

Spacing of Cytochrome Oxidase Blobs in Visual Cortex of Normal and Strabismic Monkeys

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Some models of visual cortical development are based on the assumption that the tangential organization of V1 is not determined prior to visual experience. In these models, correlated binocular activity is a key element in the formation of visual cortical columns, and when the degree of interocular correlation is reduced the models predict an increase in column spacing. To examine this prediction we measured the spacing of columns, as defined by cytochrome oxidase (CO) blobs, in the visual cortex of monkeys whose binocular vision was either normal or disrupted by a strabismus. The spatial distribution of blobs was examined in seven normal and five strabismic macaques. Tangential sections through the upper layers of the visual cortex were stained to reveal the two-dimensional (2D) pattern of CO blobs. Each blob was localized and their center-to-center spacing, packing arrangement and density were calculated using 2D nearest-neighbor spatial analyses. The mean center-to-center spacing of blobs (590 μm for normally reared and 598 μm for strabismic macaques) and the mean density of blobs (3.67 blobs/ mm^2 for normally reared and 3.45 blobs/ mm^2 for strabismic macaques) were not significantly different. In addition, the 2D packing arrangement of the blobs was not affected by strabismus. While it is clear that neural activity plays a key role in the elaboration and refinement of ocular dominance cortical modules, we conclude that it does not determine the spatial period of the pattern of CO blobs. This suggests that aspects of the neural circuitry underlying the columnar architecture of the visual cortex are established prenatally and its fundamental periodicity is not modifiable by experience.

Introduction

Our understanding of the organization of columnar systems in the visual cortex has been extended by computational models that have formalized and examined rules for the development (e.g. Swindale, 1980; Miller *et al.*, 1989) and the resulting two-dimensional (2D) pattern (Jones *et al.*, 1991) of cortical columns. Many of the developmental models are based upon the assumption that the cortex lacks inherent, pre-existing tangential organization – it is a blank slate – and that patterned retinal activity drives the formation and arrangement of cortical columns. When applied to ocular dominance column development, a key element of these models is the degree of correlation between the two eyes' inputs, where according to Hebbian rules for synaptic modification (Hebb, 1949), the strong within-eye correlations lead to strengthening and maintenance of connections, while the weaker between-eye correlations lead to competition that results in the loss of one eye's connections. Within this theoretical framework, certain predictions about the development of these patterns arise when the degree of between-eye correlation is reduced. In particular, a recent model of ocular dominance column development predicts an increase in the spacing of cortical columns when the extent of lateral interactions and the degree of binocular correlation are reduced by the introduction of a strabismus (Goodhill, 1993).

The most robust and easily visualized markers of the columnar organization in the visual cortex are the cytochrome oxidase (CO) blobs found in both monkeys and cats (Horton and Hubel, 1981; Livingstone and Hubel, 1984a; Murphy *et al.*, 1990, 1991a,b, 1995). The blobs have become central to our understanding of the columnar organization in the visual cortex because of their relationship with features such as ocular dominance columns (Fitzpatrick and Diamond, 1980; Hendrickson *et al.*, 1981; Horton, 1984; Murphy *et al.*, 1995; Hubener *et al.*, 1997), orientation columns (Livingstone and Hubel, 1984a) and spatial frequency columns (Tootell *et al.*, 1988; Born and Tootell, 1991; Shoham *et al.*, 1997). They also have an important relation to neural connectivity. Within V1 there is bias towards blob-to-blob patterns of intrinsic horizontal connections (Livingstone and Hubel, 1984b; Yoshioka *et al.*, 1996), and extrinsic corticocortical connections also exhibit biases in the pattern of projections from blob or interblob regions to specific compartments in V2 or extrastriate cortical areas (Zeki, 1976; Livingstone and Hubel, 1983, 1984b, 1987a,b; DeYoe and Van Essen, 1985, 1988; Van Essen *et al.*, 1992). Taken together, the relationship of the blobs to a large array of columnar features and neural pathways makes them an ideal marker to assess the spatial layout of functional columns in the visual cortex.

It is clear that visual experience plays a central role in the development of cortical columns (see Goodman and Shatz, 1993). Yet the question remains whether other neural mechanisms may interact with activity to guide the overall arrangement of visual cortical columns (Jones *et al.*, 1991). The prediction of some models that column spacing changes when interocular correlation is reduced provides an opportunity to examine this question further. We have measured the 2D arrangement of blobs in V1 of normal and strabismic macaques to determine whether a reduction in the degree of between-eye correlation changes the spatial layout of this cortical feature. While many aspects of the blobs and their relationship to physiological properties and other anatomical features have been studied there has not been a quantitative examination of the 2D pattern of blobs in normal or strabismic monkeys. In particular, we have tested whether the spacing and 2D arrangement of blobs changes in a manner that is consistent with the predictions of activity-dependent models of columnar development. Here we report that strabismus does not change the spacing or packing arrangement of CO blobs in macaque V1. A preliminary report included some of these data (Murphy *et al.*, 1996a).

Materials and Methods

Animals and Surgical Procedures

Twelve macaque monkeys were used to study the tangential distribution of CO blobs in V1. The monkeys were reared with either normal binocular visual experience ($n = 7$), or a strabismus surgically induced

Table 1
Summary of rearing conditions and results of analyses of CO blob spacing

Monkey	Species	Rearing condition	Deviation (°)	Ellipse aspect ratio	Average blob spacing (μm)	Blob density (mm ⁻²)
378	<i>M. fascicularis</i>	normal		1.08	552	4.17
376	<i>M. fascicularis</i>	normal		1.03	590	3.67
ZD	<i>M. nemestrina</i>	normal		1.08	588	3.66
380	<i>M. fascicularis</i>	normal		1.06	583	3.73
15478	<i>M. mulatta</i>	normal		1.06	596	3.61
XX	<i>M. nemestrina</i>	normal		1.17	634	3.14
JP1	<i>M. fuscata</i>	normal		1.04	585	3.70
356	<i>M. nemestrina</i>	strabismic	15	1.38	594	3.47
357	<i>M. nemestrina</i>	strabismic	25		591	
369 (right hemisphere)	<i>M. nemestrina</i>	strabismic	>5	1.09	595	3.46
369 (left hemisphere)	<i>M. nemestrina</i>	strabismic	>5	1.09	595	3.41
PW	<i>M. nemestrina</i>	strabismic	20	1.04	614	3.27
SY	<i>M. nemestrina</i>	strabismic	25	1.16	607	3.51

between the ages of 10 and 60 days ($n = 4$), or early onset strabismus as a result of lens-rearing ($n = 1$) (Kiorpes *et al.*, 1993). Using aseptic surgical techniques esotropia was induced in monkeys 356, 357, PW and SY, by recession of the lateral rectus and resection of the medial rectus of the left eye (Kiorpes *et al.*, 1989), in monkeys PW and SY the lateral rectus of the other eye was also cut. Normal ocular motility is maintained after this surgery (Kiorpes *et al.*, 1996) and assessment of visual acuity revealed no acuity difference in PW or SY and mild amblyopia in 356 and 357.

Histology

Monkeys were killed with a lethal injection of sodium pentobarbital (65 mg/kg, i.v.) then perfused transcardially with 0.1 M phosphate-buffered saline (PBS) (pH 7.4) and either 4% paraformaldehyde (normally reared, $n = 6$; all strabismics) or 2% glutaraldehyde (normally reared, $n = 1$) in PBS. Following perfusion, the block of brain containing the visual cortex was post-fixed in buffer containing the same aldehyde with 30% sucrose. One hemisphere from a normally reared monkey was unfolded and flattened to allow visualization of the complete tangential pattern of CO blobs in V1 following modification of the procedures used for flattening cat visual cortex (Olavarria and Van Sluyters, 1985; Murphy *et al.*, 1995). Briefly, the pia mater and white matter were carefully removed, leaving the sheet of cortical tissue and allowing the sulci and gyri to unfold prior to flattening. Cuts were made in the tissue that had been folded in the calcarine fissure to relieve the intrinsic curvature and the cortex was gently flattened between two pieces of glass. The unfolded and flattened hemisphere was sectioned on a freezing microtome tangential to the pial surface at a thickness of 50 μm, the other hemispheres were sectioned tangential to the opercular surface (40 μm thick). Cut sections were collected in 0.1 M phosphate buffer, and reacted either free-floating or mounted on gelatin-coated glass slides (for 4–7 h at 40°C) following the standard CO histochemical procedure (Wong-Riley, 1979; Horton, 1984). Stained slides were dehydrated and defatted, then coverslipped prior to being photographed. Low-magnification brightfield photographs were taken of the sections through the superficial layers of the visual cortex, where the CO blobs are most prominent, and transferred to PhotoCD format (Eastman Kodak, Rochester, NY) for subsequent quantitative analysis.

Tangential Analysis

Two-dimensional, nearest-neighbor spatial analyses were used to determine the center-to-center spacing, packing arrangement and density of CO blobs in V1 (Table 1). Nearest-neighbor spatial statistics have been developed and used to characterize and analyse a diverse array of 2D patterns (e.g. Shapiro *et al.*, 1985; Duncan and Stewart, 1991; Curcio and Sloan, 1992); our use of these spatial statistics is illustrated in Figure 1. The pattern of blobs in each section was visualized on a computer monitor (Fig. 1A), a naive observer localized the center of each blob and recorded these onto an overlay. In each case the sampling region was from a comparable region on the opercular surface of V1, away from the V1/V2 border, to minimize the influence of any regional variations in the

pattern of blobs. The 2D plot representing the centers of all the blobs in a section was entered into a computer using a high-resolution (100 lines/mm) digitizing pad (CalComp, Anaheim, CA). Nearest-neighbor statistics were then calculated to extract the center-to-center spacing, the packing arrangement and the density of blobs from within a sampling window selected to effectively eliminate edge effects (average number of blobs in the sampling window = 444). Our statistical analysis is based on a Delaunay triangulation, which links each blob to its mathematical nearest-neighbors (Guibas and Stolfi, 1985) thereby providing the angles and distances (triangle edge lengths) from a blob to its set of nearest neighbors (Fig. 1B). Using the edge lengths from the Delaunay triangulation for each hemisphere the frequency distribution and average center-to-center spacing of the blobs were determined, as was the average spacing at all orientations. Polar plots of the center-to-center spacing as a function of the angle of the Delaunay triangle edges were made and an ellipse was fitted to these data. The aspect ratio of the major to minor axis length for the best-fit ellipse was calculated to quantify the packing arrangement of the blobs. A second stage of the nearest-neighbor spatial analysis (Voronoi polygons) was performed to determine the average area of a blob-defined domain. This analysis produces a polygon around each blob (the mathematical dual of the Delaunay triangulation), with the property that every point inside the polygon is closer to the blob it encircles than any other blob in the pattern (Fig. 1C). The area of the polygons is expressed in units of mm² per blob, thus the reciprocal of the average polygon area provides an unbiased measure of the density of blobs (Curcio and Sloan, 1992). The average spacing and polygon areas are calculated from the triangle edges and polygons, respectively, that fall completely within the sampling window and thus are not biased by edge effects. These analyses provide the information necessary to characterize and compare the spacing and 2D pattern of blobs in V1 of normal and strabismic monkeys.

Results

Blob Spacing in Normal Monkeys

In normally reared macaque monkeys a number of features of the visual cortex are readily visualized in CO-stained tangential sections through the supragranular layers of V1 and V2 (Fig. 2A), namely the complete pattern of blobs in V1, the ovate shape of V1, the distinct border between V1 and V2, and the thick-pale-thin-pale stripes of V2. As has been reported previously, the 2D arrangement of the CO blobs in adult monkeys reared with normal binocular vision appears regular with blobs present throughout the full extent of V1 including both the opercular and calcarine regions. During the unfolding and flattening procedure discontinuities were introduced into the portion of V1 from the calcarine fissure. Since these discontinuities disrupt a 2D analysis, and in some animals only the opercular region was

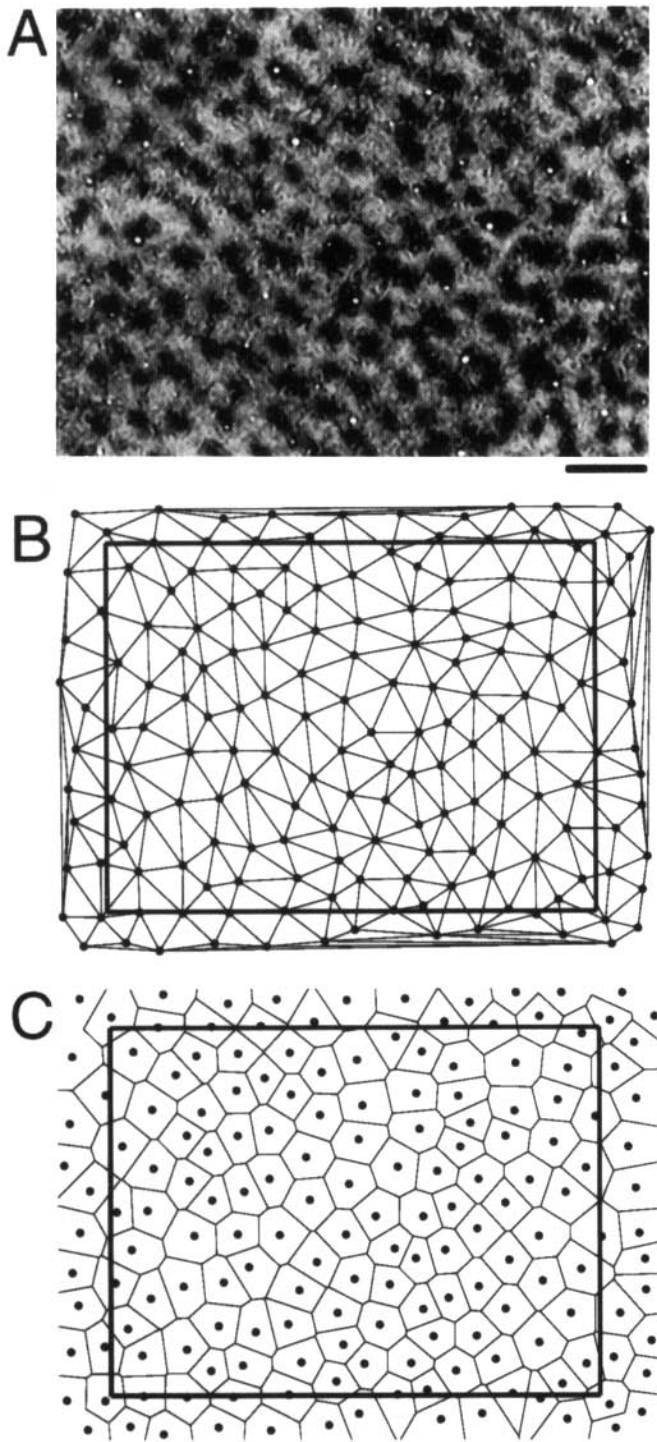


Figure 1. An illustration of the components of the 2D nearest-neighbor analysis. (A) CO blobs are visualized in a tangential section through layers 2/3 of the macaque visual cortex. Each blob is localized and the center is marked with a point. Scale bar = 1 mm. (B) The Delaunay triangulation for the array of blob centers showing the edges connecting each blob to its set of mathematical nearest neighbors. Average center-to-center spacing is determined for all of the edge lengths that fall within the sampling box to effectively eliminate edge-effects from the analysis. (C) The Voronoi polygons for the array of blobs. Each polygon represents the domain around a blob where every point inside the polygon is closer to the blob it encircles than any other blob in the pattern. The reciprocal of the polygon areas provides an unbiased estimate of density.

sectioned, the set of quantitative analyses of the 2D arrangement of the blobs were performed for the opercular region. First, each

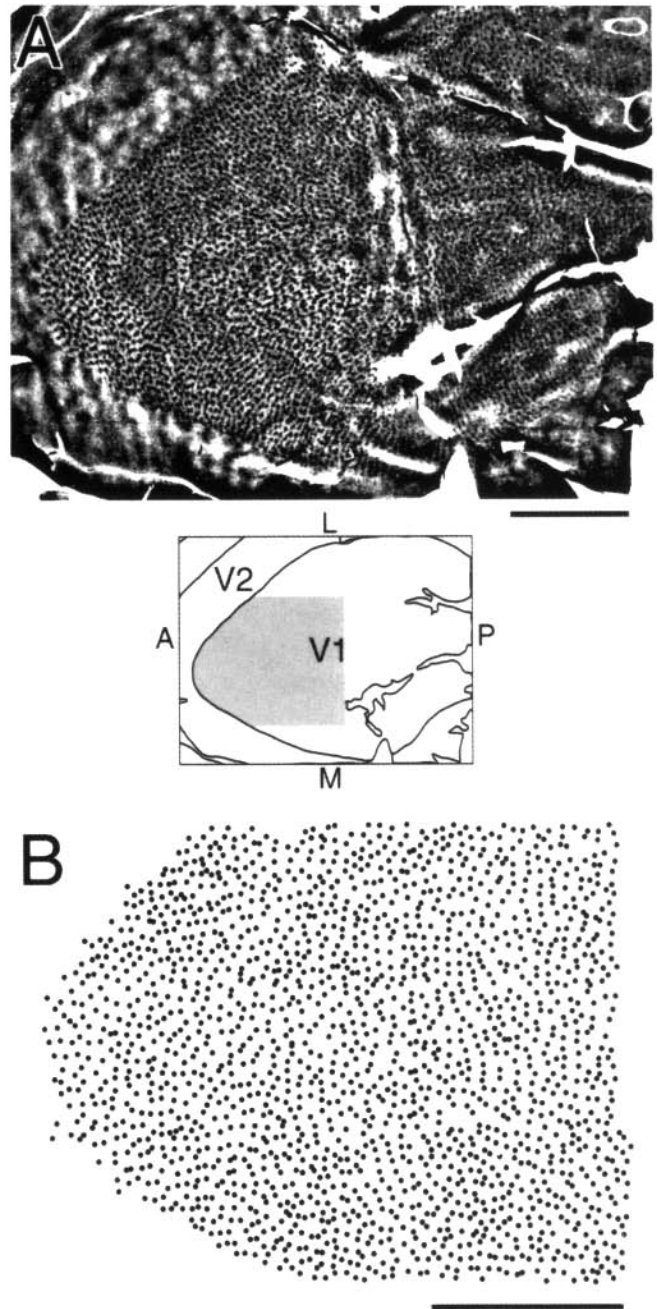


Figure 2. (A) The tangential pattern of CO blobs in a single section through the upper layers of unfolded and flattened macaque V1. The inset shows the outline of V1 and V2, the gray region indicates the area that was analyzed. (B) The array of blob centers from the opercular region of V1 (shown in A) that formed the basis for the spatial analyses. Scale bars = 1 cm.

blob was identified and the center marked to create a 2D map of the blob pattern (Fig. 2B). This map of blob centers was used for the 2D spatial analyses. For monkeys reared with normal binocular vision, the frequency histograms of blob center-to-center distances revealed that the spacing is normally distributed with a range of blob-to-blob separation from a minimum of ~200 μm to a maximum of ~1 mm (Fig. 3A). The average spacing of the blobs for the group of normal adult monkeys was 590 μm (group SD = 22.33 μm). The 2D packing arrangement of the blobs was assessed by including the orientation of the Delaunay triangle edge lengths in the analysis to determine the average blob-

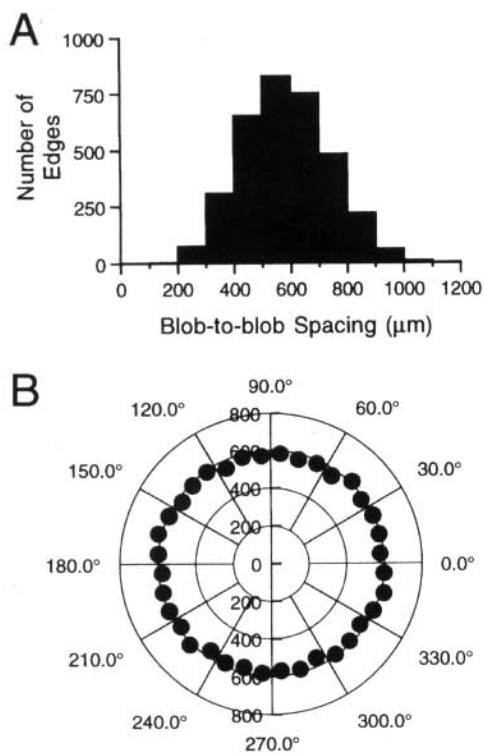


Figure 3. Normally reared macaque monkey: nearest-neighbor analysis of the 2D arrangement of blobs in the opercular region. (A) The frequency histogram of Delaunay triangle edge lengths of blob-to-blob spacing with a mean spacing of 585 μm for this example (JP1). (B) Polar plot of the average blob-to-blob spacing at all orientations of the Delaunay triangle edges with a major to minor aspect ratio of 1.04 for this example.

to-blob spacing as a function of the orientation (i.e. are blobs spaced further apart in one dimension than another?). The average aspect ratio of the major to minor axis for the best-fit ellipse to the polar plot was 1.07 (SD = 0.04), indicating that in normally reared macaque monkeys there is a small anisotropy in the spacing of blobs in V1.

The density of blobs was calculated by considering the average area of the Voronoi polygons. For the normally reared macaques this yielded an average density of 3.67 blobs/ mm^2 (SD = 0.25 blobs/ mm^2). An estimate of the total number of blobs in V1 was made for the unfolded and flattened hemisphere by measuring the area of V1 (1399 mm^2) directly from a single CO-stained section (Fig. 1A). This calculation yielded an estimate of 5134 blobs in V1 based on the assumption that the density of blobs in the opercular and calcarine regions are similar.

Blob Spacing in Strabismic Monkeys

Cytochrome oxidase blobs were readily identified in tangential sections through the superficial layers of V1 from macaques reared with a strabismus (Fig. 4). The appearance of the blobs in strabismic macaques was similar to that observed in normally reared monkeys, although in some cases the CO staining pattern clearly included thin darkened strips linking the blobs and giving the pattern the appearance of beads on a string (Fig. 4B). This is a common pattern of CO staining in the supragranular layers following strabismus. Even in these cases, however, the blobs were readily localized for quantitative 2D analysis. The distributions of center-to-center blob spacing in the strabismic monkeys (Fig. 5A) were similar to those found for normally reared macaque monkeys (Fig. 3A). The average center-to-center

spacing of the blobs was 598 μm (SD = 10.5 μm) for the macaques reared with a strabismus. To evaluate the packing arrangement of the blobs in each hemisphere polar plots were made of the average spacing as a function of orientation (Fig. 5B) and an ellipse was fit to these data. The average aspect ratio of the major to minor axis for the best-fit ellipse was 1.15 (SD = 0.13), indicating that there was a small, but consistent, anisotropy in the packing arrangement of blobs in strabismic macaques. The density of blobs, calculated from the average Voronoi polygon area, was 3.42 blobs/ mm^2 (SD = 0.04) in V1 of monkeys reared with a strabismus.

Comparison of Normal and Strabismic Blob Pattern

The spacing of CO blobs in V1 of macaques reared with either normal binocular vision or surgically induced strabismus was not significantly different ($P > 0.05$) (Fig. 6A). The average spacing of blobs in normally reared macaques was 590 μm (SD = 22.33 μm) and the average spacing in strabismic macaques was 598 μm (SD = 10.5 μm). It was also the case that the packing arrangement of the blobs did not change as a result of the strabismus since the aspect ratio for the ellipse of best fit to the polar plots of blob spacings (Fig. 6B) was not different from that observed for normal monkeys ($P > 0.05$). Finally, there was no difference in the density of blobs (Fig. 6C) between normally reared and strabismic monkeys ($P > 0.05$). None of these 2D quantitative measures of blob spacing, packing arrangement and density were significantly different between normal and strabismic monkeys.

Discussion

Our results demonstrate that strabismus early in postnatal life does not affect the spacing or arrangement of CO blobs in V1 of macaque monkeys, even though visual acuities (Kiorpes and Movshon, 1989; Kiorpes, 1992) and other aspects of the anatomical organization of V1 (e.g. Tychsen and Burkhalter, 1995) can be affected by a strabismus. The lack of change in the organization of the CO blobs indicates that factors in addition to the degree of binocularly correlated visual experience are important for determining the overall 2D spatial arrangement of blobs in macaque visual cortex. Our results extend previous reports that the development of blobs is not dependent upon retinally driven activity (Kennedy *et al.*, 1990; Kuljis and Rakic, 1990), and that aspects of synaptogenesis (Bourgeois and Rakic, 1996), lamination and the pattern of certain neurotransmitters in V1 (Rakic and Lidow, 1995) develop independent of retinal activity. Bearing in mind that the present study examined blob-defined columns, our conclusions about the factors essential for setting up the 2D pattern of blobs are similar to those drawn by a recent study for the arrangement of ocular dominance columns observed at birth in the macaque monkey (Horton and Hocking, 1996a).

Prior to the present study the 2D spacing and packing arrangement of blobs in either normal or strabismic macaque monkeys had not been analyzed quantitatively, although there have been reports of spacing in normals from local analyses. Typically, these local measurements have yielded estimates of blob spacing in the range of 550–600 μm , which is within the range determined from the 2D nearest-neighbor spatial statistics used in the present study. The 2D spatial statistics provide two additional measures that are important for characterizing and analyzing the arrangement of the blobs. First, the unbiased density of blobs was not different between normal and strabismic macaques, and was similar to an earlier report of blob

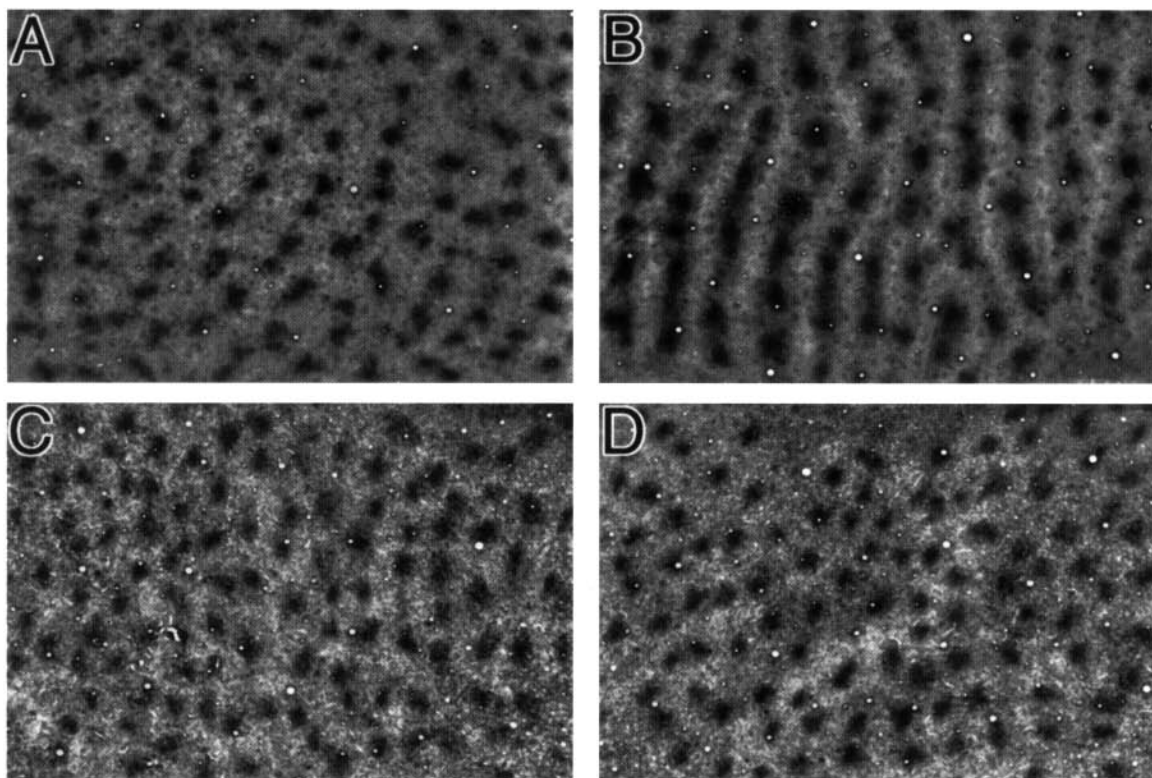


Figure 4. (A–D) Portions of the tangential patterns of CO blobs observed in four macaques reared with divergent strabismus. In B the CO staining pattern shows the darkly stained strips that often connect blobs along ocular dominance bands in strabismic macaques. Scale bar = 1 mm.

density in normal macaque monkeys that used a more traditional method for measuring density (Purves and LaMantia, 1993). Second, analysis of the average spacing between nearest-neighbor blobs at all orientations provides a measure of the global packing arrangement of the blobs. This is an important characteristic of the pattern of the blobs since it quantifies the 2D nature of their arrangement rather than collapsing it onto one dimension. This analysis showed that both normal and strabismic macaques have comparable small anisotropies in the packing arrangement of the blobs. This small, but consistent, anisotropy in the spacing of the blobs may be related to the previous observation that blob spacing is slightly smaller along as compared to across ocular dominance columns (Horton, 1984).

Visual experience during the critical period in postnatal development plays a significant role in shaping and refining the neural circuits that make up cortical columns (Wiesel and Hubel, 1963; for review see Goodman and Shatz, 1993). Some models hold that activity plays an exclusive role in the development of the functional architecture of visual cortex (for reviews see Miller, 1994; Swindale, 1996). Among the key parameters typical of the activity-dependent models applied to ocular dominance development are: the initial spatial extent of afferent arbors in layer IV; the extent of lateral interaction within the cortex; and the degree of correlation among the afferents serving the two eyes. Depending on the details of these models, changes in the degree of between-eye correlation of activity may lead to changes in the spacing of ocular dominance columns. A recent model by Goodhill (1993) predicts that reducing the level of between-eye correlation by introducing a strabismus will increase the spacing of ocular dominance columns. This prediction has received some support from reports of increased

spacing of ocular dominance columns in strabismic or alternating occlusion kittens (Lowel, 1994; Tieman and Tumosa, 1997). These results, however, are not consistent with the result from other studies where the degree of between-eye correlation was also reduced but the pattern of visual cortical columns was not changed. In humans, neither anisometropia (Horton and Stryker, 1993) nor strabismus (Horton and Hocking, 1996b) changes the pattern of ocular dominance columns. In macaque monkeys, at birth, before any visual experience, the pattern of ocular dominance columns is already adult-like (Horton and Hocking, 1996a) and postnatal monocular deprivation does not change their spacing (Hubel *et al.*, 1977), or their spatial relationship with the CO blobs (Horton and Hocking, 1997). The lack of change in the spacing, the pattern and the relationship between columnar features in these studies may reflect a difference in the mutability of visual cortical columns in primates versus cats. This possibility, however, is weakened by the results of recent studies that found no difference in the spacing of ocular dominance columns in normal, monocularly deprived or strabismic cats (Jones *et al.*, 1996; Sengpiel *et al.*, 1998).

Perhaps the discrepancy between studies that find a change (Lowel, 1994; Tieman and Tumosa, 1997), and those that do not find a change in column spacing (Hubel *et al.*, 1977; Jones *et al.*, 1996; and the present results) may be understood by considering the methodological issues that contribute to an analysis of the 2D pattern of columns. First, it is important to recognize that there is both inter-animal variability in the spacing of cortical columns, and consistent differences in the spacing of columns from different regions of V1 (Horton and Hocking, 1996c). In light of this regional variability we were careful to select a comparable portion of the opercular region in V1 for analysis. Second, the tangential patterns of blobs and ocular dominance columns are

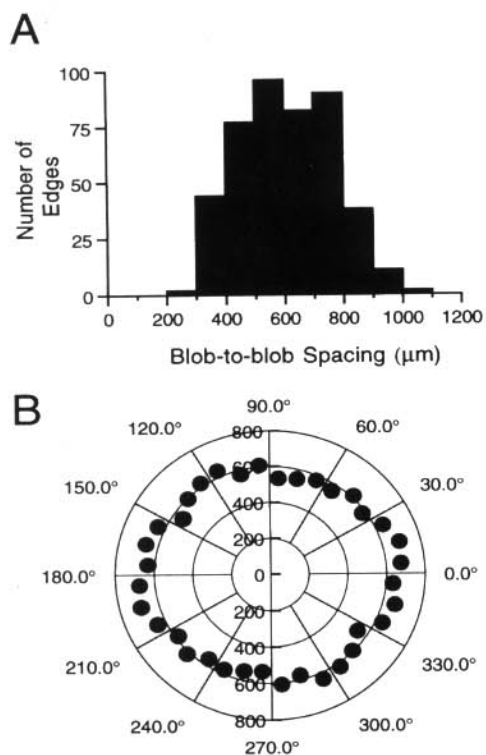


Figure 5. Strabismic macaque monkey: nearest-neighbor analysis of the arrangement of CO blobs in the opercular region. (A) The frequency histogram of Delaunay triangle edge lengths of blob-to-blob spacing with an overall mean spacing of 607 μm for this example (SY). (B) Polar plot of the average blob-to-blob spacing at all orientations of the Delaunay triangle edges with a major to minor aspect ratio of 1.16 for this example.

two-dimensional. Accurate measurement of a 2D pattern requires the use of statistical methods that are appropriate for the quantification of 2D data. Unrepresentative measurements may be obtained when radial sections or 1D analysis of tangential sections are used to quantify spacing. These errors arise because 1D techniques provide just a thin slice through a small subset of the columns and cannot take into account anisotropies in the 2D nature of the columnar pattern. Both the Lowel, and Teiman and Tumosa studies used 1D techniques to quantify the spacing of columns, although the rearing regimens that they employed led to changes in the 2D nature of the columns (patchy vs. banded). The analysis problem becomes most acute when interpreting results from 1D measurements of column spacing in animals where the staining pattern has changed as a result of altered visual experience. For example, often the 2D pattern of CO blob staining in strabismic macaques has darkly stained strips that connect the blobs along an ocular dominance band (see Fig. 4B). Because of these darkened strips a 1D analysis of spacing in strabismics that uses the rule of measuring from a labeled patch across an unlabeled patch to the next labeled patch will determine the spacing of blobs across ocular dominance bands. In normally reared macaque monkeys, however, CO blobs are not linked by darkened strips, so there is no simple way to determine which blobs are from the same ocular dominance band and which ones are from the neighboring band. As a result, applying the same rule in normal macaques will measure the spacing of blobs both across and along ocular dominance bands. Thus, the same rule and 1D technique when applied to strabismic and normal macaque monkeys will lead to the measurement of different aspects of the spacing of blobs. Since

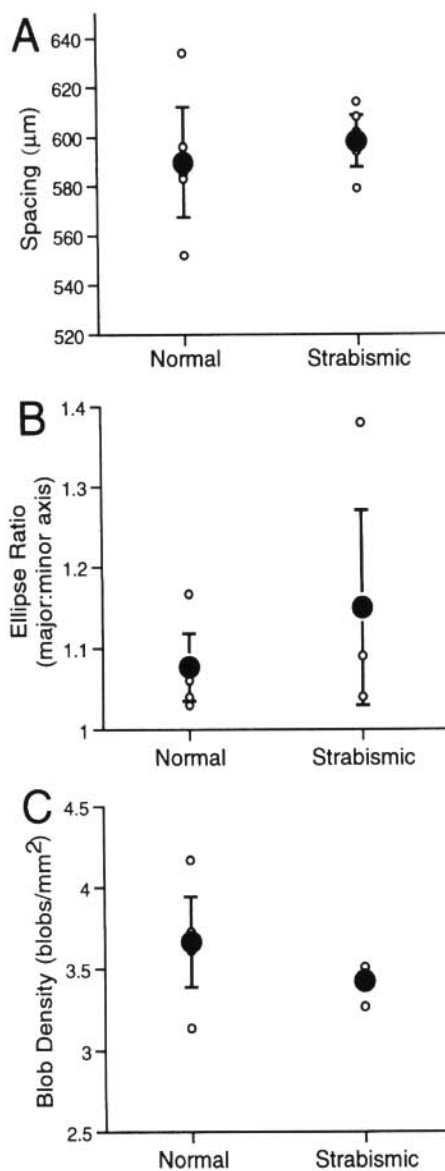


Figure 6. A comparison of: (A) the average blob-to-blob spacing; (B) the major to minor axis ratio for the ellipse of best fit to the polar plots of blob spacing; and (C) the density of blobs, for normal and strabismic macaque monkeys. The filled circles indicate the mean for the group, small open circles are the values for individual macaques and the error bars represent 1 SD. None of these measures of the arrangement of blobs were significantly different between normal and strabismic macaques. The error bars for the blob density of the strabismic macaques were too small to plot.

the 2D packing arrangement of blobs is anisotropic and the spacing appears to be smaller along an ocular dominance band than across a band (Horton, 1984), application of this type of 1D analysis could bias the results in favor of larger spacing in strabismic monkeys. In contrast, all of the blobs, regardless of whether they are connected by darkened strips, are located for the 2D nearest-neighbor statistics. This ensures that the same aspects of the spacing are measured even when the rearing regimen has changed other characteristics of the staining pattern. This fundamental difference between 1D techniques and 2D nearest-neighbor spatial statistics suggests caution in the interpretation of the spacing of columnar patterns that are derived from a 1D analysis.

The models of column development that predict an increase

in spacing are difficult to reconcile with the lack of change in blob spacing in strabismic macaques. A variety of other models, using very different constraints, can simulate a wide variety of patterns of cortical columns that appear similar to the actual biology (e.g. Rojer and Schwartz, 1990). The importance of any model, however, depends upon whether its predictions can be tested to validate the biological significance of the theoretical constraints. It has been suggested (Lowel, 1994) that the patchy pattern of the intrinsic horizontal connections may serve as a template that interacts with geniculocortical afferents during the process of segregation into ocular dominance columns, and when modified by experience (Lowel and Singer, 1992) leads to a change in the overall map of columns. This is an interesting possibility; however, in strabismic macaques the pattern of these connections is modified (Tychsen and Burkhalter, 1995), yet the pattern of blob-defined columns is not. Perhaps the mechanisms that constrain the spacing of ocular dominance columns and CO blobs are different (see Horton and Hocking, 1996d; Livingstone, 1996). The initial segregation of the magnocellular and parvocellular pathways occurs independent of retinal activity (Meissirel *et al.*, 1997), suggesting that early development of these pathways follows rules that are different from those that specify competition-driven organizations. However, in macaque V1, disruption of binocular vision by postnatal monocular deprivation does not change the spatial relationship between CO blobs and ocular dominance columns (Horton and Hocking, 1997). At some stage in development these features become linked (Fitzpatrick and Diamond, 1980; Hendrickson *et al.*, 1981; Horton, 1984; Murphy *et al.*, 1995; Hubener *et al.*, 1997) and subsequent alteration of retinal activity does not influence that relationship. Our results suggest that the initial 2D patterning of CO blobs follows the arrangement of markers that are intrinsic to the cortex and that may form an early columnar map. A variety of anatomical markers are arranged in a patchy fashion in the visual cortex during the critical period (Schoen *et al.*, 1990; Dyck and Cynader, 1993a,b), including a key postsynaptic element in activity-dependent development of the visual cortex – the NMDA receptor (Murphy *et al.*, 1996b). Computational models of column development should explore the inclusion of other factors, such as patchy receptor distributions, that can interact with activity and guide the spatial layout of nascent columns.

The development and arrangement of CO blobs is not strictly dependent upon the pattern of retinally driven activity and perhaps reflects an intrinsic organization of the cortical circuitry that is independent of the pattern of visual driven activity (Jones *et al.*, 1991). This notion is consistent with the results of demonstrations of an adult-like pattern of ocular dominance columns and CO blobs at birth in macaque monkeys (Horton and Hocking, 1996a), that monocular deprivation does not change the spacing of ocular dominance columns (Hubel *et al.*, 1977), and the immutable properties of the 2D pattern of other cortical columnar systems (e.g. Godecke and Bonhoeffer, 1996; Weliky and Katz, 1997). This suggests that certain aspects of the circuitry associated with columns in V1 are established prenatally and are not modified by postnatal visual experience.

Notes

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References

- Born RT, Tootell RB (1991) Spatial frequency tuning of single units in macaque supragranular striate cortex. *Proc Natl Acad Sci USA* 88:7066–7070.
- Bourgeois J-P, Rakic P (1996) Synaptogenesis in the occipital cortex of macaque monkeys devoid of retinal input from early embryonic stages. *Eur J Neurosci* 8:942–950.
- Curcio CA, Sloan KR (1992) Packing geometry of human cone photoreceptors – variation with eccentricity and evidence for local anisotropy. *Vis Neurosci* 9:169–180.
- DeYoe EA, Van Essen DC (1985) Segregation of efferent connections and receptive field properties in visual area V2 of the macaque. *Nature* 317:58–61.
- DeYoe EA, Van Essen DC (1988) Concurrent processing streams in monkey visual cortex. *Trends Neurosci* 5:219–226.
- Duncan RP, Stewart GH (1991) The temporal and spatial analysis of tree age distributions. *Can J Forest Res* 21:1703–1710.
- Dyck RH, Cynader MS (1993a) Autoradiographic localization of serotonin receptor subtypes in cat visual cortex: transient regional, laminar, and columnar distributions during postnatal development. *J Neurosci* 13:4316–4338.
- Dyck RH, Cynader MS (1993b) An interdigitated columnar mosaic of cytochrome oxidase, zinc, and neurotransmitter-related molecules in cat and monkey visual cortex. *Proc Natl Acad Sci USA* 90:9066–9069.
- Fitzpatrick D, Diamond IT (1980) Distribution of acetylcholinesterase in the geniculostriate system of *Galago senegalensis* and *Aotus trivirgatus*: evidence for the origin of the reaction product in the lateral geniculate body. *J Comp Neurol* 194:703–719.
- Godecke I, Bonhoeffer T (1996) Development of identical orientation maps for two eyes without common visual experience. *Nature* 379:251–254.
- Goodhill GJ (1993) Topography and ocular dominance: a model exploring positive correlations. *Biol Cybern* 69:109–118.
- Goodman CS, Shatz CJ (1993) Developmental mechanisms that generate precise patterns of neuronal connectivity. *Cell* 72:77–98.
- Guibas L, Stolfi J (1985) Primitives for the manipulation of general subdivisions and the computation of Voronoi diagrams. *ACM Trans Graphics* 4:74–123.
- Hebb DO (1949) *The organization of behavior*. New York: Wiley.
- Hendrickson AE, Hunt SP, Wu JY (1981) Immunocytochemical localization of glutamic acid decarboxylase in monkey striate cortex. *Nature* 292:605–607.
- Horton JC (1984) Cytochrome oxidase patches: a new cytoarchitectonic feature of monkey visual cortex. *Phil Trans R Soc Lond B Biol Sci* 304:199–253.
- Horton JC, Hocking DR (1996a) An adult-like pattern of ocular dominance columns in striate cortex of newborn monkeys prior to visual experience. *J Neurosci* 16:1791–1807.
- Horton JC, Hocking DR (1996b) Pattern of ocular dominance in human striate cortex in strabismic amblyopia. *Vis Neurosci* 13:787–795.
- Horton JC, Hocking DR (1996c) Intrinsic variability of ocular dominance column periodicity in normal macaque monkeys. *J Neurosci* 16:7228–7339.
- Horton JC, Hocking DR (1996d) Anatomical demonstration of ocular dominance columns in striate cortex of the squirrel monkey. *J Neurosci* 16:5510–5522.
- Horton JC, Hocking DR (1997) Timing of the critical period for plasticity of ocular dominance columns in macaque striate cortex. *J Neurosci* 17:3684–3709.
- Horton JC, Hubel DH (1981) Regular patchy distribution of cytochrome oxidase staining in primary visual cortex of macaque monkey. *Nature* 292:762–764.
- Horton JC, Stryker MP (1993) Amblyopia induced by anisometropia without shrinkage of ocular dominance columns in human striate cortex. *Proc Natl Acad Sci USA* 90:5494–5498.
- Hubel DH, Wiesel TN, LeVay S (1977) Plasticity of ocular dominance columns in monkey striate cortex. *Phil Trans R Soc Lond B Biol Sci* 278:377–409.

- Hubener M, Shoham D, Grinvald A, Bonhoeffer T (1997) Spatial relationship among three columnar systems in cat area 17. *J Neurosci* 17.
- Jones DG, Van Sluyters RC, Murphy KM (1991) A computational model for the overall pattern of cortical ocular dominance. *J Neurosci* 11:3794-3807.
- Jones, DG Murphy KM, Van Sluyters RC (1996) Spacing of ocular dominance columns is not changed by monocular deprivation or strabismus. *Invest Ophthalmol Vis Sci* 37:425.
- Kennedy H, Dehay C, Horsburgh G (1990) Striate cortex periodicity. *Nature* 348:494.
- Kiorpes L (1992) Effect of strabismus on the development of vernier acuity and grating acuity in monkeys. *Vis Neurosci* 9:253-259.
- Kiorpes L, Movshon JA (1989) Differential development of two visual functions in primates. *Proc Natl Acad Sci USA* 86:8998-9001.
- Kiorpes L, Carlson MR, Alfi D (1989) Development of visual acuity in experimentally strabismic monkeys. *Clin Vis Sci* 4:95-106
- Kiorpes, L, Kiper DC, Movshon JA (1993) Contrast sensitivity and vernier acuity in amblyopic monkeys. *Vis Res* 33:2301-2311.
- Kiorpes L, Walton PJ, O'Keefe LP, Movshon JA Lisberger, SJ (1996) Effects of early-onset surgical strabismus on pursuit eye movements and on neuronal response properties in area MT of macaque monkeys. *J Neurosci* 16:6537-6553.
- Kuljis RO, Rakic P (1990) Hypercolumns in primate visual cortex can develop in the absence of cues from photoreceptors. *Proc Natl Acad Sci USA* 87:5303-5306.
- Livingstone MS (1996) Ocular dominance columns in New World monkeys. *J Neurosci* 16:2086-2096.
- Livingstone MS, Hubel DH (1983) Specificity of cortico-cortical connections in monkey visual system. *Nature* 304:531-534.
- Livingstone MS, Hubel DH (1984a) Anatomy and physiology of a color system in the primate visual cortex. *J Neurosci* 4:309-356.
- Livingstone MS, Hubel DH (1984b) Specificity of intrinsic connections in primate primary visual cortex. *J Neurosci* 4:2830-2835.
- Livingstone MS, Hubel DH (1987a) Connections between layer 4B of area 17 and the thick cytochrome oxidase stripes of area 18 in the squirrel monkey. *J Neurosci* 7:3371-3377.
- Livingstone MS, Hubel DH (1987b) Psychophysical evidence for separate channels for the perception of form, color, movement, and depth. *J Neurosci* 7:3416-3468.
- Lowel S (1994) Ocular dominance column development: strabismus changes the spacing of adjacent columns in cat visual cortex. *J Neurosci* 14:7451-7468.
- Lowel S, Singer W (1992) Selection of intrinsic horizontal connections in the visual cortex by correlated neuronal activity. *Science* 255:209-212.
- Meissirel C, Wikler KC, Chalupa LM, Rakic P (1997) Early divergence of magnocellular and parvocellular functional subsystems in the embryonic primate visual system. *Proc Natl Acad Sci USA* 94:5900-5905.
- Miller KD (1994) Models of activity-dependent neural development. *Progr Brain Res* 102:303-318.
- Miller KD, Keller JB, Stryker MP (1989) Ocular dominance column development: analysis and simulation. *Science* 245:605-615.
- Murphy KM, Van Sluyters RC, Jones DG (1990) Cytochrome-oxidase in cat visual cortex: is it periodic? *Soc Neurosci Abstr* 16:292.
- Murphy KM, Van Sluyters RC, Jones DG (1991a) Cytochrome-oxidase blobs in cat visual cortex. *Invest Ophthalmol Vis Sci* 32:1116.
- Murphy KM, Van Sluyters RC, Jones DG (1991b) Analysis of the tangential arrangement of cytochrome-oxidase blobs in cat visual cortex. *Soc Neurosci Abstr* 17:1088.
- Murphy KM, Jones DG, Van Sluyters RC (1995) Cytochrome-oxidase blobs in cat primary visual cortex. *J Neurosci* 15:4196-4208.
- Murphy KM, Pegado VD, Fenstermaker SB, Jones DG, Kiorpes L, Movshon JA (1996a) Distribution of cytochrome-oxidase blobs in the visual cortex of normal and strabismic monkeys. *Invest Ophthalmol Vis Sci* S242.
- Murphy KM, Trepel C, Pegado VD (1996b) Non-uniform distribution of the NMDAR1 receptor subunit in kitten visual cortex. *Molecular Vision* [On-line] 2, 9. http://www.emory.edu/MOLECULAR_VISION/v2/murphy
- Olavarria J, Van Sluyters RC (1985) Unfolding and flattening the cortex of gyrencephalic brains. *J Neurosci Methods* 15:191-202.
- Purves D, LaMantia A (1993) Development of blobs in the visual cortex of macaques. *J Comp Neurol* 334:169-175.
- Rakic P, Lidow, MS (1995) Distribution and density of monoamine receptors in the primate visual cortex devoid of retinal input from early embryonic stages. *J Neurosci* 15:2561-2574.
- Roger AS, Schwartz EL (1990) Cat and monkey cortical columnar patterns modeled by bandpass-filtered 2D white noise. *Biol Cybern* 62:381-391.
- Schoen SW, Leuteneker B, Kreutzberg GW, Singer W (1990) Ocular dominance plasticity and developmental changes of 5'-nucleotidase distributions in the kitten visual cortex. *J Comp Neurol* 69:3-18.
- Sengpiel F, Godecke I, Stawinski P, Hubener M, Lowel S, Bonhoeffer T (1998) Intrinsic and environmental factors in the formation of functional maps in cat visual cortex. *Neuropharmacology* (in press).
- Shapiro MB, Schein SJ, De Monasterio FM (1985) Regularity and structure of the spatial pattern of blue cones of macaque retina. *J Am Statist Assoc* 80:803-812.
- Shoham D, Hubener M, Schulze S, Grinvald A, Bonhoeffer, T (1997) Spatio-temporal frequency domains and their relation to cytochrome oxidase staining in cat visual cortex. *Nature* 385:529-33.
- Swindale NV (1980) A model for the formation of ocular dominance stripes. *Proc R Soc Lond B Biol Sci* 208:243-264.
- Swindale NV (1996) The development of topography in the visual cortex: a review of models. *Network: Comput Neural Syst* 7:161-247.
- Tieman SB, Tumosa N (1997) Alternating monocular exposure increases the spacing of ocularity domains in area 17 of cats. *Vis Neurosci* (in press).
- Tootell RB, Silverman MS, Hamilton SL, Switkes E, De Valois, RL (1988) Functional anatomy of macaque striate cortex. V. Spatial frequency. *J Neurosci* 8:1610-1624.
- Tychsen L, Burkhalter A (1995) Neuroanatomic abnormalities of primary visual cortex in macaque monkeys with infantile esotropia: preliminary results. *J Pediatr Ophthalmol Strabismus* 32:323-328.
- Van Essen DC, Anderson CH, Felleman DJ (1992) Information processing in the primate visual system: an integrated systems perspective. *Science* 255:419-423.
- Weliky M, Katz LC (1997) Disruption of orientation tuning in visual cortex by artificially correlated neuronal activity. *Nature* 386:680-685.
- Wiesel TN, Hubel DH (1963) Single-cell responses in striate cortex of kittens deprived of vision in one eye. *J Neurophysiol* 26:1003-1017.
- Wong-Riley M (1979) Changes in the visual system of monocularly sutured or enucleated cats demonstrable with cytochrome oxidase histochemistry. *Brain Res* 171:11-28.
- Yoshioka T, Blasdel GG, Levitt JB, Lund JS (1996) Relation between patterns of intrinsic lateral connectivity, ocular dominance, and cytochrome-oxidase regions in macaque monkey striate cortex. *Cereb Cortex* 6:297-310.
- Zeki SM (1976) The functional organization of projections from striate to prestriate visual cortex in the rhesus monkey. *Cold Spring Harb Symp Quant Biol* 40:591-600.