Visual Cortical Recovery From Reverse Occlusion Depends on Concordant Binocular Experience

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Faulkner, Stuart D., Vasily Vorobyov, and Frank Sengpiel. Visual cortical recovery from reverse occlusion depends on concordant binocular experience. J Neurophysiol 95: 1718–1726, 2006. First published December 14, 2005; doi:10.1152/jn.00912.2005. The effects of early monocular deprivation on visual acuity and visual cortical responses can be reversed quickly if vision is restored to the deprived eye and the other eye is deprived instead, a procedure known as reverse occlusion. However, recovery of vision through the originally deprived eye (ODE) is not stable. Following re-opening of the recently deprived (originally nondeprived) eye (ONDE), vision in the ODE typically deteriorates rapidly, possibly because of competitive interactions, whereas vision in the ONDE also remains compromised, resulting in bilateral amblyopia, the reasons for which are unknown. Here we monitor the physiological changes in the visual cortex during recovery from reverse occlusion in a longitudinal study, using optical imaging of intrinsic signals and single-cell recording in anesthetized cats. We show that a brief period of just 4 days of concordant binocular vision intercalated between the two periods of monocular deprivation allows close to equal responses to develop through both eyes, both in terms of cortical territory and orientation selectivity. In contrast, with no binocular vision or discordant binocular experience, cortical territory dominated by the ONDE is significantly reduced, and orientation tuning of cells dominated by the ONDE is wider than that of cells dominated by the ONDE. These results support the notion that a brief period of binocular vision is sufficient to prevent bilateral acuity loss caused by reverse occlusion.

INTRODUCTION

Although an early period of monocular deprivation (MD) in kittens has severe behavioral; (Giffin and Mitchell 1978; Mitchell et al. 1977) and physiological consequences (Hubel and Wiesel 1970; Shatz and Stryker 1978; Wiesel and Hubel 1963, 1965), it is not necessarily irreversible. Substantial physiological and behavioral recovery can be achieved if vision is restored sufficiently early to that eye, i.e., during the critical period (Giffin and Mitchell 1978; Mitchell 1988; Mitchell and Gingras 1998; Mitchell et al. 1977). The effects of deprivation are indeed reversed between the two eyes when the deprived eye is reopened and the nondeprived eye is closed at the same time, a procedure known as reverse occlusion (RO) (Blakemore and Hawken 1982; Blakemore and Van Sluyters 1974; Blakemore et al. 1978; Malach et al. 1984; Movshon 1976a,b; Movshon and Blakemore 1974). While the initial effect of RO is a decrease of responsiveness through the originally nondeprived eye (ONDE), recovery of responses through the originally deprived eye (ODE) follows rapidly and within a few days results in most cells becoming dominated by the ODE (Mioche and Singer 1989).

Disappointingly, in many situations, the improvement in visual acuity seen for the initially deprived eye during RO is not maintained when normal binocular vision is restored, i.e., when the recently deprived eye is re-opened after some time. Furthermore, vision in the latter eye remains poor. Mitchell et al. (1984) and Murphy and Mitchell (1986, 1987) deprived kittens in one eye from natural eye opening for varying periods, then reverse occluded for varying periods of time. The predominant outcome of all these regimens was bilateral amblyopia. Despite this impairment of behavioral visual acuity after binocular recovery, cortical ocular dominance and binocularity appeared normal (Murphy and Mitchell 1986), suggesting that simply increasing the efficacy of cortical responses to visual stimulation did not lead to recovery of visual acuity. Therefore the spatial resolution of the most sensitive neurons, not the total number of units responsive to each eye may be a better predictor of behavioral acuity. Moreover, the LGN cells receiving input from the originally nondeprived eye have been shown to remain hypertrophied during reverse occlusion (Sloper et al. 1984). This could lead to larger receptive fields and overlapping ocular dominance columns resulting in a binocular cortex but poor visual acuity (Murphy and Mitchell 1986).

Mitchell (1991) demonstrated that if the initially nondeprived was occluded for only a few hours a day instead of continuously, such part-time RO resulted in recovery of normal acuity, contrast sensitivity, and vernier acuity in both eyes. This result suggested that a period (or periods) of concordant binocular vision may be critical in promoting normal visual acuity in both eyes of reverse-occluded animals. Murphy et al. (2002) tested this hypothesis by introducing a 4-day period of binocular vision (BV) after the initial period of deprivation and before the reverse lid-suture. They found that the acuity in the originally deprived eye (ODE) reached levels very similar to that of a normal cat (~7.5 cycle/°) in contrast to animals without the intermediate period of BV. Acuity in the ONDE also improved to a moderately high level (~3 cycle/°) when binocular vision was restored after RO. In contrast, misalignment of the eyes (strabismus) during the intermediate period of BV eliminated its beneficial effect on visual acuity in both eyes.

Earlier experiments have demonstrated that binocular vision after a period of MD leads to a faster restoration of responses through the deprived eye than RO. Kittens with binocular experience after MD begin to recover vision in the deprived eye 12–30 h before reverse-occluded subjects. However, final
visual acuity attained through the deprived eye is superior in RO kittens (Mitchell and Gingras 1998; Mitchell et al. 2001). Again concordance of binocular experience is a critical factor for the extent of recovery observed (Kind et al. 2002).

While the preceding findings suggest that incorporating a period of binocular vision into RO paradigms will improve the outcome in terms of visual acuity, the underlying physiological events remain unknown. This is the first longitudinal physiological study of the effects of RO and subsequent recovery. Using optical imaging, visually evoked potentials, and single-cell recordings, we examine whether a brief period of binocular vision, intercalated between the two periods of lid-suture, affects the cortical response to RO and whether physiological parameters parallel behavioral measures of recovery after restoration of normal vision. We further study which significance the nature of the period of binocular experience (concordant or discordant) has for whether or not recovery of responses through both eyes will be consolidated.

METHODS

Experiments were performed on 10 kittens bred in a closed colony; all procedures were carried out in accordance with UK Home Office regulations on animal experimentation [Animals (Scientific Procedures) Act 1986] and the European Communities Council Directive 86/609/EEC. Efforts were made to minimize animal suffering and to reduce the number of subjects used.

Rearing paradigms

From 2 wk of age (P14), kittens were monocularly deprived in one eye (the right eye) for 3 wk. At P35, three of these kittens were reverse sutured for 18 days (RO group) before allowing binocular recovery for a period of 3–4 wk. In another three of these kittens, a period of 4 days of binocular vision was introduced at the end of the first period of MD, before RO (4DBV). In the remaining four kittens, a period of 4 days of binocular vision was also given. However, vision was discordant through the use of goggles containing prisms each with a 4° convergent offset for each eye (equivalent to a total convergence of the visual axes by 8°; 4Ddecorr). Kittens were trained to wear the goggles without prisms for brief daily periods, several days before the end of the first period of MD. On opening of the deprived eye, the animals were kept in the dark while they recovered, then the goggles were fixed on the head (with the prisms in place). During the day, kittens were checked hourly to ensure the goggles remained in place. Some animals were fitted with an additional collar to help prevent removal of goggles. Every morning the goggles were removed for a period of 5 min. The lenses were cleaned and each eye was checked for any signs of irritation and rinsed with sterile eye drops.

The animals in the first two experimental groups (RO and 4DBV) were littersmates from three litters, whereas the animals in the 4Ddecorr group were from a further two litters.

At the end of the RO period, a chamber was implanted and optical imaging was carried out on the same day, 2–4 days (typically 3 days) later, then at weekly intervals until the final session after ≥3 wk of recovery. In one animal, imaging was carried out at the termination of the first period of MD, then again after 4 days of binocular vision, on the day of termination of RO and two further times during binocular recovery. In three kittens, all with 4 days of de-correlated vision, visual acuity was assessed by recording visually evoked potentials (VEPs) after each optical imaging session. In all cases, after the final imaging session, single-cell recordings were carried out.

We chose a period of MD long enough for maximal physiological changes to occur—these are almost saturated after 4 days (Movshon and Dürsteler 1977; Olson and Freeman 1975), whereas anatomical changes would have been limited to a retraction of deprived-eye terminals with no significant expansion of nondeprived eye terminals (Antonini and Stryker 1993, 1996). Reverse occlusion was implemented at the height of the critical period to ensure maximal effects and to guarantee that the recovery from RO would still occur during the critical period (see Mitchell 1991; Murphy and Mitchell 1986, 1987).

Optical imaging and analysis

All surgical procedures were performed under sterile conditions. Anesthesia was induced with an intramuscular injection of ketamine (25–40 mg/kg) and xylazine (3–4 mg/kg). Animals were intubated and were placed in a stereotaxic frame. They were artificially ventilated (50–60% N2O, 40–50% O2, 2.0–2.5% isoflurane, decreased to 1.5% during imaging). Electrocardiograms (ECGs), electroencephalograms (EEGs), end-tidal CO2, and rectal temperature were monitored continuously. A 4% glucose in saline solution was infused intravenously at 3 mg kg−1 h−1 throughout the experiment.

Optical imaging of primary visual cortex was performed as described previously (Bonhoeffer and Grinvald 1996). In the initial imaging session, the scalp was incised and retracted. A circular craniotomy was performed above area 17, and a titanium chamber was cemented onto the skull. The cortical surface was carefully kept clear and kept free from any traces of blood using Sugi sterile swabs (Kettenbach, Eschborn, Germany). The chamber was filled with sterile silicon oil (dimethylpolysiloxane, Sigma-Aldrich, Poole, UK) and was sealed with a glass cover-slip. To reduce invasiveness to a minimum and to protect the underlying cortex from both mechanical damage and the risk of infection, the dura was left intact for all but the terminal imaging session.

Images were captured using an enhanced differential imaging system (Imager 2001; Optical Imaging, Mountainside, NJ), with the camera focused ∼500 µm below the cortical surface and an illumination wavelength of 700 nm. Visual stimuli, produced by a stimulus generator (VSG, Cambridge Research Systems, Rochester, UK), consisted of high-contrast, sinusoidally modulated gratings (0.15–0.75 cycle/°) of four different orientations, drifting at a temporal frequency of 2 Hz, presented to the two eyes separately in randomized sequence, interleaved with trials in which the screen was blank. Activity maps were analyzed using IDL software (RSL, Boulder, CO). Single-condition responses (averages of 48–64 trials per eye and orientation) were divided by responses to the blank screen and by the sum of responses to all four orientations (“cocktail blank”) (Bonhoeffer and Grinvald 1996) to obtain iso-orientation maps. The actual signal used for subsequent quantitative analysis was reflectance change (DR/R) for each pixel, given at 16-bit precision. For illustrations, signals were range-fitted such that the 1.5% most responsive (least responsive) pixels were set to black (white), and signal amplitude was displayed on an 8-bit gray-scale. After each but the final experiment, the chamber was half-filled with agar containing an antiinflammatory steroid (0.1 ml of Dextafort, Intervet UK, Milton Keynes, UK). The rest of the chamber was filled with sterile silicone oil and sealed with a glass cover-slip; anesthesia was then suspended. Kittens were given systemic antibiotics (0.1 ml of Betamox, Norbrook Laboratories, Carlisle, UK; or 0.1 ml of Metacam, Boehringer-Ingehelm, Ingelheim, Germany) and analgesics (0.1 ml off Ketofen, Merial, Harlow, UK); these injections were given prophylactically for 5 days. Animals were allowed to recover and were then returned to their mother and littersmates. Completion of the final imaging session was followed by electrophysiological recording (see following text); afterwards, animals received an overdose of barbiturate.

Ocular dominance maps were obtained by dividing the sum of responses to all four orientations through one eye by the similar sum of responses through the other eye. Resulting maps were high-pass filtered (cut-off, 2.2 mm). This filtering was essential for removal of a global DC signal component which is superimposed on the spatially
restricted stimulus selective ("mapping") signal (Bonhoeffer and Grinvald 1996; Zepeda et al. 2004). Given that OD domains have an average size of ≤1 mm, the mapping signal was unaffected by the chosen high-pass filter. Moreover, repeated analyses with different cut-offs yielded essentially the same results as described below. To remove high-frequency noise, the images were also smoothed (Gaussian smoothing over 7 × 7 pixels). Within a region of interest that comprised the visually responsive part of the images in both cortical hemispheres, excluding blood vessel and other artifacts, pixels were assigned to the left and right eye, respectively, depending on whether their value was >1 or <1.

Orientation preference maps were calculated by vectorial addition of four blank-divided iso-orientation maps, and pseudo-color coded. Orientation selectivity indices were calculated for responses at each pixel as

$$\text{OSI} = \frac{\sqrt{(R_{0} - R_{45})^2 + (R_{45} - R_{135})^2}}{R_{0} + R_{45} + R_{90} + R_{135}}$$

where $R_{0}$, $R_{45}$, $R_{90}$, and $R_{135}$ represent the responses (reflectance changes) in each of the four iso-orientation maps (Bonhoeffer et al. 1995). The OSI represents the magnitude of the orientation preference vector divided by the sum of all responses; it is therefore normalized to values between 0 and 1.

Electrophysiology

In all experimental animals, we determined quantitative orientation/direction tuning curves of single units, recorded with glass-insulated tungsten microelectrodes, and discriminated by their spike shapes using Brainware software (TDT, Alachua, FL). Left- and right-eye responses to drifting gratings (of optimum spatial frequency) of 16 different directions in 22.5° steps were averaged over 5 trials of 1.5-s duration. Smooth tuning curves were fitted to the data points based on Fourier analysis (Wörgötter and Eysel 1987) and preferred orientation and half-width of tuning at half-height were determined from these curves. Ocular dominance was calculated as the ratio of total responses to contra- and ipsilateral eye stimulation.

A binocularity index (BI) (Murphy and Mitchell 1986) was calculated for each animal based on the number of cells placed in each OD group (using the 7 classes devised by Hubel and Wiesel 1962)

$$\text{BI} = \frac{[\text{OD}4] + 2/3 \times [\text{OD}3] + [\text{OD}5]}{N} + 1/3 \times [\text{OD}2] + [\text{OD}6] \times 100$$

where [ODx] indicates the number of cells in OD group x and N the total number of cells recorded.

Visually evoked potentials

In three animals we determined VEPs through either eye and each cortical hemisphere, recorded with a silver electrode placed on the dura. Left- and right-eye responses (low-pass, 300 Hz; high-pass off) to horizontal contrast-reversing gratings of a range of spatial frequencies from 0.05 to 6.4 cycle/° and a reversal rate of 2 Hz were averaged over 20 trials of 3-s duration, using software written in LabVIEW (National instruments, Austin, TX). Results were analyzed using IDL software (RSI, Boulder, CO). Responses to the six contrast reversals per trial were superimposed and then averaged across trials. Total VEP amplitude was defined as the peak-to-trough voltage difference of the averaged response.

RESULTS

Ocular dominance

In all three rearing paradigms, reverse occlusion had a profound effect on the cortical territory occupied by the two eyes.

In one animal, imaging was carried out after the initial period of monocular deprivation (at P35) and again after 4 days of binocular vision (Fig. 1). All other animals were imaged...
after the second period of monocular deprivation (of the ONDE; see Methods). After the initial deprivation, a dramatic loss of deprived-eye territory was seen (which occupied just 21.6 and 11.8% of the contra- and ipsilateral hemispheres, respectively). After 4 days of binocular vision, a clear increase of that eye’s cortical territory could be seen, occupying 52.4 and 31.2% of the contra- and ipsilateral hemispheres, respectively (Fig. 1, 2nd column). Thus recovery was extensive but not complete. Subsequent suturing of the originally nondeprived eye for 20 days caused a drastic reduction of that eye’s cortical territory and an almost total loss of orientation selectivity of the residual responses (Fig. 1, 3rd column). During the same time, the previously deprived eye became dominant, and its responses regained orientation selectivity. After just 3 days of binocular recovery, the ONDE regained much of the previously lost cortical territory as well as some orientation selectivity (Fig. 1, 4th column). Recovery was complete in both respects after 17 days of binocular vision (Fig. 1, 5th column).

The immediate effect of reverse occlusion was similar in all three experimental groups. A typical example of each is shown in Fig. 2, A–C (left column). In all animals, the ONDE (left eye) responses were sparse and weak on termination of the second period of deprivation, occupying only 15.8–23.8 and 15.8–18.7% of the cortex, respectively, of the hemispheres contralateral and ipsilateral to that eye. These territories were significantly smaller than values obtained for either eye of normal animals for the contralateral and ipsilateral hemispheres, respectively (Fig. 3, A and D).

It was not until after a brief period of recovery that differences between the three rearing paradigms became apparent. While significant recovery of ONDE territory was observed in all three groups, the extent varied among them. The recently deprived eye (ONDE) of all three animals with immediate RO occupied just 39.7 ± 7.9 and 27.3 ± 3.2% of the contra- and ipsilateral hemispheres. In contrast, in those animals that received an intercalated period of binocular vision, the same eye

![Figure 2](http://jn.physiology.org/)

**FIG. 2.** Recovery from reverse occlusion (RO) in 3 kittens. A: animal in which reverse lid-suture of the left eye (ONDE) immediately followed the initial period of monocular deprivation in the right eye (ODE). B: kitten with 4 days of concordant binocular vision between the periods of monocular vision. C: kitten with 4 days of discordant binocular vision. In each animal, V1 was imaged on the day of re-opening of the recently deprived (originally nondeprived) left eye, and then again 2–4 days later and again ~3 wk later. For each time point, 3 maps from the left hemispheres (ipsilateral to the ONDE) are displayed: ocular dominance maps for the ONDE as well as orientation maps obtained through the ONDE shown as “angle maps” and as “polar maps” (see Methods). Graphs plot the relative cortical territories dominated by the ONDE (- - -) and ODE (—), respectively, and the orientation selectivity of ONDE vs. ODE responses for the left hemispheres, in each case against the age of the animals. Scale bar, 1 mm.
occupied a greater portion of the cortex (Fig. 3B). In the contralateral hemisphere, the ONDE occupied 50.4 ± 9.3 and 53.3 ± 3.8% for animals with 4DBV and 4Ddecorr, respectively. In the ipsilateral hemisphere, the ONDE of animals with an intercalated period of binocular vision again occupied a similar proportion of the cortex (41.5 ± 6.2 and 42.5 ± 5.6% for groups 4DBV and 4Ddecorr, respectively. The differences between the RO group on the one hand and the 4DBV and 4Ddecorr groups on the other hand were significant in the hemisphere ipsilateral to the ONDE (P < 0.05, 2-tailed t-test) but did not quite reach significance for the contralateral hemisphere.

Figures 2A and 3C highlight the fact that after an extended recovery period (>21 days), there was only a small further increase in cortical territory occupied by the ONDE for the RO group, the average value being 37.6 ± 3.4%. In comparison, in the 4Ddecorr group, (Figs. 2C and 3C), the ONDE actually lost cortical territory after longer recovery to occupy just 41.8 ± 6.8 and 28.9 ± 0.8% of the hemispheres contralateral and ipsilateral to the ONDE, respectively. However, this loss did not reach significance (ANOVA, P > 0.05).

In contrast to RO and 4Ddecorr groups, in the 4DBV group, recovery of ONDE territory continued with further concordant binocular experience (Fig. 2B). Overall the ONDE came to occupy equal cortical territory in the ipsilateral hemisphere (49.3 ± 7.8%), much more than in RO and 4Ddecorr groups (Fig. 3C), significantly so with respect to the 4Ddecorr group (ANOVA, P < 0.05). The gain of ONDE territory was similar in the contralateral hemisphere (62.2 ± 1.7%), with the ONDE coming to occupy significantly more territory (Fig. 3C) than in the combined RO and 4Ddecorr groups (ANOVA, P < 0.05). In fact, the territory occupied by the ONDE after 3–4 wk of recovery was slightly (but not significantly) larger than the territory occupied by each eye in the contralateral hemisphere (59.5 ± 3.9%) and ipsilateral hemisphere (40.5 ± 3.9%), respectively, of four normally reared animals of a similar age (Fig. 3D).

**Orientation selectivity**

In all kittens, the weak responses that were obtained through the ONDE immediately on termination of RO were mostly lacking in orientation selectivity in both hemispheres (Figs. 1, 3rd column, and 2, A–C, 1st set of images). After termination of RO, orientation selectivity partially recovered after a few days of binocular vision. However, substantial recovery was not seen until after ≥14 days of binocular experience (Figs. 1, 5th column, and A–C, 3rd set of images). We calculated the orientation selectivity index, OSI (see METHODS) for both the ONDE and ODE and took their ratio as a relative measure of the ONDE orientation selectivity. Figure 3, E–G, summarizes the OSI data from all animals in this study. The period of RO had an almost identical immediate effect on orientation selectivity in all three rearing paradigms (OSIs of 0.51–0.57, Fig. 3E). A brief period of recovery (Fig. 3F) resulted in an increase in the OSI through the ONDE. This was more pronounced in animals that had received an intermediate period of vision than in kittens with just RO. However, the differences in the OSI ratios between the three rearing paradigms were not significant (ANOVA, P > 0.05). Orientation selectivity through the ONDE increased further with extended binocular recovery, reaching: 1.1 ± 0.03, 1.0 ± 0.07, and 1.2 ± 0.2 in the RO, 4DBV, and 4Ddecorr groups, respectively (Fig. 3G). Overall, orientation selectivity of ONDE responses was slightly (but not significantly) greater than that of ODE responses in all groups.
Recovery of neuronal response properties after RO

Single-cell recordings were carried out in all kittens at the end of the binocular recovery period. In total, 576 cells were recorded in roughly equal numbers from both cortical hemispheres in each group of animals. Most neurons (415 or 72%) were binocular in that they had at least some input from both eyes; 161 neurons (28%) were truly monocular (OD category 1 or 7) (Hubel and Wiesel 1962). In all experimental groups, we found a preponderance of cells dominated by the right ODE. The degree of binocularity was reduced compared with age-matched control animals but did not differ significantly between the three experimental groups (Fig. 4, A–D).

We determined orientation selectivity in terms of half-width at half height (HWHH) of responses to monocular stimulation. Because there were no consistent differences between the tuning curves for the two eyes in binocular cells in any group, only the responses through the eye that yielded the stronger response are described in the following. In the two experimental groups without an intercalated period of concordant binocular vision, orientation selectivity of ODE responses remained inferior to that of ONDE responses. In RO kittens, for those cells dominated by the ONDE (52 or 35%), the HWHH was 30.0 ± 1.7°, whereas the HWHH of the 96 cells (65%) responding preferentially to the ODE was significantly broader (P < 0.005, t-test) at 38.7 ± 2.1° (Fig. 4E). In 4Ddecorr kittens, for those cells dominated by the ONDE (61 or 32%), the HWHH was 29.6 ± 1.77°, whereas the tuning of cells dominated by the ODE (126 or 68%) was significantly broader (P < 0.05, t-test) at 34.5 ± 1.3° (Fig. 4G). In contrast, in those animals that received an intercalated period of concordant binocular vision (4DBV), the HWHH of cells dominated by the two eyes did not differ significantly (P > 0.05, t-test). For the 76 cells (32%) dominated by the ONDE, HWHH was 31.5 ± 1.7°. For the 165 cells (68%) dominated by the ODE, HWHH was slightly broader at 35.2 ± 1.4° (Fig. 4F). It is worth noting that we did not observe any obvious differences in response rates or variability of firing that could have contributed to differences in tuning width between the groups.

By comparison, 96 neurons recorded from two normal age-matched control animals exhibited a mean HWHH of 29.5 ± 1.4° (Fig. 4H), which was virtually identical to the tuning width of ONDE-dominated neurons in the three experimental groups, but narrower than the tuning width of ODE dominated cells (P < 0.05 4DBV and 4Ddecorr groups, P < 0.01 for RO group).

We also considered separately the minority of cells in each group of experimental animals that were strictly monocular, driven by either the ODE or ONDE. In the RO group, 39 ODE cells exhibited a mean ± SE HWHH of 44.9 ± 4.8°, 10 ONDE cells a HWHH of 27.5 ± 6.2°. In the 4DBV group, 44 ODE cells had a mean HWHH of 40.9 ± 4.1°, and 14 ONDE cells a HWHH of 32.1 ± 6.8°. Finally, among the 4Ddecorr animals, 39 ODE cells exhibited a mean HWHH of 36.5 ± 2.9°, 15 ONDE cells a HWHH of 30.8 ± 4.6°. In each of these groups, the differences in tuning width between ODE and ONDE dominated neurons was slightly more pronounced than for the total populations, but these differences were not significant.

Recovery of visual acuity

Recovery of visual acuity was assessed in the 4Ddecorr group. Visually evoked potentials were recorded from both cortical hemispheres and for each eye in three animals on the same day as optical recordings. For each eye and hemisphere, VEP amplitude was plotted against spatial frequency of the contrast reversing grating stimuli, and high-frequency cut-offs were determined (Fig. 5A).

Immediately on termination of RO, evoked responses through the ONDE were very weak, with a cut-off value of 0.5 ± 0.03 cycle/°. Through the ODE, VEP responses were strong, with a mean cut-off value of 2.7 ± 0.4 cycle/°. Typical responses from one animal are shown in Fig. 5A.

In contrast to the recovery of OD territory in the same group, recovery of VEP responses through the ONDE continued beyond the first few days after re-opening. In fact, it was not until ~7 days that substantial recovery was seen (Fig. 5B), and after ~4 wk, the high-frequency cut-off value through the ONDE reached 1.8 ± 0.1 cycle/°. In parallel, there was a decrease in cut-off value of the ODE responses. Acuity fell by nearly 40% to just 1.7 ± 1.8 cycle/° within 3 days of reintroducing binocular vision. With further binocular experience, the acuity of the ODE did not change much (Fig. 5B), finishing slightly below that of the ONDE at 1.6 ± 0.1 cycle/°.
DISCUSSION

The consequences of monocular deprivation can be ameliorated by reverse occlusion. However, in most cases functional recovery through the ODE is short-lived, whereas vision through the recently deprived (ONDE) eye is compromised, resulting in bilateral amblyopia (Mitchell et al. 1984; Murphy and Mitchell 1986, 1987). The discrepancy between poor behavioral outcome and relatively normal cortical ocular dominance distribution (Murphy and Mitchell 1986) has so far remained unexplained.

The results presented here demonstrate the importance of an intercalated period of concordant binocular input (even for as brief a period as 4 days) for physiological recovery of the recently deprived eye after a regime of reverse occlusion. Without it, the recently deprived (ONDE) eye fails to fully recover cortical territory, and the ODE continues to dominate the cortex. The long-term effect of the intercalated period of binocular vision is all the more remarkable as there is no immediate effect on visual cortical responses on termination of RO.

Despite the overall dominance over V1 by the ODE, the tuning width of individual cells driven by the ODE in animals lacking concordant binocular experience (RO and 4Ddecorr) is significantly broader than that of cells dominated by the ONDE. Overall, the physiological events during recovery from RO that we describe can for a large part account for the changes in visual acuity that have been reported.

Anatomical consequences of RO

Monocular deprivation lasting ≥7 days causes a substantial loss of geniculocortical terminals serving the deprived eye in layer 4 of V1 (Antonini and Stryker 1993, 1996; Hubel et al. 1977; LeVay et al. 1980; Shatz and Stryker 1978). However, a complementary expansion of nondeprived eye terminals is seen only after several weeks of deprivation (Antonini and Stryker 1993, 1996; LeVay et al. 1980).

RO does not, however, appear to have the same detrimental effect on arbor morphology (of the recently deprived eye) as MD. After 10 days of RO, ONDE arbours are larger and more complex than deprived-eye arbours after a similar period of MD (Antonini et al. 1998). Physiological recordings in the same study showed that despite a reversal of ocular dominance during RO, the responses through the ONDE remained much more selective for orientation than the deprived-eye responses after a single period of monocular deprivation (Crair et al. 1997). This is confirmed by our finding that although the ODE occupies a larger cortical area than the ONDE, the orientation tuning of neurons dominated by the latter is narrower. Possibly terminals from the originally nondeprived eye do not retract during the period of reverse occlusion (as shown by Antonini et al. 1998) but instead are rendered ineffective by synaptic suppression or interocular inhibition (Blakemore et al. 1982; Sillito et al. 1981). Another possibility is that the recovery of responses through the ODE does not solely depend on the re-establishment of connectivity that was present before deprivation. Intracortical circuitry may be more stable than the thalamocortical input; it may be maintained during the initial period of MD and allow rapid regeneration of selective responses during RO (Antonini et al. 1998). Such a preserved “scaffold” may also explain the finding of nearly identical orientation maps through the two eyes of RO animals despite the absence of any shared binocular visual experience (Gödecke and Bonhoeffer 1996). To explain the differences in outcome among our three experimental groups, we suggest that an intercalated 4-day period of concordant binocular vision serves to stabilize any intracortical circuitry shared between the two eyes, which will in turn allow rapid recovery of ONDE responses after RO.

Physiological consequences of RO

With MD starting very shortly after eye opening, the deprived eye is visually almost naïve. Even prolonged binocular vision after such early deprivation does not permit full recovery of cortical binocularity or orientation selectivity of cells in ferrets (Liao et al. 2004). It is therefore not surprising that in one cat, imaged immediately after the initial period of MD and again after 4 days of binocular vision (Fig. 1), recovery of ODE responses in terms of cortical territory was not as complete as in animals with MD of later onset (Faulkner et al. 2005). Moreover, there was only minimal recovery of orientation selectivity. Nevertheless, the effect of a subsequent period of RO on a cortex with a comparatively normal physiological OD distribution appears to be very different from that on an almost entirely monocular cortex (in the case of immediate RO).
The physiological outcome in animals with an intercalated period of de-correlated binocular vision is in agreement with the outcome in terms of behavioral acuity described by (Murphy et al. 2002) for the same rearing paradigm. They compared kittens in which a period of MD was followed by 4 days of normal binocular vision or 4 days of strabismic vision followed by reverse occlusion. Permanent recovery of visual acuity was observed only in case of an intercalated period of concordant binocular vision.

Poor bilateral visual acuity after RO has in the past been attributed to the possibility of a small-angle strabismus developing as a side-effect of deprivation (see Blake et al. 1974). However, this hypothesis is contradicted by the comparatively normal binocularity of cortical cells reported by Murphy and Mitchell (1986). In the present study, we too found only a slightly higher than normal incidence of monocular cells and no significant differences between our three rearing paradigms.

It is tempting to speculate that changes in N-methyl-D-aspartate (NMDA) receptor expression mediate the physiological response to RO. NMDA receptor dependent plasticity is the likely substrate of synaptic potentiation (LTP) and synaptic depression (LTD) in response to manipulations of visual input including dark-rearing, monocular deprivation and recovery from either condition (for review, see Bear 2003). Blockade of NMDA receptors prevents the shift in ocular dominance caused by MD and RO in cat visual cortex (Bear et al. 1990; Daw et al. 1999).

Interestingly, Trepel and colleagues (1998) found a transiently patchy distribution of the obligatory NMDA receptor subunit 1 (NMDAR1) in kitten V1, which was prevented by monocular deprivation. However, just 4 days of subsequent binocular vision was sufficient to express NMDAR1 patches (Trepel et al. 1998). The 4-day period of concordant binocular vision experienced by one of the groups of kittens in our study may have facilitated NMDA receptor expression during the binocular recovery period.

**Causes and prevention of bilateral amblyopia**

A picture of the underlying causes of bilateral amblyopia after RO is gradually evolving. It appears that the recovery of the originally deprived eye during the period of reverse lid suture is not complete either in anatomical or physiological terms unless an episode of concordant binocular vision is intercalated between the periods of monocular vision or some binocular experience is allowed daily during the period of reverse lid suture. This is reminiscent of our earlier finding that recovery from monocular deprivation (by simply reopening the deprived eye, without reverse lid suture) requires correlated binocular input and is far less complete if the two eyes are misaligned (Kind et al. 2002).

A broad orientation tuning of V1 cells dominated by the ODE could in part underlie the poor visual acuity in that eye, especially considering that the behavioral test of visual acuity in cats entails an orientation discrimination task. On the other hand, recovery of vision in the recently deprived (ONDE) eye appears to be compromised by the fact that it does not “hold on” to its cortical territory in the course of the recovery period unless concordant binocular experience has been provided before reverse lid suture.

Although formation of functional properties of V1 such as orientation selectivity and segregation into ocular dominance domains appears to be largely independent of early visual experience, the basic scaffold of functional connections (from both eyes) depends on concordant binocular visual input during the critical period for consolidation (Crair et al. 1998). Without such input, visual responses do not mature and eventually degrade even if normal visual inputs are restored at a later stage.

Although clinical results indicate that patching the good eye of a child is required to improve spatial resolution of an amblyopic eye, several reports demonstrate swift and substantial recovery of vision in the affected eye in infants after corrective surgery to unilateral cataract, where no patching of the good eye was used (Jacobson et al. 1983; Maurer et al. 1999). Clearly, in those cases, re-establishing concordant binocular vision sufficed to promote recovery of acuity in the deprived eye.

Part time reverse occlusion in kittens can lead to good acuity through both eyes (Mitchell 1991). Clinical practice is moving in this direction for part time occlusion therapy (of the nonamblyopic eye) in infants that have undergone corrective unilateral cataract surgery (Mitchell and MacKinnon 2002). Not only is extensive binocular vision incorporated into contemporary patching therapy regimens permitting recovery and maintenance of acuity, but also in some cases contributing to the development of stereopsis (Birch et al. 1993). This strategy is supported by recent evidence that brief daily periods of binocular vision are sufficient to fully prevent acuity loss caused by monocular deprivation (Mitchell et al. 2003).

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**References**


