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The layout of orientation and ocular dominance domains in area 17 of strabismic cats

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Abstract

In the primary visual cortex of strabismic cats, the elimination of correlated activity between the two eyes enhances the segregation of the geniculocortical afferents into alternating ocular dominance domains. In addition, both tangential intracortical fibres and neuronal synchronization are severely reduced between neurons activated by different eyes. Consequently, ocular dominance columns belonging to different eyes are functionally rather independent. We wondered whether this would also affect the organization of orientation preference maps. To this end, we visualized the functional architecture of area 17 of strabismic cats with both optical imaging based on intrinsic signals and double labelling of orientation and ocular dominance columns with [^{14}C]2-deoxyglucose and [^{3}H]proline. As expected, monocular iso-orientation domains had a patchy appearance and differed for the two eyes, leading to a clear segregation of the ocular dominance domains. Comparison of 'angle maps' revealed that orientation domains exhibit a pinwheel organization as in normally reared cats. Interestingly, the map of orientation preferences did not show any breaks at the borders between ocular dominance columns: iso-orientation domains were continuous across these borders. In addition, iso-orientation contours tended to cross the borders of adjacent ocular dominance columns at right angles. These data suggest that the basic relations between the layout of orientation maps and ocular dominance columns are not disturbed by artificial decorrelation of binocular input. Therefore in cat area 17, the orientation map does not seem to be modified by experience-dependent changes of thalamic input connections. This suggests the possibility that use-dependent rearrangement of geniculocortical afferents into ocular dominance columns is due to Hebbian modifications whereby postsynaptic responsivity is constrained by the scaffold of the orientation map.

Introduction

Strabismus eliminates correlated activity between the two eyes because the images on the two retinae cannot be brought into register. If induced early in life, this decorrelation leads to a breakdown of binocular convergence. As a consequence the segregation of geniculocortical afferents into alternating ocular dominance columns is enhanced in area 17 of strabismic as compared with normal cats (Shatz *et al.*, 1977; Löwel, 1994) and neurons become responsive almost exclusively to stimulation of either the left or the right eye (Hubel & Wiesel, 1965). In strabismic animals, monocular visual stimulation induces 2-deoxyglucose (2-DG) patterns extending in columns through all cortical layers, and these columns are in precise register with the thalamocortical afferents of the stimulated eye in layer IV (Löwel & Singer, 1993b). In addition, tangential intracortical fibres are drastically reduced between neurons activated by different eyes (Löwel & Singer, 1992). Consistent with the hypothesis that these fibres mediate the temporal coordination of distributed neuronal responses (Singer, 1995) neuronal synchronization is also severely reduced between neurons dominated by different eyes, whereas it is normal between neurons dominated by the same eye (König *et al.*, 1993). Thus, in strabismic animals, the sets of ocular dominance columns related to different eyes are rather independent of each other, raising the question whether this affects the organization of iso-orientation domains.

Recently, it has been shown that in both area 17 of ferrets and areas 17 and 18 of cats, orientation preference maps are present already 1 week after eye opening and that their layout does not seem to change dramatically in the following 2 weeks (Chapman *et al.*, 1996; Gödecke *et al.*, 1997; Crair *et al.*, 1998). In addition, orientation maps are identical for the two eyes in cats raised without binocular visual experience (Gödecke & Bonhoeffer, 1996; see also Kim & Bonhoeffer, 1994). It

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has been inferred from these studies that the layout of orientation domains develops rather independently of visual experience and is not altered while input connections reorganize due to monocular deprivation. The possibility needs to be considered, however, that the strict anatomical and physiological segregation of ocular dominance domains resulting from early onset strabismus also leads to an eye-specific segregation of the orientation map. In this case, iso-orientation domains activated by different eyes should distribute independently, i.e. they would no longer be continuous across the boundaries between different ocular dominance domains. If, however, orientation domains remain continuous across ocular dominance boundaries, this would indicate that the use-dependent rearrangement of thalamic input connections is guided by the response properties of cortical neurons which are in turn determined by intracortical interactions specifying the neuronal orientation preference. Distinction between these alternatives is of particular importance because of the squint-induced restriction of tangential connections to domains of like ocular preference (Löwel & Singer, 1992). In normal and strabismic animals, these connections link selectively columns with similar orientation preference (Gilbert & Wiesel, 1989; Schmidt *et al.*, 1997b) and have been assigned an important role in the development of iso-orientation maps. If orientation maps remain continuous across the segregated ocular dominance domains of strabismic animals this implies that tangential connections are not required for the maintenance of the sequence regularity of iso-orientation maps. To examine this question, we visualized the layout of iso-orientation and ocular dominance columns in area 17 of strabismic cats using both optical imaging of intrinsic signals and double labelling with [^{14}C]2-DG and [^{3}H]proline, and analysed the topographic relationship between the two functional systems. We selected area 17 rather than area 18 because theoretical considerations suggest that area 17 may be more susceptible to experience-dependent modifications than area 18 (Wolf *et al.*, 1996). Some of the results have been reported in abstract form (Löwel *et al.*, 1994, 1995; Schmidt *et al.*, 1994).

Materials and methods

Fifteen kittens of the institute's colony, each from a different litter, were included in this study. In 12 of them (S1–S10; BO1 and BO2), a divergent squint angle was induced surgically at postnatal days 17 or 18. Three normally raised cats (N1–N3) served as control animals. By the time of the experiments, the ages of the animals ranged from 2 months to 2.5 years (normal cats: 2 months, 10 months and 2 years). The wide age-range was chosen in order to permit detection of age-dependent variations in the parameters studied. In cats S1–S10 and N1–N3, the functional architecture of area 17 was visualized using optical imaging based on intrinsic signals while the animals were stimulated monocularly with moving gratings of different orientations. Intrinsic signal imaging exploits the fact that active cortical regions consume oxygen thereby accumulating deoxyhaemoglobin. Because deoxyhaemoglobin (compared with oxyhaemoglobin) absorbs more of the red light used to illuminate the cortex, active regions appear dark on the images (Grinvald *et al.*, 1986; Frostig *et al.*, 1990). In cats BO1 and BO2, ocular dominance and orientation columns were visualized with the transneuronal tracer [^{3}H]proline (Grafstein, 1971) and with activity-dependent uptake of [^{14}C]2-deoxyglucose (2-DG) (Sokoloff *et al.*, 1977), respectively.

Squint induction

At the age of 17–18 days, anaesthesia was induced with an intramuscular injection of ketamine hydrochloride (10 mg/kg, Ketanest®, Parke-Davis, Berlin, Germany) and xylazine hydrochloride (2.5 mg/kg, Rompun®, Bayer AG, Leverkusen, Germany). The tendon of the medial rectus muscle of the left eye was transsected to produce exotropia (divergent strabismus). To assure that all experimental animals were strabismic throughout the critical period, we used the corneal reflex method (Sherman, 1972; von Grünau, 1979) to determine eye alignment. For this purpose, each kitten was restrained manually and at least three flashlight snapshots of the animal's face were taken weekly from age 3 to age 8 weeks. The distances between the corneal reflexes and the pupils were measured on the photographs: the ratio of the reflex distance over the pupillary distance is a reliable indicator of eye alignment (Sherman, 1972; Sireteanu *et al.*, 1993). The ratios of our experimental animals were always below 0.93 (0.88–0.91) and thus in the strabismic range throughout the critical period (see Sireteanu *et al.*, 1993).

Optical imaging

The functional architecture of area 17 was visualized using optical imaging based on intrinsic signals when the animals reached an age between 2 months and 2.5 years. Because the technique has been described in detail elsewhere (Grinvald *et al.*, 1986; Bonhoeffer & Grinvald, 1993, 1996) only the essential steps of the procedure are reported here.

Surgery

Anaesthesia was induced as described above (ketamine and xylazine hydrochloride) and maintained throughout the experiment using halothane/N_2O/O_2 anaesthesia (70%N_2O/30%O_2, supplemented with 0.4–1.0% halothane, Hoechst Pharmaceuticals, Frankfurt, Germany). The ECG, pulmonary pressure, end-tidal CO_2 (3–4%) and rectal temperature (37–38 °C) were continuously monitored. The animal's head was fixed in a stereotaxic frame by means of a metal nut cemented on to the skull. A craniotomy was performed between Horsley-Clarke A3 and P13 and a stainless steel chamber was implanted over the exposed cortical region with dental cement (centred around P5). After removing the dura, the chamber was filled with silicone oil and sealed with a round glass coverslip.

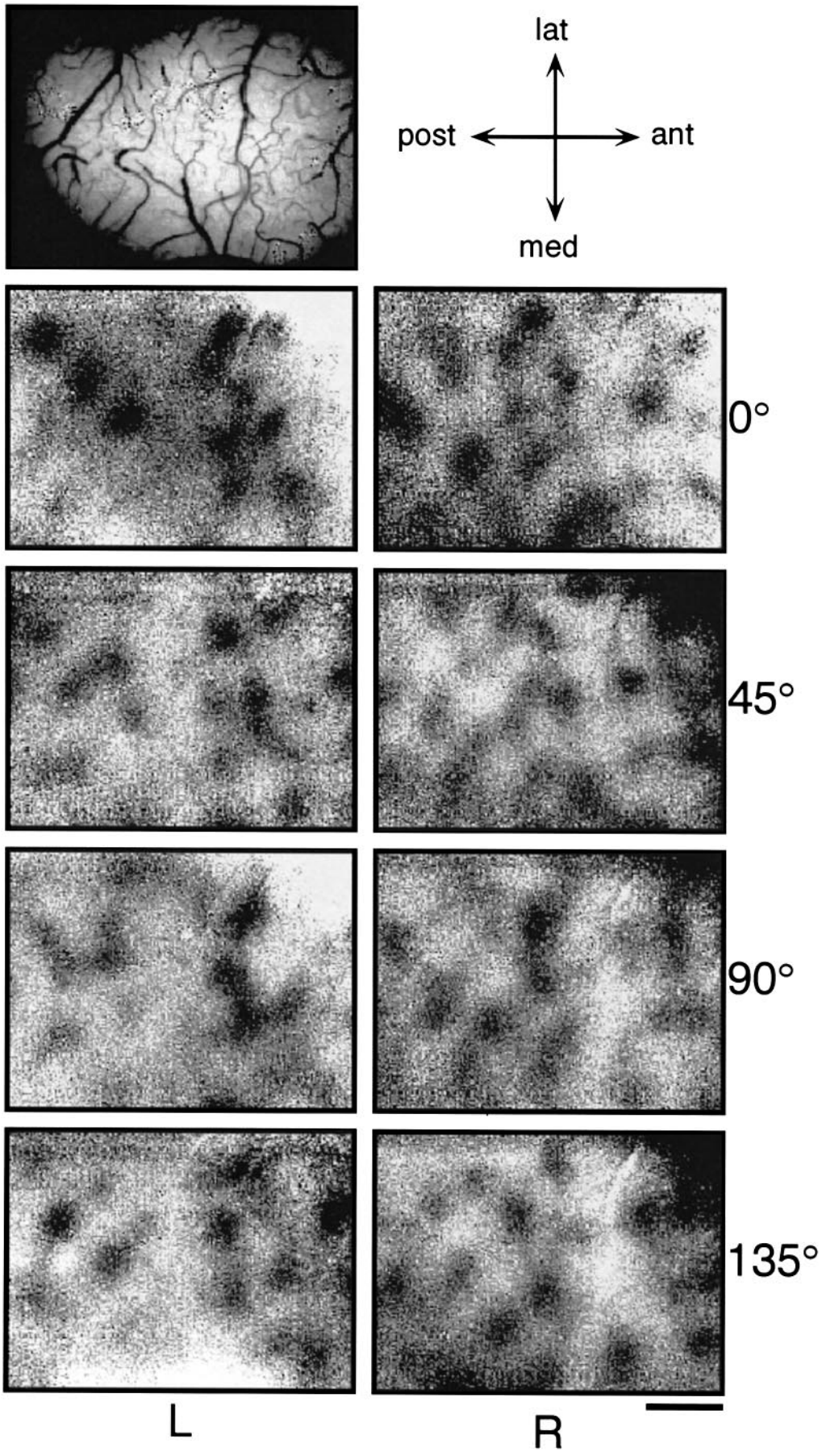
Visual stimulation

Large-field visual stimuli were presented on a monitor positioned at a distance of 25 cm in front of the animals' eyes. These were refracted appropriately using contact lenses with artificial pupils of 3 mm. Animals were stimulated monocularly with high-contrast square-wave gratings of four different orientations (0°, 45°, 90° and 135°; spatial frequency: 0.5 cyc/deg; speed: 4 deg/s; produced by STIM, developed by Kaare Christian, the Rockefeller University, New York).

Data acquisition

The cortical surface was illuminated by two adjustable light guides attached to a tungsten–halogen lamp (Zeiss), equipped with interference filters for different wavelengths. The vascular pattern of the cortex was visualized at 540 ± 10 nm (green), cortical activity maps

FIG. 1. Monocular orientation domains in the left area 17 of strabismic cat S2 (12.5 weeks old). Blood vessel pattern of the imaged cortical area (4.8 × 3.6 mm; top left). Cortical activation patterns visualized by optical imaging of intrinsic signals while the animal was stimulated through the left (L, left column) and right eye (R, right column) with orientated gratings of 0°, 45°, 90° and 135° (from top to bottom). Note that the patterns are clearly different for left and right eye stimulation. Abbreviations: med, medial; post, posterior; ant, anterior; lat, lateral. Scale bar 1 mm.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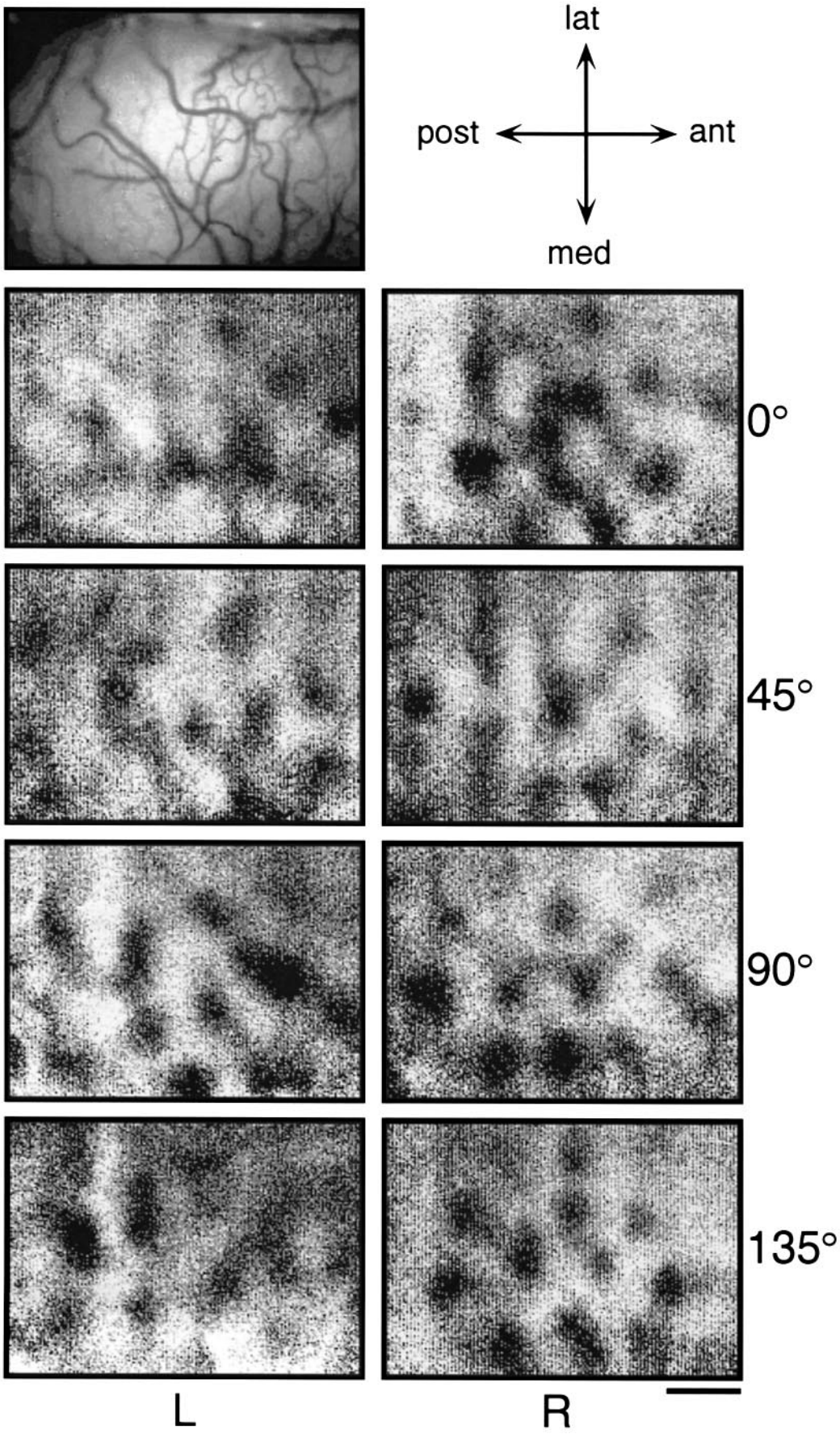
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at 605 ± 10 nm (red). A slow-scan CCD camera (System 2000, Digital Pixel) was used for collecting the intrinsic signals. To this end, every 10 s, a particular visual stimulus was presented for 3 s while five frames of 600 ms duration were acquired.

Data analysis

For data analysis, we calculated 'single condition maps' $I(\vec{x})$ which correspond to the images acquired during a particular stimulus presentation divided by the sum of all different stimulus conditions ('cocktail blank procedure'; see Bonhoeffer & Grinvald, 1993, 1996). Accordingly, ocular dominance maps were computed by summing all monocular activity maps divided by the sum of all different stimulus conditions.

For comprehensive analysis of the organization of iso-orientation domains, the single responses to all different stimulus conditions were summed vectorially on a pixel-by-pixel basis. For every point $\vec{x} = (i,j)$ in the cortex, we thus summed eight vectors, their lengths being the magnitude of the 'single condition responses' and their angles corresponding to the orientation of the gratings that produced the responses. There are several ways to display the results of the vectorial analysis, each of which emphasizes a particular aspect of the organization of iso-orientation domains. In the present analyses, the angle θ ($\theta = 0.5 \arctan\left(\frac{z_1}{z_2}\right)$) of the resulting vector is displayed and colour-coded ('angle map'; see Bonhoeffer & Grinvald, 1993, 1996), so that the respective colours indicate the preferred orientation for this piece of cortex ('orientation preference map').

Intersection angle between orientation and ocular dominance bands

To characterize the local geometric relationship between iso-orientation domains and ocular dominance borders, we analysed the statistics of their intersection angles. Ocular dominance borders are defined as the contours formed by all locations in the image where right and left eye responses are equally strong. If we define an ocular dominance pattern o by $o(\vec{x}) = I_{left} - I_{right}$, these contours are given by the condition $o(\vec{x}) = 0$. For every location $\vec{x}$ (gridpoint) on an ocular dominance border, the intersection angle with an iso-orientation contour $\alpha(\vec{x})$ is given by

$$\alpha(\vec{x}) = \arccos\left(\frac{\nabla\theta(\vec{x})\nabla o(\vec{x})}{|\nabla\theta(\vec{x})\nabla o(\vec{x})|}\right)$$

where ∇ is the spatial gradient. $\nabla\theta$(analogous for ∇o) was approximated using

$$(\nabla\theta)_{ij} = \frac{1}{2}\begin{pmatrix}\theta_{i+1,j} - \theta_{i-1,j} \\ \theta_{i,j+1} - \theta_{i,j-1}\end{pmatrix}.$$

To quantify the typical behaviour of iso-orientation domains close to ocular dominance borders, we calculated the frequency of their intersection angles in a range from [0°, 90°]. To obtain a histogram of these frequencies from the discrete grid of data points, the intersection angles were categorized into six classes K_i ($0° \leqslant \alpha \leqslant 15°$, $15° < \alpha \leqslant 30°$, ...$75° < \alpha \leqslant 90°$) (see Fig. 8). The percentage of iso-orientation contours exhibiting an intersection angle within class K_i, is then given by

$$P_i = \sum_{\alpha_{ij} \in K_i} w_{ij}$$

where

$$w_{ij} = \frac{1}{W}\left|\nabla\theta_{ij}\right|$$

is a statistical weight proportional to the local density of iso-orientation contours. W was chosen such that $\Sigma_{ij}\, w_{ij} = 1$.

This statistics of intersection angles is inherently robust against noise in the orientation preference map. The direction of iso-orientation domains is not well defined, and particularly sensitive to noise, in regions where the orientation gradient is close to 0. Such regions do not influence the statistics as defined above because the density of iso-orientation domains is proportional to the orientation gradient, i.e. w_{ij} (for an alternative method which excludes these locations from further analysis see Obermayer & Blasdel, 1993). On the other hand, pinwheels, i.e. points where the orientation gradient is large and the direction of iso-orientation contours changes strongly, are particularly emphasized in this measure. Thus, our method selectively emphasizes those parts of a map from which the most significant information about the relation of iso-orientation domains and ocular dominance borders can be obtained.

As illustrated in Fig. 8 a strong preponderance of angles between 75 and 90 degrees was observed. To exclude the possibility that this tendency for orthogonal intersections was accidental, we redetermined intersection angles after shifting the two maps in two different ways: (i) by shifting the two maps from the same animal in increments of 10 pixels in both *x*- and *y*-direction (at least 24 alternate positions compared) and (ii) we superimposed maps originating from different animals (Fig. 8, right column) as recently also described by Hübener *et al.* (1997).

Pinwheel density

Orientation centres (pinwheel centres) were determined from the vector field $z(\vec{x})$ where they coincide with the zeros of $z(\vec{x})$. The positions of putative pinwheel centres correspond to the crossings of the 0°/90° – with the 45°/135°-orientation contours (see Figs 6 and 7), calculated as the lines of $z_2 = 0$ and $z_1 = 0$, respectively. For every single point we checked by visual inspection whether it represented a proper orientation centre in the corresponding angle map. Points around which iso-orientation domains did not exhibit a clear radial organization were discarded from further analysis. Using this semiautomatic method, no orientation centres can be missed and only clear examples of pinwheel centres enter the quantitative spatial density analysis.

Pinwheel location

To analyse pinwheel location with respect to ocular dominance columns, pinwheel position was determined as described above. Next, the relative frequency of pinwheel centres in different subregions of ocular dominance maps was analysed using an approach recently introduced by Hübener *et al.* (1997). To this end, centre and border regions of ocular dominance columns were defined by way of the pixel values in the different maps: those 10% of the pixels in an ocular dominance map with the highest values were defined as corresponding to the centres of the columns of one eye, whereas the 10% with the lowest pixel values

FIG. 2. Monocular orientation domains in the right area 17 of the 7 months old strabismic cat S3. Blood vessel pattern of the imaged cortical area (top left). Cortical activity patterns visualized by optical imaging while the animal was stimulated through the left (L, left column) and right eye (R, right column) with orientated gratings of 0°, 45°, 90° and 135° (from top to bottom). Note that the patterns are clearly different for left and right eye stimulation in this 7-month-old cat (compare with Fig. 1) so that there are no age-dependent variations in the 'complementarity' of left and right eye patterns. Abbreviations as in Fig. 1. Scale bar 1 mm.

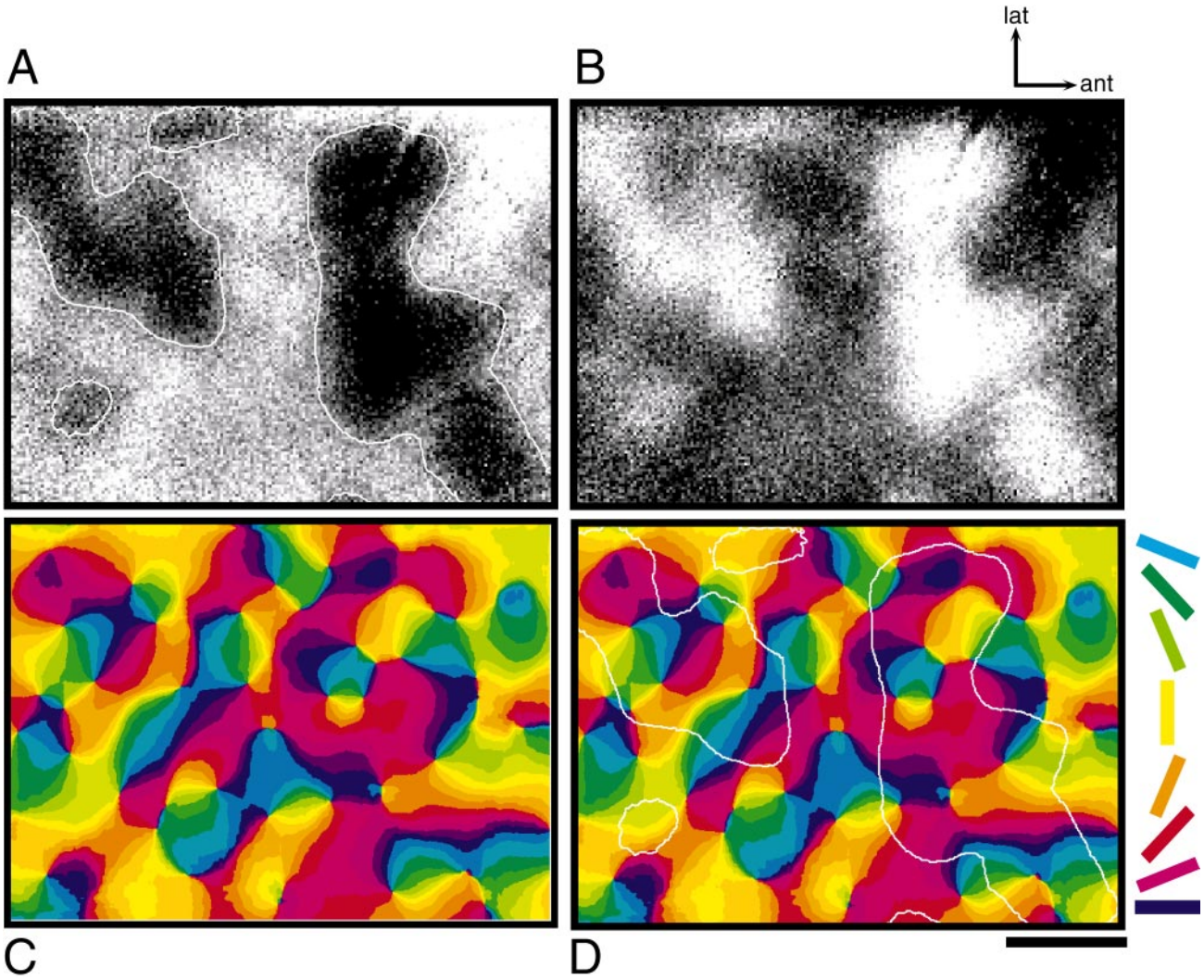

FIG. 3. Segregated ocular dominance domains and orientation preference map in the left area 17 of strabismic cat S2. The same cortical region as in Fig. 1 is visualized. (A, B) Activation patterns for the left (A) and right eye (B) are complementary: regions heavily activated by the left eye (dark regions in A, outlined in white) are only weakly activated by the right eye (light grey to white regions in B). Note that the domains of the left, ipsilateral (deviated) eye appear as dark islands on a light grey sea. (C) Orientation preference ('angle') map of the same piece of cortex: the preferred orientation for every region of the imaged cortex is colour-coded according to the scheme on the right side of the figure. Note the pinwheel-like organization of orientation domains: there are numerous singularities in the map around which all colours (orientations) appear once. (D) Topographic relationship between iso-orientation domains and ocular dominance columns. Superposition of the angle map (C) and the outlined borders of adjacent ocular dominance columns (white contours in A). Note that domains of like orientation preference labelled by the same colour in the angle map are continuous across the borders of adjacent ocular dominance domains. Scale bar 1 mm.

defined the centres of the ocular dominance columns of the other eye. Thus, the combined centre regions make up 20% of the area of an ocular dominance map. Analogous, the border regions were defined as 20% of the area of a map with intermediate pixel values (see Hübener *et al.* (1997) for details).

Double labelling with [^{3}H]proline and [^{14}C]2-DG

At an age of 3 months, cats BO1 and BO2 were subjected to double-labelling with [^{3}H]proline and [^{14}C]2-DG as described in detail previously (Löwel & Singer, 1993a,b). In these experiments, orientation domains were visualized with [^{14}C]2-DG autoradiography (Sokoloff *et al.*, 1977) after binocular visual stimulation. In the same animals, ocular dominance columns were labelled with [^{3}H]proline (Grafstein, 1971). Finally, 2-DG and proline autoradiographs derived from the same cortical flat-mount sections were superimposed to analyse the topographic relationship between orientation and ocular dominance columns.

Surgical procedures

For transneuronal labelling of ocular dominance columns, the cats were anaesthetized with a mixture of ketamine hydrochloride (10 mg/kg) and xylazine hydrochloride (2.5 mg/kg) i.m. and then received an injection of 2.5 mCi of L-[2,3,4,5-^{3}H]proline (Amersham, specific activity 95 Ci/mmol; dissolved in a volume of 50 µL) into the left squinting eye (BO1) or into the right non-squinting eye (BO2). Two weeks later, these cats were prepared for the labelling of binocular orientation domains with 2-DG. A detailed description of the experimental procedures is reported elsewhere (Löwel *et al.*, 1987). Briefly, anaesthesia was induced and maintained as described above for optical imaging. The animal's head was fixed in a stereotaxic

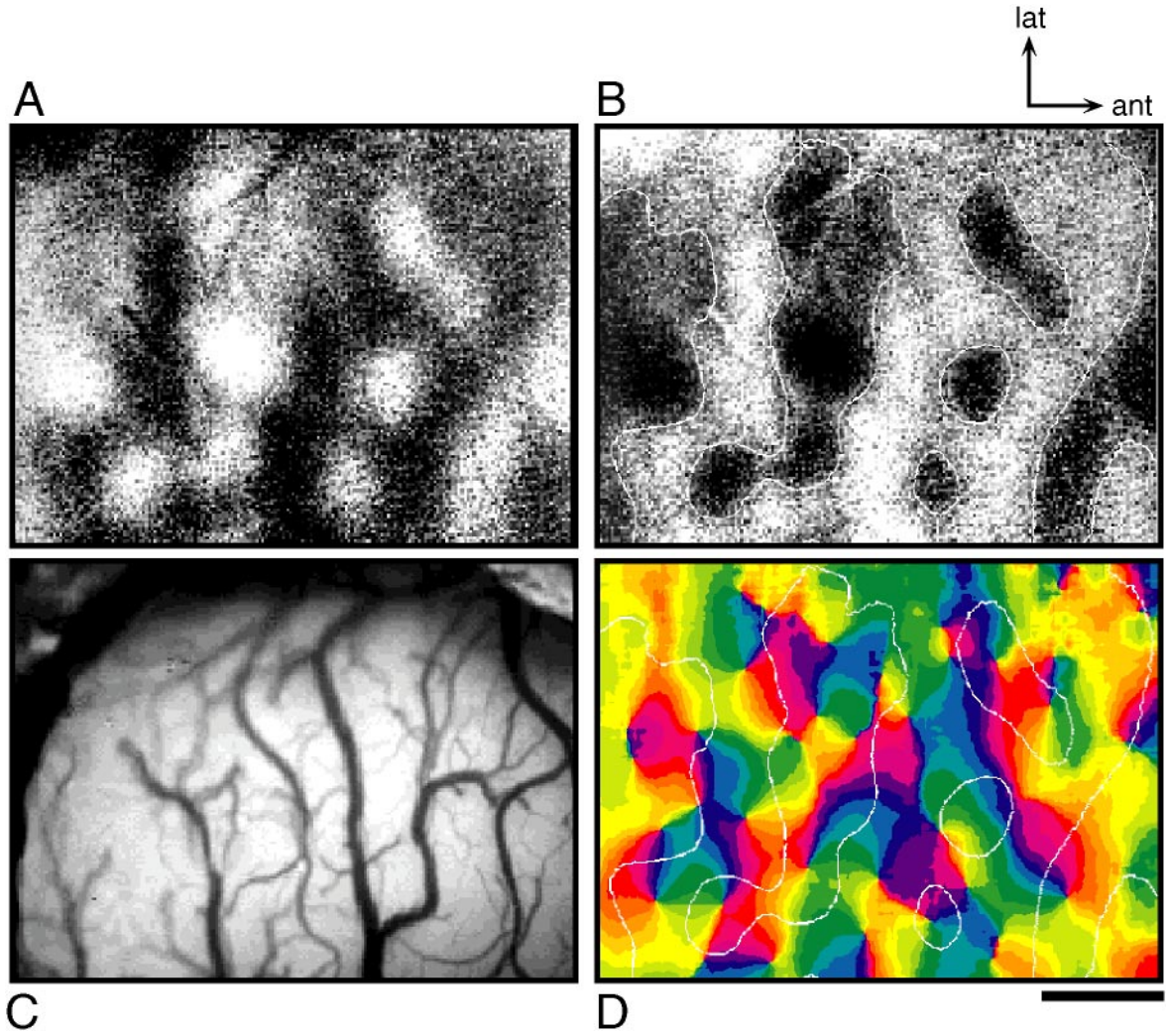

FIG. 4. Optically visualized ocular dominance and iso-orientation columns in the right area 17 of strabismic cat S1. (A, B) Cortical activation patterns for the left (A) and right eye (B). In (B), the domains of the right, ipsilateral eye are outlined in white. In contrast to Fig. 3, the left (deviated) eye is contralateral to the visualized hemisphere and tends to occupy more cortical territory than the ipsilateral eye (A). (C) Blood vessel pattern of the imaged cortical area. (D) Topographic relationship between iso-orientation domains and ocular dominance columns. Superposition of the colour-coded angle map and the outlined borders of adjacent ocular dominance columns (white contours in B). Note that domains of similar orientation preference are again continuous across the borders of adjacent ocular dominance domains. Note in addition that the very small ocular dominance domains in the right lower corner of the map contain all orientations (all colours in the map) at least once. Scale bar 1 mm. Abbreviations as in Fig. 1.

frame by means of a metal nut cemented on to the skull. Binocular stimulation with high-contrast square wave gratings of horizontal (cat BO1) or vertical (cat BO2) orientation was performed as described above. Simultaneously with the onset of visual stimulation, the animals received an intravenous injection of 2-deoxy-D-[U-^{14}C]glucose (Amersham, specific activity, 300 mCi/mmol; dose, 100 µCi/kg). After 45 min of visual stimulation, a lethal dose of penthobarbital (180 mg/kg, Nembutal) was given.

Histological procedures

The occipital poles were removed and flat-mounted before freezing the tissue on dry ice. To provide landmarks for later superposition, three holes were melted into the flat-mounts with warm needles. Subsequently, 26-µm-thick serial cryostat sections of the flat-mounts were cut parallel to the cortical surface, mounted on glass slides, immediately dried on a hot plate and exposed to X-ray films (Structurix D7 W, Agfa Gevaert, Köln, Germany) for 3 weeks to visualize the 2-DG labelling. Then, the same sections were postfixed in 4% paraformaldehyde, washed to remove all 2-DG and then re-exposed to film (Hypofilm-^{3}H, Amersham, Braunschweig, Germany) for 10 weeks to visualize the proline distributions.

Image processing

For data presentation (see Fig. 9A, upper panels), the [^{3}H]proline and [^{14}C]2-DG autoradiographs were digitized and translated into grey values. In case of large-scale inhomogeneities in the optical density distributions, a high-pass filter was applied. The images were then low-pass filtered and contrast-enhanced by expanding the grey values. To investigate the topographic relationship between the proline pattern of ocular dominance columns and the 2-DG-labelled binocular orientation domains, the respective patterns derived from the same flat-mount sections were computed as

binary images (Fig. 9A, middle panel). Because proline labelling is visible only in layer IV of areas 17 and 18 single proline autoradiographs usually contain only parts of the labelled areas despite flat-mounting. Therefore, sections with large labelled portions of central area 17 were selected for image processing. For the ocular dominance distribution a threshold was selected that assigned 50% of the pixels to one eye assuming an equal distribution of the left and the right eye domains. In case of the patterns of binocular orientation columns, the 2-DG-labelled area was defined to cover 40% of the cortical surface and displayed in green, the remaining area was displayed in white. This threshold was adjusted to fit a naive observer's impression of labelled domains in the originals. Therefore, the range of orientation tuning contained in the labelled area in Fig. 9A (middle panel, right) can be assumed to cover $\pm$ 35°. Superposition of the binary images of both columnar systems was performed with the help of the three needle holes. The regions of overlap showing both proline and 2-DG labelling were displayed in blue (Fig. 9A, lower panel, Fig. 9B,C).

TABLE 1. Pinwheel densities in area 17 of strabismic and normal cats

Cat	Age	Pinwheel density (1/mm^2)	Cortical area a(mm^2)
S1	7 weeks	2.7	12.5
S2	12.5 weeks	2.7	10.4
S3	7 months	2.6	10.1
S4	8 weeks	2.5	7.2
S6	7 weeks	2.8	8.3
S7	2.5 years	2.7	7.7
S8	2 years	2.7	11.3
N1	8 weeks	2.6	10.0
N2	2 years	2.6	8.0
N3	10 months	2.5	8.0

Cat, 'name' of the animal. Age, age of the cats. Pinwheel density [1/mm^2], density of pinwheel centres per mm^2 cortical surface. Cortical area [mm^2], size of the cortical area used for quantitative analyses of pinwheel densities.

Results

Activity maps in area 17 of strabismic cats were recorded in 3.6 mm $\times$ 4.8 mm large regions in the central visual field representation at the junction of the lateral to the posterolateral gyrus of the brain (both ipsi- and contralaterally to the squinting eye). Examples of the blood vessel pattern of the imaged cortical areas are illustrated in Figs 1A, 2A and 4C.

Layout of ocular dominance columns

Monocular visual stimulation with contours of a single orientation caused activity patterns consisting of rather isolated patches (Figs 1 and 2). These resembled closely the activation patterns revealed with 2-DG after similar stimulation conditions (Schmidt *et al.*, 1997b).

As expected for strabismic animals (e.g. Löwel & Singer, 1993b), the orientation columns activated through the right and left eye differed (Figs 1 and 2) and ocular dominance domains can readily be visualized with optical recording (Figs 3 and 4). In normally raised cats, the visualization of ocular dominance domains becomes increasingly difficult after a few weeks of life (see Crair *et al.*, 1997a; Hübener *et al.*, 1997) because maps induced through the two eyes become nearly identical (Crair *et al.*, 1998). This is contrasted by the situation in strabismic cats where also in adult animals the activity maps of the two eyes are clearly different (compare Figs 1 and 2) and ocular dominance columns are complementary and well segregated: Regions activated by the left eye in cat S2 (dark grey to black in Fig. 3A) appear almost inactive (light grey to white in Fig. 3B) with stimulation of the right eye. Another example of the complementarity of left and right eye domains in strabismic cats is illustrated in Fig. 4A,B.

Ocular dominance domains of the ipsilateral eye (Figs 3A and 4B) appear as 'dark islands on a light grey sea' and the reverse is true for the domains of the contralateral eye (Figs 3B and 4A). These observations are in accordance with previous anatomical and electrophysiological results (e.g. Hubel & Wiesel, 1962; Blakemore & Pettigrew, 1970; Albus, 1975; Shatz & Stryker, 1978; Tieman & Tumosa, 1983; Löwel & Singer, 1987; Löwel, 1994), indicating that the contralateral eye tends to occupy more cortical territory than the ipsilateral eye. This was the case irrespective of whether the contralateral eye was the deviated (operated) or non-deviated eye (compare Figs 3A,B and 4A,B). Consist-

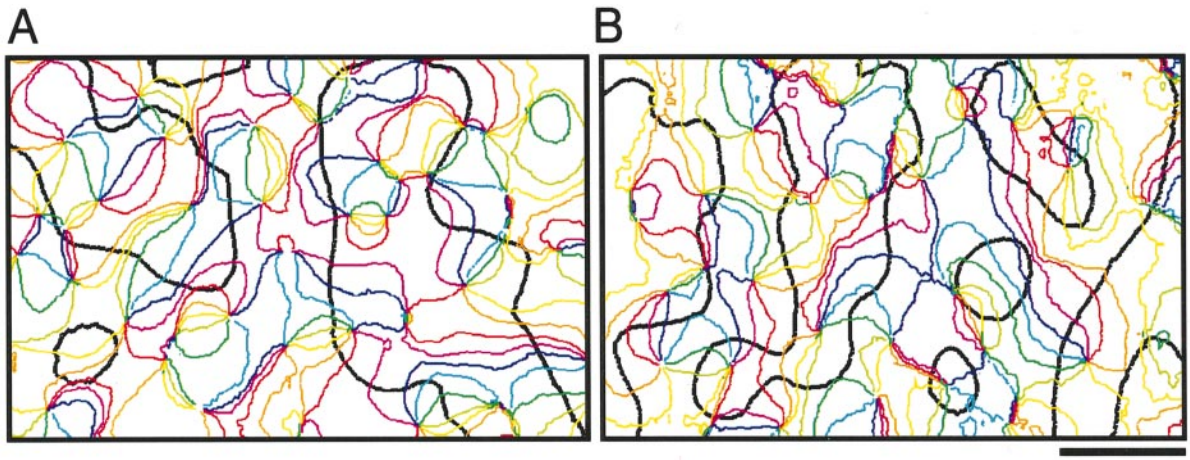

FIG. 5. Topographic relationship between iso-orientation and ocular dominance columns. Iso-orientation contours (coloured) superimposed on the borders between adjacent ocular dominance columns (black contours) in area 17 of cats S2 (A, see also Fig. 3D) and S1 (B, see also Fig. 4D). The iso-orientation contours were colour-coded according to the scheme illustrated in Fig. 3. Note that iso-orientation contours tend to intersect ocular dominance borders at right angles. Scale bar 1 mm.

ent with previous electrophysiological and anatomical experiments (i.e. Hubel & Wiesel, 1965; Löwel & Singer, 1992; König *et al.*, 1993; Schmidt *et al.*, 1997b) our optical imaging data thus give no indication of a disadvantage of the deviated compared with the non-deviated eye in divergently squinting cats (Figs 1–4).

Layout of orientation preference maps

To analyse the geometrical relationship between iso-orientation domains and ocular dominance columns, orientation preference maps were computed by vectorial summation of the responses to the different stimulus conditions (see Methods). In these 'angle maps' (Blasdel & Salama, 1986; Bonhoeffer & Grinvald, 1993), a colour-code is used to display the orientation that elicited the maximal response at a particular cortical region. In all our strabismic animals, the 'angle-maps' demonstrated a pinwheel-like organization of iso-orientation domains (Figs 3C, 4D and 6A) – as described previously for normally raised macaque monkeys, cats (see also Fig. 7A), ferrets and tree shrews (Blasdel & Salama, 1986; Bonhoeffer & Grinvald, 1993; Chapman *et al.*, 1996; Bosking *et al.*, 1997).

To further investigate the layout of the functional domains we determined the spatial density of the pinwheel centres. To this end, we performed a semiautomatic analysis of the angle maps as described in detail in the Methods section. Figure 6 illustrates one example of this analysis in a strabismic cat: pinwheel centres are numbered consecutively from left to right in the angle map and their spatial density is computed. In the illustrated case, the pinwheel density was 2.7/mm^2 cortical surface. The analysis of six additional angle maps in strabismic cats that were of sufficient quality to unambiguously determine pinwheel centres revealed an average density of 2.7 ± 0.1/mm^2 cortical surface ($n = 7$; see Table 1). This value is between 29% and 50% higher than the 1.8 resp. 2.1 pinwheel centres/mm^2, recently reported by Rao *et al.*

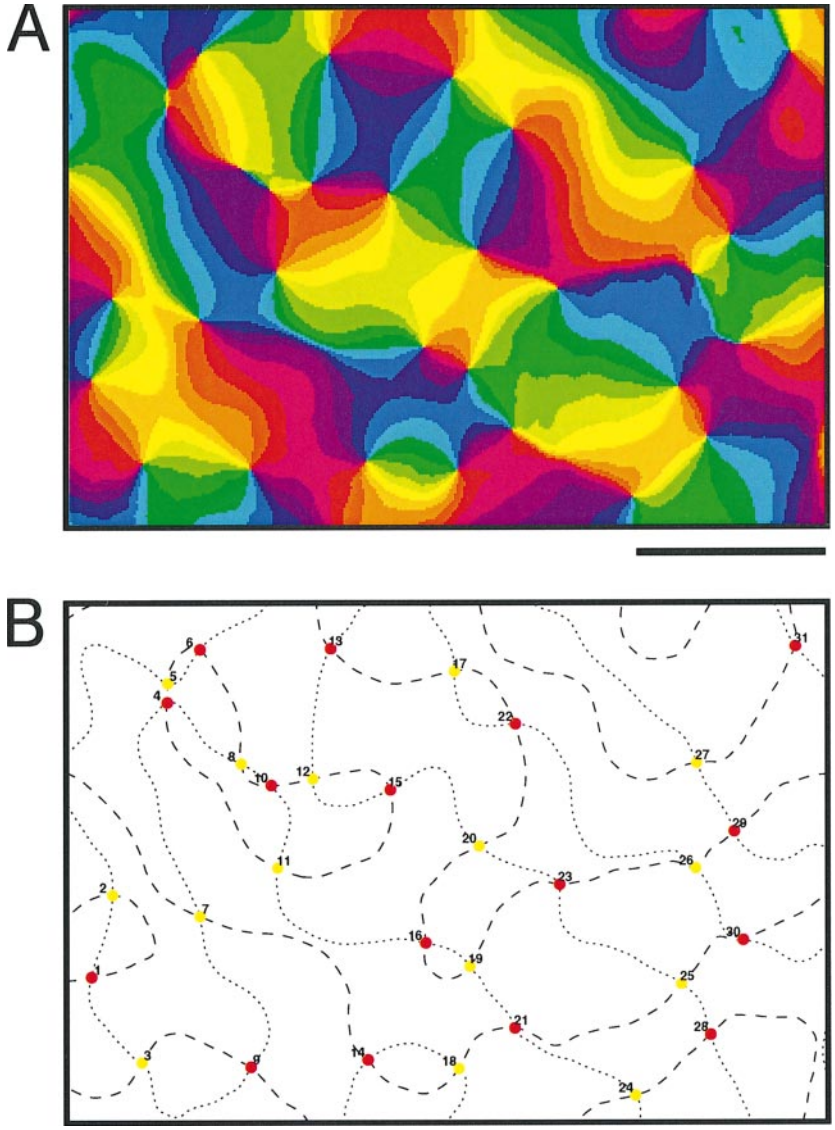

FIG. 6. Quantitative analysis of the distribution and spatial density of orientation (pinwheel) centres in area 17 of cat S8. (A) The angle map of the analysed visual cortical region is 11.3 mm^2 in size. (B) Locations and numbers of pinwheel centres: The pinwheel centres correspond to the crossings of the dotted with the broken lines (the lines of $z_1 = 0$ and $z_2 = 0$, see Methods). Pinwheel centres are numbered consecutively from left to right in the figure. Red resp. yellow dots correspond to pinwheel centres of opposite chirality (red: + 1/2, yellow: – 1/2). In cat S8, the angle map contains 31 pinwheels corresponding to a pinwheel density of 2.7/mm^2 cortical surface. Scale bar 1 mm.

(1994) and Bonhoeffer *et al.* (1995) in area 17 of normally raised cats. Both groups, however, used a different algorithm to quantify pinwheel densities. We therefore analysed orientation preference maps in three additional, normally raised cats from our institute's colony. An example of such an angle map in normal cats is illustrated in Fig. 7. Average density of pinwheel centres in area 17 of these animals was $2.6 \pm 0.1/mm^2$ cortical surface ($n = 3$; see Table 1) and is thus in the same range as the values observed in our strabismic cats. There was no age-dependent variation in the measured pinwheel density.

Topographic relationship between ocular dominance and iso-orientation columns: angles of intersection between orientation and ocular dominance columns

Comparing orientation with ocular dominance maps revealed that iso-orientation domains were continuous across the borders of ocular dominance columns (Figs 3D, 4D and 5). To analyse this continuity quantitatively, we determined the angle of intersection between orientation and ocular dominance columns. Iso-orientation contours tended to cross the borders between ocular dominance columns at steep angles (see the contour-plots in Fig. 5). Figure 8 shows histograms of intersection angles in five strabismic cats, revealing a strong preponderance of angles between 75 and 90 degrees. Between 26.0% and 33.3% of all intersection angles fell into this range (Fig. 8, left column). To exclude that this tendency for orthogonal intersections was merely an accidental feature we redetermined intersection angles after shifting the two maps from the same animal or after superimposing maps of different animals (Fig. 8, right column). In both cases, the distribution of intersection angles was even. The mean of the average angle for all cats was $55.8 \pm 2.2°$ ($n = 5$; Fig. 8), whereas in the shifted maps it was $45.7 \pm 1.5°$ ($n = 5$) ($P < 0.05$; Wilcoxon signed-rank test), a value very close to the expected average angle of 45°. These data are nearly identical to the average angles of 51.7% (original) resp. 45.8% (shifted) that have recently been reported by Hübener *et al.* (1997) for area 17 of normally raised cats. This indicates that iso-orientation and ocular dominance columns are not independent but exhibit a systematic topographic

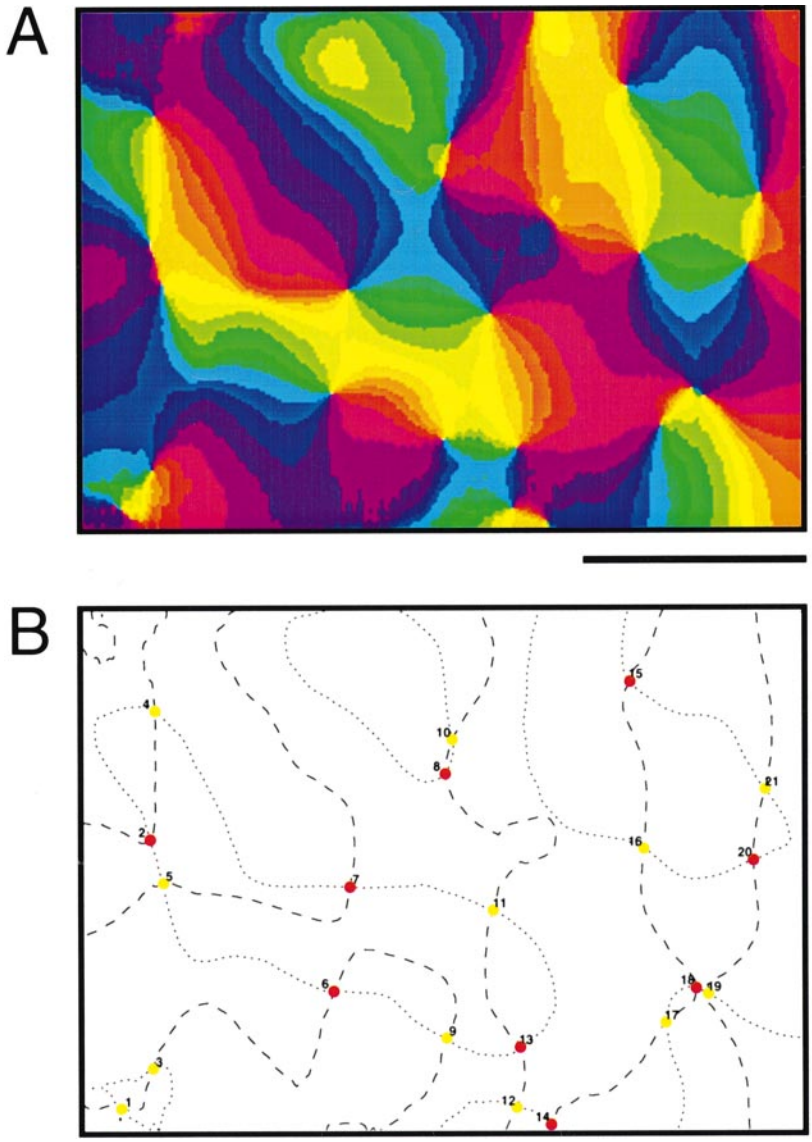

FIG. 7. Quantitative analysis of the distribution and spatial density of orientation (pinwheel) centres in area 17 of a normally raised control cat (N2). (A) The angle map of the analysed visual cortical region is 8.0 mm^2 in size. (B) Locations and numbers of pinwheel centres: the angle map contains 21 pinwheels (numbered consecutively from left to right) corresponding to a pinwheel density of $2.6/mm^2$ cortical surface. Nomenclature as in Fig. 6. Scale bar 1 mm.

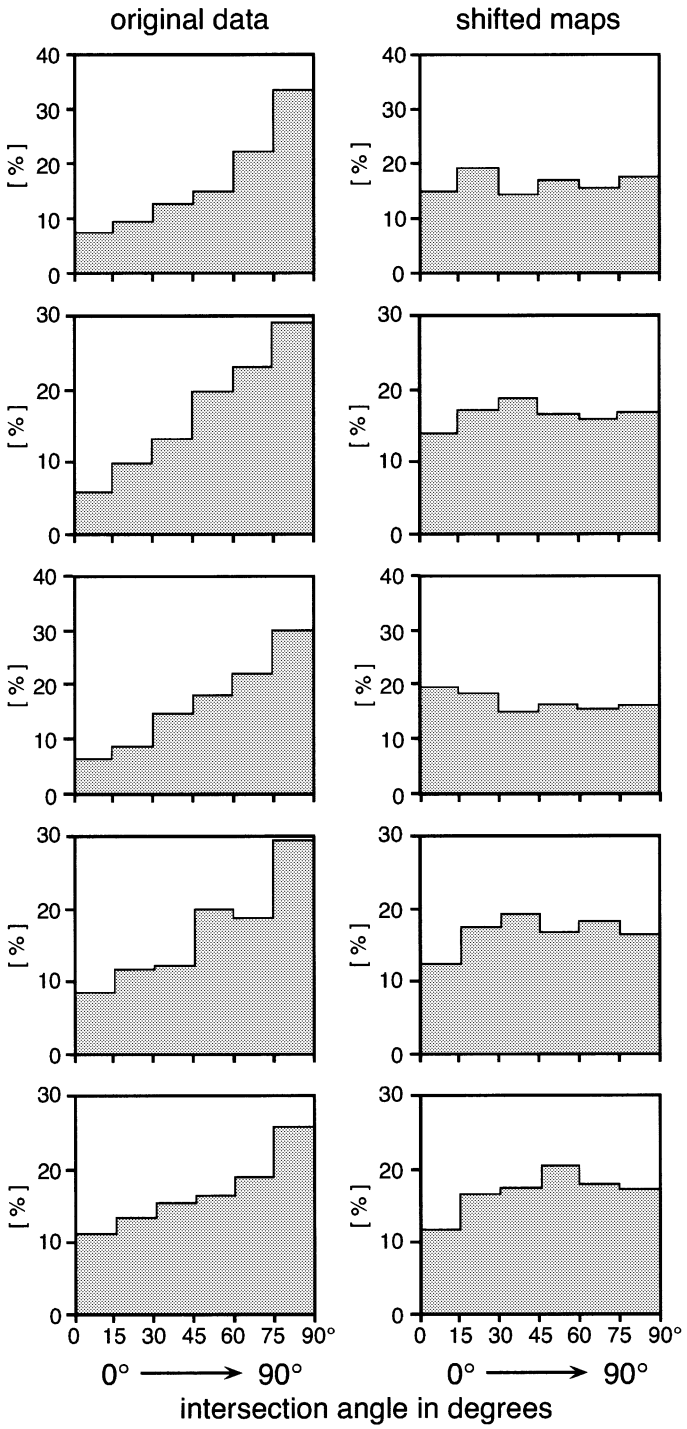

FIG. 8. Histograms of intersection angles between iso-orientation and ocular dominance columns in five strabismic cats (cats S1 to S5, ages: 7 weeks, 12.5 weeks, 7 months, 8 weeks, 14 weeks, from top to bottom). *x*-axis, intersection angle in degrees from 0 to 90°, divided into six classes (0–15°, 15–30°, 30–45°, 45–60°, 60–75°, 75–90°). *y*-axis, percentage of intersection angles in the respective class. *Left column*, original data. *Right column*, 'shifted' maps: iso-orientation contours of one animal superimposed with the ocular dominance borders of another animal. Note that intersection angles between 75° and 90° are most abundant in the original data of all cases, irrespective of the age of the animals. Note in addition that the histograms are always flat after shifting the maps.

relationship as originally suggested by Hubel & Wiesel (1977) on the basis of electrophysiological studies.

Topographic relationship between ocular dominance and iso-orientation columns: superposition of [^{14}C]2-DG-labelled binocular orientation columns with [^{3}H]proline-labelled ocular dominance columns

To corroborate our observation that iso-orientation domains are continuous across ocular dominance borders we labelled the two columnar systems with complementary techniques. Iso-orientation columns were visualized with [^{14}C]2-DG autoradiography after binocular stimulation with contours of either horizontal (BO1) or vertical orientation (BO2) and ocular dominance columns were labelled with the transneuronal tracer [^{3}H]proline injected in either the squinting (BO1) or the non-squinting eye (BO2). The 2-DG labelled binocular orientation domains extended in columns through the entire cortical thickness and resembled those of autoradiographically labelled binocular iso-orientation domains of normal cats (Albus, 1979; Singer, 1981; Löwel *et al.*, 1987; Löwel & Singer, 1993a; Schmidt *et al.*, 1997b). As in normals, the orientation domains appeared as beaded continuous bands indicating that orientation columns of the left and the right eye are not isolated from each other (Fig. 9A). We already showed in a former study in squinting cats that monocular stimulation with one orientation produces rather isolated patches of 2-DG uptake (Schmidt *et al.*, 1997b). In the flat-mount sections through layer IV, large segments were labelled transneuronally by proline. Therefore the original [^{3}H]autoradiographs could be used without reconstruction to reveal topographic relationships in central parts of area 17. Features of the layout of ocular dominance columns were similar as described elsewhere in detail (Löwel, 1994). The superposition of the 2-DG-labelled orientation maps with the proline labelled ocular dominance columns revealed essentially similar results as the optical imaging experiments (Fig. 9): there were no 'breaks' in the pattern of orientation columns at ocular dominance borders (crossings from green to blue in Fig. 9A, lower panel) nor isolated orientation domains of one eye. Rather, there was again a tendency of iso-orientation domains to cross adjacent ocular dominance borders orthogonal to their main course. This leads to vertically orientated 'zebra-crossings' of blue and green stripes in the middle of the lower right panel of Fig. 9A or to more diagonally running crossings in the upper right part of the same panel. The segments of the other three flat-mount maps (Fig. 9B,C) are smaller because sections through layer IV are less parallel to the cortical lamination but they still cover a larger area than the optical recording maps. These cases confirm that the relations between ocular dominance and orientation columns are essentially the same irrespective of whether the ocular dominance columns are labelled from the ipsi- or contralateral or the normal or the squinting eye (compare Fig. 9A,B,C).

Topographic relationship between ocular dominance and iso-orientation columns: localization of pinwheel centres

In normally reared macaque monkeys (Blasdel, 1992; Swindale, 1992) and kittens (Crair *et al.*, 1997a,b; Hübener *et al.*, 1997), pinwheel centres have a tendency to lie in the centres of ocular dominance domains so that all orientations (all colours in the angle maps) appear at least once per ocular dominance column. Visual inspection of our angle maps suggests that this also might be the case in the strabismic cats as even very small ocular dominance domains (see for example the small domains in Fig. 4D) contain all orientations. However, quantitative analysis demonstrates that the tendency of pinweel centres to be located in the middle of ocular

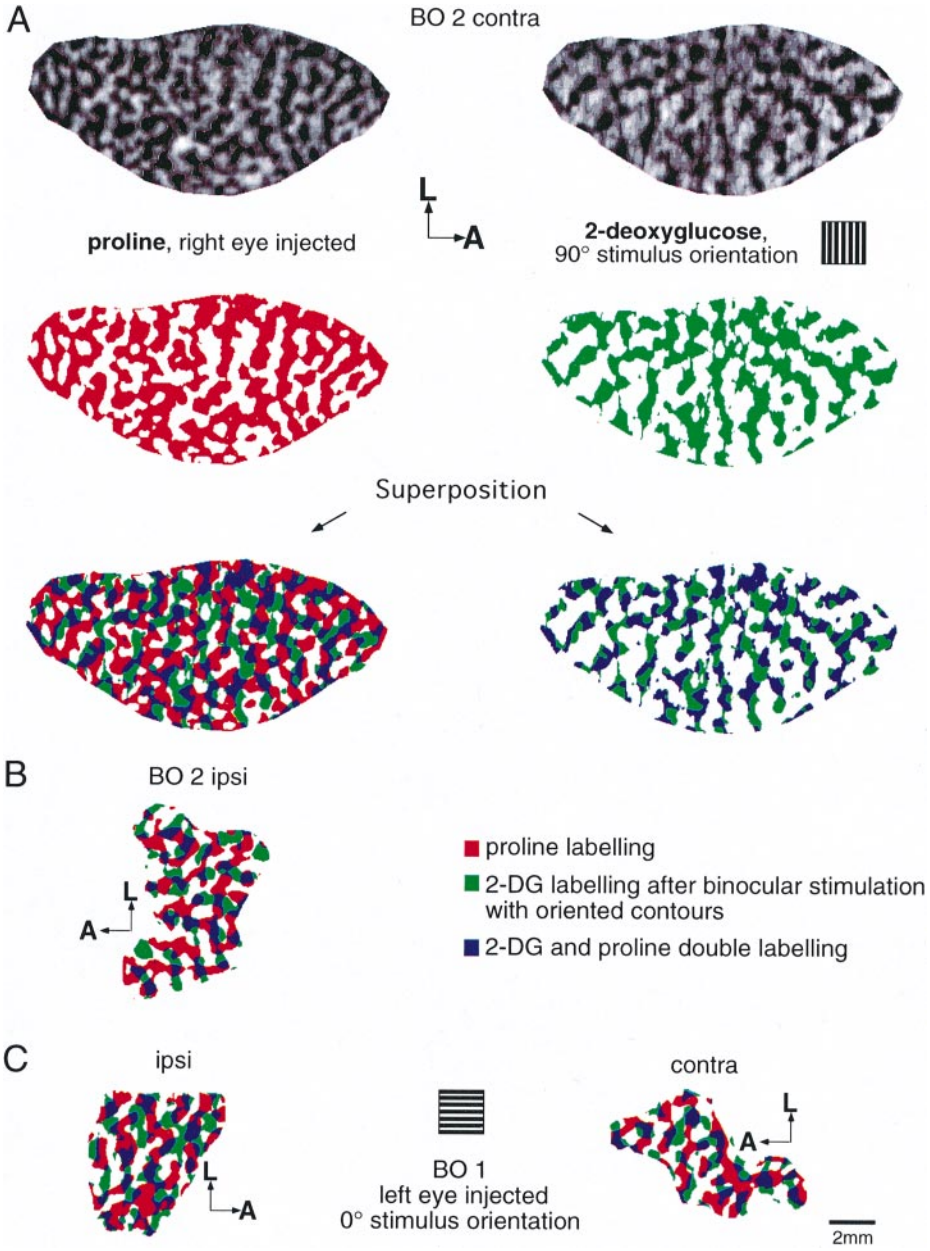

FIG. 9. Superposition of [^{14}C]2-deoxyglucose (2-DG) labelled orientation and [^{3}H]proline labelled ocular dominance columns in strabismic cats. (A) Patterns of [^{3}H]proline labelling and [^{14}C]2-DG uptake (black regions) and their superposition in autoradiographs derived from the same flat-mount section through cortical layer IV. The left visual cortex, contralateral to the injected non-squinting eye of cat BO2 is illustrated. Upper panel: contrast-enhanced large segment of continuous proline labelling of right eye domains (left) and the corresponding 2-DG-labelled binocular orientation domains after stimulation with vertical (90°) contours (right). Large parts of the central visual field representation are included. As inferred from the columnar pattern of the 2-DG autoradiograph outside the documented segment, the area 17/18 border is close to the upper right edge of the selected section. Middle panel: binary colour-coded images of the segments shown in the upper panel. To compute the ocular dominance domains (red domains, left panel) a threshold value was selected that assigned 50% of layer IV to the right (contralateral) eye. 2-DG-labelled binocular orientation domains were defined to cover 40% of the cortical surface (green domains, right panel). Note that orientation domains appear as beaded but uninterrupted bands. Lower panel, left: superposition of the right eye columns (red) and the binocular orientation domains (green). The double labelled regions are displayed in blue. In the right panel, the red fraction of the superposition image is discarded to demonstrate the topographic relation between orientation domains dominated by the right (blue) and left eye (green), respectively. Note that there are almost no isolated blue or green domains nor domains of one colour completely surrounded by another colour. Rather, there are 'zebra-crossings' of green and blue stripes. (B) Colour-coded superposition of ocular dominance and orientation columns in the right visual cortex, ipsilateral to the injected non-squinting eye in cat BO2. (C) Cat BO1: superpositioned patterns in selected segments with continuous proline labelling of the left visual cortex (left panel) ipsilateral and in the right visual cortex (right panel) contralateral to the injected left squinting eye. During 2-DG uptake, cat BO1 was stimulated with moving horizontal (0°) contours. Although the three lower panels show much smaller segments because sections through layer IV were less parallel to the cortical lamination, the area covered is still larger than that analysable with conventional optical imaging. Abbreviations: A, anterior; L, lateral; ipsi, ipsilateral; contra, contralateral.

dominance columns is statistically not significant in strabismic cats ($n = 5$; Fig. 10) ($P > 0.1$; χ^2 test). As in primates pinwheel centres located in different but adjacent ocular dominance domains tend to be connected by iso-orientation contours (Figs 3D, 4D and 5).

Discussion

The major finding of the present study is that in strabismic cats, iso-orientation domains remain continuous across the borders of ocular

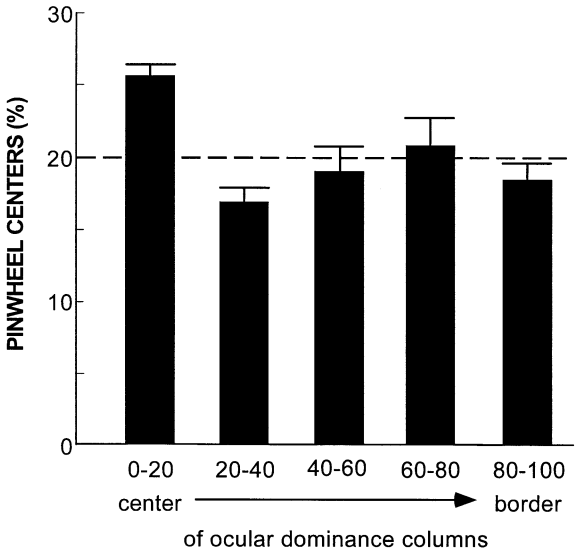

FIG. 10. Relative frequency of pinwheel centres in different subregions of ocular dominance maps. The maps ($n = 5$; the same maps as in Fig. 8 were analysed) were divided into five regions of equal area, with the 0–20 percentile corresponding to the centre and the 80–100 percentile corresponding to the border regions of the ocular dominance columns (error bars are SEM). The dashed line indicates the expected value (20%) if the pinwheel centres were distributed randomly. Note that pinwheel centres appear to be preferentially located in the centre regions of ocular dominance columns. This tendency, however, is not statistically significant.

dominance columns. This was observed both with optical imaging of intrinsic signals and with double labelling of orientation and ocular dominance columns with [^{14}C]2-DG and [^{3}H]proline. The observation is remarkable in view of the nearly complete structural and functional segregation of these columns in strabismic animals.

The most likely explanation for this observation is that in cat area 17 the basic layout of orientation preference maps is specified *before* the age at which we had induced strabismus and that the subsequent rearrangement of thalamic input and of tangential intracortical connections occurred within the scaffold of the already fixed map of orientation preferences. This possibility is supported by several observations. First, orientation selective neurons can be found already in area 17 of 8-day-old kittens, i.e. before eye opening (Hubel & Wiesel, 1963). Second, maturation of orientation selective neurons is rather independent of visual experience up to the third week of age (Leventhal & Hirsch, 1980; Mower *et al.*, 1981; Frégnac & Imbert, 1984; for a recent review see Henry *et al.*, 1994) and also the sequence regularity characteristic for a columnar arrangement gets expressed in the absence of experience (Wiesel & Hubel, 1974). Third, iso-orientation maps can develop in the absence of visual experience and remain unchanged even if thalamocortical input connections get rearranged as a consequence of manipulated visual experience (Mioche & Singer, 1989; Kim & Bonhoeffer, 1994; Gödecke & Bonhoeffer, 1996; Gödecke *et al.*, 1997). Fourth, a recent optical imaging study reveals orientation maps that are strongly dominated by the contralateral eye already in 2-week-old cats (Crair *et al.*, 1998). Because maps developed similar in normal and binocularly deprived animals until 3 weeks of age early pattern vision seems unimportant for the initial establishment of the functional domains (Crair *et al.*, 1998).

This does not imply that neuronal activity plays no role in organizing orientation maps. One possibility is that spontaneous temporally and spatially patterned activity of the retina (Galli & Maffei, 1988; Meister *et al.*, 1991) contributes to the organization of orientation maps. However, the recent evidence that orientation maps are identical for the two eyes in area 18 of cats raised without binocular visual experience (Gödecke & Bonhoeffer, 1996) argues against this possibility because the spontaneous retinal activation patterns are with all likelihood not correlated between the two eyes (see also Bonhoeffer & Kim, 1995). However, the possibility needs to be considered that intrageniculate interactions mediated by feedback from nucleus reticularis thalami and/or corticofugal fibres coordinate the activity across laminae served by different eyes. Another possibility is that the layout of orientation maps is determined by intracortical interactions that are mediated by tangential connections. The topology of these connections (Fitzpatrick, 1996; Bosking *et al.*, 1997; Schmidt *et al.*, 1997a) begins to exhibit its characteristic periodic clustering at the developmental stage at which orientation selective neurons first appear (Callaway & Katz, 1990; Galuske & Singer, 1996; Ruthazer & Stryker, 1996). Spontaneous activity waves could then serve to 'read' these anisotropies, to promote the full expression of the orientation map and to select appropriate sets of converging thalamocortical afferents. Finally, there is the possibility that travelling waves of cortical (Huttenlocher, 1967) or thalamic (Kim *et al.*, 1995; McCormick *et al.*, 1995) origin codetermine both the early clustering of tangential connections and the layout of orientation maps. The recent evidence that blockade of cortical but not of retinal activity prevents the initial development of clustered horizontal connections in area 17 of ferrets is compatible with this scenario (Ruthazer & Stryker, 1996). However, as the present data indicate, the orientation map appears to be resistant to subsequent reorganization of both tangential connections and thalamic afferents as iso-orientation domains extend smoothly across territories with different eye preference that are no longer interacting through tangential connections and no longer receive shared subcortical input.

If then the orientation map serves as scaffold for the rearrangement of thalamic input connections, the pattern of ocular dominance columns should be influenced by the layout of the pre-existing orientation map (Hoffsümmer *et al.*, 1996). Our data show that each ocular dominance domain contains a full representation of all orientations. Such an arrangement is indispensible in strabismic animals because there is very limited cross-talk between the domains of the two eyes. Assuming that the orientation map is invariant, such an arrangement can be achieved if the spatial periodicity of the ocular dominance columns gets adapted to that of the orientation map. Comparison of ocular dominance columns in normal and strabismic cats revealed differences in columnar spacing that are compatible with this view. In area 17 of strabismic animals, the spacing of adjacent ocular dominance columns is about 20–30% larger compared with that in normally raised cats (between 1100 and 1300 μm; Löwel, 1994; see also Tieman & Tumosa, 1996, 1997; but see Jones *et al.*, 1996), and resembles the spacing of the iso-orientation domains (1000–1300 μm; Löwel *et al.*, 1987; LeVay & Nelson, 1991). The increased columnar spacing in strabismic cats may thus be regarded as an adaptive response to achieve a topographic arrangement between orientation and ocular dominance columns that assures optimal coverage of monocular representations with all orientations. Furthermore, it supports the hypothesis that the orientation preference of cortical neurons at a particular site is determined by intracortical factors and that thalamic input connections are selected as a function of cortical response properties (Rauschecker & Singer, 1979, 1981; Mioche & Singer, 1989).

However, the possibility should not be dismissed that the orientation map also adapts to the altered input constellation. In this case, one might expect either a change in pinwheel density or in pinwheel location. Unfortunately, published data on pinwheel density in area

17 of normally reared adult cats differ from those in our own controls. Rao *et al.* (1994) and Bonhoeffer *et al.* (1995) have reported values of 1.8/mm^2 and 2.1/mm^2 of cortical surface, respectively. In our strabismic cats, the average pinwheel density was 2.7/mm^2 of cortical surface and thus at least 30% higher than the recently reported values. However, in our own controls that were matched for age and cortical location, average pinwheel density turned out to be 2.6/mm^2, and was thus in the same range as the values of our strabismic animals. The most likely reasons for the diverging results on pinwheel density are methodological differences and possibly also recording location. Because area 18 contains 1.2 pinwheels/mm^2 (Bonhoeffer & Grinvald, 1993) and because the transition of the maps between the two cortical areas is smooth (Bonhoeffer *et al.*, 1995), lower pinwheel density could result if measurements were from regions closer to the 17/18 border. It appears thus – at least from our own control data – that the space constants of the orientation map are not altered by strabismus and the concomitant rearrangement of thalamocortical and intracortical connections. Concerning pinwheel location, our quantitative analyses in area 17 of strabismic cats indicate that pinwheel centres do have a tendency to lie in the middle of ocular dominance columns as already observed in macaque monkeys and normally raised and monocularly deprived cats (Blasdel, 1992; Swindale, 1992; Crair *et al.*, 1997a,b; Hübener *et al.*, 1997). However, this tendency is statistically not significant, most probably due to a pronounced interindividual variability. Therefore the possibility needs to be considered that the distribution of pinwheel centres with respect to ocular dominance columns is more heterogeneous in strabismic compared to normally raised cats. In the latter, pinwheels tend to lie in regions in which ocular dominance is most biased towards one eye, i.e. in the centre of ocular dominance columns (Crair *et al.*, 1997a; Hübener *et al.*, 1997). In strabismic cats, by contrast, these regions span the entire width of a column and hence pinwheel centre location might be less constrained.

The observations that iso-orientation contours intersect the borders between adjacent ocular dominance columns at steep angles further indicate a systematic topological relation between the two maps as originally suggested by Hubel & Wiesel (1977). A similar geometry of the functional domains has been described previously for macaque monkey striate cortex (Bartfeld & Grinvald, 1992; Obermayer & Blasdel, 1993) and recently also in kitten area 17 (Crair *et al.*, 1997a; Hübener *et al.*, 1997). This arrangement is well adapted because it permits coverage of a particular point in the visual field with all relevant combinations of orientation preference and ocular dominance in the smallest possible volume of cortex (Swindale, 1991; Hübener *et al.*, 1997).

Taken together, accumulating evidence suggests that in kittens, the maps of orientation preference are resistant against experience-dependent reorganization of thalamic input when this reorganization is imposed around the age of 3 weeks. This indicates that the selection of input connections during rearrangement is guided by the already stabilized orientation map. In the cat, segregation of thalamic afferents into ocular dominance columns occurs only after birth and after eye opening. This segregation is caused by activity-dependent competition between the two sets of afferents for cortical territory (for review see Stryker, 1991), and, because it occurs after eye opening, can be influenced by manipulating visual experience. In the macaque monkey, however, the segregation of ocular dominance columns is completed at birth (Horton & Hocking, 1996). Thus, visual experience is not a necessary prerequisite for the formation of ocular dominance columns. It appears then, as if both, orientation and ocular dominance maps and their specific mutual relations can develop without structured visual input. If, however, alterations are imposed on the ocular dominance system by later manipulation of experience, the new layout adapts to the constraints set by the orientation map, the latter remaining stable despite the breakdown of intracortical tangential connections. This indicates that the maintenance of a once established orientation map becomes independent of the specific arrangement of thalamocortical and, quite unexpectedly, also of part of the intracortical connections. The possibility needs to be considered therefore that orientation selectivity, once the map has been established, is determined by local interactions within the respective columns.

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