Brief daily binocular vision prevents monocular deprivation effects in visual cortex

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Abstract

Even short periods of early monocular deprivation result in reduced cortical representation and visual acuity of the deprived eye. However, we have shown recently that the dramatic deprivation effects on vision can be prevented entirely if the animal receives a brief period of concordant binocular vision each day. We examine here the extent to which the cortical deprivation effects can be counteracted by daily periods of normal experience. Cats received variable daily regimens of monocular deprivation (by wearing a mask) and binocular vision. We subsequently assessed visual cortex function with optical imaging of intrinsic signals and visually evoked potential recordings. Regardless of the overall length of visual experience, daily binocular vision for as little as 30 min, but no less, allowed normal ocular dominance and visual responses to be maintained despite several times longer periods of deprivation. Thus, the absolute amount of daily binocular vision rather than its relative share of the daily exposure determined the outcome. When 30 min of binocular exposure was broken up into two 15-min blocks flanking the deprivation period, ocular dominance resembled that of animals with only 15 min of binocular vision, suggesting that binocular experience must be continuous to be most effective. Our results demonstrate that normal experience is clearly more efficacious in maintaining normal functional architecture of the visual cortex than abnormal experience is in altering it. The beneficial effects of very short periods of binocular vision may prevent any long-term effects (amblyopia) from brief periods of compromised vision through injury or infection during development.

Introduction

The mammalian cerebral cortex displays remarkable experience-dependent plasticity, in particular during a critical period early in life. Wiesel and Hubel showed in their classic experiments (Wiesel & Hubel, 1963, 1965) that an early period of monocular deprivation (MD) causes neurons in the primary visual cortex (V1) to become driven almost exclusively by the open, non-deprived eye. This physiological effect is mirrored anatomically by shrinkage of the deprived eye's ocular dominance (OD) columns (Shatz & Stryker, 1978). Vision through the deprived eye is severely degraded or lost altogether (Giffin & Mitchell, 1978; Mitchell, 1988) in this animal model of deprivation amblyopia (Mitchell, 1989).

Although it is economical to allot more of the available neuronal resources to the processing of information from the good eye instead of wasting half on the deprived one, such dramatic changes to cortical function and visual capability after only transient periods of MD would appear to be maladaptive. Transient conditions such as injury or infections of lid margins or conjunctiva could have disastrous consequences, effectively rendering one eye useless. Although even periods of deprivation as short as 6 h can cause significant shifts in ocular dominance (Frank *et al.*, 2001), we and others have recently shown that following a period of continuous monocular exposure, recovery is rapid (within a few days) and substantial, if the deprived eye is simply re-opened and the subsequent binocular experience is concordant (Mitchell & Gingras, 1998; Mitchell *et al.*, 2001; Kind

et al., 2002). Here we investigated how successive periods of monocular and binocular vision each day are weighted in terms of their influence on visual cortical function. If all types of visual experience were equally instructive, then one would expect to observe cortical deprivation effects of graded severity proportional to the ratios of monocular and binocular visual exposure. A recent behavioural study indicated that this might not be the case. Mitchell *et al.* (2003) found that 2 h of binocular experience per day allowed kittens to develop normal grating acuity for both eyes despite the animals receiving 5 h of monocular vision each day. In this context, it made no difference whether the period of daily mixed monocular and binocular experience followed a month of dark-rearing from birth, or whether it was preceded by a month of normal binocular visual experience, which presumably led to the establishment of a normal, predominantly binocular V1.

The intriguing behavioural consequences of mixed normal and abnormal experience pose questions concerning its neural basis and, in particular, the role of visually driven activity in development of the visual cortex. In an earlier single-cell study (Olson & Freeman, 1980), kittens that had experienced daily regimens of 4 h of monocular and 14 h of normal binocular vision exhibited, perhaps unsurprisingly, normal ocular dominance distributions. We set out to determine the minimum requirements for maintaining normal V1 function. Kittens were reared with various regimens of daily periods of monocular and binocular exposure. The V1 ocular dominance architecture was assessed through optical imaging of intrinsic signals, a technique that gathers information from a very large number of cells and thus overcomes the problem of sampling bias inherent to single-cell recordings (Bonhoeffer & Grinvald, 1996; Zepeda *et al.*, 2004). We

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determined the relative cortical area of ocular dominance domains dedicated to the two eyes, as well as visually evoked potentials as a physiological measure of acuity (Berkley & Watkins, 1973; Freeman *et al*., 1983).

Materials and methods

Rearing details

All procedures were approved by local ethical review and covered by UK Home Office licences. Forty-seven cats were used in this study. Selective rearing was carried out for about 3 weeks, starting at the peak of the critical period between postnatal days 30 and 35. Subjects were subdivided into two cohorts: 23 subjects were permitted 7 h of visual exposure per day, the other group of 24 subjects only 3.5 h. An additional three subjects received just 0.25 h of visual exposure, all of which was binocular. For the remainder of the day animals were kept in complete darkness, together with their mother. The room in which they were reared contained cardboard boxes, toys, and furniture for environmental enrichment. Animals were encouraged to play to keep them awake and active during the period of visual exposure.

Each animal was assigned to one of various rearing regimens, which determined the order and duration of MD and binocular exposure (BE) it received on a daily basis (see Table 1). The period of BE was 0 h, 0.25 h, 0.5 h, 1 h or 2 h and either preceded or followed the period of MD. Six subjects were permitted two binocular periods of 0.25 h which flanked the monocular period. Four subjects in each cohort served as controls and received binocular vision only. Deprivation was carried out by means of completely opaque eye patches made from surgical face masks that were fastened with velcro bands.

During the period of visual exposure the animals were monitored regularly to readjust the masks, if necessary. Whenever a mask slipped and permitted binocular experience this was recorded and the average maximal time of binocular experience was calculated for each subject at the end of the rearing period. After the first few days subjects

TABLE 1. Rearing conditions and actual daily binocular exposure of all animals included in this study

Group	N (OI)	N (VEP)	VE (h)	BE (h)
0/3.5	1	1	3.5	0.08 –
0.25/3.5	5	5	3.5	0.28 ± 0.01
0.5/3.5	4	5	3.5	0.56 ± 0.04
1/3.5	4	4	3.5	1.04 ± 0.02
2/3.5	1	1	3.5	2.02 –
3.5/3.5	4	4	3.5	3.52 ± 0.02
0/7	4	3	7.0	0.08 ± 0.03
0.25/7	1	1	7.0	0.32 –
0.5/7	3	3	7.0	0.58 ± 0.03
1/7	4	4	7.0	1.09 ± 0.06
2/7	3	4	7.0	2.05 ± 0.01
7/7	4	4	7.0	6.98 ± 0.02
2 × 0.25/3.5	3	4	3.5	0.54 ± 0.02
2 × 0.25/7	3	3	7.0	0.56 ± 0.02
0.25/0.25	3	3	0.25	0.25 ± 0.00

The first column (labelled 'Group') gives the nominal rearing condition in hours of binocular exposure per hours of total daily visual exposure. The animals which received two equal periods of binocular exposure flanking a period of monocular exposure are listed, respectively, as $2 \times 0.25/3.5$ and $2 \times 0.25/7.0$. The second and third columns give the number of animals per group for which optical imaging maps or VEP data were obtained. The fourth column (VE) gives the total daily visual exposure in hours. The final column (BE) gives the actual daily binocular exposure averaged across all animals in each experimental group (±SEM).

usually adapted well to wearing the eye patches, and any unintentional binocular exposure caused by removal of a mask rarely exceeded a few minutes.

Optical imaging

On the day of data collection, animals did not receive any visual exposure. At the end of the dark-rearing period, anesthesia was induced by i.m. administration of ketamine (20–40 mg/kg) and xylazine (4 mg/kg). Atropine (0.2 mg/kg i.m.) was given to reduce mucus secretion. A tracheotomy was performed and the animals were intubated and placed on a heating blanket in a stereotactic frame. Subjects were artificially ventilated with an N_2O/O_2 mixture (60 : 40) and isoflurane (2–3% during surgery; 1–1.5% during data collection). End-tidal CO_2 (3.5–4.0%), rectal temperature (37.5–38.0 °C), electrocardiogram (150–200 b.p.m.) and electroencephalogram were monitored throughout the experiment and adequate measures taken if any of the values diverged from the described target values. Atropine and phenylephrine were administered to the eyes, which were fitted with gas-permeable contact lenses, to protect them and to focus the animal's vision onto the stimulus display.

An intravenous catheter was inserted in one of the hind legs for administration of drugs and for a continuous infusion of 4% glucose in saline at a rate of 3 mL/kg/h; the infusion solution also contained dexamethasone (Dexafort, Intervet, UK; 0.2 mg/kg/h) for prevention of cortical oedema, and gallamine triethiodide (Sigma, UK; 10 mg/kg/h) for prevention of eye movements. The posterior portion of the lateral gyrus, containing the central visual field representation of the primary visual cortex, was exposed in both hemispheres through craniotomy. For some animals the dura was also removed because its opacity and vascularization would have compromised data collection. The cortical surface was carefully cleared and kept free from any traces of blood using Sugi sterile swabs (Kettenbach, Eschenburg, Germany). A titanium chamber was cemented to the skull and sealed on the inside with dental wax. The chamber was filled through an inlet with silicone oil and closed with a cover slip (Bonhoeffer & Grinvald, 1996).

Initially, the exposed brain was illuminated with green light and a reference image of the surface vascular pattern was taken. Subsequently, the cortex was illuminated with red light at 700 nm. Intrinsic signals were recorded using an enhanced differential imaging system (Imager 2001, Optical Imaging Inc., Mountainside, NJ, USA), with the camera focused approximately 500 μm below the cortical surface. Images were therefore obtained primarily from layers 2/3, which are thought to play a key role in initiating cortical plasticity in response to altered visual experience (Trachtenberg *et al*., 2000; Trachtenberg & Stryker, 2001). The imaged area subtended about 12 mm by 9 mm.

The animal's eyes were focused on a 21-inch computer monitor (distance, 33 cm), on which stimuli were displayed by a visual stimulus generator (VSG Series 3; Cambridge Research Systems, Rochester, UK). They consisted of high-contrast sinusoidal or square-wave gratings (0.1–0.6 cycles per degree; mean luminance, 38 cd/m^2) of four different orientations (0°, 45°, 90° and 135°), drifting at a temporal frequency of 2 Hz, randomly interleaved with trials in which the screen was blank. Activity maps were analysed using IDL software (RSI, Boulder, CO, USA). Single-condition responses (averages of 48–64 trials per eye and orientation) were divided (a) by responses to the blank screen, and (b) by the sum of responses to all four orientations ('cocktail blank'; Bonhoeffer & Grinvald, 1996) to obtain iso-orientation maps. Orientation preference maps were calculated by vectorial addition of four blank-divided iso-orientation maps, and pseudo-colour coded.

Stimuli were presented to the two eyes separately in randomized sequence by means of shutters placed in front of the animal. Ocular dominance maps were calculated by dividing all responses to one eye by the responses to the other. The actual signal used for subsequent quantitative analysis was reflectance change ($\Delta R/R$) for each pixel, given at 16-bit precision. For analysis of the relative strength of responses through the two eyes, images were only low-pass filtered (smoothed). For analysis of areas responding preferentially through one or the other eye (see below), images were additionally high-pass filtered well above the periodicity of ocular dominance domains (cut-off, 200 pixels = 7.8 mm) to level the image intensity across the region of interest. For illustrations, signals were range-fitted such that the 1.5% most responsive (least responsive) pixels were set to black (white), and Gaussian averaging over 6 pixels was applied to remove high-frequency noise. Signal amplitude was displayed on an 8-bit grey-scale.

To quantify cortical territory occupied by the two eyes, for each hemisphere a region of interest (ROI) was defined using IDL software (RSI). We manually excluded blood vessel and other artefacts using an image of the cortical surface taken under green-light illumination for guidance. Based on differential responses to gratings of high (0.4–0.6 cycles per degree) and low (0.1–0.2 cycles per degree) spatial frequency, analysis was restricted to V1 (Bonhoeffer *et al.*, 1995). In order to minimize subjectiveness in defining the ROI, IDL software shifted the ROI by ± 10 pixels in *x*- and *y*-coordinates and calculated mean results across all shift conditions. The ratio of the numbers of pixels responding more strongly to the left and the right eye, respectively, were calculated. Finally, the percentages of pixels responding to the deprived eye and non-deprived eye were averaged across both hemispheres.

Visually evoked potential (VEP) recording

After imaging data acquisition was completed, the chamber was reopened and the silicone oil replaced with saline for VEP recording. A silver ball electrode was placed on the surface of the primary visual cortex near the representation of the area centralis (approximate Horsley–Clarke coordinates, P4 L2). The recorded signal was amplified by a factor of 20 000 and low-pass filtered (cut-off, 300 Hz). Usually, four recordings were made, recording from each hemisphere and stimulating each eye separately. Stimuli were displayed on a computer screen at a distance of 100 cm and consisted of phase-reversing square wave horizontal gratings of 98% contrast that varied only in spatial frequency, typically from 0.14 to 2.26 cycles per degree. For animals that showed a very pronounced deprivation effect, an additional set of low frequencies (0.05–0.4 cycles per degree) was used for the deprived eye. Gratings reversed contrast at a rate of 1 Hz and drifted upwards at a velocity of 0.1 cycles/s. Moreover, a blank screen was used to measure the baseline response. Stimuli were presented to the left and right eye separately for 3 s, corresponding to six contrast reversals, with interstimulus intervals of 3 s. Responses were averaged across the six contrast reversals per presentation and across 20 presentations of each stimulus using software written in LabVIEW (National Instruments, Austin, TX, USA). The resulting signals therefore constituted the responses to 120 contrast reversals each per stimulus. The total amplitude of the VEP signal was defined as the difference in voltage between the signal peak and the subsequent trough within a 500-ms window.

As a physiological measurement of visual acuity, the VEP cut-off point was determined from the VEP amplitude vs. spatial frequency curve. We fitted a straight line through the final 3–4 descending data points, and calculated the spatial frequency of its intersection with a line corresponding to the blank response plus standard error. Additionally, a ratio of the VEP amplitudes through the two eyes was calculated by dividing the sum of the amplitudes in response to the four lowest spatial frequencies for the deprived eye by the same sum obtained for the non-deprived eye.

After all data collection was completed, the animal was killed via an i.v. overdose of barbiturate.

Results

Effects of rearing regimens on ocular dominance maps

The relative representation of the two eyes in V1 as a function of daily binocular exposure was determined from ocular dominance maps generated by optical imaging. Figure 1A shows typical examples from the first cohort, which was permitted 7 h of total daily visual experience. The top row of maps shows responses through the deprived eye (dark patches), and the bottom row of maps from the
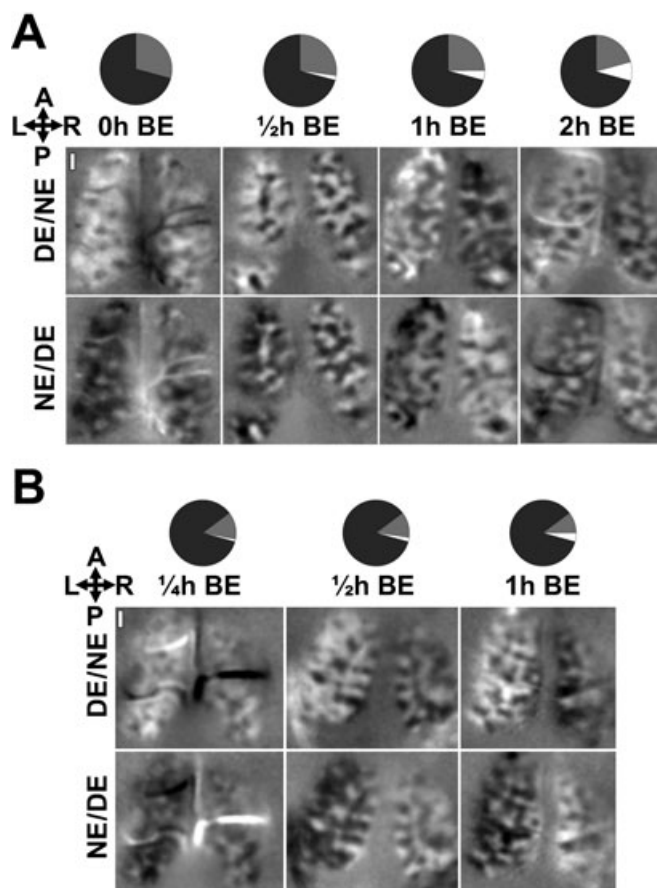

FIG. 1. Ocular dominance maps of representative subjects from various rearing conditions. For each subject, the experimental condition is denoted by the icon above the pair of ocular dominance maps obtained from V1 for left- and right-eye stimulation, respectively. In the row of maps labelled DE/NE, dark areas correspond to cortical domains activated by the deprived (left, DE) eye, and in the row labelled NE/DE dark areas represent cortical areas responding to the non-deprived (right, NE) eye. Because of the way the OD maps are calculated (see Methods), the images in the two rows are 'negatives' of each other; they are both shown in order to facilitate by-eye comparisons between the effects of the different rearing conditions. (A) Subjects from the cohort with 7 h of total daily visual exposure. (B) Subjects from the cohort with 3.5 h of daily vision. Scale bar, 1 mm.

same animals shows responses through the non-deprived eye. It is immediately apparent that the longer the period of daily binocular exposure (from left to right, 0, 0.5, 1 and 2 h) the larger was the area responding to deprived-eye stimulation. Conversely, in the animal with 0 h of binocular exposure most of the imaged cortex responded exclusively to non-deprived eye stimulation but this over-representation decreased dramatically in the 0.5-h condition and further still in the 1- and 2-h conditions.

The subject displayed from the 0-h condition (i.e. no binocular exposure) exhibited an ocular dominance pattern typical of a kitten monocularly deprived by lid suture (Faulkner *et al.*, 2005), with the deprived eye dominating only 21.1% of the cortical surface (9.7% of cortical territory in the hemisphere ipsilateral to the deprived eye and 32.5% in the contralateral hemisphere). In total, five animals were raised without any binocular exposure (see Table 1). In these animals, the deprived eye dominated, on average 20.3% (± 3.3%, SEM) of the V1 surface, compared with 16.2% reported previously in animals of similar age deprived by lid suture (Faulkner *et al.*, 2005). If anything, the patching regimen appeared to have a slightly less (but not significantly so) detrimental effect on the cortical representation of the affected eye than monocular lid suture.

The animal that was permitted 0.5 h of BE exhibited a slightly reduced representation of the deprived eye, which was dominant for 35.7% of the cortical surface. However, in marked contrast, the two subjects that received 1 or 2 h of BE per day, respectively, exhibited ocular dominance maps typical of normally reared kittens. A contralateral bias was evident in both hemispheres of the latter animals (i.e. the left hemisphere being dominated by the right eye and vice versa), and the deprived eye dominated, respectively, 53.5 and 46.7% of the cortical surface. Therefore, a relatively short daily period of normal visual experience was sufficient to offset completely a much longer period of abnormal, monocular vision (6 and 5 h, respectively).

In order to assess whether the absolute amount of daily binocular vision or the ratio of binocular to monocular experience was the critical factor for the physiological outcome, we examined a second cohort of animals that were allowed 3.5 h of daily vision. Figure 1B shows representative maps from subjects that received, respectively, 0.25, 0.5 and 1 h of binocular exposure. Whereas the first exhibited a marked effect of monocular deprivation on OD architecture (with only 23.3% of the cortex dominated by the deprived eye), the maps from the latter two animals appeared quite normal despite the brevity of daily binocular vision (0.5 h BE, 51.0% deprived-eye territory; 1 h BE, 52.6% deprived-eye territory). By comparison, animals reared with only 0.25 h BE per day, but no MD ($n = 3$) exhibited normal ocular dominance maps (Fig. 2) with roughly equal cortical territory representing the two eyes (46.8 ± 2.0% left-eye territory, compared with 46.7 ± 6.1% in controls from the 3.5-h cohort and 51.2 ± 4.0% in controls from the 7-h cohort, which received, respectively, 3.5 and 7 h of daily binocular exposure).

Quantitative analysis of images from all animals confirmed that brief daily periods of binocular vision offset much longer periods of monocular vision so that the latter had virtually no effect on OD representation in V1. Analysis of surface area data for each cortical hemisphere separately revealed the same principal result; 0.5 h of daily BE was sufficient to maintain a share of territory that was not significantly different from those for the contralateral or ipsilateral eye, respectively, in normal animals (Fig. 3A and B). Of course, because of the contralateral bias in the cortical representation of the two eyes, absolute values differed considerably between the two hemispheres. For further analysis, the results from both hemispheres were averaged for each animal. Figure 3C and D shows for both the 3.5- and 7-h cohorts the mean cortical territory dominated by the deprived eye for
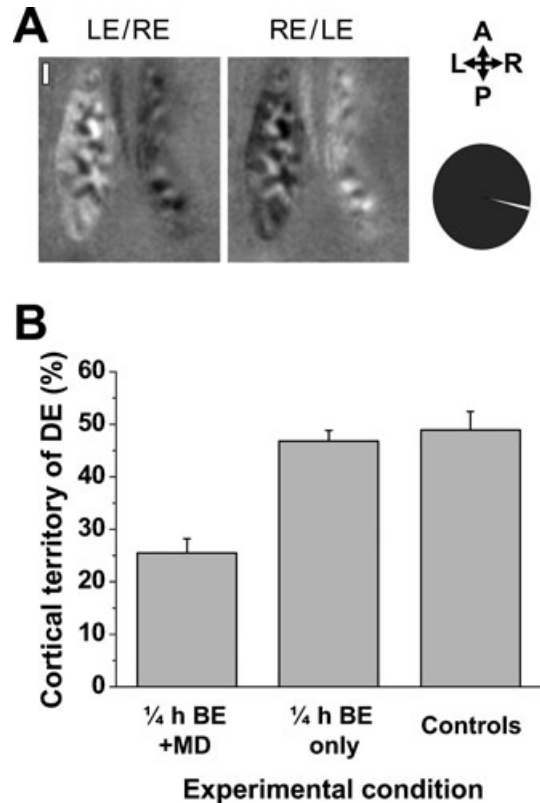

FIG. 2. (A) Ocular dominance maps from a subject with only 0.25 h of binocular exposure per day (and no monocular exposure). Left eye and right eye ocular dominance map are shown. Note that on the right hemisphere, only the medial part of the lateral gyrus could be exposed for imaging. Scale bar, 1 mm. (B) The average cortical territory occupied by either eye in three kittens that had 0.25 h of BE only (and no other, monocular experience) was close to 50%, as in eight control animals that had 3.5 h or 7 h of BE. In contrast, for six animals that had experienced 0.25 h of BE and either 3.25 h or 6.75 h of MD (BE + MD, see Table 1), the deprived eye occupied on average 25.5% of cortical territory.

each condition plotted against either the absolute or the relative duration of binocular exposure. In both cohorts, one-way analysis of variance revealed a statistically significant effect of experimental condition on the mean cortical territory occupied by the deprived eye across both hemispheres (7-h group, $F_{4,10} = 7.52$, $P < 0.01$; 3.5-h group, $F_{4,10} = 6.73$, $P < 0.01$).

As little as 0.5 h of daily binocular vision resulted in only a small deficit in the cortical representation of the deprived eye compared with the non-deprived eye, irrespective of whether the daily total visual experience was 3.5 or 7 h. The fact that similar short absolute binocular exposures in both groups led to the development of near-normal ocular dominance domains (Fig. 3C) suggests that the outcome is determined by the absolute amount of daily BE. This point is further reinforced by the displacement of the 3.5-h group data shown in Fig. 3D to the right of that from the 7-h group when the data are plotted with the BE expressed as a proportion of the total exposure. Goodness-of-fit analysis for the plots of deprived-eye territory as a function of absolute daily BE (Fig. 3C) yielded a χ^2 value of 4.95 ($P > 0.1$), while the same analysis for the deprived-eye territory plots as a function of the daily proportion of BE (Fig. 3D) yielded a χ^2 value of 11.62 ($P < 0.01$). Additionally, when data were grouped according to their absolute daily BE no significant difference between cohorts (two-way ANOVA, $P > 0.1$) was found, but data grouped by their relative daily BE differed significantly between cohorts (two-way
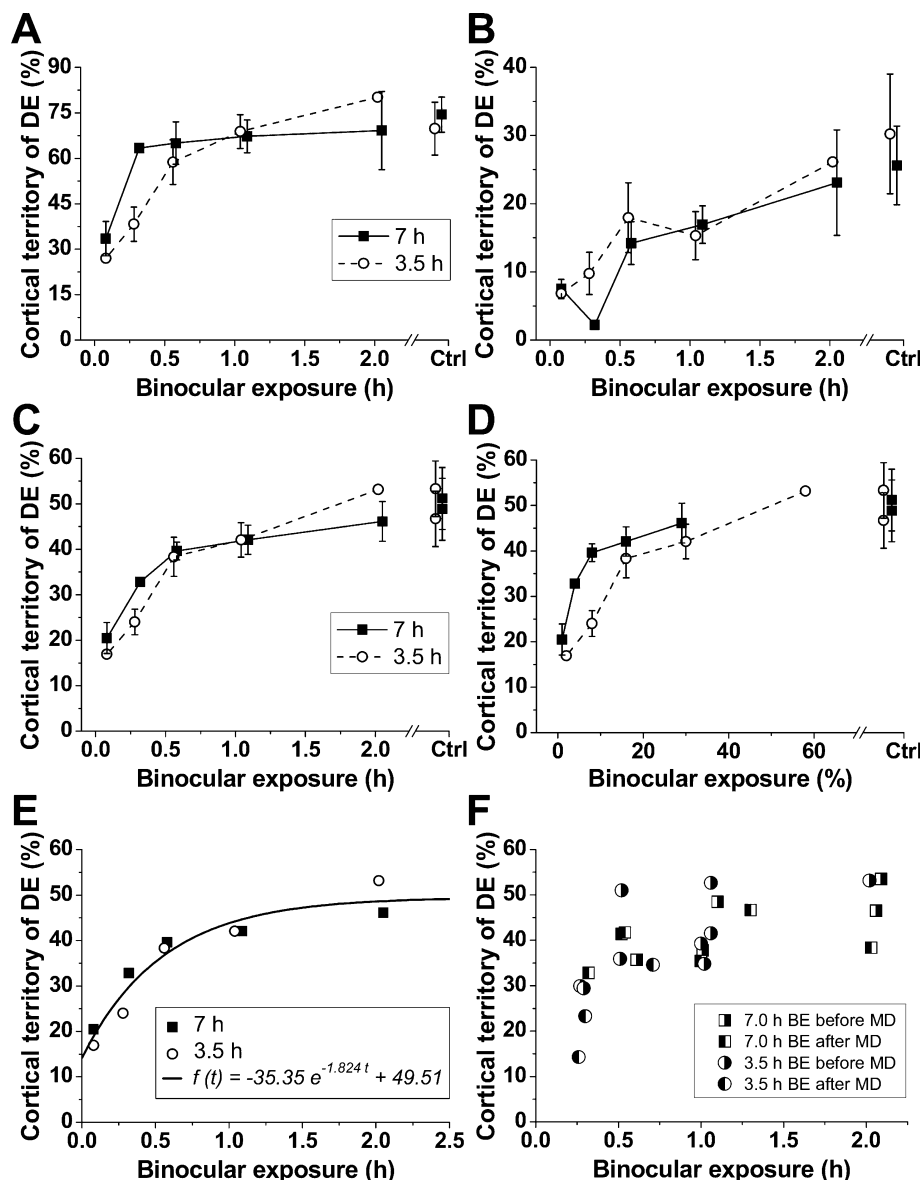
FIG. 3. Ocular dominance balance depends on daily binocular experience. The percentage cortical area dominated by the deprived eye (DE) in each experimental condition is plotted against the amount of binocular vision provided. (A) Data for the hemispheres contralateral to the DE; (B) those for the ipsilateral hemispheres. Data from control animals, which did not receive any monocular experience, are displayed as the mean of two hemispheres obtained when the left or the right eye was taken as the deprived one. C and D depict the mean values averaged across both cortical hemispheres. In C, data are plotted against the absolute duration of daily binocular vision; in D, data are plotted against the percentage of binocular vision relative to total daily vision. Data from control animals are plotted separately for the left or the right eye, because none of the eyes was deprived. Filled squares: 7-h cohort. Open circles: 3.5-h cohort. All data represent mean ± 1 SEM; absence of error bars indicates $n = 1$. In E, pooled data from the 7-h and 3.5-h cohorts are plotted against the absolute duration of daily binocular vision, and an exponential function (see inset) is fitted to the data (solid line). In F, data from all individual animals from both the 7-h and 3.5-h cohorts are shown that received mixed daily binocular and monocular vision, with the shading of symbols indicating whether binocular experience preceded or followed monocular deprivation (see inset).

ANOVA, $F_{3,24} = 5.31$, $P < 0.05$). Taken together this suggests that the absolute amount of binocular exposure is critical in terms of the resulting cortical ocular dominance. We therefore pooled data from the 7- and 3.5-h cohorts and plotted deprived-eye territory against absolute daily BE (Fig. 3E). The data were well fitted with an exponential function ($r^2 = 0.93$), which allowed extrapolation of the amount of BE needed to reduce the deprivation effect by 50% ($t_{50} = 0.39$ h) and by 95%, respectively ($t_{95} = 1.81$ h).

There was no significant difference (two-way ANOVA, $P > 0.1$) in terms of cortical territory occupied by the deprived eye between animals in which the daily period of binocular exposure had preceded the period of monocular deprivation and those in which it had followed it (Fig. 3F).

Because it could be argued that the first effect of brief or intermittent MD on cortical ocular dominance is a weakening of responses through the deprived eye rather than shrinkage of deprived-eye territory, and that this effect would be obscured by our image processing, we also analysed response amplitudes across the previously defined ROIs in both cortical hemispheres in images that were not high-pass filtered, such that overall responsivity (DC level) differences were preserved. Although results inevitably displayed greater variability than those obtained after high-pass filtering, the
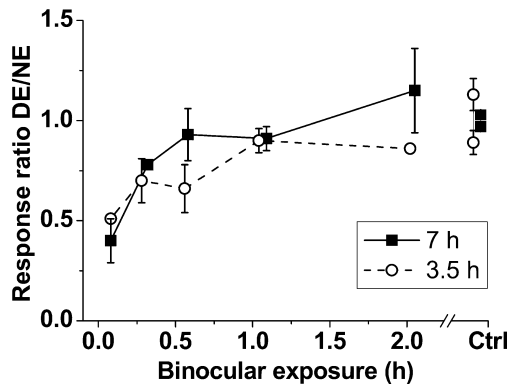
FIG. 4. Ratio of absolute strength of responses through the deprived and non-deprived eyes for the 3.5-h cohort (open circles, dashed lines) and the 7-h cohort (filled squares, solid lines), as determined from the regions of interest in unfiltered images, averaged across both cortical hemispheres (see text).

trend confirmed the results obtained from the area analysis presented above (Fig. 4). In both the 3.5- and the 7-h cohort, the responsivity through the deprived eye was within the normal range for 1 h or 2 h of BE. In the 3.5-h cohort, reduced strength of responses through the deprived eye was observed for animals that had experienced only 0.25 or 0.5 h of daily binocular vision; a more marked reduction in responsivity was seen in both cohorts in the absence of any binocular experience.

Effects of rearing regimens on orientation maps

The effects of our selective rearing regimen on the ocular dominance maps were reflected by similar changes to orientation preference maps obtained through the deprived eye (Fig. 5). Whereas full-time deprived subjects or those receiving just 0.25 h of binocular vision showed little to no orientation-selective responses through the deprived eye (Fig. 5C), in animals with 0.5 h of binocular exposure orientation maps for the deprived eye were normal, albeit weaker than through the non-deprived eye (Fig. 5B), and in animals with 1 h or more of daily binocular vision the maps for the two eyes were qualitatively indistinguishable (Fig. 5A). The lack of orientation maps through the deprived eye in animals with no or very brief binocular experience was primarily due to an overall loss of responsiveness through the deprived eye (see Fig. 4) rather than persistence of strong but non-orientation-selective responses as observed in MD animals with subsequent discordant binocular experience (Kind *et al.*, 2002). These results again indicate that a very brief period of binocular vision each day prevents the loss of orientation selectivity normally associated with monocular deprivation.

Effects of rearing regimens on VEPs

We were interested in establishing whether mixed normal and abnormal visual experience has a similar effect on visual function as it does on cortical ocular dominance. We recorded VEPs as there are several reports (Berkley & Watkins, 1973; Campbell *et al.*, 1973; Harris, 1978; Freeman *et al.*, 1983) that they provide an electrophysiological estimate of visual acuity. Figure 6 displays VEP amplitudes over a range of spatial frequencies for two individual animals as well as population data for the high-frequency cut-off points, which were taken as a measure of visual acuity, and the amplitude ratios for the two eyes. As expected, for a subject monocularly deprived for the

whole of the daily 7-h period of visual experience, large differences in VEP amplitude and cut-off point between responses through the two eyes were observed (Fig. 6B); in fact, in the hemisphere contralateral to the deprived eye, no significant responses could be elicited through that eye at any spatial frequency. By contrast, for an animal that was permitted just 2 h of BE in a total of 7 h of visual exposure per day, these differences were minimal (Fig. 6A).

The difference in VEP cut-off frequency between the two eyes is the most appropriate measure of the acuity deficit in the deprived eye. We found that there was a good correlation overall between cortical territory dominated by the deprived eye and the difference in acuity between the eyes as estimated from the VEP recordings ($r = 0.51$, $P < 0.001$; see Supplementary material Fig. S1). Population data for both cohorts of animals (7 h and 3.5 h per day total visual experience, respectively) are plotted against the absolute and relative daily binocular exposure in Fig. 6C and D. As was found for cortical ocular dominance, very brief daily epochs of 0.5 to 1 h of normal binocular vision were sufficient nearly to eliminate the effects of much longer periods of monocular deprivation. Although for both the 3.5- and the 7-h cohorts only the groups without any BE (0 h BE) differed significantly from the control group in Tukey-Kramer *post-hoc* analysis ($P < 0.05$), the 0.25 h BE group in the 3.5-h cohort also showed significantly reduced acuity in the deprived eye compared with the control group in a one-tailed *t*-test ($P < 0.05$). There was a statistically significant main effect of the duration of binocular exposure on the severity of the impairment of the deprived eye for both the 7-h cohort (ANOVA, $F_{5,13} = 4.67$, $P < 0.02$) and the 3.5-h cohort ($F_{5,14} = 17.16$, $P < 0.001$).

The VEP signal amplitudes reflected the findings from our image analysis. We plotted the ratio of normal and deprived-eye amplitudes against the absolute (Fig. 6E) and relative (Fig. 6F) duration of binocular exposure. These data showed a statistically significant main effect of binocular exposure for the 3.5-h cohort (ANOVA, $F_{5,14} = 8.50$, $P < 0.001$) and the 7-h cohort ($F_{5,13} = 5.01$, $P < 0.01$). Only subjects without any binocular exposure in the 7-h cohort and subjects with 0 h or 0.25 h of BE in the 3.5-h cohort showed responses to stimulation of the deprived eye that were significantly (Tukey-Kramer *post-hoc* analysis, $P < 0.05$) reduced compared with those through the normal eye. As was the case for the OD data, it appears to be the absolute amount of daily BE that determines VEP amplitudes through the deprived eye (Fig. 6E), as indicated by the displacement of the 3.5-h group data to the right of those for the 7-h group when the data are plotted with the binocular exposure expressed as a proportion of the total exposure (Fig. 6F).

### Single vs. split daily periods of binocular exposure

To assess whether the period of binocular exposure must be continuous to prevent deprivation-induced effects or whether it can be accumulated within a 24-h period, we imaged six animals that received 0.25 h (15 min) of BE both before and after the period of MD, giving a total 0.5 h of BE per day. The results are illustrated in Fig. 7. The OD maps from kittens that received 2 × 0.25 h of BE per day exhibited a pronounced deprivation effect and thus were comparable with those from animals which were given a single binocular period of 0.25 h per day rather than to those which were given 0.5 h of BE, resulting in relatively normal OD maps. Because no differences were observed between the 7-h and 3.5-h cohorts, data from the two cohorts were pooled. One-way analysis of variance comparing the 0.25 h of continuous BE, the 2 × 0.25 h of split BE and the 0.5 h of continuous BE conditions revealed a significant main
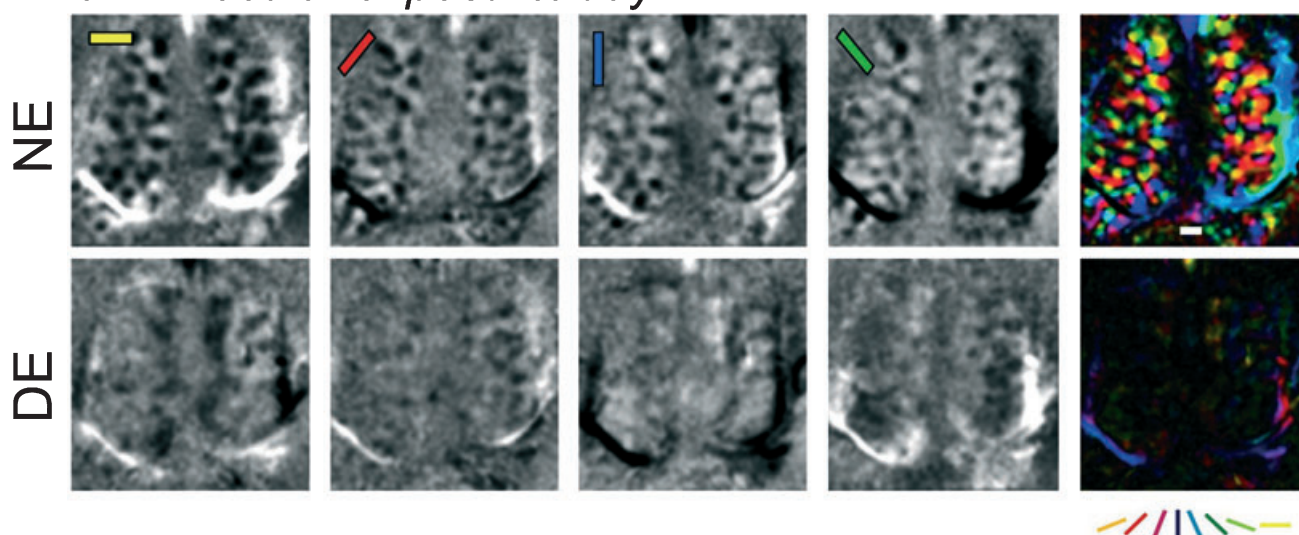
A *2h Binocular exposure/day*
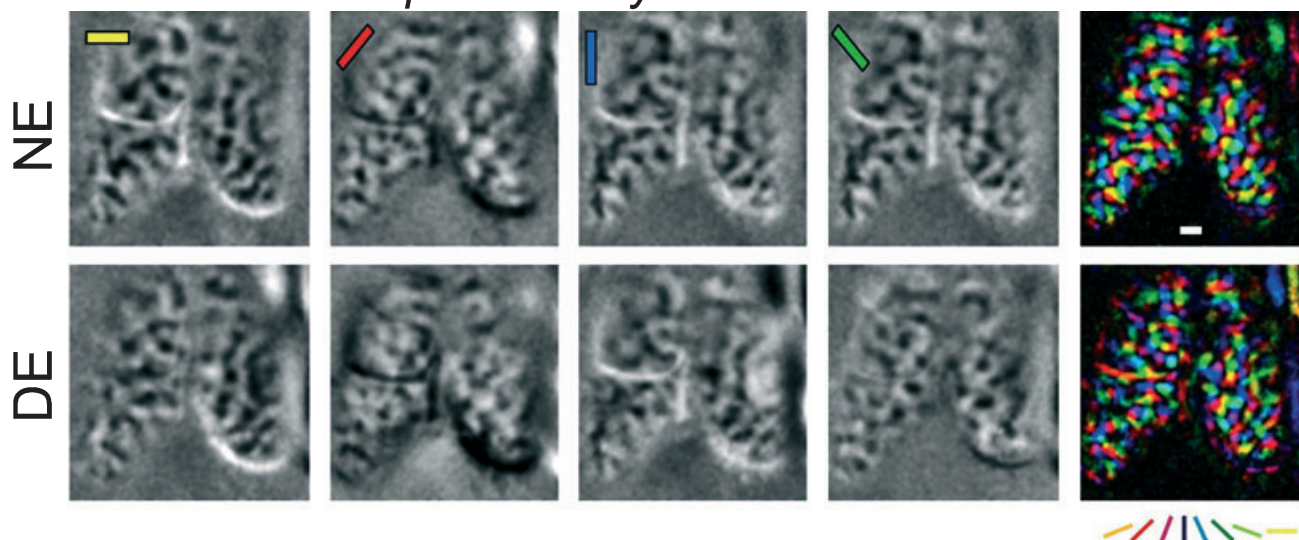
B *0.5h Binocular exposure/day*
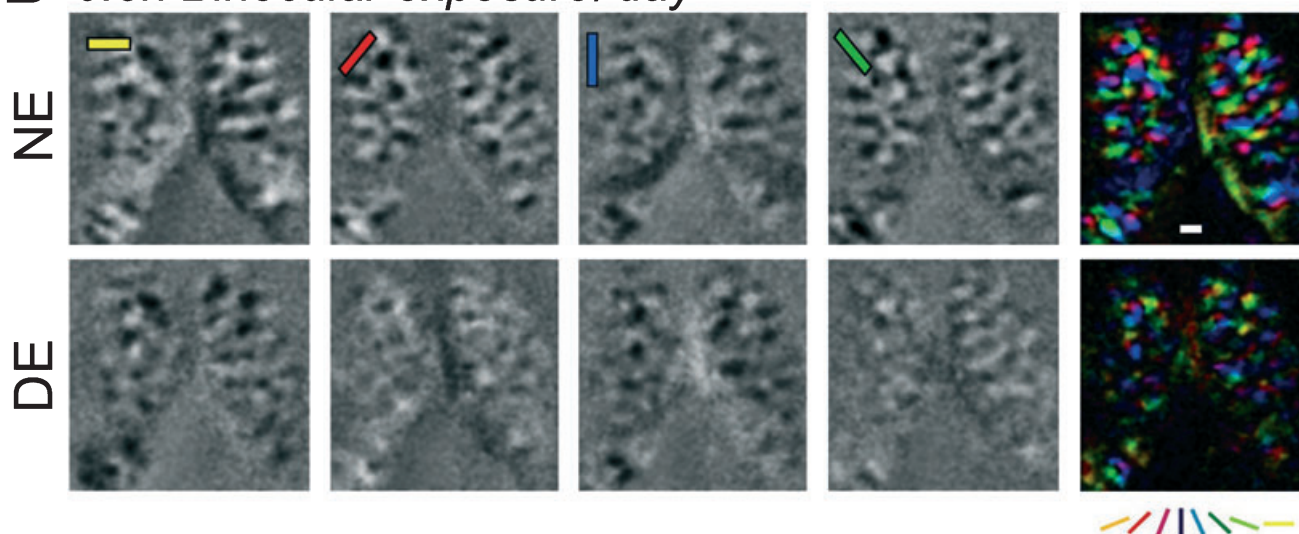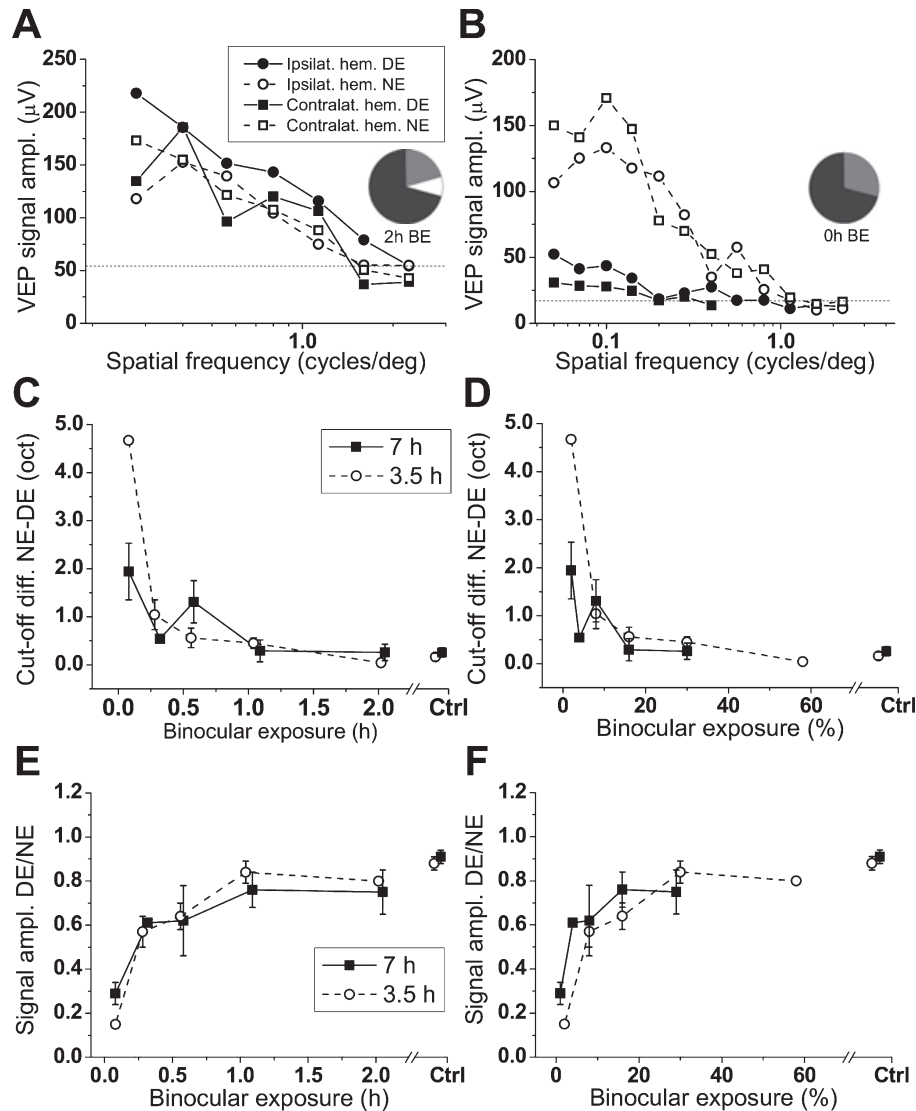
C *0h Binocular exposure/day*

FIG. 6. Visual acuity based on visually evoked potentials (VEPs). (A and B) VEP signal amplitude for two subjects plotted against the spatial frequency of the stimulus. The subject in A received 2 h of binocular vision and 5 h of monocular vision each day. The animal in B was monocularly deprived for the whole 7 h of visual exposure per day. The mean response to the blank screen stimulus plus 1 standard error is indicated by the dotted horizontal line. Filled symbols: deprived eye (DE) responses. Open symbols: non-deprived eye (NE) responses. Circles: ipsilateral hemisphere. Squares: contralateral hemisphere. (C and D) Difference in spatial-frequency cut-off points between the two eyes (in octaves) is plotted against binocular exposure. In C, binocular exposure is plotted in terms of absolute duration of daily binocular vision and in D as percentage of total daily visual exposure. (E and F) Interocular VEP amplitude ratios plotted against binocular exposure. In E, binocular exposure is plotted in terms of absolute duration of daily binocular vision, and in F as percentage of total daily visual exposure. For control animals, which did not receive any monocular experience, the eye eliciting the weaker response was defined as the deprived one. Filled squares: 7-h cohort. Open circles: 3.5-h cohort. All data are mean ± 1 SEM; absence of error bars indicates $n = 1$.

effect of experimental condition on the cortical territory occupied by the deprived eye ($F_{2,16} = 7.02$, $P < 0.01$). Tukey-Kramer *post-hoc* analysis showed a significant difference ($P < 0.05$) between the 0.5 h of BE group and the other two, but no difference between the 0.25 h of continuous BE group and the one that received two separate periods of 0.25 h of BE.

As the VEP amplitudes and high-frequency cut-off points were not significantly different between the animals with 0.25 h of BE and those with 0.5 h of BE, the VEP data from the animals with

2×0.25 h of BE did not allow any reliable conclusions to be drawn (data not shown).

Discussion

Our imaging results support the notion suggested by previous behavioural studies (Mitchell *et al.*, 2003, 2006) that different types of sensory input differ in their influences on cortical development.

FIG. 5. Orientation maps from three subjects that received 2 h (A), 0.5 h (B) and 0 h (C) of daily binocular exposure, respectively. The first four columns of maps show single-condition, cocktail-blank divided iso-orientation maps obtained by stimulation with the orientation depicted by the coloured bar in the top left corner. The right-most column shows 'polar' maps in which orientation-selective domains are colour-coded (see colour key below) and response strength is indicated by intensity. The top row of maps in each figure panel shows maps obtained through the non-deprived eye, and the bottom row shows maps obtained through the deprived eye. Scale bar, 1 mm.
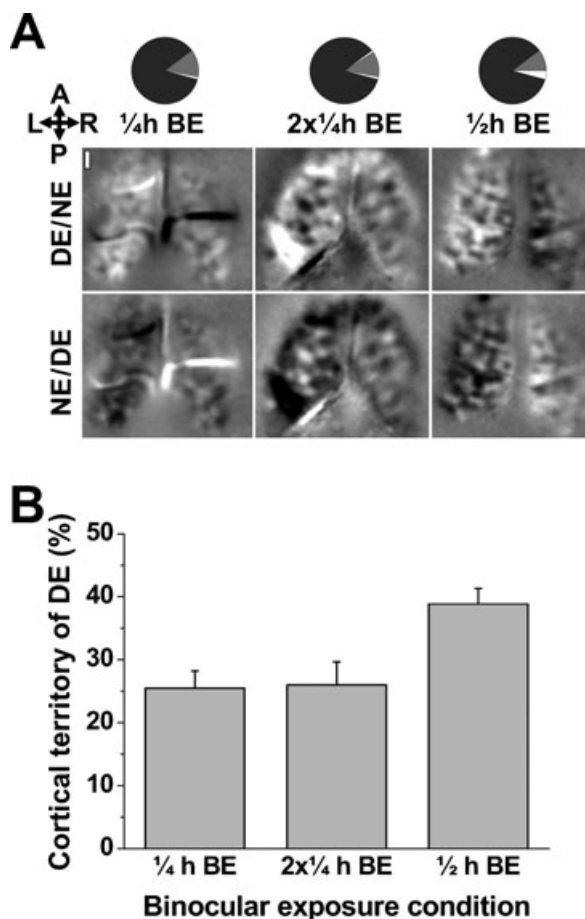
FIG. 7. Ocular dominance in split binocular exposure conditions. (A) Ocular dominance maps from subjects that received 0.25 h of continuous binocular exposure (and 3.25 hours of MD), two periods of 0.25 h of binocular exposure flanking a 3-h period of MD, and 0.5 h of continuous binocular exposure following 3 h of MD, respectively (as indicated by the icons above). Maps in the top row, labelled DE/NE, show ocular dominance domains of the deprived eye as dark patches, whereas maps in the bottom row, labelled NE/DE, show non-deprived eye domains as dark patches. (B) Cortical territory occupied by the deprived eye in three experimental groups (data combined from the 7-h and 3.5-h cohorts), having either 0.25 h or 0.5 h of continuous binocular exposure, or two periods of 0.25 h of binocular vision flanking the monocular period. All data represent mean ± 1 SEM.

Comparatively short daily periods of normal binocular visual experience override almost completely longer periods of abnormal experience to allow the maintenance of both normal vision and normal V1 architecture. A conservative estimate based on the slightly different figures obtained from the analyses of functional images and VEPs is about 30 min of BE a day. The unique design of our study, with its manipulation of both the total daily visual exposure as well as its partition into normal (BE) and abnormal (MD) components, allowed us to address an additional important issue, namely whether the outcome was dictated by an absolute amount of binocular exposure as opposed to the proportion of the total daily exposure that was binocular. Our finding that the absolute amount of daily binocular experience has a greater bearing on the cortical territory occupied by the two eyes supports the conclusion that a certain minimum of daily normal vision is necessary and sufficient to maintain a normal V1 architecture. Two previous studies that reported a beneficial effect of daily binocular experience employed either a single predominantly binocular exposure paradigm (Olson & Freeman, 1980) or a limited

number of exposure paradigms (with a fixed total daily exposure) followed by a 3-year period of recovery before physiological recording, which further complicated the interpretation of the data (Sakai *et al.*, 2006).

From a teleological perspective, the sort of input integration we observed is highly beneficial, as it ensures that rather minor and transient impairments of vision in one eye do not compromise vision in the longer term. Only when patterned visual input is lost altogether to one eye does an ocular dominance shift occur. The preferential weighting of binocular over monocular experience may in fact reflect the 'inertia' of the visual cortex to change a previously established functional architecture. It is now quite clear that at the onset of visual experience, the visual cortex is not a *tabula rasa*; on the contrary, ocular dominance and orientation columns of an adult-like periodicity are already present (Horton & Hocking, 1996; Crair *et al.*, 1998, 2001; Crowley & Katz, 1999, 2000). Even dark-rearing up to the beginning of the critical period [around postnatal day (P)20 in cats] has little effect on binocularity in V1, and orientation selectivity is reduced only slightly, in particular through the ipsilateral eye (Crair *et al.*, 1998). As our selective visual exposure started only at P30–35, one could argue that a 'normal' functional architecture had been stabilized by the preceding period of binocular vision. However, our earlier study (Mitchell *et al.*, 2003) showed that kittens dark-reared from birth up to the start of the period of selective visual exposure (at 4 weeks of age) were no more susceptible to monocular deprivation and required no more daily binocular vision to maintain normal visual acuity through the deprived eye than their light-reared littermates.

Our results have important implications both in terms of the general learning mechanisms at play in the visual cortex and in terms of the time course of synaptic events presumed to underpin visual cortical plasticity. First of all, we have to reject a purely instructive role of visual experience, as different types of input clearly differ in their effects on cortical development. The functional architecture of V1 is, at least to some extent, selective for concordant binocular input, such that even a small amount of normal visual experience allows ocular dominance patterns and binocularity to be stabilized, regardless of the nature of visual experience during the remaining time. Indeed, a binocular V1 may be considered the default state. Second, the compensation of long periods of monocular deprivation by brief periods of binocular vision suggests a much slower time course for the synaptic depression of deprived-eye inputs, which is widely believed to be the initial response to MD during the critical period, than for the potentiation induced by re-opening the deprived eye. These processes probably correspond to NMDA receptor-mediated long-term depression and long-term potentiation, respectively (Roberts *et al.*, 1998). Recent work has shown NMDA receptor-mediated plasticity to be bidirectional, and more importantly, the observed time constants are in good agreement with our findings. Moving a dark-reared rat into the light causes very rapid changes in synaptic transmission, within less than 2 h, while dark-rearing of a previously light-reared animal induces much slower changes, taking days (Quinlan *et al.*, 1999). The molecular basis of this experience-dependent plasticity is a change in NMDA receptor subunit composition. Visual experience decreases the proportion of receptors containing NR2B and increases the number of those containing NR2A, while visual deprivation exerts the opposite effect; both effects are reversible (Philpot *et al.*, 2001).

It has been argued that VEP cut-off frequencies provide a physiological measure of visual acuity (Berkley & Watkins, 1973; Freeman *et al.*, 1983), an interpretation that has been reinforced by the similarities in the estimates obtained in normal animals with those measured by use of behavioural techniques. The fact that the estimates obtained from VEP data were somewhat lower than behaviourally

measured acuities in animals reared under identical exposure conditions (Mitchell *et al.*, 2003, 2006) may be a consequence of a number of factors that include the possibility that the VEP data may not have sampled the activity of the most sensitive neurons in young kittens that mediate behaviourally determined acuity values. Nevertheless, the conclusions that can be drawn from the VEP data mirror closely those obtained earlier on the basis of behavioural measurements. Importantly, short periods of daily binocular experience outweighed far longer periods of monocular experience to lead to the development of normal acuities in both eyes whether acuity was determined from VEP data or from behavioural measurements.

In contrast to our imaging results, Mitchell *et al.* (2003) reported a longer critical daily binocular exposure (up to 2 h) to protect against the effect of monocular deprivation. However, ocular dominance architecture is unlikely to show a perfect correlation with visual acuity. The reduced size of deprived-eye domains compared with the non-deprived eye's territory reflects numbers of neurons dominated by the two eyes, but the behavioural measurement of grating acuity is more likely to depend on the 'best' cells, those responding to the highest spatial frequencies. It is possible that those are more vulnerable to MD. Alternatively, of course, behavioural performance may depend on the response characteristics of neurons beyond V1, at a stage where the representation of visual stimuli is integrated into a behavioural response. It is worth noting that similarly rapid recovery (within 0.5–2 h) following re-opening of an eye deprived by lid suture has been reported for ferret V1, albeit only for the hemisphere contralateral to the deprived eye; in the ipsilateral hemisphere, recovery took about 4 days (Krahe *et al.*, 2005). It has been shown that the rapid form of recovery can occur independent of protein synthesis (Krahe *et al.*, 2005).

Our finding that the absolute amount of daily binocular experience is a stronger driving force of ocular dominance plasticity than its relative share of the overall experience is corroborated by earlier studies on the recovery from MD. It appears that such recovery primarily depends on the absolute level of visual evoked activity in deprived-eye afferents, and not on competition between the afferents from the two eyes: mere hours after reopening of the deprived eye there is substantial recovery of vision in that eye (Mitchell & Gingras, 1998). The initial speed of recovery is even greater when visual experience is binocular than when the experienced fellow eye is closed (Mitchell *et al.*, 2001). Similarly rapid recovery of vision following surgical treatment has been observed in human infants who had been deprived of patterned visual input by congenital cataract (Maurer *et al.*, 1999).

Thus, while the afferents arriving from the deprived eye appear slowly to lose synaptic weight during monocular viewing, high activity levels in cortical neurons when binocular vision is restored can quickly and effectively reverse this change. The daily binocular episodes in our paradigm can be regarded as very brief recovery periods, which immediately counteract the deprivation effect during the preceding period of monocular viewing. Alternatively, one could argue that each daily period of binocular experience leaves a memory trace that enhances the effectiveness of similar inputs on subsequent days, analogous to the recently reported effects of repeat MD in mouse visual cortex (Hofer *et al.*, 2006). It is worth noting that neither our study nor the behavioural study by Mitchell *et al.* (2003) found evidence of an order effect: it appears to make no difference to the eventual cortical architecture and visual acuity whether the daily period of binocular exposure follows that of monocular exposure or vice versa. This confirms an earlier study of alternating monocular occlusion (Freeman & Olson, 1980). Sleep has been proposed to consolidate experiences into memory (Stickgold *et al.*, 2000) and may

also consolidate the effects of monocular deprivation (Frank *et al.*, 2001). Therefore, the type of exposure which occurs at the end of the day might be expected to be more effective in driving plasticity. However, the absence of any order effect in our data does not preclude the possibility of a consolidative property of sleep, as it may be the overall balance of binocular and monocular experience in a day which is consolidated during sleep. Moreover, we did not observe the animals' sleep patterns and cannot therefore be sure whether they were more likely to sleep in the beginning of the dark period than at other times. Our results do, however, indicate that the second exposure period in our rearing regimen is no more capable of driving cortical plasticity than the first one.

The present study demonstrates that visual cortical development in early life is biased towards a normal outcome supporting binocular vision. Even brief periods of binocular experience can outweigh the effects of much more prolonged monocular deprivation, at least if vision has developed normally until vision in one eye becomes compromised. Although remarkable plasticity exists in the postnatal brain, this does not come at the cost of economically sensible development. The kind of stimulation most likely to occur under normal circumstances is favoured by the visual system. In this context, it may be important that binocular experience is presented within an enriched environment in order to provide maximal sensory stimulation and therefore to ensure maximal effectiveness (Cancedda *et al.*, 2004). This allows a hopeful outlook for the treatment of ocular defects in infants as brief amounts of daily binocular exposure may be sufficient for normal visual development. In fact, patching regimens similar to those employed in the present study are now routinely used in human patients (Mitchell & MacKinnon, 2002). Assuming that mechanisms of plasticity are similar in the human and cat visual cortices, our results suggest that in children who need to wear a patch over one eye for a longer period of time, normal vision will be maintained in that eye if the patch is removed for about an hour a day to permit normal binocular visual experience.

Supplementary material

The following supplementary material may be found on http://www.blackwell-synergy.com
Fig. S1. Correlation of visual acuity deficit and cortical territory occupied by the deprived eye.

Acknowledgements

Abbreviations

BE, binocular exposure; MD, monocular deprivation; OD, ocular dominance; ROI, region of interest; V1, primary visual cortex; VEP, visually evoked potential.

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