± 0.003 cpd at P34–P35. In contrast, age-matched CDR mice exhibited a significantly reduced spatial threshold (0.34 ± 0.004 cpd, $^{**}p = 0.004$, Mann–Whitney test; Fig. 2*B* left).

Because the OPT response is a behavioral assay system that reflects a combination of subcortical and cortical function (Prusky et al., 2006), we also assessed visual acuity using the VEP recorded directly from primary visual cortex (V1). VEP in response to abrupt high contrast reversal of square gratings were recorded from populations of neurons located in layers 3–4 of binocular V1. Consistent with previous reports (Gianfranceschi et al., 2003), we found that CDR until P34–P35 disrupted maturation of visual acuity (CDR 0.35 ± 0.01 vs LR 0.46 ± 0.02; $^{*}p = 0.016$, Mann–Whitney test; Fig. 2*B*, left). To test whether spatial vision remained immature even into adulthood, we then measured CDR mice at P55–P65. Surprisingly, both OPT and VEP responses exhibited a significant improvement compared with LR control levels (vs LR, respectively, $p = 0.2934$, t test; $p = 0.96$, t test; Fig. 2*B*, right).

We therefore compared the developmental trajectory of spatial frequency threshold in LR and CDR mice from P18–P62 (Fig. 3). The average OPT response of control mice (white circles) around the time of eye-opening (P12–P14) was 0.145 cpd. Over the next 10 d, the average OPT response increased in the light, reaching the adult level of 0.4 cpd by P25. In contrast, the development of the OPT response for CDR mice (black circles) was delayed. At P18, OPT spatial threshold in CDR mice was significantly lower than LR control mice (0.19 ± 0.01 cpd vs 0.27 ± 0.05 cpd; $^{***}p = 0.0001$, t test). However, even in CDR mice, the average OPT response increased with age, albeit at a slower rate than controls, reaching 0.34 ± 0.004 cpd by P27. OPT threshold in these visually deprived mice continued to increase after P27, finally reaching the adult level at P55, >25 d after control LR mice.

The increase in OPT threshold of CDR mice was best described with a double-exponential function with a relatively fast time constant of 3 d as well as a slower one of 67 d. Our finding that sensory deprivation delays, but does not halt, the development of both OPT threshold and visual acuity was unexpected. Previous studies in cat and rat had demonstrated that prolonged visual deprivation from birth resulted in stagnation of cortical development (Cynader and Mitchell, 1980; Timney et al., 1980; Bartoletti et al., 2004). However, our results suggested that visual deprivation in mice only delays development of spatial vision and that spontaneous activity is sufficient for the eventual establishment of some aspects of visual circuitry and function.

Delayed critical period plasticity in CDR mice

Evidence supporting the canonical view that CDR leaves the cortex in a permanently immature state also comes from studies showing functional OD plasticity extending into older ages in CDR cats, rats and mice (Cynader, 1983; Mower et al., 1983; Fagiolini et al., 1994; Gianfranceschi et al., 2003; Bartoletti et al., 2004). We then re-examined the effects of CDR on this CP plasticity, by performing 4 d MD starting at different ages during CDR. Visually deprived mice were anesthetized in the dark, and one eye was sutured. After surgery, animals were placed into a normal light/dark cycle. Such MD typically weakens responsiveness in the deprived eye in favor of the open eye only during a restricted period in juvenile life (Wiesel and Hubel, 1963; Hubel and Wiesel, 1970; Movshon and Dürsteler, 1977; Blakemore et al., 1978; Dräger, 1978; Olson and Freeman, 1980; Fagiolini et al., 1994; Gordon and Stryker, 1996; Issa et al., 1999; Fagiolini and Hensch, 2000) and produces an enduring loss of visual acuity (Dews and Wiesel, 1970; Giffin and Mitchell, 1978; Fagiolini et al., 1994; Daw, 1998; Kiorpes et al., 1998; Prusky et al., 2000). We therefore assayed both in CDR mice.

First, we mapped OD plasticity using single-unit recordings in LR mice. Consistent with previous results, short-term MD induced a significant shift in favor of the nondeprived eye only between P24–P35 (Fig. 4*A*, open columns; Gordon and Stryker., 1996). The OD shift can be summarized as a CBI, which is ~0.7 in LR mice and 0.5 after MD (Fig. 4*B*, open circle). In contrast, CDR mice did not yet exhibit OD plasticity at this age range (Fig. 4*A*, solid columns, right). The OD distribution started to shift toward the open, ipsilateral eye after MD only 1 week later in CDR mice (>P35). Moreover, this plasticity persisted at least until P55–P65 (Fig. 4*A*, solid columns, right). When these adult CDR mice were exposed to normal vision, OD plasticity rapidly decreased within a week, suggesting that the closure of the CP requires visually evoked neuronal activity (Fig. 4*B*).

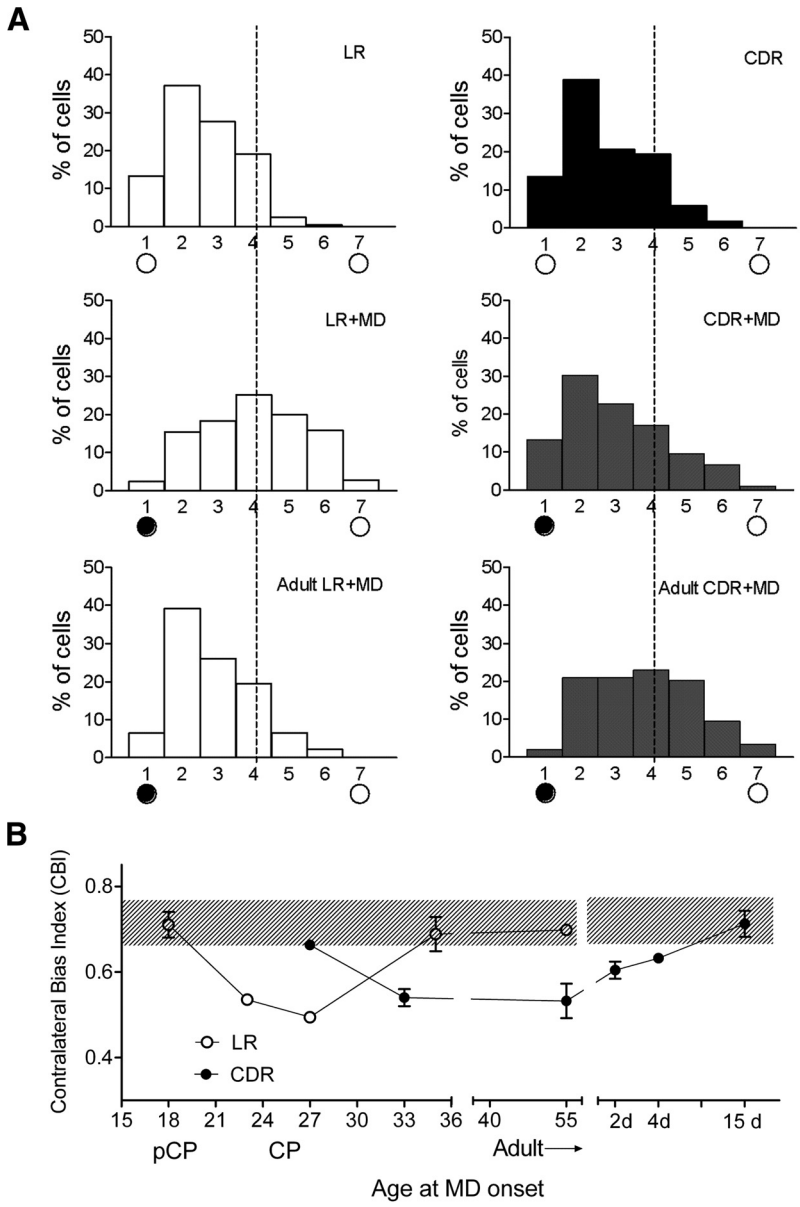


Figure 4. Onset of critical period for OD plasticity is delayed in CDR mice. ***A***, Both LR and DR exhibit the typical response bias toward contralateral eye input (ocular dominance Groups 1–3) in the binocular zone of mice (χ^2 test, $p = 0.13$, LR vs CDR, 210 and 172 cells; 8 and 6 mice respectively). OD distribution is robustly shifted in favor of the ipsilateral open eye (Groups 5–7) following MD during the CP but not in adulthood in LR mice (χ^2 test, $p < 0.0001$, LR vs LR + MD, 246 cells, 10 mice; $p = 0.42$, vs Adult LR + MD, 92 cells, 3 mice). On the contrary, CDR mice do not display a significant OD shift toward the open ipsilateral eye at the peak of CP (χ^2 test, $p = 0.37$, CDR vs CDR + MD, 106 cells, 4 mice; $p < 0.0001$; vs adult CDR+, 148, 5 mice, respectively) but they do instead in adulthood (χ^2 test, $p < 0.0001$, CDR vs adult CDR + MD, 172 and 148 cells; 6 and 5 mice; $p < 0.0001$; adult CDR + MD vs adult LR + MD, 148 and 92 cells; 5 and 3 mice, respectively). ***B***, CBI over postnatal development in LR and CDR mice. Histograms are quantified as a weighted average (CBI) which ranges from 0 to 1 for complete ipsilateral or contralateral eye dominance, respectively. One week after eye opening at P13–P14, sensitivity to brief MD rapidly appears and persists for ~10 d, as measured by single unit recordings. The immature pre-CP phase is extended by CDR such that the overall profile is delayed to yield plasticity starting from P33 (*t* test, vs LR: $p = 0.15$, CDR + P27MD, 106 cells, 4 mice; $p < 0.002$, CDR + P33MD, 82 cells, 3 mice; $p < 0.003$, CDR + P55MD, 148 cells, 5 mice) until adulthood when mice are exposed for the first time to light. Shaded region is the range of nondeprived LR mice. Some error bars are smaller than symbol size. (*t* test $p < 0.01$; CDR + 2 d light + MD, 151 cells, 5 mice; $p < 0.04$, CDR + 4 d light + MD, 88 cells, 3 mice; $p = 0.8$, CDR + 15 d light + MD, 81 cells, 3 mice vs LR, respectively).

To determine whether OD plasticity was accompanied by amblyopia, we recorded VEPs after 4 d MD starting at P24. As expected for the peak of the CP in LR mice, acuity through the deprived eye was reduced (Fig. 5*A*; $^{**}p = 0.01$, *t* test). Instead, CDR mice did not respond to MD at P24–P30 ($p = 0.6$, *t* test), possibly due to their still lower baseline acuity at this age (Fig. 2) and lack of OD plasticity (Fig. 4). Consistent with a delayed CP, MD at an older age (>P45) robustly reduced acuity in CDR mice (Fig. 5*A*, right; $^{*}p = 0.016$, Mann–Whitney test), but surprisingly, amblyopia was no longer induced at later stages. By P55–P60, visual acuity was insensitive to MD despite the fact that OD shifts were still possible in CDR mice (Fig. 4*B*; 5B, $p = 0.5$, Mann–Whitney test). Thus, the two cortical properties are dissociable in the dark.

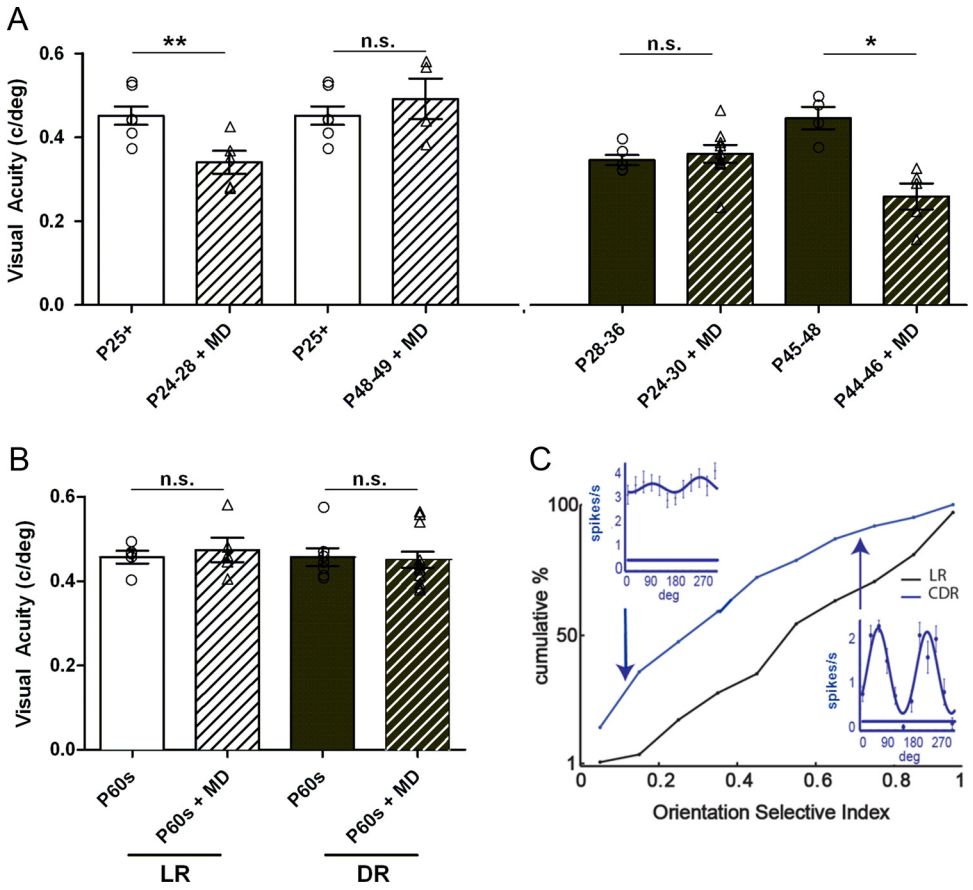


Figure 5. Uncoordinated maturation of receptive field properties in CDR mice. Effects of 4 d MD on acuity (VEPs) during development (***A***) and in the adult (***B***). Comparison of MD to control in P24–P30 mice: LR ($n = 5$–7, $^{**}p = 0.01$), CDR animals ($n = 6$–9); in P44–P49 mice: LR ($n = 4$–7), CDR ($n = 4$–5, $^{*}p = 0.02$); and in the adult LR ($n = 5$); CDR animals ($n = 7$–12). ***C***, Orientation selectivity index is significantly reduced in CDR mice ($n = 80$ cells) when compared with LR mice ($n = 67$ cells; Kolmogorov–Smirnov test, $p = 0.01$). Inset, Examples of orientation tuning curve for high (0.71) and low OSI (0.0853) visual cortical neurons in CDR mice.

As a further control, we turned to orientation selectivity (OS). Previous studies in cat have demonstrated that experience-independent mechanisms first establish a rudimentary map of orientation preference (Crair et al., 1998). However, fully refined OS is only maintained with normal visual experience. In mice, development of orientation selectivity is complete by P24, an age just before OD plasticity (Fagiolini and Hensch, 2000). We found that CDR severely degraded OS (Fagiolini et al., 1994, 2003). By P55, the OS tuning curve was broader in CDR than in LR mice (data not shown) and the distribution of OS indices was shifted to the left (Fig. 5*C*). Thus, CDR uncouples the trajectory of different RF properties in visual cortical circuits, notably allowing for the maturation of visual acuity and a CP for amblyopia without sensory experience.

Rapid recovery of acuity by light exposure in older CDR mice

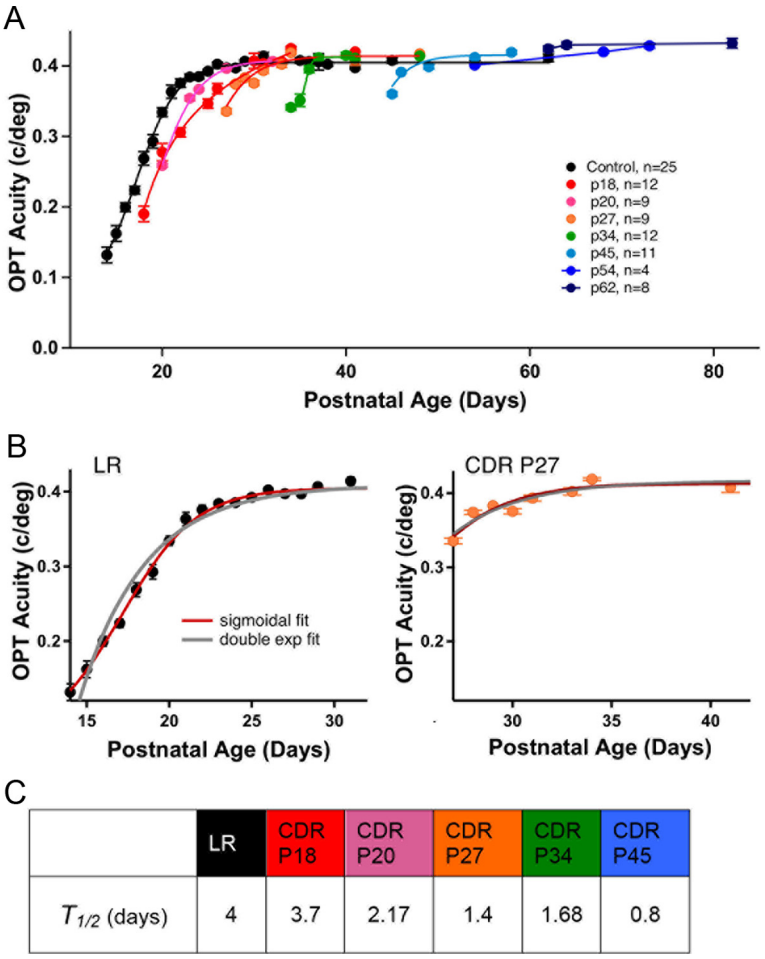
Next, we examined the time course of cortical maturation in CDR mice upon exposure to light. P18–P62 CDR mice were brought into a lighted room for OPT testing, then returned to an environment on a 12 h light/dark cycle. The OPT response for each mouse was then monitored longitudinally over 1–2 weeks. Figure 6*A* shows the summary of the average OPT response of these mice over days after light exposure. The potential for plasticity in the cortical circuitry is still present in CDR mice. Interestingly, CDR mice exposed to light for the first time exhibited a faster rate of maturation than that of LR mice.

Notably, the time course of sensory-dependent OPT threshold maturation depended on the age of first light exposure. This time course was quantified as the time to reach half of the total OPT threshold improvement for each age group of CDR mice ($T_{1/2}$; Fig. 6*C*). The $T_{1/2}$ for OPT threshold increase in LR mice was 4 d. In contrast, the $T_{1/2}$ for CDR mice exposed to light at P20 and P34 were 2.17 and 1.68 d, respectively. This acceleration in maturation cannot simply be explained by the fact that there has already been partial development of the circuit in the absence of visual stimulation. For example, P34/35 CDR mice exhibit a reduced OPT threshold of 0.34 cpd comparable to that of P20 LR mice (Fig. 2). However, OPT threshold reaches adult levels over the course of 3 d in CDR mice, in contrast to the 6–7 d (from P20) in LR mice (Fig. 6*A*).

We found that the time course of OPT threshold maturation in normally reared mice is best fit with a sigmoidal function consistent with cooperative interactions during development (Fig. 6*B*). Single- or double-exponential functions provide poor fits of the normal developmental time course as assessed by χ^2 test (Fig. 6*B*, $\chi^2 = 40$ vs 123.6 for sigmoidal vs double exponential fit, respectively). In contrast, χ^2 values for sigmoidal and exponential fits describing spatial vision development of CDR mice exposed to light are similar (as an example, $\chi^2 = 54$ and 57.5 for sigmoidal vs double exponential fit, respectively; Fig. 6*B*, right). These findings indicate that the visual circuit undergoes different connectivity "states" during development, and that circuit refinement after prolonged visual deprivation does not follow the same path or process as in normal development.

Discussion

Our data demonstrate that visual deprivation from birth (CDR) disrupts spatial vision, OS and OD plasticity to different degrees, and at distinct developmental time points. Notably, CDR does not halt maturation of both OPT threshold and visual acuity but rather slows it down. Ongoing spontaneous activity is sufficient to drive particular circuit maturation (Rochefort et al., 2011), whereas other RF functions can rapidly reach adult levels only upon the introduction of vision. The different experimental assays that we use are weighed toward specific visual system circuits, namely OPT reports largely subcortical function whereas VEP reflects mainly thalamocortical function and single unit recordings report intracortical function. Under LR conditions, these circuits develop in a coordinated fashion. Darkness from birth still allows subcortical circuits to develop, although at a slower rate. On the other hand, cortical circuits are more severely affected and retain plasticity when re-exposed to light (Fagiolini et al., 2003). Thus OPT threshold and VEP acuity increase in the dark and lose their plasticity in response to MD in adulthood, whereas intracortical circuits remain plastic. Consequently, subcortical and cortical maturation is not coordinated over development in CDR mice (Wang et al., 2010).



	LR	CDR P18	CDR P20	CDR P27	CDR P34	CDR P45
$T_{1/2}$ (days)	4	3.7	2.17	1.4	1.68	0.8

Figure 6. Exposure to light triggers rapid maturation of visual function in CDR mice. ***A***, Mean OPT response of CDR mice upon exposure to light at different ages. ***B***, Comparison of sigmoidal (red) versus double exponential (gray) fits to the time course of OPT threshold during normal development (left, LR) and for CDR mice after exposure to light at P27 (right, CDR P27). $\chi^2 = 40, 23, 2.2, 54, 2.45, 21.6$ vs $123.6, 18.4, 4.2, 57, 5, 2.74, 2.38$ for sigmoidal vs double exponential fit, respectively, for the following conditions: LR, P18 CDR, P20 CDR, P27 CDR, P34 CDR, and P45 CDR. ***C***, $T_{1/2}$ of maturation from different ages.

Experience-independent visual circuit maturation

The finding that spatial vision can develop without sensory experience is surprising given previous studies in other species. Behavioral testing of acuity in CDR cats, involving a learned jumping task, shows no evidence of maturation at 4–10 months (Timney et al., 1978, 1980). Apart from the added confound of learning, no VEPs have been performed to corroborate these behavioral findings, although recordings from V1 neurons are sluggish or unresponsive (Mower et al., 1983; Mower, 1991). In cat, the CP for OD plasticity begins ~3 weeks, and tapers over 4–8 months (Cynader et al., 1980). After CDR, single-unit recordings demonstrate that OD plasticity is extended up to 12 months consistent with a permanently immature state (Cynader and Mitchell, 1980; Mower et al., 1981, 1983). In CDR rats, single-unit and VEP recordings similarly exhibit immature neuronal function, reduced acuity and persistent OD plasticity up to P60 (Bartoletti et al., 2004).

One potential explanation for differences across species is that the time course of cortical development is significantly prolonged in cat and rat as compared with mice. The window of OD plasticity is known to scale with species lifespan (Berardi et al., 2000). Longer periods of DR may then be needed to detect maturation of acuity independent of vision in higher mammals, in addition to other species differences. However, alternative explanations involving distinctions between behavioral assays or different mechanisms driving visual system development in various species cannot be ruled out.

Dissociating ocular dominance plasticity and amblyopia in the dark

Our findings of degraded OS and persistent OD plasticity in favor of the nondeprived eye in adult CDR mice are consistent with previous findings in higher species (Cynader and Mitchell, 1980; Mower et al., 1983; Crair et al., 1998). Unexpectedly, in striking contrast to the plasticity found in visual cortical neurons, amblyopia was induced by MD only during a surprisingly short critical period (P45–P60).

During the classical CP, MD produces a rapid shift of neuronal spiking response in favor of the open eye, which is detected by single-unit electrophysiology from the primary visual cortex (V1). These functional changes are accompanied by spine pruning and regrowth, and rewiring of horizontal connections and thalamic afferents (Antonini and Stryker, 1993, Antonini et al., 1999; Trachtenberg and Stryker, 2001). MD is always followed by loss of visual acuity and contrast sensitivity in the deprived eye that cannot be recovered after the CP has closed (Dews and Wiesel, 1970; Giffin and Mitchell, 1978; Fagiolini et al., 1994; Kiorpes et al., 1998; Mower and Kaplan, 1999; Prusky et al., 2000; Morishita et al., 2010).

One possible explanation for the dissociation of OD shift and loss of acuity is that the two properties reflect distinct cortical circuits with differential dependence on experience. OD, re-

corded at the level of single cells, is evaluated across all cortical layers, although binocularity is more pronounced in the output layers II/III (Shatz and Stryker, 1978). On the other hand, visual acuity, quantified by VEP recordings, is assessed in the time domain by measuring the peak-to-trough amplitude and peak latency of the principal negative component (N1) which is heavily weighted toward deep layer III and thalamo-recipient layer IV. We hypothesize that CDR may have a larger effect on intracortical circuitry than on thalamocortical projections.

Anatomical studies support this model. Thalamocortical afferents innervate OD columns even without retinal input (Crowley and Katz, 2000), and thalamocortical arbors no longer remodel in response to MD in adult CDR cats (Mower et al., 1985). Functionally, the CP in LR animals ends earlier in layer IV than in other layers (Mower et al., 1985; Daw et al., 1992) and OD plasticity is more pronounced outside layer IV (Shatz and Stryker, 1978; Gordon and Stryker, 1996; Beaver et al., 2001). Neurons in more superficial layers retain the ability to change RF properties, as well as exhibit LTP and LTD, throughout life (Kirkwood et al., 1996; Trachtenberg et al., 2000). Together, if our model is correct, we would predict that MD after prolonged CDR should not induce amblyopia in cats.

The susceptibility of different circuits to sensory experience may also reflect distinct molecular signaling pathways for plasticity in excitatory versus inhibitory neurons (Hensch, 2005; Maffei and Turrigiano, 2008). The classical delay of OD plasticity by CDR can be prevented by directly enhancing inhibition in the dark: by infusion of benzodiazepines (Iwai et al., 2003), Otx2 homeoprotein (Sugiyama et al., 2008), or genetic overexpression of BDNF (Gianfranceschi et al., 2003). It will be of great interest to see whether these manipulations also impact the maturation of acuity in the dark.

Recovery rates in CDR mice suggest altered trajectory of acuity development

Upon light exposure, kittens dark-reared for 4 months exhibit progressive improvement of visual acuity over several months (Timney et al., 1978). This is a similar time course as that of normal acuity development in LR animals (Giffin and Mitchell, 1978). However, kittens dark-reared for longer periods of time (6–10 months) exhibit an even slower improvement of vision that never reaches the acuity levels of normally reared cats. In striking contrast, we found the opposite change in the rate of OPT threshold maturation upon light exposure of CDR mice (Fig. 6*C*). With longer periods of CDR, recovery of OPT threshold accelerated with the age of initial light onset. Unlike cats, the rate of improvement is faster in mice visually deprived for >20 d than for LR animals.

These results support the idea that circuit development in the dark follows a different trajectory than in LR mice. Maturation in LR animals follows a sigmoidal time course suggesting that circuit development passes through different states or involves distinct mechanisms that interact cooperatively. In contrast, the time course of CDR maturation is distinctly different from that of LR animals; a double-exponential fit describes the trajectory much better than a sigmoidal relationship (Fig. 3). In addition, experience-dependent maturation of previously CDR animals exposed to light also does not follow the same trajectory as LR animals: at some ages, an exponential relationship can describe the maturation as well as a sigmoidal function (Fig. 6*B*).

Overall, this suggests that certain states/mechanisms are bypassed or lost in sensory-deprived animals because of dissociable development of different features of visual function. Microarray and proteomic studies during experience-dependent development and cortical plasticity support our finding that CDR does not simply delay cortical development but may activate signaling pathways that specifically maintain or increase the plasticity potential of V1 (Majdan and Shatz, 2006; Tropea et al., 2006; Dahlhaus et al., 2011). Differential impact of CDR on the "molecular brakes" that would normally limit CP development may need to be considered further.

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