Intrinsic and environmental factors in the development of functional maps in cat visual cortex

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Abstract

In the mammalian visual cortex, key neuronal response properties such as orientation preference and ocular dominance (OD) are mapped in an orderly fashion across the cortical surface. It has been known for some time that manipulating early postnatal visual experience can change the appearance of the OD map. Similar evidence for developmental plasticity of the orientation map has been scarce. We employed optical imaging of intrinsic signals to examine the contribution of intrinsic and environmental factors to the development of cortical maps, using the paradigms of strabismus, reverse occlusion and rearing in a single-orientation environment ('stripe-rearing'). For several weeks after induction of strabismus, the pattern of OD domains remained stable in young kittens. The isotropic magnification of the OD map matched the postnatal growth of the visual cortical surface during the same period. In reverse-occluded and in stripe-reared kittens, orientation preference maps obtained through the left and the right eye were very similar, although the two eyes had never shared any visual experience. We suggest that the geometry of functional maps in the visual cortex is intrinsically determined, while the relative strength of representation of different response properties can be modified through visual experience. © 1998 Elsevier Science Ltd. All rights reserved.

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1. Introduction

A long-standing debate in neuroscience, and in biology in general, concerns the issue pointedly phrased as 'nature versus nurture': To what extent are structure and function of the nervous system innately predetermined, and to what degree can they be shaped through experience in the environment during early life? During the past 30 years, numerous neurophysiological and neuroanatomical studies have investigated the plasticity of the visual cortex. While the two most prominent emergent properties of the primary visual cortex (V1), binocularity and orientation selectivity, are to some extent present prior to any visual experience (Hubel and Wiesel, 1963; Blakemore and Van Sluyters, 1975; Frégnac and Imbert, 1978), they can be influenced

dramatically by altering visual experience during the so-called critical period. Binocularity breaks down in strabismic animals (animals whose visual axes are misaligned; Hubel and Wiesel, 1965; Van Sluyters and Levitt, 1980; Sengpiel et al., 1994), and the balance of ocular dominance (OD) shifts towards the experienced eye following monocular deprivation (Wiesel and Hubel, 1965; Hubel and Wiesel, 1970; Blakemore, 1976) and again after reverse lid-suture (Blakemore and Van Sluyters, 1974; Movshon, 1976; Mitchell et al., 1977). Similarly, it has been reported that exposure to lines of just one orientation ('stripe-rearing') results in a preponderance of neurons preferring that orientation (Blakemore and Cooper, 1970; Cynader and Mitchell, 1977; Stryker et al., 1978; Rauschecker and Singer, 1979).

Transneuronal tracing and 2-deoxyglucose mapping techniques have revealed changes in OD and orientation columns that parallel the physiological changes

observed at the neuronal level. In monocularly deprived animals, columns representing the open eye expand at the expense of those representing the deprived eye (Shatz and Stryker, 1978; LeVay et al., 1980; Swindale et al., 1981). In stripe-reared animals, the same seems to be true for the experienced orientation as compared with other orientations (Singer et al., 1981). In strabismic cats, where activity in the two eyes is decorrelated, the segregation of OD columns in V1 is enhanced (Shatz et al., 1977; Löwel and Singer, 1993). Segregation is weaker or absent under conditions in which neuronal activity is reduced in both eyes (Wiesel and Hubel, 1965; Swindale, 1981; Stryker and Harris, 1986).

While it is widely believed that principles of self-organization based on activity-dependent competition govern these changes (e.g. Stryker, 1991; Goodman and Shatz, 1993), it has also been shown that functional maps can form prior to any visual experience. For example, OD maps can even be demonstrated in V1 of neonate macaque monkeys delivered in the dark (Horton and Hocking, 1996a). Similarly, orientation preference maps develop in the visual cortex of binocularly deprived kittens (Gödecke et al., 1997; Crair et al., 1998). It has therefore been suggested that the structure of these maps is determined by factors intrinsic to the cortex itself (Wiesel and Hubel, 1974; Jones et al., 1991). Consequently, it may not be too surprising that the overall periodicity of each columnar system appears to be invariant to experimental manipulations of visual input (e.g. Shatz and Stryker, 1978; LeVay et al., 1980; Singer et al., 1981; Swindale et al., 1981). However, this notion has recently been challenged, first theoretically (Goodhill, 1993) and then experimentally: Löwel, (1994) employed transneuronal labelling and 2-deoxyglucose mapping techniques and found the spacing of OD columns in strabismic cats to be wider than in normal animals. Such an experience-dependent change in columnar periodicity would be evidence that even the processes determining map geometry are activity-dependent.

While until recently, all studies examining the plasticity of functional maps have had to rely upon inter-individual comparisons, optical imaging of intrinsic signals has now provided a tool for longitudinal studies. This is advantageous as it allows one to monitor developmental changes of the cortical functional architecture in individual animals. We therefore employed this method to address the question of which features of functional cortical maps can and which cannot be altered by visual experience, using the paradigms of strabismus, reverse occlusion and stripe-rearing in young kittens.

2. Methods

The experiments were performed on 16 kittens bred in the institute's colony.

2.1. Optical imaging

Surgery and optical imaging techniques have been described in detail elsewhere (Bonhoeffer and Grinvald, 1993; 1996). All recording sessions were performed under sterile conditions. Anaesthesia was induced with an i.m. injection of ketamine (20–40 mg/kg) and xylazine (Rompun, Bayer, 2–4 mg/kg). Animals were intubated and placed in a stereotaxic apparatus. They were artificially ventilated with a 60:40 mixture of nitrous oxide and oxygen, supplemented with 1–1.5% halothane as required to maintain adequate anaesthesia. Electrocardiogram, end-tidal carbon dioxide, arterial oxygen saturation and rectal temperature were monitored continuously.

In the initial recording session for each kitten, the scalp was incised and retracted. A circular craniotomy with a diameter of ~12 mm was performed around A2-P2 (Horsley-Clarke) in order to expose area 17 (strabismic and stripe-reared kittens) or area 18 (reverse-occluded animals). A titanium chamber was implanted on the skull with dental cement, filled with silicone oil and sealed with a glass cover-slip. In all experiments, the dura was left intact for as long as possible; it was removed only in the final imaging session or when imaging through the dura had become impossible due to overgrowth of connective tissue.

Animals were refracted and fitted with gas-permeable contact lenses to focus their eyes on a 21 in. monitor or on a frosted glass screen at a distance of 33 cm or 1 m, respectively. Visual stimuli were produced by custom-made software (STIM, Rockefeller University, NY) or by a visual stimulus generator (VSG Series Three, Cambridge Research Systems, Rochester, UK). Stimuli consisted of high-contrast square-wave gratings of 0.15–0.5 cycles/degree, drifting back and forth at 2 cycles/second and presented at four different orientations (0, 45, 90, or 135°). Computer-controlled occluders in front of both eyes were used for monocular stimulation.

The cortex was illuminated using bandpass-filtered light of 707 ± 10 nm. Images of the intrinsic signals produced by neuronal activity in response to the different visual stimuli were captured using a cooled slow-scan CCD camera or an enhanced differential imaging system (ORA 2001 or Imager 2001, Optical Imaging, Germantown, NY), focused ~500 μm below the cortical surface. Five frames of 600 ms duration were collected during each 3 s stimulus presentation, followed by a 9 s interstimulus interval during which the next stimulus was presented stationary. For analysis, the

first frame was discarded. In some experiments, three frames of 600 ms each were collected immediately prior to stimulus motion onset for 'first-frame' analysis: the average of these frames was subtracted from each of the five subsequent frames in order to minimize slow biological noise (see Bonhoeffer and Grinvald, 1996).

Following each but the final recording session, the dura or cortex was covered with a layer of agar containing a broad-spectrum antibiotic (Paraxin, Bayer). The chamber was then filled with silicone oil and sealed with a cover-slip. The kitten was allowed to recover from the anaesthesia before being returned to its mother and litter-mates. After the terminal imaging experiment, the animal was euthanized with an overdose of barbiturates.

2.2. Strabismus

In seven kittens from three different litters, a divergent squint (exotropia) was induced surgically between postnatal days P 21 and P 29, subsequent to the first successful imaging experiment (see Section 2.1). In all animals, the medial rectus muscle of the left eye was severed. The animals were allowed to recover and then were returned to their mother and litter-mates. Further imaging experiments were carried out at intervals of 6–19 days in order to monitor changes in the layout of OD and orientation preference maps.

2.3. Reverse occlusion

In five kittens, the right eye was sutured prior to natural eye-opening. Animals were anaesthetized with ketamine (20–40 mg/kg i.m.) and xylazine (2–4 mg/kg i.m.). The eyelids were separated, their margins trimmed and sutured shut with fine silk. The seam was inspected every day in order to close any developing holes immediately. Before the first imaging experiment, which was carried out at around 5 weeks of age, the deprived eye was re-opened. After the experiment, the previously open eye was sutured, and the animal was allowed to recover. A second optical imaging was carried out 1–2 weeks later.

2.4. Stripe-rearing

From the day of birth, four kittens and their mothers were reared in a darkroom. In each kitten, the right eye was sutured prior to natural eye-opening (see Section 2.3). Starting at P16/17, they were placed in cylinders (2 m tall, 65 cm in diameter), which were painted with evenly spaced black and white stripes (3 or 4 cm wide) of a single orientation (0, 45, 90, or 135°). Cylinders with horizontal or vertical stripes contained a non-reflectant glass plate at a height of 50 cm, on which the kittens were placed. The animals were exposed to this visual environment for 4–5 h a day, until a total exposure of 70–102 h was reached. At this point, the sutured eye was opened and the first optical recording was performed (see Section 2.1). After the experiment, the animals underwent reverse occlusion (i.e. the left eye was sutured shut), and were allowed to recover. They were then exposed to stripes orthogonal to the previous ones for another 2 weeks (55–82 h), before a second optical imaging experiment was performed.

2.5. Image analysis

Signal averaging across 128–192 stimulus presentations was used to reduce noise in the acquired images. Iso-orientation maps (0, 45, 90, 135°) were produced by dividing single-condition maps either (1) by the response to a 'blank' screen of zero contrast, or (2) by the 'cocktail blank' consisting of the sum of the images obtained in response to all four orientations. The latter should represent a homogeneous activation of the imaged region of cortex. Division by the 'cocktail blank' will remove activity or response components that are common to all images and will therefore result in maps of orientation-selective responses (for details, see Bonhoeffer and Grinvald, 1993; 1996).

'Angle' maps were calculated in order to combine images obtained for each of the four orientations into a single colour-coded orientation preference map, using vectorial addition on a pixel-by-pixel basis. The vector angle is displayed as the hue (colour) of each pixel, indicating the preferred orientation (see Bonhoeffer and Grinvald, 1993; 1996, for details).

The degree of similarity between maps recorded at different ages from the same animal (or, for control purposes, from different animals) was determined by 2D cross-correlation analysis. Lowpass-filtered activity maps were aligned with the help of blood-vessel patterns obtained before the respective recording sessions. As many of the recordings were performed through the intact dura, a perfect alignment was sometimes difficult to achieve. We therefore calculated 2D cross-correlations in which the cross-correlation between the maps was calculated for every offset between -300 μm and $+300$ μm (-20 to 20 pixels). We then searched for maxima and minima of the cross-correlation coefficients. The absolute maxima were always found at positions with small offsets ($< \pm 10$ pixels) in either dimension.

3. Results

3.1. Strabismic animals

From five animals we obtained orientation and OD maps in area 17 that could be compared quantitatively
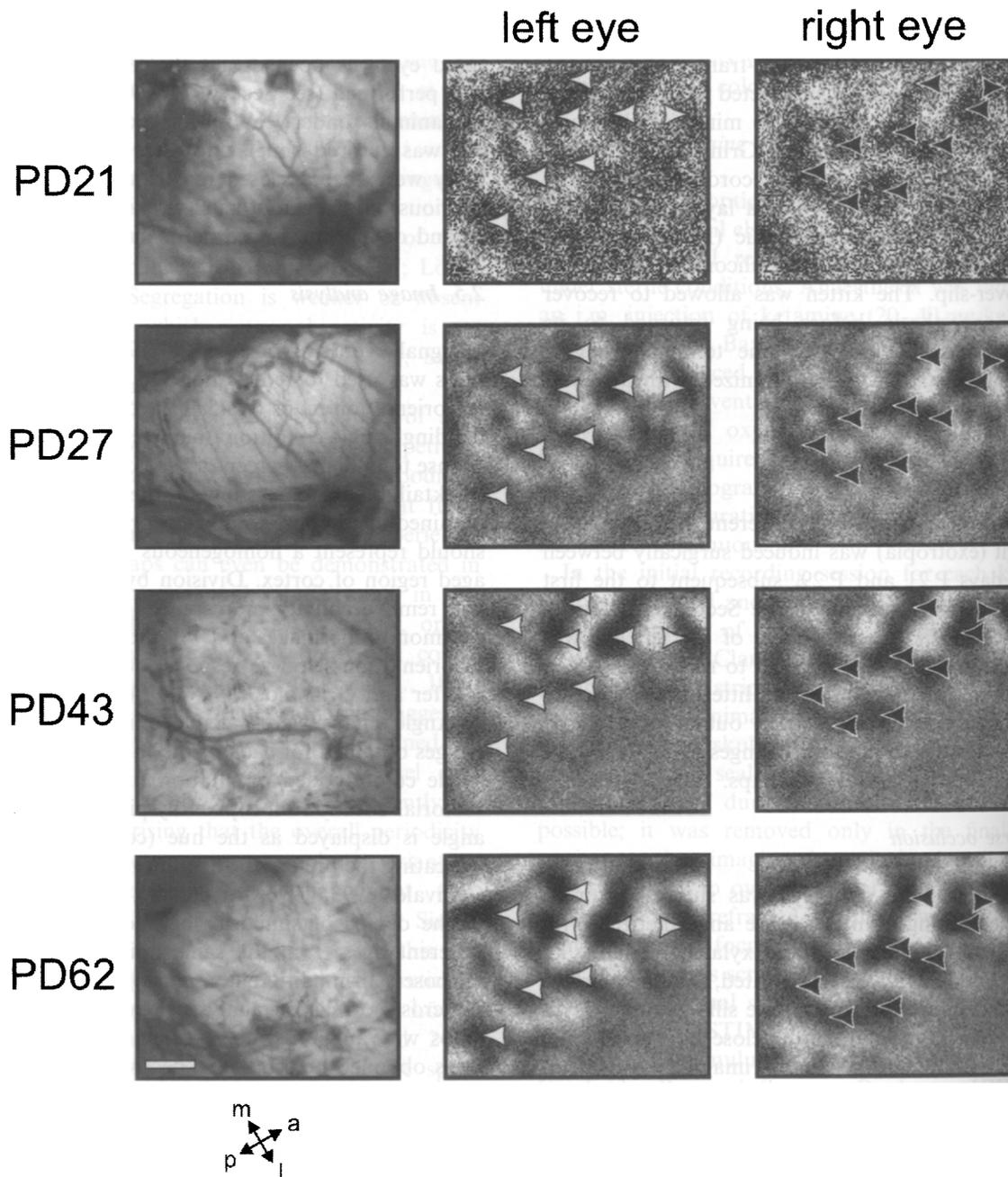
Fig. 1. Ocular dominance maps in area 17 of a kitten before and after induction of strabismus. Exotropia was induced in the left eye on PD 21. Subsequent imaging experiments were carried out on PD 27, PD 43 and PD 62. The left column shows the blood vessel patterns of the imaged region; the dura was left intact in all experiments. The centre and right columns display ocular dominance patterns. In the centre column, dark areas represent regions dominated by the left eye, in the right column, regions dominated by the right eye. To facilitate comparison, corresponding points are marked with arrows. The pattern of OD domains remains essentially unchanged for 6 weeks after the onset of strabismus. Scale bar, 1 mm.

between recordings carried out before and up to 41 days after induction of strabismus. For one animal, OD maps recorded on postnatal days (PD)21 (when strabismus was induced), PD 27, PD 43 and PD 62 are shown in Fig. 1. Maps were aligned on the basis of the blood-vessel patterns shown on the left. It can be seen immediately that position and size of patches of cortex dominated by

one eye or the other change little over a period of 6 weeks, even though signal strength increases considerably. Results for a second kitten that was imaged over a period of 5 weeks from PD 26 to PD 60 are displayed in Fig. 2. Again, the pattern of OD domains remains essentially unchanged. For this animal, angle maps of orientation preference are also shown (see below).
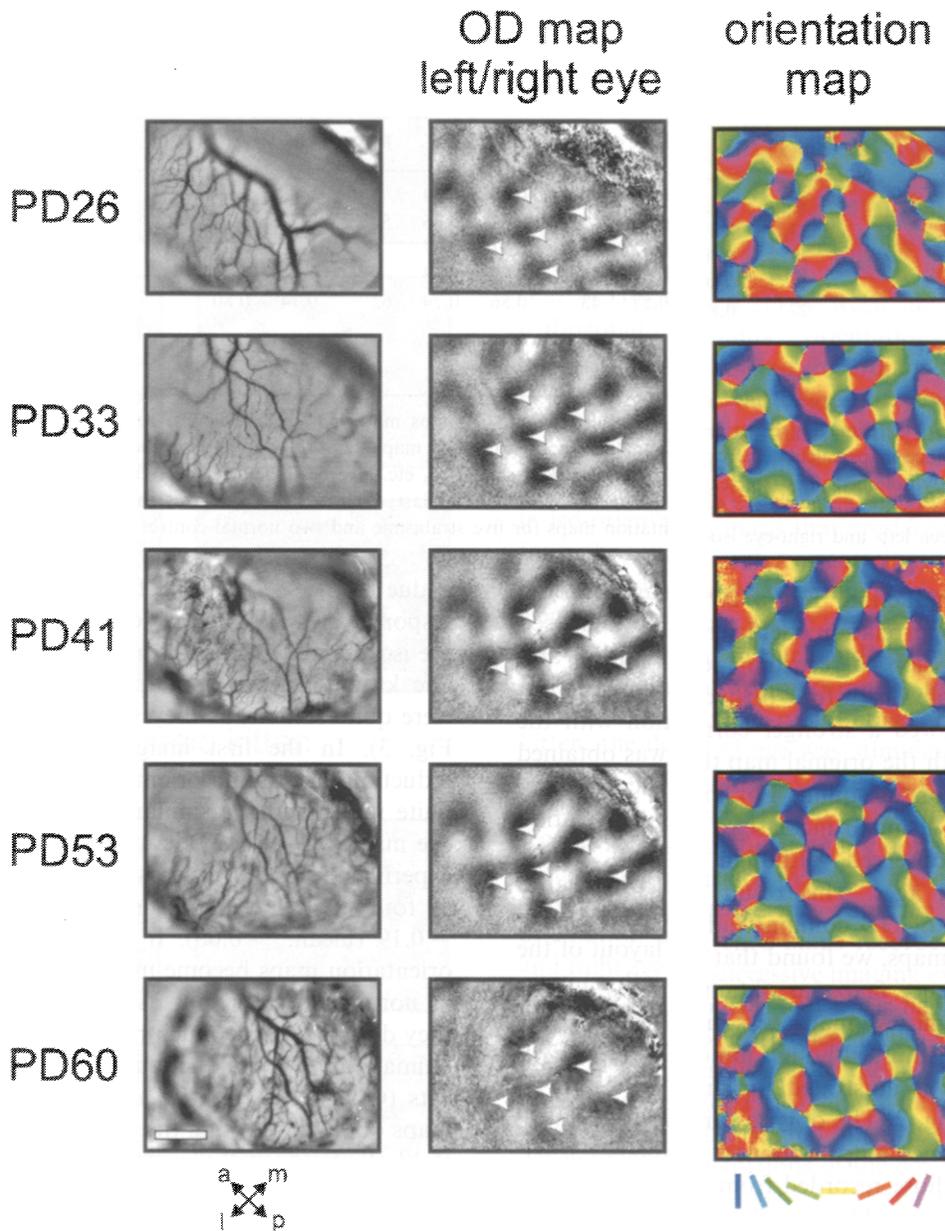
Fig. 2. Ocular dominance and orientation preference maps in area 17 of a second kitten before and after induction of strabismus. Exotropia was induced in the left eye on PD 26. Subsequent imaging experiments were carried out on PD 33, PD 41, PD 53 and PD 60. The left column shows the blood vessel patterns of the imaged region; the dura had been removed in the first experiment. The centre column displays ocular dominance patterns. Dark areas represent regions dominated by the left eye, light areas those dominated by the right eye. To facilitate comparison, corresponding points are marked with arrows. Angle maps of orientation preference (see Section 2) are shown in the right column, the colour code of preferred orientations below. Scale bar, 1 mm.

In all animals, the first maps were obtained immediately before squint induction (between PD 21 and PD 29), and the second experiment was performed 6–11 days later. In each case, 2D cross-correlation analysis yielded coefficients of 0.70–0.80 (see Table 1), confirming the high degree of similarity that qualitative inspection of the maps revealed. This similarity was found to be preserved in all subsequent imaging sessions: as late as 5 to 6 weeks after the induction of exotropia, the correlation coefficient for the cross-corre-

lation of the first and last obtained maps was always greater than 0.6. However, it should be noted that correlation coefficients decreased over time in four out of five animals. In view of the fact that an increase of OD column width by about 30% has been reported for strabismic cats (Löwel, 1994), we also computed correlation coefficients for cases where the OD map recorded before strabismus surgery was magnified isotropically by 15, 20, 25 or 30%. Ideally, this magnification should compensate for an expansion in OD column spacing

Table 1
Results of cross-correlation analysis for strabismic kittens

Kitten number	1st experiment	2nd experiment			3rd experiment			4th experiment			5th experiment			$r_{left,right}$
	PD	PD	$r_{1,2}$	$r_{1m,2}$	PD	$r_{1,3}$	$r_{1m,3}$	PD	$r_{1,4}$	$r_{1m,4}$	PD	$r_{1,5}$	$r_{1m,5}$	
26	26	33	0.75	0.75	40	0.64	0.79							-0.29 ± 0.11
27	26	33	0.77	0.55	41	0.67	0.66	53	0.79	0.74	60	0.64	0.76	-0.05 ± 0.13
28	27	38	0.70	0.67	51	0.71	0.67							-0.36 ± 0.05
34	29	37	0.80	0.57	51	0.61	0.63							-0.19 ± 0.20
36	21	27	0.82	0.57	43	0.86	0.74	62	0.74	0.80				-0.08 ± 0.10
Control 1														0.58 ± 0.08
Control 2														0.40 ± 0.16

PD, postnatal days on which imaging experiments were carried out. Columns marked $r_{1,2}, r_{1,3}$ etc. give best coefficients of cross-correlation between ocular dominance maps calculated from 2D analysis, comparing OD maps from the first experiment (before induction of squint) with each of the subsequently obtained ones (see text). Columns marked $r_{1m,2}, r_{1m,3}$ etc. give best coefficients of cross-correlation between OD maps after magnifying the map obtained in the first experiment by 20%. The last column ($r_{left,right}$) gives mean ($\pm$ S.D.) best coefficients of cross-correlation between left- and right-eye iso-orientation maps for five strabismic and two normal control animals.

after strabismus induction. For a 20% magnification, we obtained cross-correlation coefficients that increased from recording session to recording session in all animals but one (Table 1). In four of five kittens, the last recorded map showed a stronger correlation with the expanded than with the original map that was obtained before the induction of squint. For magnification factors of 25 or 30%, a similar increase of cross-correlation coefficients was observed, although absolute values were lower at all ages. This result indicates that a modest expansion of OD columns did take place.

Just as for OD maps, we found that the layout of the orientation preference maps, as obtained with summation of left- and right-eye maps, was preserved after the induction of strabismus, although minor changes could be observed. This is illustrated for one animal in the right column of Fig. 2. The obvious difference between orientation preference maps obtained from the strabismic animals and those recorded from two normal control animals of comparable age relates to the degree of similarity between maps obtained through the left and the right eye, respectively, with monocular stimulation (see also Löwel et al., 1994, 1998). In the normal animals, left- and right-eye maps obtained for the same orientation were always very similar; averaged cross-correlation coefficients for all four single-orientation maps were + 0.58 and + 0.40, respectively (Table 1). In contrast, in the strabismic animals, there was little correlation between left- and right-eye maps obtained for the same orientation, as iso-orientation domains often showed clear offsets when comparing maps for the two eyes (Fig. 3). Averaged cross-correlation coefficients for all four single-orientation maps ranged from − 0.08 to − 0.36 in the five strabismic animals (mean, − 0.19; Table 1). However, this does not indicate inter-ocular differences in preferred orientation but merely a displacement of domains of strongest activation, which

is due to the segregation of areas of left- and right-eye responsiveness. Therefore, despite the dissimilarities of the iso-orientation maps for the two eyes in the strabismic kittens, the angle maps of orientation preference were quite similar, as they were in normal animals (see Fig. 3). In the first imaging experiment before the induction of squint, orientation maps were generally quite weak, and the similarity between left- and right-eye maps was only moderately higher than in the later experiments. Averaged cross-correlation coefficients for all four single-orientation maps ranged from + 0.06 to − 0.19 (mean, − 0.06). It therefore appears that iso-orientation maps become more similar for the two eyes in normal development, as binocularity increases, but they do not become much more dissimilar in strabismic animals after the induction of squint. Like in normal cats (Gödecke et al. 1997), the orientation preference maps obtained through the two eyes remain remarkably stable throughout the critical period.

3.2. Reverse-occluded animals

Cortical maps from three kittens were evaluated quantitatively; the results were confirmed qualitatively for another two kittens (see also Gödecke and Bonhoeffer, 1996). Fig. 4 presents a summary of our principal findings for a kitten that was monocularly deprived on PD 7 and reverse-sutured on PD 35. The first imaging experiment was carried out on PD 35, the second on PD 48, 13 days after reverse occlusion. Single-condition maps (i.e. responses to each one of the four orientations, 0, 45, 90, and 135°) were analysed in two ways. First, they were divided by the response to a 'blank' screen; second, they were divided by the sum of responses to all four orientations ('cocktail blank', see Section 2.5).

A

left eye   right eye

0°

m
p←↗a
↙↘
    i

90°

Strabismic kitten

B

0°

a↖↗m
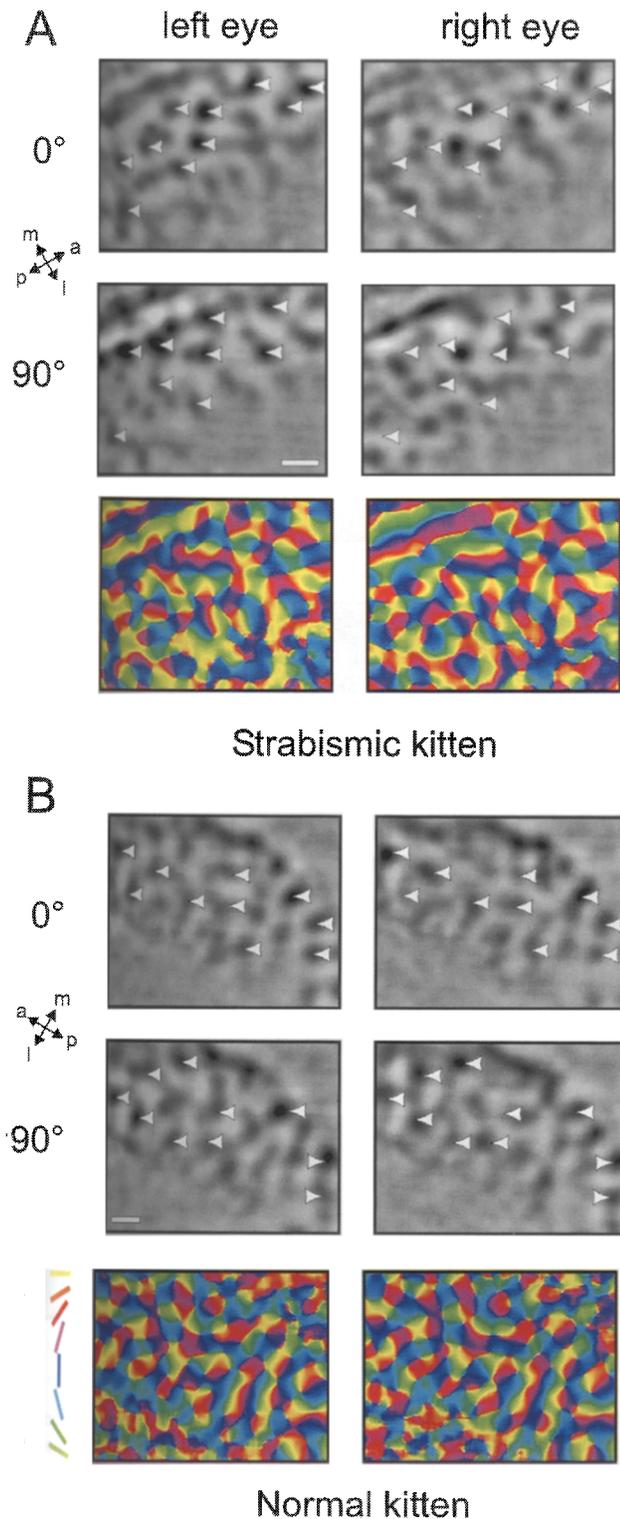   ↙↘
i   p

·90°

Normal kitten

Fig. 3. Iso-orientation domains and angle maps of orientation preference in area 17 recorded through the left eye (left column) and the right eye (right column), respectively, of a strabismic kitten ((A), same animal as in Fig. 1), and of a normal kitten imaged on PD 56 (B). In the strabismic kitten, exotropia had been induced on PD 21, and the recording experiment illustrated here was performed on PD 62. To facilitate comparison, all maps were low-pass filtered; arrowheads are placed in corresponding positions in the left- and right-eye maps. Scale bars, 1 mm.

Single-condition maps divided by the blank response display some similarity, indicating that regions of cortex were activated to some degree by gratings of any orientation through the open eye. This was found to be true for maps obtained after the first period of monocular experience (first column of Fig. 4) as well as for maps recorded after the period of reverse occlusion (third column). Cross-correlation analysis confirmed this observation: there was just a weak negative correlation ($r = -0.08$ to -0.21) between images obtained with orthogonal stimulus orientations (0 vs. 90° and 45 vs. 135°) in the first recording session. Following reverse occlusion, the degree of correlation was more variable between animals, with the cross-correlation coefficient ranging from -0.33 to $+0.61$. In contrast, dividing responses by the cocktail-blank naturally results in iso-orientation maps that are largely complementary for orthogonal stimulus orientations (second and fourth column). The corresponding cross-correlation coefficients ranged from -0.62 to -0.85 in all cats. It is worth noting that in our hands both the cocktail-blank corrected and, more importantly, the blank corrected images showed no sign of patterned activity when, in one kitten, the deprived eye was stimulated after the initial period of monocular lid-suture (see Crair et al., 1997). In all animals, the experienced eye both in the first and the second experiment functionally dominated virtually the entire cortical surface that was being imaged.

In all kittens, there was a striking similarity between orientation preference maps recorded through the left and right eyes in successive imaging experiments (Fig. 4) although the two eyes had never had any shared visual experience. Minor differences in the fine structure of the maps may be attributable to the fact that the first set of images was recorded through the intact dura, which was removed before the second imaging session, or to slight developmental distortions of the cortical surface. In the three kittens that were examined quantitatively, 2D cross-correlation analysis was performed on preference maps for all four orientations (see Section 2). The best correlations were $r = 0.86 \pm 0.03$ (mean $\pm$ S.D.) for the animal shown in Fig. 4, $r = 0.71 \pm 0.03$ and $r = 0.77 \pm 0.06$ for the other two kittens. For control purposes, we also calculated 2D cross-correlations for pairs of maps taken from different animals; none of these correlations was significantly different from zero (see Gödecke and Bonhoeffer, 1996).

3.3. Stripe-reared reverse-occluded animals

Like in the kittens that had been monocularly deprived and reverse-sutured, the first period of exposure to a single-orientation environment through the initially open (left) eye resulted in the generation of an orientation preference map for this eye that showed an essentially normal layout. Blank-corrected iso-orientation
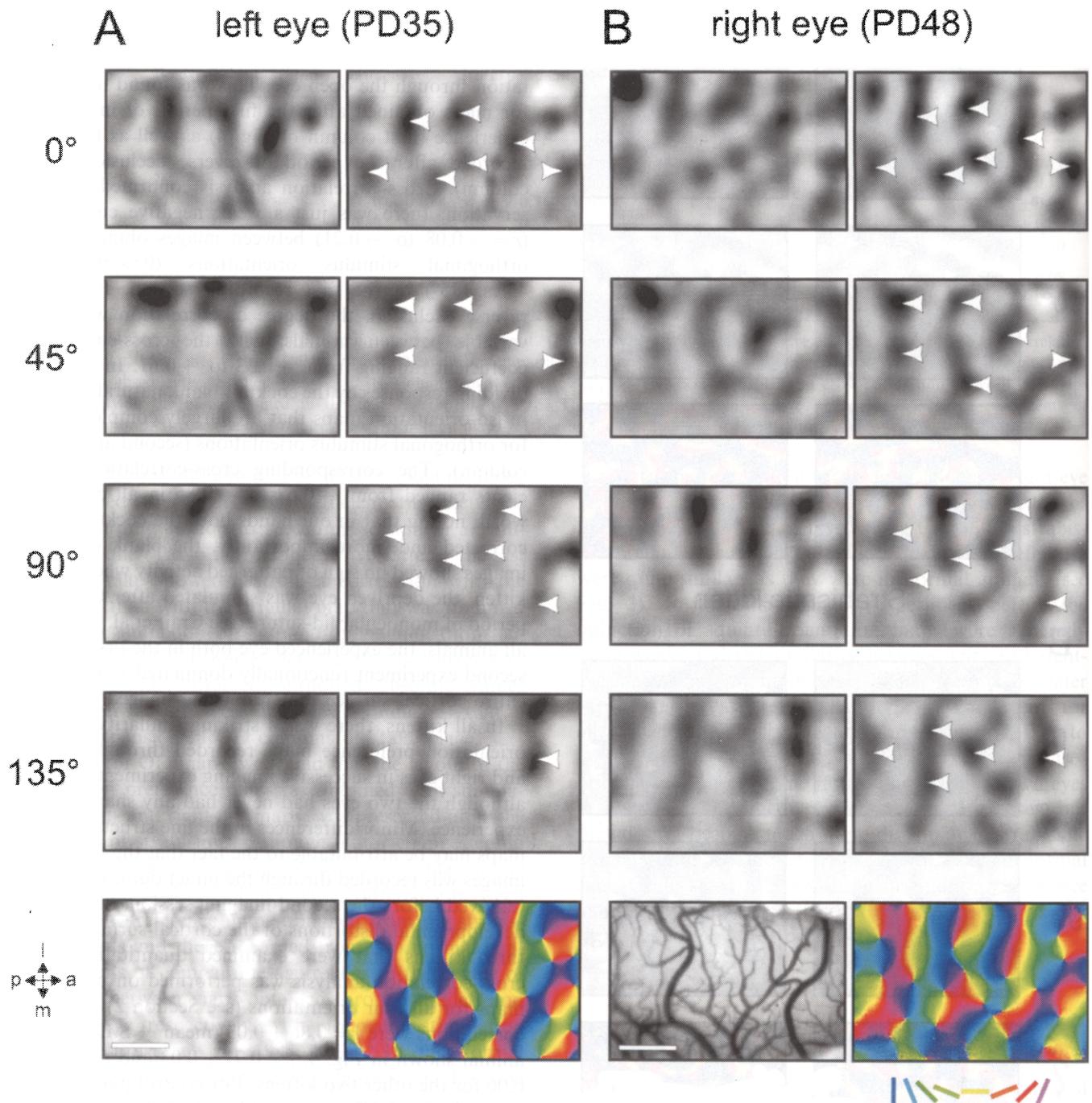
Fig. 4. Iso-orientation maps from the left and right eye of a kitten raised with reverse occlusion. The right eye was sutured shut before natural eye-opening. Images were obtained from the left hemisphere, ipsilateral to the initially open eye. The two columns on the left (A) show iso-orientation maps obtained from area 18 through the left eye on PD 35, before reverse eyelid suture was performed. The kitten then had visual experience through the right eye until PD 48, when a second imaging experiment was carried out. The iso-orientation maps obtained are displayed in the two columns on the right (B). The bottom row shows blood-vessel patterns and angle maps of orientation preference for the two experiments. The dura was left intact during the first optical recording and was removed before the second. In the first and third column, low-pass filtered single-condition maps, divided by responses to the blank screen, are shown. The second and fourth columns display iso-orientation maps obtained after division by the cocktail blank (see Section 2). To facilitate comparison, all iso-orientation maps were low-pass filtered; arrowheads are placed in corresponding positions in these maps. Scale bars, 1 mm.

maps for orthogonal stimulus orientations were at least partly complementary (Figs. 5 and 6; first columns). Cross-correlation analysis revealed weak negative cor-

relations ($r = -0.20 \pm 0.12$, mean $\pm$ S.D.; see Table 2), similar to the reverse-occluded animals. However, in all animals raised in that manner, the experienced orienta-
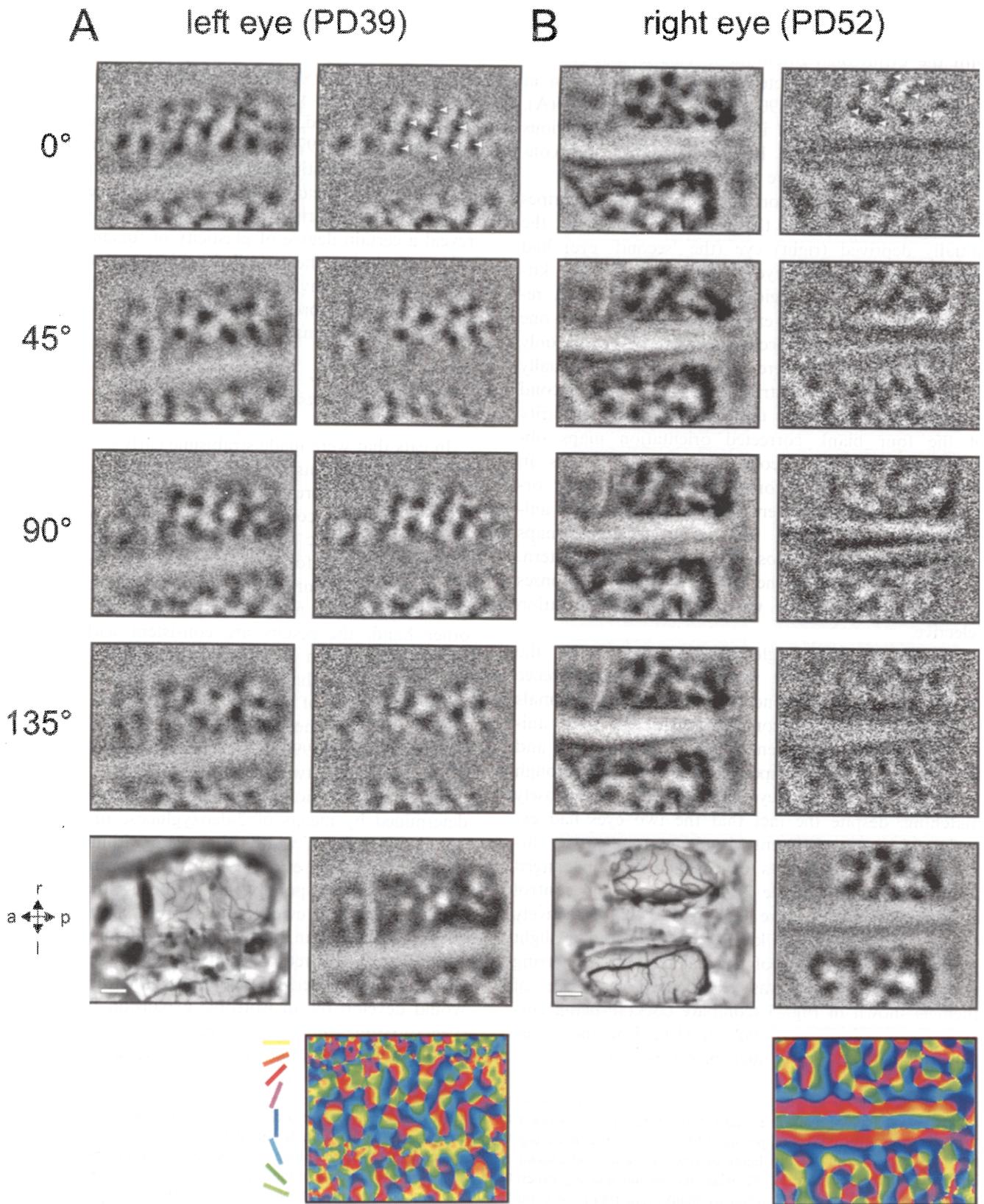
Fig. 5.

tion was over-represented in terms of the cortical territory occupied by it, in particular when compared with the orthogonal orientation. This is best seen in the cocktail-blank corrected maps (second column in Figs. 5 and 6: compare 90 and 0° maps in Fig. 5(A), and 45 and 135° maps in Fig. 6(A)). Any obvious differences between the hemispheres ipsi- and contralateral to the open eye were not observed.

After reverse occlusion and exposure to stripes that were orthogonal to the previously seen ones, the initially deprived (right) eye (the 'second' eye) had become the dominant eye in three out of four kittens, although some regions of cortex remained responsive to the initially experienced (left) eye. In one animal where reverse occlusion was performed only at PD 43, both eyes were represented nearly equally at PD 56. The most striking result of the second imaging experiment was the high degree of similarity of the four blank corrected orientation maps obtained through the second eye (third columns in Figs. 5 and 6). Cross-correlation coefficients for orthogonal orientations were highly positive in all animals ($r = 0.78 \pm 0.09$). All iso-orientation maps corresponded quite closely to the OD pattern. This indicates that the newly gained responses through the second eye were largely non-orientation selective.

Division of the single-condition images by the 'cocktail-blank' revealed weak orientation preference maps through the second eye in the three animals where this eye had become dominant. In these animals, orientation preference maps from the first and the second imaging experiments (obtained through the left and the right eye, respectively) were closely matching, despite the fact that the two eyes had experienced a very different visual environment at different times. Coefficients of cross-correlation ranged from 0.45 to 0.64 (Table 2). For two normal control animals, the values were 0.58 and 0.40, respectively (see Table 1). For two kittens, we observed a slight predominance in terms of cortical territory preferring the orientation that was experienced last: one of them is shown in Fig. 5 (compare cocktail-blank corrected maps for 0 and 90° in (B)). For the kitten illustrated in Fig. 6, no such bias was seen.

4. Discussion

Most of the experimental manipulations described in this study (induced strabismus, stripe-rearing) were performed at an age when the first cortical maps had already been formed, independently of visual experience (Gödecke et al., 1997; Crair et al., 1998). The geometry of the (already existing) OD and orientation preference maps did not appear to be altered by induced strabismus and stripe-rearing. However, these paradigms did reveal a certain degree of plasticity of functional cortical maps during the so-called critical period. Specifically the visual environment can modify the relative strength of the representation of the two eyes and of different orientations.

4.1. Effect of strabismus on OD maps

In cats that were made strabismic early in the critical period, OD patterns recorded before the induction of squint are highly preserved afterwards over a period of several weeks. A complete rearrangement of OD domains in strabismic animals can therefore be ruled out as an explanation for the previously reported observation that OD columns appear to be about 30% wider in strabismic than in normal cats (Löwel, 1994). On the other hand, the results are consistent with a slight isotropic expansion of OD columns by about 15–20%. One might argue that this expansion, had it continued beyond the time of the final optical recording, would have resulted in the unusually wide OD columns observed by Löwel (1994) in strabismic cats of 8–12 weeks of age. Indeed, in two of the five animals in our study, in whom the periodicity of OD columns was later determined by means of 2-deoxyglucose mapping, we found OD column widths that were within the range reported by Löwel et al. (1994) for strabismic cats.

However, at this point we do not believe that our data provides sufficient evidence to support the notion that strabismus causes an increase in OD column periodicity. First, from a theoretical point of view, it seems rather unlikely that wider OD columns in strabismic cats would develop by an isotropic expansion from an already existing 'normal' pattern, and moreover, that the centre of such an expansion would happen to lie in the

Fig. 5. Iso-orientation maps from the left and right eye of a stripe-reared kitten raised with reverse occlusion. This animal was exposed through the left eye to vertical (90°) stripes until PD 38, when the first imaging experiment was carried out. Iso-orientation maps for left-eye stimulation (A) are shown after division by blank (leftmost column) and cocktail-blank (second column). The kitten was then reverse-sutured and exposed to horizontal (0°) stripes until PD 52, when the second imaging experiment was performed. Iso-orientation maps for right-eye stimulation (B) are shown in the third column (division by blank) and rightmost column (division by cocktail blank). Note the high degree of similarity between blank-divided maps for all four orientations after the second period of exposure. Nevertheless, the layout of the (weak) orientation preference maps (images divided by cocktail blank) is largely preserved (compare second and fourth columns; arrowheads mark corresponding positions in the 0° maps). The bottom row shows blood-vessel patterns and ocular dominance maps for the experienced eye for both experiments. Below the OD maps, angle maps of orientation preference are also shown. The dura was left intact during the first optical imaging and was removed before the second. Scale bars, 1 mm.

Fig. 6.
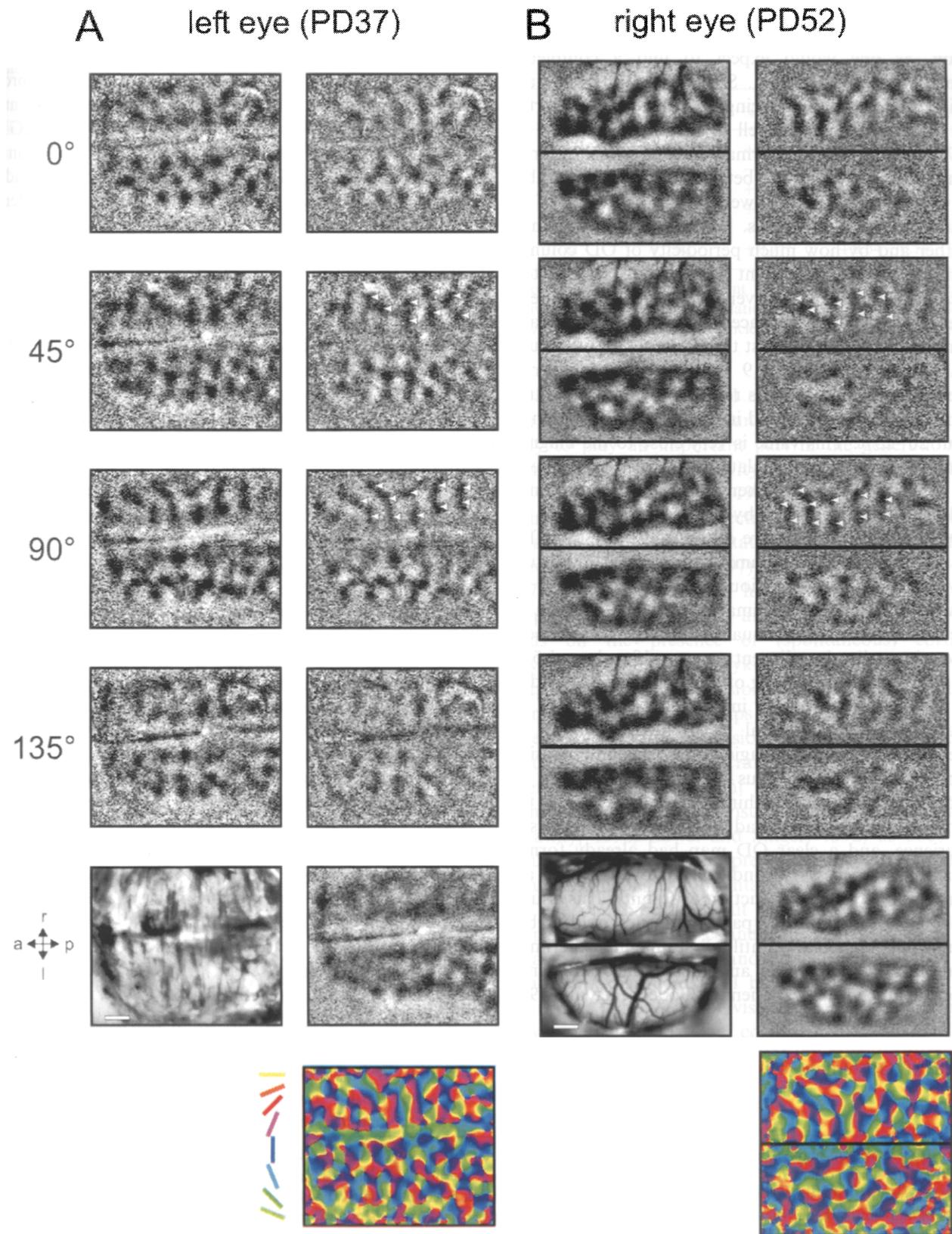
rather small region of area 17 that was imaged. If the centre of expansion were further away from the imaged region, as one would expect to find a completely changed columnar pattern. Second, the intrinsic variability of OD column spacing is, in macaques (Horton and Hocking, 1996b) as well as in cats (Hübener et al., 1997), greater among normal animals from different litters, than the difference between normal and strabismic cats in the study of Löwel (1994) where most of the squinters were litter-mates. Third, we do not know whether and by how much periodicity of OD columns changes during development of normally raised kittens of comparable age. However, very recent experiments have shown that the surface area of cat V1 increases isotropically during the first three months of life (Duffy et al., 1998). From 4 to 9 weeks of age (the period covered by the experiments reported here), V1 expands both in anteroposterior and in mediolateral direction by about 20–25%. This value is very close to the magnification factor that we calculated for the OD columns in our strabismic kittens. Therefore, our observation is likely explained primarily by cortical growth.

On the other hand, there are reasons why the effect of strabismus on OD column periodicity in this study may have been less obvious than that reported by Löwel (1994). First, we imaged the part of area 17 where the centre of the visual field is represented, i.e. a region immediately adjacent to area 18, where Löwel did not observe a widening of OD columns. The widening may be more obvious in the representation of the paracentral and peripheral visual field, which is not accessible for optical imaging. Second, and possibly more important, strabismus was induced later (in the fourth rather than in the third postnatal week). At this point, the animals had had about 2 weeks of visual experience, and a clear OD map had already formed (which was a necessary condition for our studying any changes caused by the induction of squint). This issue is difficult to resolve in our paradigm; one could at best deprive cats binocularly until a first imaging experiment at about 3 weeks of age and then induce strabismus prior to any visual experience (Gödecke et al., 1997; Crair et al., 1998).

Recent evidence appears to support the view that the layout of OD columns is intrinsically determined. In particular, neonate macaque monkeys exhibit OD patterns of adult-like periodicity prior to any visual experience (Horton and Hocking, 1996a). Moreover, in ferret area 17, clustering of geniculocortical afferents according to lamina of origin may occur even if the eyes are enucleated before layer IV is innervated by the LGN (on PD 8), i.e. in the absence of functional retinal inputs (Crowley and Katz, 1997). Our experiments indicate that strabismus does not alter this innate pattern but further research is required.

4.2. Effect of reverse-occlusion on orientation preference maps

In all reverse-occluded kittens, whether with or without visual experience restricted to a single orientation, orientation preference maps through the left and right eye were remarkably similar although the two eyes had never shared any visual experience. It is worth noting that the cross-correlation coefficients for iso-orientation maps obtained through left and right eye were even higher in the reverse-occluded animals (without stripe-rearing) than in normal kittens of similar age (Tables 1 and 2). Cross-correlation coefficients were slightly lower in stripe-reared and normal animals simply because responses through the two eyes did not occupy exactly the same regions of cortex. In the stripe-reared animals, this was due to the fact that the initially open eye did not lose all of its input into area 17 after reverse lid-suture and exposure to stripes that were orthogonal to those experienced first (see Figs. 5 and 6).

Two conclusions can be drawn from our findings. First, manipulating the visual environment can shift the balance of representation of the two eyes and, to a lesser extent, of different orientations in the visual cortex, but it does not affect the geometry of orientation maps (Gödecke and Bonhoeffer, 1996). Secondly, following monocular exposure and reverse lid-suture, inputs from the second eye do not have the same capacity to shape neuronal responses, especially orientation selectivity, as afferents from the initially experienced eye had. Apparently, selectivity for a particular orientation can only develop in regions of cortex that had been selective for that orientation before (as it does in the reverse-occluded animals reared in a normal

Fig. 6. Iso-orientation maps from the left and right eye of a stripe-reared kitten raised with reverse occlusion. This animal was exposed through the left eye to stripes oriented at 45° until PD 37, when the first imaging experiment was carried out. Iso-orientation maps for left-eye stimulation (A) are shown after division by blank (leftmost column) and cocktail-blank (second column). The kitten was then reverse-sutured and exposed to stripes of 135° until PD 52, when the second imaging experiment was performed. Iso-orientation maps for right-eye stimulation (B) are shown in the third column (division by blank) and rightmost column (division by cocktail blank). Note the high degree of similarity between blank-divided maps for all four orientations after the second period of exposure. However, the layout of the (weak) orientation preference maps (images divided by cocktail blank) remains unchanged (compare second and fourth columns; arrowheads mark corresponding positions in the 45 and 90° maps). The bottom row shows blood-vessel patterns and ocular dominance maps for the experienced eye for both experiments. Below the OD maps, angle maps of orientation preference are also shown. The dura was left intact during the first optical imaging and was removed before the second. Scale bars, 1 mm.

Table 2
Results of cross-correlation analysis for reverse-occluded kittens

Kitten number	Stripe-rearing	1st experiment			2nd experiment			$r_{left,right}$
		PD	$r_{0,90}$	$r_{45,135}$	PD	$r_{0,90}$	$r_{45,135}$	
01	No	34	−0.11	−0.11	47	+0.22	+0.30	+0.71 ± 0.03
02	No	35	−0.16	−0.21	48	−0.33	−0.12	+0.86 ± 0.03
04	No	34	−0.16	−0.08	42	+0.59	+0.60	+0.77 ± 0.06
17	90/0°	38	−0.25	−0.01	52	+0.80	+0.85	+0.45 ± 0.05
18	135/45°	44	−0.14	−0.29	57	+0.84	+0.84	+0.64 ± 0.04
26	45/135°	37	−0.14	−0.31	52	+0.62	+0.71	+0.54 ± 0.14

Regimens of stripe-rearing are stated, where applicable. PD, postnatal days on which the first experiment (left eye open) and the second experiment (right eye open) were carried out. Columns marked $r_{0,90}$ and $r_{45,135}$ give coefficients of cross-correlation between maps for orthogonal orientations obtained through the experienced eye. The last column ($r_{left,right}$) gives mean (± S.D.) best coefficients of cross-correlation between left- and right-eye iso-orientation maps (see Text).

environment), or it fails to develop properly. The role of experience is to modify response properties such as orientation preference and ocularity, not to set them up.

This interpretation offers an explanation for our data from stripe-reared kittens. While their visual experience was limited to a single orientation (or at least a narrow range of orientations, considering the possibility of head rotations), V1 in these animals exhibited regions of preference for orientations to which they had never been exposed, although these areas were reduced in relative size. The most straightforward explanation of this finding is that the preferred orientations of these regions were pre-specified. Interestingly, there is apparently only limited scope for experience-driven recovery of orientation-selective responses through the second eye after reverse occlusion. The now open eye does gain overall dominance and regions of cortex that responded in an orientation-selective manner through the initially open eye lose selectivity, but there is no complementary increase in orientation-selective response through the second eye. This result is reminiscent of the loss of visual acuity observed in both eyes after monocular deprivation and reverse occlusion (Murphy and Mitchell, 1987). Very recently, it has been shown that monocular deprivation up to the age of 6 weeks results in a substantial loss of the NMDA receptor subunit NMDAR1 in particular in that part of V1 where the central visual field is represented (Duffy et al., 1997), i.e. in the region which was imaged in the present study. Given that the NMDA receptor is widely believed to be essential in mediating synaptic plasticity in the brain, including V1 (for a review, see Collingridge and Singer, 1990), this finding may explain why the visual cortex acquires neither normal orientation selectivity nor normal spatial resolution through the newly experienced eye.

If one assumes that the layout of an orientation map depends on factors intrinsic to the cortex, then the horizontal intracortical circuitry is a good candidate for the underlying anatomical substrate. It is likely to play an important part in the generation of cortical orientation selectivity (e.g. Douglas and Martin, 1991; Crook et al., 1997), and anisotropies of intrinsic horizontal connections are correlated with the axis of preferred orientation of sites in V1 of tree shrews (Bosking et al., 1997) and cats (Schmidt et al., 1997). Crude clustering of horizontal connections develops in the absence of any retinal input but depends on the presence of (spontaneous) cortical activity (Ruthazer and Stryker, 1996). Such clustering might therefore be responsible for the development of orientation preference maps in kittens that are deprived of any patterned vision by binocular lid suture (Gödecke et al., 1997; Crair et al., 1998). On the other hand, the refinement of clustered intracortical connections depends on visual experience (Katz and Callaway, 1992; Löwel and Singer, 1992) as does the maturation of orientation preference maps. In reverse-occluded animals, an orientation map set up by the first eye could be read out by the second eye from the pattern of intrinsic connections that persists in the visual cortex (Bonhoeffer and Kim, 1995).

Further experiments will have to answer the question which factors, if not visual experience, determine the geometry of functional cortical maps.

Acknowledgements

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