[FINAL ACCEPTED VERSION]

Title: Functional Organization of Visual Cortex in the Prosimian Bush Baby Revealed by Optical Imaging of Intrinsic Signals

Authors: Xiangmin Xu1*, William H. Bosking2**, Leonard E. White2,5, David Fitzpatrick2
Vivien A. Casagrande1,3,4

1Departments of Psychology, 3Cell & Developmental Biology and 4Ophthalmology & Visual Sciences, Vanderbilt University, Nashville TN
Departments of 2Neurobiology and 5Community & Family Medicine, Duke University, Durham NC
*Current address: Systems Neurobiology Laboratories, Salk Institute for Biological Studies, La Jolla, CA
**Current address: Department of Neuroscience and Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX

Running Title: Organization of bush baby visual cortex

Key Words: Striate cortex, V2, Prosimian, Visuotopic map, Orientation preference, Ocular dominance, CO blobs

Pages: 51

Figures: 10

Address all correspondence and reprint requests to:
V. A. Casagrande, PhD
Department of Cell & Developmental Biology
Vanderbilt Medical School
U3218 Learned Lab
Nashville, TN 37232-8240

Phone: (615) 343-4538
Fax: (615) 343-4539
Email:vivien.casagrande@vanderbilt.edu

Copyright © 2005 by the American Physiological Society.
Functional Organization of Visual Cortex in the Prosimian Bush Baby Revealed by Optical Imaging of Intrinsic Signals

Abstract

Cells in primary visual cortex (V1) of primates and carnivores respond most strongly to a visual stimulus presented to one eye, in a particular visual field location, and at a particular orientation. Each of these stimulus attributes is mapped across the cortical surface, and, in macaque monkeys and cats, strong geometrical relationships exist between these feature maps. In macaque V1 and V2, correlations between feature maps and cytochrome oxidase (CO) rich modules have also been observed. To see if such relationships reflect a conserved principle of V1 functional architecture among primate species, we examined these maps in the prosimian bush baby, a species that been proposed to represent the ancestral primate organization. We found that the layout of individual feature maps in bush baby V1 is similar to that of other primates, but we found an entirely different organization of orientation preference in bush baby V2, compared to that reported in simian primates. Another striking distinction between bush baby and simian species is that we observed no strong relationships among maps of orientation, ocular dominance and CO blobs in V1. Thus, our findings suggest that precise relationships between feature maps are not a common element of the functional organization in all primates, and that such relationships are not necessary for achieving basic coverage of stimulus feature combinations. In addition, our results suggest that specific relationships between feature maps in V1, and the subdivision of V2 into functional compartments, may have arisen comparatively late in the evolution of primates.
Introduction

The responses of cells in primary visual cortex (V1) depend on the eye to which the stimulus is presented, the location of the stimulus in the visual field, and the orientation of the stimulus. In most primates and carnivores, cells with similar eye preference, receptive field location, and preferred orientation are grouped together into radial columns or domains (Hubel and Wiesel, 1977; Blasdel and Salama, 1986; LeVay and Nelson, 1991; Bartfeld and Grinvald, 1992; Bonhoeffer et al., 1995; Landisman and Ts’o, 2002). In addition to columns related to these stimulus features, staining visual cortex for the activity of the metabolic enzyme cytochrome oxidase (CO) reveals an additional columnar structure: CO blobs or patches (Horton and Hubel, 1981; Horton, 1984; Livingstone and Hubel, 1984). Thus, viewed from the cortical surface, the superficial layers of V1 contain at least four distinct but overlapping columnar systems: a map of visual space, a map of orientation preference, a map of ocular dominance, and a mosaic of patches that are alternately rich and poor in cytochrome oxidase.

Specific relationships between these columnar systems have been observed in some cases. The most striking map relationships have been observed in macaque V1, where, for example, singularities in the orientation preference map tend to lie near the center of ocular dominance domains, and iso-orientation contours tend to run orthogonal to ocular dominance boundaries (Blasdel and Salama, 1986; Bartfield and Grinvald, 1992, Obermayer and Blasdel 1993). In addition, CO blobs are found aligned over the centers of ocular dominance bands (Horton and Hubel, 1981; Blasdel and Salama, 1986; Bartfeld and Grinvald, 1992). While not as strong, similar relationships between ocular dominance domains, orientation preference maps and cytochrome oxidase blobs have been reported in the cat area 17/18 (Murphy et al.,
A relationship between compartments defined by CO and columnar organization for orientation selectivity has also been observed in V2 of the macaque (Livingstone and Hubel, 1984; Ts'o et al., 1990; Roe and Ts'o, 1995), squirrel monkey (Malach et al., 1994), and owl monkey (Xu et al., 2004a).

Given the striking relationships among columnar systems found in both macaque and cat V1, and the evolutionary distance between the primate and carnivore lines of descent, one is tempted to conclude that systematic relationships among maps are a general feature of cortical organization and of fundamental importance for the representation of multiple features within the same functional area. Indeed, it has been argued that systematic map relationships may be important for achieving uniform coverage, which is insuring that all combinations of stimulus features are represented uniformly for all regions of visual space (Swindale, 2000; Swindale et al., 2000). There are, however, reasons to question the idea that map relationships are common to all species that exhibit these columnar systems, and thus are critical for achieving uniform coverage. For example, in another carnivore, the ferret, little evidence was found for a relationship between the map of orientation preference and the map of ocular dominance (White et al., 2001). Similarly, in a different primate, the squirrel monkey, CO domains do not adhere strictly to the center of ocular dominance domains as they do in the macaque (Horton and Hocking, 1996). Currently, however, no study has provided quantitative evidence on the structure and relationships of all four of these columnar systems in any primate other than the macaque.

The goal of the present study was to examine the map of visual space, the map of orientation preference, the map of ocular dominance, and CO staining in another primate, the
prosimian bush baby. We found robust spatial organization for each of these columnar systems in V1, but little evidence for strong relationships among maps. Our results suggest that precise relationships among stimulus feature maps in V1 are not a general feature of cortical organization in primates, and that the relationships observed in the cat and the macaque may have evolved independently. Moreover, assuming the properties of extant prosimians can be taken to resemble those of early ancestral primates (Purvis, 1995), map relationships appear to be a more recently acquired trait, one that is not essential for either the development or proper function of circuits in V1. Some of the results reported here were presented previously in abstract form (Bosking et al. 1996; Emeric et al., 2003).

**Materials and Methods**

*Subjects*

Twenty bush babies of both sexes were handled according to the approved protocols from the Institutional Animal Care and Use Committees at Vanderbilt University and Duke University. Twelve animals were used for experiments at Duke University, and the other 8 were used at Vanderbilt University. In these animals, V1 was successfully imaged in 16 hemispheres; V2 was imaged in 11 hemispheres.

Additional data from two owl monkeys imaged as part of a previous study (Xu et al., 2004a) were used to compare the organization of V2 in bush babies and owl monkeys. The owl monkeys and bush babies were imaged in the same set up under identical conditions (see Figure 6).

*General preparation*
The general preparation differed slightly for experiments conducted at the two institutions. At Duke University, anesthesia was induced with a mixture of ketamine and xylazine and maintained at surgical levels with a 2:1 mixture of NO₂ and O₂ and 1-3% halothane. Animals were placed in a stereotaxic frame, intubated, paralyzed (intravenous pancuronium bromide, 0.2 mg kg⁻¹ h⁻¹), and artificially ventilated with the gas mixture at a depth and rate which kept the expired CO₂ near 4.0%. Skin and muscle were reflected and a craniotomy was performed exposing parts of V1, V2, and adjacent visual areas. A stainless steel chamber was secured to the skull using dental acrylic, the dura removed, and then the chamber was filled with saline and sealed with a glass top. Anesthesia was then lowered for optical imaging to a 1:1 mixture of NO₂ and O₂ and 0.5-1% halothane.

At Vanderbilt University, the animals were initially anesthetized with isofluorane (2-4% in O₂) and, after tracheal intubation and implantation with two femoral catheters, were mounted in a stereotaxic apparatus. Neuromuscular blockade was initiated by i.v. injection of 0.7-1.0 mg kg⁻¹ vecuronium bromide. Animals then were artificially ventilated with a 3:1 mixture of NO₂ and O₂ delivered at a rate sufficient to maintain the peak end tidal CO₂ level at around 4 %. Paralysis was maintained by intravenous infusion of vecuronium bromide (0.1-0.3 mg kg⁻¹ hr⁻¹) mixed in 5% dextrose lactated Ringer's. Propofol (2,6-di-isopropylphenol: 10 mg kg⁻¹ hr⁻¹) was used for anesthesia during these experiments. An opening was made in the skull over visual cortex and was sealed with 1% agarose in saline under a cover glass.

Pupils were dilated with 1% atropine eye-drops and clear contact lenses with artificial pupils 3 mm in diameter were used to render the retina conjugate with the viewing screen 28.5 or 57 cm distant. During experiments the optic disks and areae centralii (ACs) were
plotted on the screen using either a reversible ophthalmoscope or by back reflection of the retinal image from the surface of the tapetum using a fiber optic light source.

Optical Imaging and visual stimuli

Intrinsic optical imaging signals were acquired with the differential video-enhancement imaging system and data acquisition software from Optical Imaging Inc. (Mountainside, NJ). Similar imaging procedures have been described in detail previously (Bosking et al., 1997; Bosking et al., 2002; Xu et al., 2004a,b).

Reference images of cortical vasculature were acquired with a 540 nm green light. The cortex was illuminated with a 611 or 689 nm light during data acquisition. We obtained consistent results or equivalent image quality with either a 611 or a 689 nm light, although 689 nm tended to reduce blood vessel artifacts in some cases. In order to map the visuotopic organization of V1, topographically limited horizontal or vertical grating stimuli were presented monocularly through the contralateral eye either within narrow rectangular windows [1º - 2º width x full screen height (54º) or full screen width (74º) x 1º - 2º height] or 2º circular patches (see the insets in Figures 1 and 2) at eccentricities ranging from 0º to 15º. High-contrast square wave gratings (fundamental spatial frequency 0.5-1.0 cyc/deg, drift velocity 2 Hz, duty cycle 20%) were moved back and forth within the windows at different locations. At each location mapped, stimuli consisted of two orthogonally oriented gratings. A single trial consisted of data acquisition during continued presentation of the drifting grating stimulus for 8 sec and an interstimulus interval of 12 sec using a blank screen of mean luminance (~30 cd/m²). Stimulus sets were repeated 10-20 times.

To examine the size and distribution of activity evoked in cortex by stimuli confined to very small regions of visual space we examined the “line image” for stimuli that were
similar to those described above but now restricted to very thin vertical or horizontal windows on the stimulus screen. This approach was similar to previous studies that used small visual stimuli to examine ‘point spread’ or “line spread” of optical imaging signals (Grinvald et al., 1994; Das and Gilbert, 1995; Bosking et al., 2002).

To study the organization of orientation preference, high-contrast rectangular gratings (fundamental spatial frequency 0.5 cyc/deg, drift velocity 2 Hz, duty cycle 20%) of four orientations at intervals of 45° were displayed on the full screen and presented binocularly. In some blocks, all orientation conditions and a blank control were presented in a randomized order. On other blocks, only orthogonal pairs of orientations (e.g., 0/90°, 45/135°) were presented in separate trials. Stimulus sets were made up of 20-40 blocks.

To determine the map of ocular dominance, four or eight oriented gratings were presented while either the left eye or right eye was blocked using a hand held occluder or electrical eye shutters. Data acquired during presentation of all orientations to the left eye were summed, as were data acquired during presentation of all orientations to the right eye, and the two data sets were then subtracted from each other or divided by each other to form an ocular dominance map.

Video images were acquired at a rate of 30 frames/sec, but all frames acquired for each condition during the 8-sec period of stimulation were summed together for 8 data frames before further analysis. Individual data frames were either 744 x 480 pixels, with a resolution of either 87 pixels/mm or 174 pixels/mm, or 655 x 480 pixels with a resolution of ~75 pixels/mm.

*Visuotopic map analysis*
In order to create visuotopic maps, all images associated with stimuli of the same orientation were summed and divided by the “pure blank” obtained by summing the images of the blank control to create single-condition maps using Winmix or TVMIX software (Optical Imaging Inc.), unless specified otherwise. The resulting maps were routinely “clipped” at 1-2 STD, smoothed by using a mean-filter kernel or Gaussian-filter kernel at a radius of 3-7 pixels and scaled in the range of 0–255 gray levels for appropriate display, using Matlab (MathWorks, Natick, MA). In addition, to quantify the activation pattern sizes in response to topographically restricted stimuli, the distinctive modular foci of activation on the resultant topographical map which contained pixels above 1 STD from the image mean of mask-filtered maps (see below) were detected by a custom Matlab program. We then outlined activation contours manually and measured across the extracted activation contour to get the width, orthogonal to the major axis of cortical activation.

Visual field maps were constructed based upon a series of activation patterns produced by topographically limited horizontal and vertical grating stimuli in different locations of the visual field. The midlines of these activation patterns were plotted to create iso-azimuth or iso-elevation lines using the following procedure: (1) The low-pass filtered single condition image was thresholded to include only the top 15 -20% darkest pixels in order to show the central points of each activation patch. (2) The same image was also used to create contours of the total activation zone by reducing the number of gray levels to 8 or 16 discrete levels. The latter (4th or 8th gray level) helped to determine the outer edges of the activation zone (see Blasdel and Campbell, 2001). (3) Data obtained from the center points of activation (1) and the outermost contour of the total activation zone (2) were combined to determine the midline of the activated area in cortex. The intersections of the iso-azimuth and
iso-elevation midlines were used to determine individual retinotopic points. Some of these points were also verified by examining the location of activation patterns produced by the 2° circles of stimulation presented at different eccentricities.

The cortical magnification factor (CMF) was measured based upon the visual field maps and calculated as the average linear distance in cortex representing 1° in visual space (mm/deg) at a particular eccentricity (Daniel and Whitteridge, 1961; Van Essen et al, 1984). Specifically, the distance between two adjacent intersection points of iso-azimuth and iso-elevation lines on the topographical maps of cortex (in mm) was divided by the corresponding distance in degrees of visual space.

*Orientation map analysis*

Data acquired during presentation of one orientation were divided by or subtracted from data acquired during presentation of the orthogonal orientation to produce difference images using Winmix or TVMIX software. Custom programs written in NIH Image (as an extension to the public domain National Institutes of Health Image program, at [http://rsb.info.nih.gov/nih-image/](http://rsb.info.nih.gov/nih-image/)) and Matlab were used to further process the data. In most cases, to reduce vascular artifacts, we used a stack of images from the same data set to create a mask indicating the location of the major blood vessels. The grayscale value for each pixel in the data images that was located in the mask was replaced by the mean of the grayscale values of the appropriate surrounding pixels (5-35 pixels) outside the mask (Bosking et al., 2000; Blasdel and Campbell, 2001). In most cases, this process altered about 10% of the pixel values in the region of V1 that we imaged. Quantitative measures were made in regions outside of the masked areas. Resulting difference images were smoothed using a 7 x 7 pixel mean filter kernel. Low frequency noise was reduced by convolving the image with a
80 x 80 pixel mean filter kernel and subtracting the result from the original image. These difference images were "clipped" at 1.5-2 STDs around the mean of the image pixel distribution and scaled in the range of 0-255 gray levels. Difference images were also combined by vector summation to produce orientation preference maps. In this paper, orientation preference maps where we show only the angle information from this vector summation we term angle maps, those maps where only the magnitude of the vector is depicted we term magnitude maps, and those maps where angle and magnitude information are combined we term polar maps. Maps showing rate of change of orientation preference were also constructed for the same regions of cortex with the highest rate of change of orientation represented as white and the lowest as black.

Ocular dominance map analysis

As described earlier, ocular dominance maps were constructed by comparing the images acquired during the stimulation of one eye (with full field gratings at 4 or 8 orientations) with images acquired during the stimulation of the other eye so that regions dominated by different eyes appear dark and light, respectively. The ocular dominance maps were band-pass filtered in the same way as described above for orientation difference maps. The same images were also used to create contours of ocular dominance by reducing the number of gray levels from 256 to 8-16 discrete levels, and subsequently determining the geometric centers of ocular dominance domains.

Quantitative analyses of map relationships

Orientation preference vs. CO: The outlines of CO blobs (manually drawn or determined by a custom program) were transferred (see next section) to maps of orientation and ocular dominance. Orientation pinwheel centers were identified based upon high rate of
change regions in the map of orientation preference, and confirmed by visual inspection of orientation preference maps and difference images. Pinwheel locations were then transferred to ocular dominance maps and CO maps from the same cortical region. We then quantified the number of pinwheel centers found in different regions of the ocular dominance map and in the blob and interblob regions. To quantitatively examine the degree of orientation response selectivity between compartments (i.e., V1 CO blobs and interblobs), we calculated the index of orientation response selectivity on the 8-bit magnitude map by dividing the grey scale value of each pixel (the resultant vector strength of four stimulus orientations) by the maximum grey value, 255. The orientation selectivity index ranged from 0 to 1 (lowest to highest).

Orientation preference vs. ocular dominance: Color-coded iso-orientation contours were created from orientation preference maps and superimposed over contours of the ocular dominance map to examine how iso-orientation contours intersected with ocular dominance contours. In addition, we quantified the relationship between orientation preference pinwheels and ocular dominance centers from two cases by performing a nearest neighbor analysis. In each case, we first examined the distribution of distances to the nearest neighboring pinwheel for each pinwheel in the analysis region. We also computed nearest neighbor distances using a random placement of the same number of pinwheels in the same cortical area. This procedure was repeated for 1000 sets of randomly placed pinwheels and the mean cumulative probability distribution and 95% confidence intervals on this distribution were calculated. The same procedure was carried out for ocular dominance centers. In addition, we calculated the minimum distance between each pinwheel and the nearest ocular dominance center. We compared this distribution to the same distribution
calculated using random placement of pinwheel centers. Again this procedure was performed for 1000 sets of random pinwheel placements and the mean and 95% confidence interval were calculated.

**Histology and alignment**

At the termination of each experiment, the animal was deeply anaesthetized with an overdose of sodium pentobarbital and perfused transcardially with a saline rinse followed by 2% paraformaldehyde in 0.1 M phosphate buffer. The brain was removed and the imaged area of cortex was separated and flattened. The imaged piece of cortex was then frozen and cut with the surface vascular pattern preserved in the first 100 -150µm section. Subsequent sections were cut at 50 µm. Cytochrome oxidase (CO) staining was performed using methods described previously (Boyd and Matsubara, 1996).

Surface and radial blood vessels were the primary landmarks used to align histological sections to the optical reference images of the imaged area. Differences between images and sections due to distortion or tissue shrinkage (about 10 -15%) were handled by global scaling and rotation. After the optical images were aligned with the histological data, we examined the corresponding locations or patterns of the imaged brain areas to compare CO staining patterns with map structures of orientation preference and ocular dominance and to see if the anatomical delineation of each visual area was compatible with the optical imaging results.

**Results**
We first describe our results obtained, examining the structure of individual maps of visual space, orientation preference, and ocular dominance. We then turn to relationships between feature maps and the relationship of these maps to CO staining patterns.

**Visuotopic organization**

Our goal was to use optical imaging with stimuli restricted to small regions of the visual field to examine the population response to simple stimuli, and to examine the structure of the map of visual space. We focused on the most accessible portion of bush baby V1 for optical imaging, which represents approximately the central 10° of the visual field (Rosa et al., 1997).

Topographically restricted stimuli produced large, patchy, activation patterns (Figures 1, 2). The patchy nature of the activation pattern was expected since we used oriented gratings within topographically restricted windows. For stimulus gratings confined to 2° windows, this activation pattern was approximately 2.8 ± 0.36 mm in width (mean ± STD, N = 5 cases). Stimuli as small as 0.5° produced activation patterns that were 1.34 ± 0.2 mm in width (N = 2 cases, data not shown). Movement of the stimulus window by as little as 1° on the screen produced highly overlapping but discretely shifted cortical activation patterns. For example, as the stimulus was moved from the vertical meridian to more eccentric positions, the stripe of activation moved from anterior to posterior in V1 (Figures 1,2). As the stimulus was moved from above the horizontal meridian into the lower visual field, the band of activation moved from lateral to medial in V1. The same size stimuli appeared to activate slightly more cortical territory when presented close to the *area centralis* (e.g., Figures 1H, 2D).
By using a series of stimuli in both the vertical and horizontal axes spaced at 1° we were able to construct complete maps of visual space for V1 in two cases (Figures 2H and 3). Although in some cases we could see a band of activation in V2 (arrow, Figure 1E), the strength of the signal in V2 was not sufficient to construct a map of visual space for this area. The validity of our maps produced using this technique was confirmed by examining the activation foci produced by small circular patch stimuli presented at specified locations. For example, for the case shown in Figure 2, a 2° circular stimulus patch located 4° from the vertical meridian (VM) and 1° below the horizontal meridian (HM), produced cortical activation at the location predicted by the intersection of the contours shown in Figure 2H.

[Figures 1, 2 about here]

The location of the V1/V2 border predicted by our imaging with topographically restricted stimuli was consistent with the border determined by differences in CO staining between V1 and V2 (see below). The V1/V2 border also could be distinguished by differences in structure in the orientation preference map such as larger domains in V2 (see below).

We obtained the cortical magnification factor (CMF) by measuring the distance (mm) between two adjacent intersection points of iso-azimuth and iso-elevation lines of the visual field maps and dividing this number by the corresponding distance in degrees of visual space (see Figure 1K). The CMF values we obtained for eccentricities of 1- 8° using optical imaging (Figure 3A) matched the CMF vs eccentricity function previously generated by physiological recordings (Figure 3A, dashed line, CMF = 2.36 ⋅ (E (eccentricity) + 0.73) ^{-0.8}, Rosa et al.,
Our measurement of the average CMF at eccentricities of 1-8º (N = 4 cases) was 0.77mm/º, which matches the average extrapolated value (0.76 mm/º) of Rosa et al. (1997) at the same eccentricities. The CMF for the representation of central vision in bush babies is much less than that reported in macaque monkeys (Figure 3B, dotted line).

We found anisotropy in the CMF not reported based upon reconstructions from microelectrode mapping (Rosa et al., 1997). CMF measured along the iso-azimuth dimension (parallel to the vertical meridian) was greater than that measured along the iso-elevation dimension (parallel to the horizontal meridian), which was indicated by the rectangle-like (not square) pattern of grids seen in the visual field map (Figure 1K, Figure 2H) defined by the iso-elevation and iso-azimuth lines. The mean ratio of the iso-azimuth vs iso-elevation dimension for eccentricities between 0º and 8º was 1.16 ± 0.03 (mean ± STD, n = 4).

[Figure 3 about here]

Orientation preference in V1 and V2

In V1, optical imaging of intrinsic signal revealed a highly regular and continuous map of orientation preference (Figure 4). The orientation preference map was similar to those found in other species, and contained regions where orientation cycles around point singularities (pinwheel centers), regions where orientation preference changes in a straight line (linear zones) and regions where orientation preference changes slowly (saddle points) (e.g. cat, Bonhoeffer and Grinvald, 1991; Shmuel and Grinvald, 2000; macaque, Blasdel, 1992; squirrel monkey, Malach et al., 1994; ferret, Chapman et al., 1996; tree shrew, Bosking et al., 1997; owl monkey, Xu et al., 2004a). The average center-to-center spacing of iso-
orientation domains in V1 is $0.69 \pm 0.1$ mm (mean \pm STD). Areas of high rate of change in the orientation preference map are largely confined to pinwheel centers (Figure 4D). We found both pinwheel centers with clockwise progression of orientation preference (Figure 4E, blue squares), and pinwheel centers with counter-clockwise progression of orientation preference (Figure 4E, red squares). Regions with low magnitude of orientation selectivity (dark areas in Figure 4B) occupy only a small fraction of the map and largely correspond to the centers of pinwheels and to short thin bands that interconnect adjacent pinwheel centers (Figure 4D).

Clear compartments of high and low orientation selectivity have been described in V2 of both diurnal simians (i.e. macaque monkey and squirrel monkey) and the nocturnal simian (owl monkey) which appear to correlate either with high or low CO activity (Livingstone and Hubel, 1984; Ts'o et al., 1990; Malach et al., 1994; Roe and Ts'o, 1995; Xu et al., 2004a). Surprisingly no such compartments were seen in V2 of bush baby. Figures 5 and 6 compare the organization of V2 in bush babies and owl monkeys imaged in the same set up under identical conditions. As can be seen in Figure 5, the map of orientation preference in bush baby V2 was largely uniform whereas in owl monkey (Figure 6) there were clear stripes of low and high orientation selectivity in V2 (see also Xu et al., 2004a). As reported by others, we also saw no evidence of CO bands in bush baby V2 (see Figure 5H; also see Condo and Casagrande, 1990; Collins et al., 2001). Owl monkey V2, however, clearly has bands in CO-stained sections (Figure 6D).
The structure of the map of orientation preference in V2 was similar to that found in V1 although the overall response magnitude in V2 was lower (Figure 5). Iso-orientation domains in V2 were larger than their counterparts in V1 (see Figure 5A-C, E-F), which has also been reported for the V2 domains in high orientation selectivity regions in other primate species (Ts’o et al., 1990; Malach et al., 1994; Xu et al., 2004a). Consequently, the density of pinwheel centers was significantly lower in V2 than in V1 (Figure 5G). The mean pinwheel density in V2 was $3.7 \pm 0.5$ (mean ± STD) mm$^{-2}$, while it is $6.4 \pm 0.4$ mm$^{-2}$ for V1.

[Figures 5 and 6 about here ]

Ocular dominance map in V1

Consistent with both previous anatomical and physiological evidence for ocular dominance columns in bush baby V1 (Glendenning et al. 1976; DeBruyn et al., 1993), we found evidence for the existence of ocular dominance domains in 8 out of 9 cases. In the ocular dominance map shown in Figure 7, dark regions correspond to one eye activation and light regions to the other eye activation; white and black dots indicate the geometric centers of ocular dominance domains. Ocular dominance domains found in the bush baby tended to be less regular, more ovoid, and less stripe-like than those observed in the macaque (e.g., see Blasdel, 1992; Shtoyerman et al., 2000; Blasdel and Campbell, 2001). The average distance (center to center) between adjacent left and right eye columns was $0.53 \pm 0.12$ mm (mean ± STD). The ocular dominance patterns that we observed were confined to V1 (Figure 7C and D).
Relationship between ocular dominance and orientation preference

The relationship between the map of orientation preference and the map of ocular dominance was investigated in detail for 2 cases. We used three methods to examine this relationship. The first method was to obtain iso-orientation contours and compare these to the map of ocular dominance (Figure 8A). A majority of iso-orientation contours tended to run orthogonal to the borders of ocular dominance domains (Figure 8A). This impression was quantified by creating frequency histograms of the distribution of intersection angles between iso-orientation contours and ocular dominance contours (Figure 8B). The mean intersection angle, pooled from two cases, was 64.4 ± 29.9 (mean ± STD), with around 55% of the intersection angles falling in the range of 68º-90º. The relationship between orientation and ocular dominance contours is less regular than reported in the macaque and more similar to that seen in cats (Blasdel and Salama, 1986; Bartfeld and Grinvald, 1992; Obermayer and Blasdel, 1993; Hübener et al., 1997; Kim et al., 1999).

The second method we used was to examine the location of pinwheel centers relative to ocular dominance maps that had been divided into 8 gray levels. Unlike the organization described for the macaque (Blasdel and Salama, 1986; Bartfeld and Grinvald, 1992, Obermayer and Blasdel, 1993), pinwheels did not appear to be preferentially located over the darkest or lightest regions of the ocular dominance map (e.g., see Figure 8A). Overall, only 31.5 ± 6.3 % of pinwheel centers (mean ± STD, N = 3 cases) were located in the center of ocular dominance domains; ocular dominance centers as measured here occupied about 20% of the measured V1 area.
Finally, the third method we used to quantitatively assess the relationship between the map of orientation preference and the map of ocular dominance was to examine the distribution of distances between pinwheel centers and ocular dominance centers. For each case, we first examined the cumulative distribution of distances between neighboring pinwheel centers (Figure 9A). The cumulative distribution for our real data for pinwheel centers was significantly shifted to the right compared to a similar distribution generated by calculating nearest neighbor distances after random placement of pinwheels. This indicates that the placement of pinwheels is highly non-random. Pinwheel centers tended to be spaced more uniformly than would be expected for a random arrangement, with many nearest neighbors found at distances of approximately 300 µm. A similar result for the distribution of pinwheel centers has been obtained in the cat and the ferret (Muller et al., 2000).

Our nearest neighbor analysis for the map of ocular dominance produced similar results. Again the cumulative distribution for the real data was shifted to the right of that expected for random placement of ocular dominance centers in the same cortical area (Figure 9B). Nearest neighbors in the ocular dominance map were found at a distance of approximately 500 µm.

Although based on this nearest neighbor analysis each individual map was arranged in a highly non-random fashion, we found no evidence for a relationship between the two maps. The relationship between maps was assessed by calculating the distance between each pinwheel center and the nearest ocular dominance center. This procedure was also performed for data sets where pinwheels were placed randomly. In this case, we found no difference between the cumulative distribution generated from our real data and the one generated using random pinwheel placement (Figure 9C). Had there been a strong tendency for pinwheel
centers to lie near the center of ocular dominance domains, we would have expected a leftward shift of the cumulative distribution.

[Figures 8 and 9 about here]

**Relationship between CO blobs and ocular dominance and orientation preference maps**

The pattern of CO staining was examined in tangential sections in seven bush babies. As seen in the macaque, and previously reported for the bush babies (Condo and Casagrande, 1990), dark CO staining in superficial layers of V1 appeared as a periodic series of "blobs" (see Figures 5 and 10). Figure 10 shows the CO staining pattern, angle map, magnitude map, and ocular dominance map for the same region of cortex from the same case, with the stained tissue aligned to the optical reference image. We first analyzed the number of pinwheels found in blob and interblob areas. In Figure 10 B and C, for this case, 33.6% of cortex was covered by blobs, and 31.5% of the pinwheels were found in blobs, which suggested that there is no tendency for pinwheels to be located preferentially in the blob areas. Similar relationships were obtained in two other animals. We also examined the magnitude of orientation selectivity in blob and interblob regions. The average orientation selectivity index from 24 CO blobs pooled from two cases was $0.51 \pm 0.01$ (mean \pm SE); the average index from 20 interblobs was $0.45 \pm 0.03$. These two values were not significantly different.

To examine the relationship between the map of ocular dominance and the pattern of cytochrome oxidase staining, outlines of blobs were created and placed over the map of ocular dominance (Figure10E). Although there are some blobs centered on ocular dominance domains, many blobs are situated at the borders of columns and straddle adjacent ocular
dominance domains. Overall, we did not see a clear relationship between the distribution of CO blobs and the ocular dominance domains.

Discussion

Our results for functional organization of V1 in the bush baby include: 1) An orderly map of visual space, 2) systematic representation of orientation preference, ocular dominance, and CO domains, and 3) no strong relationships between these individual maps. In V2, we find robust maps of orientation preference but no apparent functional compartments based on CO staining or level of orientation selectivity. In our discussion, we will first compare our results on the structure of the map of visual space and the structure of individual feature maps to results in other species. Then we will consider the importance of the lack of relationships between these systems for understanding the development and function of visual cortex.

Visuotopic organization

We found that even stimuli restricted to small portions of the visual field evoked large patterns of activity over a millimeter in width in V1. This result is similar to the results from previous studies that have attempted to determine the point image, or the amount of cortex activated by restricted stimuli in other species (Grinvald et al., 1994; Blasdel and Campbell, 2001; Bosking et al., 2002). The systematic changes in the V1 activation pattern we observed with small movements in the stimulus in either the horizontal or vertical axis suggest that the
map of visual space is nevertheless organized in a smooth and continuous fashion. This result is also similar to results from other primates (Tootell et al., 1988; Blasdel and Campbell, 2001), and to results obtained in the tree shrew (Bosking et al., 2002), but differs from at least some reports for the cat (Das and Gilbert, 1995). The combination of large population responses to restricted stimuli and orderly representation of visual space has important consequences for considering how coverage is obtained in visual cortex (see below).

The cortical magnification factor for central vision (eccentricities of 0 - 10°) was significantly smaller in the bush baby (~0.8 mm/°) than that observed in diurnal primates such as the macaque monkey (~2.2 mm/°) and the squirrel monkey (~2.3 mm/°, Blasdel and Campbell, 2001), but is comparable to that found in other nocturnal primate and non-primate species such as the owl monkey (~0.9 mm/°, unpublished observations) and the cat (~0.9 mm/°, Tusa et al., 1978). This result is not surprising given the much smaller amount of retinal specialization for central vision found in the retina of the bush baby as compared to diurnal species such as the macaque (DeBruyn et al., 1980; Perry and Cowey, 1985; Wässle et al., 1990).

We found a small amount of overall anisotropy in the cortical magnification factors for the two axes of visual space with approximately 1.2 times as much cortical space devoted to representing 1° of visual space along an iso-azimuth line as compared to 1° of visual space along an iso-elevation line. This type of asymmetry in the mapping of visual space has also been found in macaque and squirrel monkeys, and in these species it has been suggested that this expansion of the map of the visual field on one axis is related to the layout of ocular dominance bands or domains (Tootell et al., 1988; Blasdel and Campbell, 2001). We did not
attempt to resolve whether this is the source of the magnification factor anisotropy in the bush baby.

*Spatial organization for orientation, ocular dominance, and CO*

An organized orientation preference map is a robust feature of bush baby V1, as it is in all carnivores (Bonhoeffer and Grinvald, 1991; Chapman et al., 1996; Shmuel and Grinvald, 2000; White et al., 2001) and primates (Blasdel, 1992; Malach et al., 1994; Xu et al., 2004a) that have been examined, as well as for some other mammalian species such as the tree shrew (Bosking et al., 1997). The map of orientation preference is organized according to similar principles in all of these species despite large differences in the overall functional organization observed in V1 of different mammals including differences in the size of V1, the cortical magnification factor, the number of other stimulus features represented in V1, and the presence or absence of ocular dominance domains and CO blobs. There are also differences in the actual sets of connections that are likely to provide the anatomical basis for orientation tuning in each species. For example, orientation selectivity first arises in layer 4 of the cat and bush baby (Hubel and Wiesel, 1962; DeBruyn et al., 1993), but is not found until more superficial layers in the macaque and tree shrew (Hubel and Wiesel, 1968; Blasdel and Fitzpatrick, 1984; Chisum and Fitzpatrick, 2004). Overall, these observations demonstrate striking similarities among species in the layout of the map of orientation preference in upper layers of V1, despite possible variation in the neural mechanisms that first instantiate orientation selectivity in the cortical network. These findings also suggest that cortical maps of orientation preference in V1 may be a natural consequence of activity dependent mechanisms of cortical development that are constrained by orientation bias elsewhere in the retino-geniculostriate pathway (Xu et al., 2002; Chisum and Fitzpatrick, 2004). It seems
unlikely that one specific genetic plan for generating an orientation preference map in V1 has
been conserved across all the mammals that exhibit these maps. Nevertheless, the universal
presence of orientation selectivity at the first telencephalic level in both birds and mammals
suggests that this feature is essential to higher order processing of visual features (see Medina
and Reiner, 2000).

We found no evidence for regional variation in the magnitude of orientation
selectivity in V1. Areas of poor selectivity and high rate of change in orientation preference
were largely confined to pinwheel centers. This result is consistent with observations from
the cat (Bonhoeffer and Grinvald, 1991; Shmuel and Grinvald, 2000), tree shrew (Bosking et
al. 1997), macaque (Blasdel and Salama, 1986; Blasdel, 1992), and owl monkey (Xu et al.,
2004a). However, a recent study has noted that when orientation selectivity is evaluated by
examining the height and width of tuning curves instead of using the magnitude of a vector
addition that larger areas of cortex are found that have a low height of the orientation tuning
curve (Swindale et al., 2003). We did not examine this issue in the bush baby, but it is clear
in the bush baby and in other species that orientation tuning width appears to remain at near
normal values within small distances of pinwheel centers and that the map of orientation
selectivity remains continuous across any areas of low tuning height that may exist
(Maldonado et al., 1997; Swindale et al., 2003).

We found a robust spatial organization for ocular dominance in the bush baby. In
primates, much more variation is seen in the strength and organization of the ocular
dominance map than is observed for orientation selectivity (Horton and Hocking, 1996). For
example owl monkeys do not have robust ocular dominance columns (Kaas et al., 1976;
O’Keefe et al., 1998; Blasdel and Campbell, 2001; unpublished optical imaging results from
our lab). Individual squirrel monkeys show strong variation in ocular dominance organization (Horton and Hocking, 1996). In marmosets, it has been possible to demonstrate ocular dominance columns only in animals that have had long periods of monocular suture or in neonates (DeBruyn and Casagrande, 1981; Spatz, 1989; Sengpiel et al., 1996). The organization of the ocular dominance patterns that we observed in the bush baby is more similar to the patchy pattern found in cats (Hübener et al., 1997, Löwel et al., 1998; Issa et al., 1999) than to the more stripe-like pattern found in macaque monkeys (Blasdel, 1992; Shtoyerman et al., 2000, Blasdel and Campbell, 2001), although the density of anatomically defined dominance columns in cats is lower (~2/mm², Shatz et al., 1977; Hubel and Wiesel, 1977) than found in bush babies (~4/mm², Glendenning et al., 1997). It is not clear why different primate species exhibit differences in the strength and pattern of ocular dominance organization (see also Adams and Horton, 2003).

Finally, in this report, as in other reports for the bush baby, we found a robust and highly regular pattern of staining for CO in V1 (Condo and Casagrande, 1990; Collins et al., 2001). With small differences in the shape and distribution of CO blobs (see Condo and Casagrande, 1990), the pattern of CO staining in the bush baby in V1 is similar to that observed in macaques (Horton and Hubel, 1981; Livingstone and Hubel, 1984) and squirrel monkeys (Malach et al., 1994).

Map relationships

Despite the robust and regular pattern of each individual columnar system in the bush baby, we found very little evidence for precise relationships between maps. The only robust relationship that we did find was a tendency for contours in the map of orientation preference to cross the borders of ocular dominance domains at right angles. This particular result is
similar to results obtained for quantification of intersections between contours in these two maps in the cat and macaque (Blasdel and Salama, 1986; Bartfeld and Grinvald, 1992; Obermayer and Blasdel 1993; Hübener et al., 1997; Kim et al., 1999). However, it is clear that the relationship between these two maps is not as constrained in the bush baby as it is in the macaque or cat, since when we examined the location of pinwheel centers relative to ocular dominance centers we found no systematic relationship.

Although we only examined the relationship between ocular dominance and CO domains using qualitative methods it is clear that the relationship between these columnar systems is also not the same as in the macaque. In the macaque CO blobs have been found to precisely align with the center of ocular dominance bands (Blasdel and Salama, 1986; Bartfeld and Grinvald, 1992). In the bush baby, we find instead that individual CO blobs can lie entirely within one ocular dominance domain or can be found directly spanning the border between left and right eye domains. This type of organization has also been observed in the squirrel monkey (Horton and Hocking, 1996). A lack of relationship between these two maps poses an interesting problem in the bush baby given that the direct LGN input to CO blobs is all monocular, coming from K cells related either to the left or right eye (Lachica et al., 1992; Lachica and Casagrande, 1992; Ding and Casagrande, 1997). A preliminary study in bush babies, nevertheless, showed that even though ocular dominance columns are clear, injection of tracer into one eye labeled every CO blob (Olivaria et al., 1997). This would suggest that all CO blobs receive K monocular inputs from both eyes in bush babies even though ocular dominance columns innervated by either P or M cells remain monocularly segregated (Florence and Casagrande, 1987; 1990). Presumably this is not the case for macaque monkeys since each blob appears to be dominated by the monocular input represented in the
ocular dominance column in which it lies (Horton and Hubel, 1981; Blasdel and Salama, 1986; Bartfeld and Grinvald, 1992).

Finally, we found no evidence for a relationship between compartments defined by CO in V1 and the map of orientation selectivity. Our first measure of this relationship was an assessment of the number of pinwheels in blob and interblob areas as compared to the percentage of V1 occupied by these two regions. As some have questioned the accuracy and precision of optical imaging for addressing this question (Polimeni et al., 2005), we also evaluated the magnitude of orientation selectivity in blob versus interblob regions. By this measure we also found no difference between blob and interblob regions. This result is in accord with previous physiology from the bush baby (DeBruyn et al., 1993). Whether orientation selectivity is lower in the blobs in the macaque has been somewhat controversial (Livingstone and Hubel, 1984; Ts'o and Gilbert, 1988; Lennie et al., 1990; Leventhal et al., 1995). What has been clear in both species, however, is that the orientation preference map is continuous across blob boundaries, and that no large areas of poor orientation tuning width exist in V1 (Blasdel and Salama, 1986; Bartfeld and Grinvald, 1992; Blasdel, 1992). Optical imaging has been used to demonstrate variation in the magnitude of orientation selectivity in V2 of owl monkeys and macaques (Ts'o et al., 1990; Roe and Ts'o, 1995; Xu et al., 2004a), so we have reason to believe that we should be able to see these regions in V1 if they exist. Thus, CO blobs do not serve to strictly segregate V1 into regions which either exhibit orientation selectivity or fail to exhibit orientation selectivity.

Compartments in V2

In the macaque monkey, squirrel monkey and owl monkey, relationships between columnar systems for orientation selectivity and CO staining are found in V2 (Livingstone
and Hubel, 1984; Blasdel and Salama, 1986; Ts'o et al., 1990; Bartfeld and Grinvald, 1992; Malach et al., 1994; Roe and Ts'o, 1995; Xu et al., 2004a). In the bush baby, we found no evidence for functional compartments in V2 as defined by either CO staining or regions of poor orientation selectivity. This suggests that compartments of this type are not a common feature of all primates, and that they are not required for generation of the basic response properties found in V2. This result, however, should not be construed as providing evidence that there are no functional subdivisions of any sort in bush baby V2. Stripe-like regional variation in the input and output connections of V2 is still observed in the bush baby, and it is likely that this reflects real differences in response properties across the layout of V2 (Boyd et al., 1997; Colins et al., 2001).

*Lack of map relationships*

A lack of relationships between columnar systems, or feature maps in V1, had already been shown for particular combinations of maps in the ferret (White et al., 2001), squirrel monkey (Horton and Hocking, 1996), and in the tree shrew (Bosking et al., 2002). Our results in the bush baby extend this observation to another primate, one that has particularly striking representation of each individual columnar system. Thus our results demonstrate that precise relationships between feature maps are not a common element of functional organization in all primates. Furthermore, it seems likely that such relationships are not required in order to obtain coverage of all feature combinations. It is important to note that complete coverage of all combinations of feature preferences is neither required nor ultimately even possible. Instead adequate coverage is most likely obtained by a combination of broad tuning of individual cells for each response property, orderly and continuous mapping of visual space and orientation preference, and periodic mapping of features such as
ocular dominance and blobs. The result of this combination of factors is that even a small stimulus of a particular orientation activates a large set of neurons above baseline, even if that stimulus is not exactly the preferred stimulus for any one neuron in the active population. Thus, the cortical point spread evoked by a minimal stimulus encompasses elements of multiple columnar systems within a local cortical region; provided that this is so, the constituent columns need not be in systematic relation with one another to ensure coverage. Our results for topographically restricted stimuli are in accord with just such a model for obtaining coverage, and are in accord with a previous attempt to understand coverage for the mapping of position and orientation preference in the tree shrew (Bosking et al., 2002).

If precise map relationships are not required for adequate coverage of the features that we studied, it is interesting to ask why such relationships have evolved in the cat and macaque. The first possibility is that when more stimulus features are represented in one area it becomes necessary to have such relationships (Swindale, 2000; Swindale et al., 2000). A second possibility is that such relationships reflect particular developmental constraints. Such constraints could include the cortical layer in which orientation selectivity is first obtained, the amount of lateral interaction during various stages of development, or different time courses for the development of particular connections related to each feature map. A third possibility is that precisely aligned feature maps are not required for basic coverage of these features, but do enable a richer set of computations on the same set of features for each point in the map of visual space.

Hubel and Wiesel were the first to address the concept of coverage by asking what set of circuitry is required for processing of all types of visual information for one point in the visual field, a unit they referred to as a hypercolumn (Hubel and Wiesel, 1977). Research
over the last two decades has progressively refined our understanding of the structure of a hypercolumn in the cat, macaque, and other species. While at first glance it may seem that our results from the bush baby are quite different from those obtained in the cat and macaque, several striking similarities should be recognized. In each species, approximately one square millimeter of V1 appears sufficient for encoding all attributes of a visual stimulus. Within this region, multiple stimulus attributes may be organized in a columnar fashion. And finally, no discrete borders exist between hypercolumns, or between the functional domains within each hypercolumn (Bartfeld and Grinvald, 1992; Landisman and Ts’o, 2002). Instead, each feature is mapped in a continuous fashion across the cortical surface. All mammals appear to share this basic plan, and the remaining challenge is to understand why in some species more specific relationships have evolved among the columnar systems.
References

**Adams DL and Horton JC.** Capricious expression of cortical columns in the primate brain.  

**Bartfeld E and Grinvald A.** Relationships between orientation-preference pinwheels, cytochrome oxidase blobs, and ocular-dominance columns in primate striate cortex.  


Crair MC, Ruthazer ES, Gillespie DC and Stryker MP. Ocular dominance peaks at 
pinwheel center singularities of the orientation map in cat visual cortex. *J Neurophysiol* 
77: 3381-3385, 1997b.

Daniel PM and Whitteridge D. The representation of the visual field on the cerebral cortex 

Das A and Gilbert CD. Long-range horizontal connections and their role in cortical 
reorganization revealed by optical recording of cat primary visual cortex. *Nature* 375: 

Debruyn EJ, Wise VL and Casagrande VA. The size and topographic arrangement of 

DeBruyn EJ and Casagrande VA. Demonstration of ocular dominance columns in a New 

DeBruyn EJ, Casagrande VA, Beck PD and Bonds AB. Visual resolution and sensitivity of 
single cells in the primary visual cortex (V1) of a nocturnal primate (bush baby): 
correlations with cortical layers and cytochrome oxidase patterns. *J Neurophysiol* 69: 
3-18, 1993.


**Horton JC and Hocking DR.** Anatomical demonstration of ocular dominance columns in striate cortex of the squirrel monkey. *J Neurosci* 16: 5510-5522, 1996.


Figure legends

**Figure 1. Activation maps produced by topographically limited stimuli (Case 1)**

A shows a surface reference image. B-E are a series of activation patterns evoked by topographically limited vertical gratings [2° x full screen height (54°)] at different locations parallel to the vertical meridian (VM): VM 0°, VM +1°, VM+2°, VM+4° (+, to the right of VM in the contralateral hemifield). F-J are a series of activation patterns evoked by topographically limited horizontal gratings [full screen width (74°) x 2°] parallel to the horizontal meridian (HM): HM+2°, HM+1°, HM0°, HM-1°, HM-2° (+, above HM; -, below HM). By mapping both vertical and horizontal dimensions of the visual field, we were able to construct a visual field map. The small insets in lower left corners (B-J) indicate the mapping stimulus positions relative to the display monitor. The arrow in E points to the band of activation in V2. K is a visual field map of V1 constructed based upon the series of activation patterns shown in B-J, superimposed on a cortical surface image from the imaged region. For the visual field map, H -2° and H +2° are 2 degrees below and above the horizontal meridian (HM) representation, respectively; V +2° and V +4° are 2 and 4 degrees eccentric from the vertical meridian (VM) representation, respectively. A, anterior, M, medial.

**Figure 2. Activation maps produced by topographically limited stimuli (Case 2)**

A-C are a series of activation patterns resulting from stimulation with topographically limited vertical gratings (2° x 54°) at VM +3°, VM +4°, VM +6°. D-F are a series of activation patterns resulting from stimulation with topographically limited horizontal gratings (74° x 2°).
at HM 0°, HM-2°, HM-6°. G is an activation map produced following stimulation with 2°
circular patch located at (x: +4°, y: -1° relative to area centralis ). H shows the same
activation map as shown in G with the visual field map (derived from the activation maps in
A-F) superimposed. The black dashed lines are iso-azimuth lines derived from activation
patterns using the vertical grating windows at 2°, 4° and 6° from vertical meridian; the white
dashed lines are iso-elevation lines derived from activation patterns using the horizontal
grating windows at +2°, 0°, -2° and -4° from horizontal meridian. The white star indicates the
center of activation produced by a 2° circular patch located at (x: +4°, y: -1° relative to area
centralis). The insets in either lower left or right corners (A-G) indicate the mapping
stimulus positions on the display monitor. Other conventions are as in Figure 1. The images
in G-H have been contrast-enhanced to better show the activation pattern. A, anterior, M,
medial.

Figure 3. Cortical magnification factors (CMF) measured by optical imaging and
microelectrode recordings

A. The CMF measured using optical imaging at different eccentricities matches the CMF
versus eccentricity (E) function (dashed line) from Rosa et al. (1997): CMF = 2.36 · ( E +
0.73) -0.8. CMF was calculated three ways: 1) using the iso-azimuth lines determined from
presentation of narrow vertical stimuli at different azimuths (solid black diamonds), 2) using
the iso-elevation lines determined from presentation of narrow horizontal stimuli at different
elevations (solid black circles) and 3) using diagonal lines taken between the intersection
points made by the iso-elevation and iso-azimuth lines (open triangles) (also see the
Methods). B. Comparison of our data with data obtained with microelectrode mapping.
Dotted line shows CMF results obtained from microelectrode mapping in macaque monkeys from Van Essen et al. (1984); CMF = 10 · (E + 0.82)−1.14 compared with results obtained in bush babies using microelectrode mapping (dashed line) from Rosa et al. (1997).

**Figure 4. Spatial arrangement of orientation preference in V1.**

A shows difference images obtained for four stimulus orientations (0°, 45°, 90° and 135° shown in the inset of each panel) from V1 of one animal. Dark areas of each image indicate areas of cortex that were strongly activated by the indicated stimulus, white or light gray areas indicate regions of cortex that were strongly activated by the orthogonal stimulus. B is a polar map of orientation preference obtained by vector summation of the four images in A. The angle of orientation preference is color-coded according to the key shown below the panel. The magnitude of orientation selectivity is coded by the lightness of the image; areas with strong orientation selectivity appear as bright regions, those with weak orientation selectivity are dark. C is an angle map for a portion of the region shown in A and B. In this map, magnitude of selectivity information has been discarded and color is used to code for orientation preference angle according to the key beneath the panel. D is a rate of change map for the same region of cortex. Areas with high rate of change of orientation preference are shown in white, those with low rate of change are shown in black. E shows location of pinwheel centers determined by thresholding the rate of change map shown in D. Blue squares indicate pinwheel centers with clockwise progression of orientation preference, red squares indicate pinwheel centers with counter-clockwise progression of orientation preference.
Figure 5. Orientation preference maps in V1 and V2.

A-D are from one case; E-H are from a different case. A and B are orientation difference images of 0°/90° and 45°/135°, respectively. C and D show a color-coded orientation preference map and a magnitude map, respectively. The scale in B is for A-D. The red or white lines in A-D indicate the V1/V2 border based upon visuotopic mapping done in the same case. E-G show an orientation difference image of 0°/90°, a polar map and an orientation rate of change map, respectively. Orientation preference for C and F is color coded as indicated by the left and right color keys at the bottom of the figure, respectively. For the magnitude map (D) and the polar map (F), areas of strong orientation selectivity are bright while those showing poor orientation selectivity are dark. In G, areas of low rates of change in orientation selectivity are shown in black, those with high rates of change are shown in white. H shows a CO stained section from the imaged region in the case shown in E-G. The section has been aligned to the optical imaging data. The V1/V2 border determined based on CO staining is shown in red in E and G, and in white in F. The scale in H is for E-H. Other conventions are as in Figure 4. A, anterior, L, lateral.

Figure 6. Orientation preference maps in owl monkey V1 and V2.

A and B are two orientation difference images (45°/135° and 90°/0°) showing the organization of orientation in owl monkey V1 and V2. C is a magnitude map from the same region as shown in A and B. Unlike bush baby, there are stripes of high and low orientation selectivity in owl monkeys (compare to Figure 5; also see Xu et al. 2004a). The thin white lines indicate the V1/V2 border. D is a CO stained section showing V1 and V2 from a different owl
monkey case. Other conventions are as in Figure 4. The scale in C is for A-C; and the scale in D is for D only.

**Figure 7. The ocular dominance map in V1.**

A and B are from one case; C and D are from a second case. A shows the pattern of ocular dominance seen in a large region of the dorsal part of V1 in one animal. The V1/V2 border is just beyond the right hand side of the image. Dark areas of the image indicate areas that responded most strongly to the left eye, light areas indicate areas that responded more strongly to the right eye. B shows a band-pass filtered pattern of ocular dominance from the white square region of A; in B ocular preference is indicated in 16 shades of gray with black indicating strong preference for the left eye and white indicating strong preference for the right eye. White and black dots indicate the geometric centers of ocular dominance domains (see the Methods for details). Similarly, C is an ocular dominance map; and D is the same ocular dominance map as C, but illustrated in 16 shades of gray, with white and black dots indicating centers of the right and left ocular dominance domains, respectively. The white dashed lines in C and D indicate the V1/V2 border based upon visuotopic mapping. L, lateral; A, anterior. Scale bars = 1mm.

**Figure 8. Relationship between iso-orientation contours and ocular dominance contours**

A shows iso-orientation contours from an orientation preference map superimposed over an ocular dominance map from the same V1 region. The ocular dominance map is displayed as
contours of 8 gray levels, with black indicating strong preference for the left eye and white indicating strong preference for the right eye. Each iso-orientation contour is color coded using the scheme under A. Many iso-orientation contours appear to intersect with ocular dominance contours at roughly right angles. B shows a frequency histogram of the intersection angles between iso-orientation contours and ocular dominance contours from two cases. Four intervals are plotted (0°-23°, 24°-45°, 46°-67°, 68°-90°), with each bar representing mean percentage ± standard error (SE).

**Figure 9. The nearest neighbor analysis for orientation pinwheel centers and ocular dominance centers**

A-C show the nearest neighbor analysis for pinwheel centers and ocular dominance centers in two different cases. Specifically, A shows the cumulative distribution for the distance between neighboring pinwheel centers. The dashed line and gray shaded region show the mean and 95% confidence intervals for this distribution when pinwheels are arranged randomly. The thick black line indicates the actual distribution found in real data. B shows the cumulative distribution for the distance between neighboring ocular dominance centers. The dashed line and gray shaded region show the mean and 95% confidence intervals when this distribution is calculated for randomly arranged ocular dominance centers. The thick black line shows the actual distribution found in real data. C shows the cumulative distribution for the distance between pinwheel centers and the nearest ocular dominance center. The dashed line and gray shaded region show the mean and 95% confidence intervals when this distribution is calculated using randomly placed pinwheels. The thick black line
shows the distribution when the real pinwheel and ocular dominance centers are used. See text for details.

**Figure 10. Relationship between cytochrome oxidase (CO) blobs and functional maps**

*Figure 10.* illustrates the procedure used to align the histological data to the optical imaging data. The grayscale image in the background is the reference image taken during optical imaging. Shown in red is a drawing of the tangential and radial blood vessel outlines found in the first section that has been aligned to the reference image using global changes in scaling, rotation, and translation. Shown in blue is a drawing of the radial vessel profiles from the tangential section shown in *B* that has been aligned to the reference image and drawing of the first section. *B* is a scanned image of a cytochrome oxidase stained section that has been aligned to the reference image using the same transforms used to align the radial vessel profiles shown in *A*. The radial vessels used for alignment are visible in this image and the “blobs” have been outlined in red. *C* is a color-coded orientation preference map with outlines of blobs transferred from *B* (shown in black). *D* is a magnitude map with outlines of blobs transferred from *B* (shown in red). Areas of cortex with strong selectivity are shown in white, those with poor selectivity are shown in black. *E* is the ocular dominance map for the same region with outlines of blobs transferred from *B* (shown in red). Regions that were dominated by the left eye are shown in black and regions that were dominated by the right eye are shown in white. Other conventions are as in Figure 4.