The Organization of Orientation Selectivity Throughout Macaque Visual Cortex

A double-label deoxyglucose technique was used to study orientation columns throughout visual cortex in awake behaving macaques. Four macaques were trained to fixate while contrast-reversing, stationary gratings or one-dimensional noise of a single orientation or an orthogonal orientation were presented, during uptake of $[^{14}\text{C}]$deoxyglucose ($[^{14}\text{C}]$DG) or $[^{3}\text{H}]$DG, respectively. The two orthogonal stimulus orientations produced DG-labeled columns that were maximally separated in the two isotope maps (interdigitated) in four areas: V1, V2, V3 and VP. The topographic change from interdigitated to overlapping columns occurred abruptly rather than gradually, at corresponding cortical area borders (e.g. VP and V4, respectively). In addition, the data suggest that orientation column topography systematically changes with retinotopic eccentricity. In V1, the orientation columns systematically avoided the cytochrome oxidase blobs in the parafoveal representation, but converged closer to the blobs in the foveal representation. A control experiment indicated that this was unlikely to reflect eccentricity-dependent changes in cortical spatial frequency sensitivity. A similar eccentricity-dependent change in the topography of orientation columns occurred in V2. In parafoveal but not foveal visual field representations of V2, the orientation columns were centered on the thick cytochrome oxidase stripes, extended into the adjacent interstripe region, but were virtually absent in the thin stripes.

**Introduction**

Orientation selectivity in single cortical neurons, and its anatomical segregation into orientation columns, has been a topic of great interest in visual cortex of primates (Hubel and Wiesel, 1968, 1974; Poggio et al., 1975; Schiller et al., 1976; Bartfeld and Grinvald, 1992; Blasdel, 1992). Recent experiments furnished invaluable information on the circuitry underlying this emergent cortical property (Sillito et al., 1980; Chapman et al., 1991; Reid and Alonso, 1996; Chung and Ferster, 1998), and the topographic arrangement of orientation columns relative to maps of other variables in V1 and V2 (Livingstone and Hubel, 1984; Lennie et al., 1990; Ts'o et al., 1990; Leventhal et al., 1995). However, visual cortex encompasses ~60% of the cortical surface area in macaques, and there has not yet been a systematic mapping study of orientation throughout the additional ~30 visual areas in the cortex. Here we used the double-label deoxyglucose (DG) technique to address such remaining issues.

For instance, it is widely recognized that orientation-selective neurons exist in many visual areas – but in which of these areas (beyond V1 and V2) are such neurons anatomically grouped into orientation columns? Even in V1, where orientation columns have been most well studied, questions remain. For instance, there have been claims (Livingstone and Hubel, 1984) and counterclaims (Lennie et al., 1990; Leventhal et al., 1995) that orientation selectivity is reduced or absent in neurons in the cytochrome oxidase blobs of V1. The orientation maps shown here may resolve some of that discrepant literature by revealing that both the orientation selectivity, and its relationship to the blobs, is eccentricity-dependent.

Significant technical issues also arise with regard to orientation maps. Essentially, all previous orientation maps, and many previous single unit experiments on orientation, have been done using anesthetized animals [but see e.g. (Bauer et al., 1983; Vogels and Orban, 1991; Snodderly and Gur, 1995; Vnek et al., 1999)]. For technical reasons, the oriented stimuli in such studies have almost always been presented while moving. Thus, such experiments unavoidably confound manipulations of orientation with manipulations of the axis-of-motion, and introduce additional complications such as the motion-aperture problem. Here, the awake monkeys fixated on stationary gratings, so the interpretation of the results was more straightforward.

We used the DG technique to label the columns, because DG yields maps of high spatial resolution and high contrast, from each layer and area, unconfounded by vascular artifact, throughout the brain. The additional advantage conferred by having two activity labels in the same animal made it possible to distinguish true orientation columns (corresponding to interdigitated columns in the two isotope maps), from columns of high activity irrespective of the stimulus orientation (corresponding to overlapping columns in the two isotope maps). We are not aware of any other technique which could have accomplished these goals.

**Materials and Methods**

**Surgery and Training**

Procedures in this experiment were similar to those described previously (Geesaman et al., 1997; Vanduffel et al., 2000). Four male rhesus monkeys (Macaca mulatta) were restrained in a primate chair. The head of each monkey was fixed in place with a stainless steel device. Eye positions were measured using the scleral eye-coil technique.

All operations were performed under ketamine anesthesia (10 mg/kg Ketalar® i.m., Parke-Davis, Brussels), supplemented with xylazine (0.5 mg/kg Rompun®, Bayer, Brussels). During the eye-coil surgery, anesthetics (0.2 mg Unicaine®, Bournonville Pharma, Braine-l’Alleud), antibiotics (150 mg Lincomycin®, Upjohn, Puurs), corticosteroids (2 mg Celestone®, Schering-Plough, Brussels), and a vasoconstrictor (5 µg Levorinine®, Sterop, Brussels) were locally administered. Antibiotics (50 mg/kg i.m. Kefzol®, Lilly, Brussels) were given daily during the week following each surgery. The fourth monkey received analeptics for 3 days after the implantation of the catheter (5 mg/kg i.m. Tramadol, Dolzam®, Zambon, Brussels). The surgical procedures conformed to the American and European Guidelines for the care and use of laboratory animals.

The monkeys were water-deprived during the period of testing, and behavioral control was achieved using operant conditioning. The monkeys were rewarded with drops of apple juice for maintaining fixation within a square-shaped central fixation window (1-2 on a side for training, 3 during the DG experiment). Multiple rewards within a given trial of maximally 6 s were given at gradually decreased intervals to encourage longer fixation by the monkey. The goal of this fixation paradigm was to have the monkeys viewing the CRT screen as consistently as possible during the period of DG uptake. The health status of the monkeys was closely monitored throughout the deprivation period.
Visual Stimuli
The monkeys were trained in a room, in which the only source of illumination was the CRT screen. When the monkeys were fixating the central point (the rewarded task), oriented stimuli (e.g. gratings) were presented on the remainder of the CRT screen. Whenever the monkey's gaze was directed outside the fixation window, the CRT screen was immediately blackened, to minimize artificial DG uptake in response to uncontrolled room stimuli. One hundred milliseconds after any eye movement excursus outside the central fixation window, the fixation point (but not the associated stimuli) reappeared until the monkey began fixating again. Fifty milliseconds after the monkey began fixating again, the visual stimuli also reappeared.

In three animals, the stimuli were black/white (contrast ~97%, mean luminance of 4 cd/m²) square wave (1 cycle/deg.) gratings presented on a Brilliance 21A Philips monitor (70 Hz refresh rate), positioned 44 cm from the monkey's eyes. The stimuli subtended the full extent of the rectangular screen (50° × 36°). The stimulus phase was selected at random and reversed at a temporal rate which was continuously varied between 1 and 8 Hz.

In the remaining four animals which had been used in other experiments (Schoups et al., 2001), three stimuli were used. The portion of these stimuli relevant to the present study was oriented, one-dimensional (1-D) noise (size = 5.3° × 12.8°, mean luminance = 5.6 cd/m², contrast = 96%), designed to have almost equal power across a broad region of its 1-D spectrum (ranging from 2 to 12 cycles/deg.). A stimulus was selected from a pool of 40 1-D noise stimuli. As in the first three monkeys, the phase of the stimuli was reversed in consecutive stimulus presentations. In addition, we showed the same stimuli at two and four times the original spatial frequency. After this sequence of six stimuli was presented, we randomly selected another 1-D noise pattern. Thus we presented 240 different 1-D noise stimuli for a particular orientation. In the monkeys viewing the horizontal, the two orthogonal oblique orientations, 45° to vertical and 45° to horizontal, were presented. In the third and the fourth monkeys, the orientations were horizontal versus vertical. In the monkeys viewing the same grating orientations, stimuli were presented in opposite order.

Double-label DG Experiment
Approximately 1 week before the DG experiment, after the monkeys had mastered the fixation paradigm, a bilumen catheter (Deltacath, Becton Dickinson, 7 French × 15) was implanted in the external jugular vein, with its distal ends fixed to the headholder, under surgical anesthesia (see above). Later, secondary catheters were connected to the bilumen catheter so as to make injections from outside the testing room.

During the final DG session, the monkeys were given 5–10 min to reach stable performance levels on the fixation task, using the first stimulus. Then, the [3H]DG (American Radiolabelled Chemicals, St Louis, MO; 13–16.0 µCi/kg body wt; 40 Ci/mmol sp. act.) was slowly (1–2 min) injected through one lumen of the indwelling catheter. It was then flushed with 2–3 ml of heparinized saline (500 units heparin/ml) to ensure that all of the isotope was injected. During the ensuing 45 min period, ~95% of the [3H]DG was taken up from the bloodstream (Sokoloff et al., 1977), while the grating was presented at the first orientation. Approximately 35 min after the injection of the [3H]DG, 15 000–25 000 units of heparin sodium were slowly injected, in preparation for the cardiac perfusion later.

Forty-five minutes after the [3H]DG injection, while the monkey continued to fixate, the stimulus orientation was changed to the second (orthogonal) orientation. After 1–2 min of adaptation to the new orientation, a bolus injection of [14C]DG (ARC; 17–20 µCi/kg body wt; 55 mCi/mmol sp. act.) was given through the second lumen of the catheter. Ten minutes later, an overdose of sodium pentobarbital (100 mg/kg) was injected, also as an intravascular bolus. This 'early' sacrifice abbreviated the second period of DG uptake, thus preventing recirculation and/or loss of the first isotope. After physical examination (absence of heart beat and muscle tonus, pupil dilatation) it was confirmed that the barbiturate overdose was effective, the monkey was removed from the restraining chair and perfused transcardially (for 1–2 min) with: (i) phosphate-buffered saline including 18% sucrose; and (ii) the above solution with 1% paraformaldehyde and 1.5% glutaraldehyde added. The perfusion removed unbound 14C isotope from the bloodstream, added a cryoprotectant, and lightly fixed the tissue. The brain was quickly removed and dissected. Brain regions of interest were flattened and frozen as described elsewhere (Vanduffel et al., 2000), and stored at −80°C for later sectioning.

Histology and Autoradiography
The tissue was cut in a cryostat, in a plane parallel to the flattened surface, using a slice thickness of 40 µm as described by Vanduffel et al. (Vanduffel et al., 2000).

Sections were exposed twice, against two sets of film. One film was optimized for 3H-sensitivity ([3H]Hyperfilm, Amersham, exposure time ~1 month). With the 800:1 ratio of isotope concentrations used here, the number of 3H decays far outweighed the number of 14C decays. This helped to produce an image due almost entirely to the 3H on the Hyperfilm.

Next, the sections were exposed for 3–4 months against Polaroid 891 film. This film consists of three adjacent emulsion layers, with a different color dye-coupling in each layer. The beta particles emitted by the decaying tritium are of low energy (19 keV) and can only energize the silver halide in the outermost (blue dye-coupled) emulsion layer of this film. The beta particles emitted by the 14C are of much higher energy (156 keV), so they can energize the emulsion in all three emulsion layers. Thus the 3H produces a pure blue image, and the 14C produces a pinkish-white image. By imaging the resultant sections through color filters passing long wavelengths (Kodak Wratten no. 12, a yellow filter), the image components due to the 3H decay (blue) were removed.

During this experiment the Polaroid 891 films became commercially unavailable. Therefore, we exposed the sections of the fourth monkey for a second time on 3H-Hyperfilms in order to detect the 14C signals. During this second long exposure duration (172 days), a thin Mylar sheet (19 µm) was positioned between the sections and the film (Friedman et al., 1989). This Mylar sheet blocks all beta particles emitted by tritium. The 14C and 3H signals (from two different sets of Hyperfilms) were digitized as described for the Polaroid films, except for the use of a color filter.

The combination of all these procedures yielded two activity-based images which were estimated to be ~95% independent of each other (R.B.H. Toottell, unpublished observations). In the present experiment, we determined the actual cross-contamination of the 14C signal on the 3H-sensitive Hyperfilms. To this end, we measured the [14C]DG uptake in 20 3H-labeled orientation columns of area V2. We chose V2, since column spacing is larger in V2 than in area V1 (see below). The 14C concentration was measured on the 3H-sensitive layer of the Polaroid film, or the Hyperfilm in combination with the Mylar sheet) at these spatial locations corresponding to the width at half maximum of the 3H-labeled orientation column. This concentration was compared to [14C]DG uptake in the two region of the same size flanking both sides of the former region. This comparison revealed a cross-contamination of 4.07 ± 1.06% of 14C in the 3H signal. Using similar procedures, no cross-contamination of the 3H signal was measured in 20 14C-labeled columns. Thus the Mylar sheet and the upper layers of the Polaroid films blocked all beta particles emitted by the 3H (see also Friedman et al. (Friedman et al., 1989)).

After autoradiographic exposure, selected tissue sections were stained for cytochrome oxidase activity (Silverman and Tootell, 1987).

Imaging and Analysis
The autoradiographs corresponding to the 3H-dominated images (Hyperfilm) were digitized using a computerized imaging system (Analysis Imaging Concepts, or MCID Imaging, Inc.). This image was corrected by image subtraction of the background. The resultant image was shown at half-contrast while the corresponding 14C image was carefully aligned on-line and then digitized (Geesaman et al., 1997; Vanduffel et al., 2000). The alignment of the two functional images from one section was done very carefully using a micromanipulator which allowed fine translation and rotation adjustments. After digitizing the 3H image of one section (with a resolution of 520 × 480 pixels, or 650 × 512 pixels), this digitized image was shown at inverted contrast and semi-transparently while the corresponding 14C image of the same section was
also presented at half-transparency. This latter ('live') image was aligned on-line with the digitized $^3$H-image using features present in the isotope patterns, tissue border and tissue artifacts (Vanduffel et al., 2000).

To increase the signal-to-noise ratio, we aligned and averaged signals from two to five consecutive sections. This procedure is illustrated in Figure 1. In Figure 1A and B, we show $^3$H signals from a portion of two consecutive sections through the supragranular layers of V1. When the contrast of one of the images is reversed and when these images of opposite contrast are overlayed and aligned, the resulting image is homogeneously gray (as shown in Fig. 1C). Displacements can easily be detected when one of the two images (the 'live' image) is moving during the realignment procedure relative to the digitized image. In this example, we artificially translated one of the two images by 52 up to 208 $\mu$m (Fig. 1D–F) and digitized the 'imperfectly' realigned signals.

**Figure 1.** Alignment procedure for DG images in consecutive sections. (A) and (B) normalized $[^3]$H DG signals (see Materials and methods) of two consecutive sections through layers 2 and 3 of V1 (subject M3, left hemisphere). (C) signals of (A) superimposed onto the inverted image of (B), after equalizing the mean gray value of the two $[^3]$H DG images to 128 (in a total range of 256 gray values). (D–F) Image of (A) superimposed on the inverted image of (B), translated over 52 $\mu$m (D), 104 $\mu$m (E) or 208 $\mu$m (F). Notice the increasing heterogeneity from (D) to (F) until the columns of the two consecutive sections were almost 'interdigitating'. Small movements of the second 'live' image during the realignment procedure (which cannot be illustrated in a figure) considerably enhanced the detection of misalignment compared to the static images shown in (D–F).
gradual larger displacement of one of the two images yields a gradually more heterogeneous signal. Spatial displacements larger than 15 µm between the two functional images of the same section were detectable when using the relative motion cues induced by slightly moving the live image, and could be corrected.

To normalize the signals from the two isotopes, we first applied a Gaussian filter (radius 10 µm) to the [3H] images. This compensated for the differences in photographic grain between the [3H] and [14C] images. Using calibrated [3H] and [14C] standards (Amersham), we normalized histograms of the digitized gray values reflecting the optical density, for each film type. In autoradiographic images of the gray matter, the histograms of the gray values from the two isotopes were shifted relative to each other, but had similar gain. The constant shift between both sigmoid shapes was added to the gray value of each pixel of the [3H] images to obtain a normalized [3H] gray value. This normalization procedure (which was based on standards) did not assume equal [3H] and [14C] signals in the autoradiographs. Although laminar modulations of the columnar dimension were also analyzed and described. For convenience, we describe such DG-labeled variations simply as columns. To define the column amplitude, width and spacing, we plotted the gray-level distributions through 20 columns in each area of each subject. This yielded nearly Gaussian distributions of gray-levels with maximal intensities at the middle of a column and minimal intensities approximately halfway between two consecutive columns. Column amplitude was defined as the difference in gray levels between peak DG uptake within a column and the lowest DG uptake in the region between the orientation columns. Column width was measured at half height, based on the gray levels. Column ‘spacing’ is defined as the mean distance between the peak of a given column relative to the peak of the nearest neighboring column. Laminar borders were based on cytochrome oxidase staining patterns. Eccentricity measurements were based upon Tootell et al. (Tootell et al., 1988c) and unpublished retinotopic double-label DG data [similar data as in Vanduffel et al. (Vanduffel et al., 2000); Figure 3A].

Results

Behavior

The percentage of time that each monkey spent fixating was monitored continuously. During the four DG experiments reported here, the monkey’s gaze was within the central fixation window either 85.3, 88.2, 91.8 or 85.0% of the time (average values, for each of the four experiments, respectively). There were no significant differences in the average time the monkey spent fixating between the two isotope uptake periods.

Striate Cortex (Area V1), Overall

In the autoradiographs from V1, the orientation ‘columns’ actually showed considerable laminar variation (see Fig. 2). Columns were faintly present in layer 1, and they gradually increased in contrast from there to the bottom of layer 3. Below layer 3, the contrast of the DG patterns changed abruptly at each laminar border.

The most obvious orientation columns were located in layer 4α, which receives the bulk of the input from the magnocellular lateral geniculate nucleus (LGN) layers. In 4β, which receives the bulk of the input from parvocellular LGN layers, orientation columns were faint or absent, even though overall DG uptake in that layer was relatively high (see Fig. 2). This is consistent with previous reports from anesthetized, paralyzed animals (Blasdel and Fitzpatrick, 1984; Livingstone and Hubel, 1984), and it supports the general idea of a magnocellular stream bias in orientation processing.

Labeling in layers 5 and 6 was quite light. The light labeling in layer 6 was quite different from that reported earlier from anesthetized paralyzed macaques, using moving stimuli (Hubel et al., 1977; Livingstone and Hubel, 1984). In those studies, the DG pattern in layer 6 was very similar to that in 4Cα (i.e. high uptake, high contrast).

Variations with Retinotopic Eccentricity, V1

Under our experimental conditions, we observed several interrelated, graded changes in the orientation columns, with corresponding changes in the cortical representation of visual field eccentricity. Towards more foveal visual field representation (i) the contrast of the DG columns decreased, (ii) the DG columns labeled by the two isotopes become progressively more coincident, and (iii) the DG columns coincided increasingly with the cytochrome oxidase blobs in layers 2 and 3. Thus, towards the fovea, the DG ‘orientation columns’ were actually less orientation-specific than they were at more peripheral representations. These differences with eccentricity may help to explain discrepancies in prior reports (see Discussion).

Typical eccentricity-related variations in the raw autoradiographic contrast (single label) are shown in Figure 3A. Clear columns are present in the parafovea (e.g. 2–6 eccentricity, rightmost in the figure), but the column contrast decreased towards the foveal representation (leftmost). Such gradients were seen in both sets of film, in all monkeys tested. Quantitatively, the average amplitude of the DG uptake in the orientation columns decreased by 41 ± 7%, when measurements were compared from −6° relative to −0° eccentricity (for the first three monkeys).
The increased convergence of the two sets of orientation-related columns can be seen in the pseudocolor rendering of Figure 3B. One orientation map is shown in red, and the other in green. Towards the right of the figure (parafoveal representations), the two orientation maps clearly interdigitated in alternating stripes of red/green pseudocolor. Towards the left of the figure (foveal representation), the increased convergence of the regions of high DG uptake appeared as alternating stripes of lighter and darker yellow (since overlapping red plus green equals yellow).

The increasing overlap between orthogonal orientation columns at smaller eccentricities is documented quantitatively in the plots of Figure 3C. These $^{13}$C: (red) and normalized $[^{3}H]DG$ (green) concentrations were measured along a line (100 µm width) from foveal towards more peripheral visual field representations (in the same sections as those shown in Fig. 3B). Note that in this graph, the amplitude of DG uptake in orientation columns in the foveal visual field representation was reduced by 35% (median for the two labels) compared to more peripheral visual field representations.

Figure 3D,E shows magnified views of the two DG maps at foveal and parafoveal eccentricities (respectively), with the topography of the cytochrome oxidase blobs added (after applying a difference-of-Gaussian filter with an excitatory kernel size of 400 µm). In the parafoveal sample (Fig. 3E), the maps of orthogonal orientations alternated systematically in red and green as in Fig. 3B. The cytochrome oxidase blobs (shown in blue) systematically avoided the maxima of DG uptake. Thus there was very little overlap between the red, green and blue.

However towards the fovea, the DG 'orientation' columns converged not only with each other, but also on the blobs (see Fig. 3D). The increased overlap of red, green and blue (i.e. the pseudocolor codes for two orientation maps and the cytochrome oxidase blobs, respectively) produced the whitish patches in Figure 3D. Few such white regions were found parfoveally (see Fig. 3E).

This was also borne out quantitatively. First, the DG and the cytochrome oxidase maps were thresholded to obtain a 25/75% ratio for blobs/interblobs, as suggested by Farias et al. (Farias et al., 1997) and a 50/50% ratio for the two orientation maps, in both foveal and parafoveal samples. Then, we measured the cortical surface of the DG columns which covered either the blobs or the interblob region. The overlap between the DG columns and the blobs was 50% (of the total orientation column surface) in the foveal sample, compared with 16% in the parafoveal sample.

The increased overlap between columns labeled by orthogonal grating orientations towards the fovea is further illustrated for three monkeys in Figure 4 (for the supragranular layers of V1). In this figure, amplitude-normalized $^{13}$C: and $[^{3}H]DG$ concentrations are plotted for foveal (<1.5° eccentricity) and more peripheral (>3° eccentricity) visual field representations. For the three monkeys, peaks of higher $^{13}$C: and $[^{3}H]DG$ uptake (corresponding to DG uptake within the orientation columns) were largely overlapping at foveal visual field representations (see Fig. 4A–C). On the other hand, DG uptake was almost completely complementary to $[^{14}C]DG$ uptake at more peripheral visual field representations: high $[^{3}H]DG$ uptake (columnar activity) occurred at locations with low $[^{14}C]DG$ uptake (inter-columnar activity), and vice versa.

We also measured the width and spacing between the orientation columns in striate cortex (see Methods). At ~6 eccentricity, the orientation columns had a mean width of 297 µm (SD 51 µm), and a mean column spacing of 760 µm (SD 90 µm).

**V1, Spatial Frequency Control**

The apparent foveal decrease in orientation specificity could be interpreted in at least two ways. Most simply, it supports previous evidence suggesting that orientation-selective cells are systematically underrepresented in the foveal representation in V1, but more prevalent parfoveally, as suggested by single cell results (Poggio et al., 1975; Zeki, 1983). Alternatively, this DG result could reflect the change in spatial frequency sensitivity with eccentricity, relative to the constant spatial frequency spectrum of the stimulus across the retina.

It is known that cells in the interblobs respond to a higher range of spatial frequencies, compared to cells in the blobs (Tootell et al., 1988; Born and Tootell, 1991). Furthermore, the range of spatial frequencies to which we are sensitive changes profoundly with eccentricity (Koenderink et al., 1978). Although the spatial frequency spectrum of square wave gratings is quite broad it could nevertheless be argued that the spatial frequency spectrum of the stimulus was effectively a ‘high’ spatial frequency at eccentricities of 2 and more peripherally, thus producing maximal DG uptake between the blobs. Nearer to the cortical representation of the fovea, it could be argued that the same grating would be effectively a ‘lower’ spatial frequency, thus producing more activity in the blobs. The spatial-frequency model would also produce less orientation-selective signals in the fovea, if the blobs are indeed nonselective for orientation (Livingstone and Hubel, 1984).

To distinguish between these two interpretations, we did an additional experiment using oriented, 1-D noise instead of square wave gratings. The spatial frequency spectrum of these 1-D noise stimuli was approximately flat across the range of 2–12 cycles/deg. (see Fig. 5B). This range is well within the acuity limits at 0 eccentricity (~43 cycles/deg.) and 5 eccentricity (~20 cycles/deg.) as reported for macaque monkeys (Merigan et al., 1993).

This stimulus was less effective than the grating stimuli used in the main experiments. Nevertheless it also produced ‘orientation’ columns (see Fig. 5C), and these orientation columns also showed eccentricity-dependent topographic features, similar to those produced by the gratings. In Figure 5C, one orientation map is shown in red and the other in green. In parafoveal visual field representations (~5° eccentricity; towards the right of Fig. 5C), the two orthogonal orientations produced interdigitating red and green color-coded orientation columns. Near the foveal visual field representation, the two orientation maps overlapped (as indicated by the yellow color towards the left of Fig. 5C). The increased overlap between the two sets of DG columns near the fovea is also documented in Figure 4C. Furthermore, in this control experiment, the overlap between the DG columns and the blobs was 45% (of the total orientation column surface) in the foveal sample, compared with 28% in the parafoveal sample. Thus, the eccentricity-dependent variations in the DG blob maps appeared to be a genuine ‘orientation-related’ effect, rather than an artifact of eccentricity-dependent variations in spatial frequency sensitivity.

**Area V2**

In V2, there have been contradictory claims about whether or not orientation-selective neurons, and orientation columns, are preferentially located in the thick cytochrome oxidase stripes [for example (DeYoe and Van Essen, 1985; Gegenfurtner et al., 1996)]. As in V1, it is conceivable that some of this controversy in V2 may be explained by eccentricity-dependent variations in the relationship between the orientation columns and the three cytochrome oxidase compartments (see below).
First we will describe our results in the more classically mapped region, outside the representation of the fovea. In parafoveal V2, we found a functional organization similar to that described previously, based on different functional mapping techniques in paralyzed anesthetized animals (Tootell and Hamilton, 1989; Ts’O et al., 1990; Roe and Ts’O, 1995).

**Figure 3.** Eccentricity-dependent variation in the relationship between orientation maps and the cytochrome oxidase blobs in area V1. (A) Portion of a single-isotope ([3H]DG) normalized autoradiograph (average of two consecutive sections, M3, left hemisphere) produced during viewing of a horizontally oriented grating, from layer 3 of the flattened occipital operculum. From left to right, the panel includes the representation of foveal (0°) through parafoveal (to ∼7°) eccentricities. (B) The relationship between the two orientation-linked maps in pseudocolor (same cortical section as in A). One isotope map, produced by a horizontally oriented grating, is coded in pseudocolor such that high > low DG uptake corresponds to red > black. In the other isotope map from the same section, uptake was produced by a vertical grating, and it is coded green > black over the same range of uptake values. In both DG maps a difference-of-Gaussian filter with an excitatory kernel of 400 µm was applied to enable a comparison with the cytochrome oxidase blobs (see D, E, F). (C) Plots of 14C- (red) and [3H]DG (green) concentrations measured along a line (100 µm width) from foveal towards more peripheral visual field representations (0–1.5° eccentricity, A–C) and more peripheral visual field representations (3–7° eccentricity, D–F) derived from the same sections. Data from M1 (A, D) and M4 (C, F) right hemisphere, M3 left hemisphere (B, E). Scale bars = 500 µm.

**Figure 4.** The relationship between orthogonal orientation maps in foveal and parafoveal representation of area V1. Amplitude-normalized 14C- (red) and [3H]DG (green) concentrations were measured along a line (100 µm width), spanning seven to nine orientation columns, at foveal visual field representations, (0–1.5° eccentricity, A–C) and more peripheral visual field representations (3–7° eccentricity, D–F) derived from the same sections. Data from M1 (A, D) and M4 (C, F) right hemisphere, M3 left hemisphere (B, E). Scale bars = 500 µm.
Figures 6 and 7 show such maps from the present study, produced by orthogonal orientations, extending from the representation of $\sim2–8$ eccentricity. In the pseudocolor combination of the two isotope maps (Fig. 6), and especially in the gray-level difference images of the two isotope maps (Fig. 7), one can discern alternating stripe-shaped regions of: (i) diffuse, orientation-nonselective uptake, and (ii) more columnar, orientation-selective DG uptake. Comparison to the cytochrome oxidase staining pattern from the same sections showed that the regions of prominent orientation columns were centered on the thick, medium-dark cytochrome oxidase stripes (see Fig. 7). However, the orientation-specific columns also extended beyond the thick stripes, about halfway into the intervening pale stripe compartment (the ‘pale’ or ‘inter’ stripes). The orientation columns were mostly absent from the thin dark cytochrome oxidase stripes.

We also found consistent variations with eccentricity which were strikingly similar to those described above for V1 (see Fig. 8). From parafoveal towards more foveal representations, two changes take place. First, the orientation nonselective DG uptake in those ‘thin’ dark stripe compartments increased progressively. Secondly, the truly orientation-selective components (in the pale and thick-stripe cytochrome compartments) decreased in contrast. At least with regard to the pale-stripe compartments, this is consistent with (i) our finding of a greater concentration of DG columns in the interblob compartments of V1, together with (ii) the known projections from the V1 interblobs to the V2 pale stripes (Livingstone and Hubel, 1984).

At the foveal representation of V2, the DG maps were dominated by high and diffuse uptake in the thin cytochrome oxidase stripes, with very little orientation-selective uptake in inter-
digitating columns between the thin stripes. This is consistent with (i) the relative convergence of the DG ‘orientation’ columns on the foveal blobs of V1, described above, and (ii) the known projection from the V1 blobs to the V2 thin stripes (Livingstone and Hubel, 1984).

Within each of the orientation-selective compartments of V2, the orientation-specific columns had a mean column width of 450 µm (SD 60 µm), and a mean column spacing of 950 µm (SD 200 µm).

In V2, the contrast of the DG patterns was highest in layer 4, becoming progressively fainter throughout lower layers, and especially faint in the upper layers (see Fig. 9). In other extrastriate areas, the DG-labeled ‘columns’, which were not interdigitated (see below), extended further into the upper layers.

**Figure 7.** Alternating stripes of high-versus-low orientation selectivity in V2, relative to the cytochrome oxidase stripes. (A) Difference image between the two single-isotope, single-orientation autoradiographs (from two consecutive sections from layer 4, normalized 3H signal; M3, right hemisphere). The image was created by first equalizing the gray value range from each film, then subtracting one image from the other. Regions responding most selectively to the horizontal grating are shown in black, and regions showing selective activity to the vertical grating are shown in white. Regions showing more equal DG uptake (either low, high or intermediate levels) appear gray. Black triangles indicate orientation-selective columns. (B) Cytochrome oxidase staining pattern from the same sections, in topographic alignment with the autoradiographic image. As pointed out earlier (Tootell et al., 1989), the thick cytochrome oxidase stripes stained less darkly than the thin stripes, especially in this layer. (B, D) Estimated position of the thick and thin cytochrome oxidase stripes (enclosed red and blue lines, respectively) superimposed on the images of (A) and (C), respectively.

**Areas VP and Ventral V4**

Immediately adjacent to ventral V2 lies area VP (Newsome et al., 1986). VP has a long, thin topography, extending ~4–5 mm anterior to the V2 border, including the posterior portion of the anterior bank of the inferior occipital sulcus. Anterior to VP lies ventral V4.

Figure 10 shows a flattened view of the anterior bank of the inferior occipital sulcus, extending onto the prelunate gyrus. The DG labeling was robust and columnar in this region. In the posterior half of this region, the columns produced by each orientation were interdigitated (i.e. orientation-selective). The anterior terminus of the V2 stripes (the anterior border of V2) was revealed by the cytochrome oxidase staining in the same sections (e.g. Fig. 10.A,B). Within that anterior portion of V2, the DG columns were interdigitated, as one would expect from the...
Figure 8. Variations with eccentricity in the activity maps from V2 (M3 left hemisphere). (A) Cytochrome oxidase staining of the posterior bank of the lunate sulcus. (B) Single-orientation (horizontal), normalized [3H]DG map (average of two consecutive sections through layer 4). The abscissa indicates the eccentricity; white arrowheads indicate the thin cytochrome oxidase stripes.

data in the rest of V2 (Figs 6 and 7). However, the interdigitated DG-labeled orientation columns also extended for another 4–5 mm anterior to this border, consistent with the location and topography of area VP (see Fig. 10A). Anterior to these orientation-selective columns in presumptive VP, there was a sharp transition to a region in which columns were coincident rather than interdigitated (i.e., not DG orientation-selective). At this border, the cytochrome oxidase staining also became lighter (see Fig. 10B). All evidence suggests that this border, where both the DG patterns and cytochrome oxidase patterns change coincidentally, marks the transition from VP to ventral V4.

The topographic relationship shown in Figure 10 was similar in all cases examined. The combined results argue quite strongly that orientation-selective columns exist in parafoveal VP. These VP orientation columns had a mean width of 440 µm (SD 70 µm), and a mean spacing of 880 µm (SD 210 µm). In parafoveal V4v, the DG-labeled columns were not orientation-selective, i.e. they were overlapping.

Areas V3, V3A and Dorsal V4
Anterior to dorsal V2 lies area V3. Topographically, V3 is long and thin (typically 2–4 mm), even thinner than VP (Burkhalter and Van Essen, 1986; Newsome et al., 1986). V3 extends along, and just anterior to, the fundus of the lunate sulcus. Anterior to V3 lies V3A. Histologically, V3 shows cytochrome oxidase activity which is relatively dark and topographically uniform
(Tootell and Taylor, 1995) allowing a distinction from adjacent area V3A (which shows lighter cytochrome oxidase staining) and from V2 (in which the staining shows alternating dark/light stripes arranged perpendicular to the length of V3).

The results in V3 were very similar to those in VP. Figures 11 and 12 show flattened views of this region, from the fundus of the lunate sulcus to the crown of the prelunate gyrus. Again, the DG maps from each isotope showed robust and columnar labeling, especially in the posterior portion of the region (e.g. Fig. 11, gray-level inset). The columns were orientation-specific in a thin band which lay parallel with, and just anterior to, the fundus of the lunate sulcus. Anterior to that, the columns became coincident (not orientation-specific). The location of the orientation-specific columns coincided with a continuous band of dark cytochrome oxidase staining (e.g. Fig. 12). If this staining result had been due to an extension of the V2 stripes across the fundus, it would have appeared as a periodic alternation in density (as in the V2 stripes in Figs 7 and 10), rather than the continuous pattern found here.

The continuous band of dark cytochrome staining, its thin width, and the location of the columns on the medial bank of the lunate sulcus, all suggest that orientation columns are present in V3, but absent in V3A and more anterior areas. In V3, the orientation-specific columns had a mean width of 390 \( \mu m \) (SD 80 \( \mu m \)), and a mean spacing of 1060 \( \mu m \) (SD 370 \( \mu m \)).

As in ventral V4, columns were also produced in V3A and
dorsal V4, but these were labeled coincidentally (i.e. not orientation specific) in both DG labels.

Orientation-specific columns have been reported in V4 using optical recording (Ghose and Ts’O, 1997). Those columns were described as ‘similar’ in size and arrangement to those in V2, and confined to ‘foveal’ V4. A possible explanation for this apparent discrepancy between our results and those of Ghose and Ts’O might be the large size of our stimuli. Such large stimuli may engage more profound surround suppression mechanisms in area V4 (Desimone and Schein, 1987). This might have obscured the detection of orientation-specific columns in this region. However, finding orientation-selective columns confined to the foveal representation of V4 remains puzzling, since at least for the stimuli used in the present experiment, we found a distinct decrease of orientation-selective activity in the foveal representation in prior areas V1 and V2 (see Figs 3, 4, 5 and 8). Furthermore, even in foveal V4, columns in the present study were completely nonspecific for orientation (see bracketed region in Fig. 11).

MT
Macaque area MT is generally regarded as sensitive to the direction of stimulus motion, rather than to its orientation. However, some residual sensitivity exists for the orientation of non-moving stimuli in single unit reports from MT (Albright, 1984; Marcar et al., 1995). In fact, in optical recordings from MT in New World Aotus monkeys, the functional organization was dominated by the orientation, rather than the direction, of grating stimulus motion (Malonek et al., 1994).

It was thus of interest to examine the DG labeling patterns in MT. The gratings and oriented noise stimuli used here did produce DG columns. However, as in area V4, the columns were coincident (i.e. not orientation-specific) in the two isotope maps (see Fig. 13). Like those in most extrastriate areas, the columns extended through all cortical layers (see Fig. 9). The difference with the earlier optical imaging study (Malonek et al., 1994) could either be due to the stimulus (stationary in the present study, and moving in the previous study) or to the species. Orientation may be represented more robustly in MT of owl monkeys, as suggested by single unit reports (Zeki, 1980).

The orientation nonspecific columns in MT of the macaque may represent homologues of the band-interband columns (Born and Tootell, 1992), or the cytochrome oxidase ‘patches’ (Tootell et al., 1985), or perhaps columns specific to stimuli near zero disparity (DeAngelis and Newsome, 1999). Because such MT columns were not produced in other double-label DG studies in which visual stimulation was ~80% shorter compared with that in the present study (e.g. (Vanduffel et al., 2000)), the columns in this study appear to reflect the activity of a subset of MT neurons which were better activated by the stationary stimuli used here (for whatever reason), compared to neighboring cells in MT. Thus they are unlikely to reflect variations in endogenous metabolic activity, as reflected by the patches of cytochrome oxidase activity in MT (Tootell et al., 1985).

Additional Areas
We looked systematically for columns (either coincident or interdigitated) throughout visual cortex, including the intraparietal sulcus, inferior temporal cortex, and the anterior bank of the superior temporal sulcus. Coincident DG-labeled columns were found in area TEO, similar to those described above in V4 and MT (see Fig. 13). However, columns were not obvious in additional cortical areas, with the stimuli used here, as measured for VIP and AIT (see Fig. 15).

Discussion
Variation in Orientation Columns with Retinotopic Eccentricity
In V1 and V2, our results suggest a systematic topographic variation of the orientation columns relative to each other, and to the cytochrome oxidase blobs and stripes. In the representation of parafoveal and more peripheral eccentricities, where the first evidence for orientation columns was obtained (Hubel and Wiesel, 1974), we found a segregation of the two orientation columns from each other, and from the blobs. This fits with the optical imaging results of Bartfeld and Grinvald obtained at 6–8° eccentricity. These authors found that 78% of the pixels that corresponded to blobs exhibited poor orientation selectivity. However, towards the foveal representation, peak activity in each DG map shifted towards the center of the cytochrome oxidase blobs, so that all three of these metabolic labels coincided more closely. Orientation columns have been repeatedly reported in optical imaging studies of primate V1 (Bartfeld and Grinvald, 1992; Blasdel, 1992; Shoyerman et al., 2000; Blasdel and Campbell, 2001). The position of the recording chamber suggests that most if not all recordings come from dorsal V1 at eccentricities exceeding 3°, as confirmed by a recent study explicitly stating the eccentricities explored (Blasdel and Campbell, 2001). It would indeed be difficult to record optically
from foveal V1 because of the curvature of the cortex at that level. Hence our DG results at peripheral eccentricities are in clear agreement with these optical imaging experiments. In a control experiment using oriented 1-D noise stimuli, we demonstrated that variation of spatial frequency sensitivity with eccentricity is unlikely to explain the reduced foveal DG-orientation selectivity. The changes in orientation sensitivity with eccentricity suggested by our results may resolve apparently contradictory reports of orientation selectivity outside and within blobs [e.g. (Livingstone and Hubel, 1984) vs (Lennie et al., 1990; Leventhal et al., 1995)]. Several changes in neuronal properties could underlie these eccentricity dependent changes in orientation columns. Specifically, in foveal compared to parafoveal visual field representations there may be fewer orientation selective neurons, or alternatively, highly orientation selective neurons may be less segregated from each other. Single-unit studies (Poggio et al., 1975; Zeki, 1983) suggest that the first alternative is the most plausible explanation for our observations. Zeki reported a decrease in the proportion of orientation selective neurons from 70% in peripheral visual field representations to 40% in the central visual field representation (Zeki, 1983). This decreased orientation selectivity towards foveal visual field representations within the visual cortex might be needed to accommodate processing of eccentricity-dependent attributes such as color or disparity. Future electrophysiological and/or optical recording experiments are required to address the exact nature of the eccentricity-dependent variations we observed in the DG blob maps.

**Magno/Parvo Influences in Orientation Tuning**

Several lines of evidence suggest that magnocellular-stream input is tapped preferentially in the emergence of orientation tuning, in striate and extrastriate cortex. In our DG patterns in V1, the orientation columns were most prominent in magnocellular-recipient layer 4Ca, and they were virtually absent in parvocellular-recipient layer 4Cβ. A similar conclusion arises from single-unit recordings and single-label DG studies, which reported more orientation selective neurons or higher contrast orientation columns in layer 4Ca, compared to 4Cβ (Blasdel and Fitzpatrick, 1984; Livingstone and Hubel, 1984; Hawken et al., 1988).

If this distinction between magnocellular and parvocellular ‘streams’ is meaningful, one might expect to see a corresponding orientation bias in at least a portion of extrastriate regions which receive prominent magnocellular stream input. Such regions include the thick stripes of V2 (Livingstone and

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**Figure 12.** Topography of orientation-selective columns relative to cytochrome oxidase variations, in V3 and adjacent areas. Data are averages from two consecutive sections extending from the fundus of the lunate sulcus (rightmost) towards the crest of the prelunate gyrus (leftmost), after normalization of the [1H]DG signal (from M3, left hemisphere). (A) Subtractive comparison of the two autoradiographic maps: areas with higher sensitivity to the horizontal grating are darker, and higher sensitivity to the vertical grating is coded in white. Orientation-selective columns were robust in V3, but not in more anterior areas such as V3A. (B) Topography of the cytochrome oxidase staining, with visual area borders indicated. As reported previously (Tootell and Taylor, 1995), area V3 is distinguished by higher cytochrome oxidase staining.
Hubel, 1987; Levitt et al., 1994), area V3 (Burkhalter et al., 1986) and MT (Shipp and Zeki, 1985; Maunsell and Van Essen, 1983). Here and in previous functional imaging studies (Tootell and Hamilton, 1989; Ts’O et al., 1990; Roe and Ts’O, 1995), orientation columns were centered on the thick cytochrome oxidase stripes of V2. Furthermore, we also demonstrated orientation columns in V3, which also receives prominent magnocellular-stream input from V1.

V3 and VP
A high percentage of orientation-selective cells has been reported previously in macaque V3 (Felleman and Van Essen, 1987; Gegenfurtner et al., 1997). Here we showed that such orientation-selective neurons are organized into columns. We also found that similar orientation columns are present in VP, though the percentage of orientation-selective single units reported in that area is somewhat lower (Burkhalter et al., 1986).

Retinotopically, VP is complementary to V3 (Burkhalter et al., 1986; Newsome et al., 1986; Felleman and Van Essen, 1987). In fact, some authors consider V3 and VP to be conjoined segments of the same contiguous visual area, called V3 (Gattas et al., 1988; Boussaoud et al., 1990). The presence of DG orientation columns in both V3 and VP, as well as the uniformly higher cytochrome oxidase staining in both areas relative to their anterior neighbors, supports the idea that they are actually two parts of the same visual area. However, the original distinction between V3 and presumptive VP was based on additional functional and connectional differences (Burkhalter et al., 1986).

V4, TEO
Throughout V4 and TEO, the gratings produced numerous columns, but the columns were never specific for the orientation of the grating. Such DG columns could represent either: (1) aggregates of orientation non-selective cells; (2) orientation-selective cells clustered irrespective of their preferred orientation; or (3) cells which have higher metabolic activity irrespective of the visual stimulus.

Although we cannot rule it out completely, variation in endogenous metabolic activity (no. 3) is unlikely to account for all the columnar uptake in V4. In previous studies of V1 and V2, it was initially thought that the endogenously higher metabolic capacity in the cytochrome oxidase blobs and stripes reflected endogenously higher neural activity, which could produce endogenously higher DG uptake (Humphrey and Hendrickson, 1983). Subsequent evidence suggested this effect to be small, perhaps negligible (Tootell et al., 1988a). Furthermore, in other double-label DG studies of awake behaving macaques, columns identical to those reported here were not found in V4, TEO or MT (Vanduffel et al., 2000). Thus in this experiment, such columns were apparently produced by viewing the more prominent gratings stimuli.

Other evidence suggests that possibility no. 2 is more likely. Electrophysiological studies from V4 report classical orientation-

![Figure 13. Topography of DG uptake in additional cortical visual areas. Patterns of DG uptake are illustrated for areas MT, VIP, V4, TEO and AIT, in rows arranged from top to bottom, respectively. For each area, portions of the raw autoradiographs from each isotope-orientation pair are shown in the middle and right columns, produced during viewing of horizontal or vertical gratings, respectively. The red–green pseudocolor comparisons of these two isotope maps are shown in the left column. All patterns were reproduced at the same contrast, from the same animal (M3). DG data were taken from individual sections after normalizing the [3H]DG signal. Scale bars = 5 mm.](image)
selectivity in a majority of neurons [(e.g. 57% in (Maunsell et al., 1991); see also (Desimone and Schein, 1987)]. These reported percentages of orientation-selective V4 cells are ample to accommodate the sparsely distributed V4 columns described here.

**Overall**

Why is it necessary to segregate neurons tuned to specific orientations in as many as four different visual areas, spanning three different stages in the cortical hierarchy? Other examples of functionally specific columns, such as the ocular dominance columns in V1, and the direction columns in MT (Albright et al., 1984; Geesaman et al., 1997), are confined to just one or two cortical areas.

It seems that the orientation specificity for a given region in space is present in V1 and carried along in the same architectural form (i.e. orientation columns) through V2, V3 and VP, for integration and transformation within larger receptive fields. Those 'later stages' may include area V4 and/or TEO – where single cells still show orientation specificity, and where the gratings are still effective stimuli, but this information is no longer segregated into orientation-selective columns. Orderly arrangement of neurons with a wide range of orientation selectivities might be a requirement to enable emergence of more complex feature selectivities in these later stages (Wilson et al., 1997), such as selectivity for angles and curves (Pasupathy and Connor, 1999) or polar or hyperbolic gratings (Gallant et al., 1993). The later stages may also include MT, where orientation may be used to disambiguate the processing of motion direction (Movshon et al., 1985). Interestingly, our results suggest that regions where no columns were observed at all (e.g. TE and VIP), might represent a level beyond the middle level areas such as V4, TEO and MT.

**Notes**

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