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Topographic Organization of the Orientation Column System in Large Flat-Mounts of the Cat Visual Cortex: A 2-Deoxyglucose Study

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ABSTRACT

We developed a flat-mount technique in order to visualize, without additional reconstruction, the system of orientation columns in the cat visual cortex by using 2-deoxyglucose-autoradiography.

Experimental animals were injected with 2-deoxyglucose and then stimulated for 45–60 minutes either with vertical or horizontal or oblique gratings alone or with vertical and horizontal gratings presented in alternation.

In both areas 17 and 18 stimulation with either vertical or horizontal or oblique stripes produced similar and highly ordered patterns of parallel bands of increased 2-deoxyglucose uptake that were perpendicular to the boundaries of the areas. In area 17 they occasionally extended without interruption from the 17/18 border on the top of the lateral gyrus to the monocular segment in the splenial sulcus. Superposition of serial sections revealed that these bands were present in all cortical layers and in precise register along lines orthogonal to the lamination. The center-to-center spacing of the bands was 1.0–1.1 mm in area 17 and 1.2–1.4 mm in area 18. Stimulation with alternating vertical and horizontal contours led to a pattern the general organization of which resembled that induced by a single orientation but the spacing of which was reduced by a factor of 0.5. This strongly supports the concept that orientation is mapped in a system of parallel bands and argues against a recently formulated hypothesis that iso-orientation bands extend like spokes from centers that lack orientation selectivity (Braitenberg and Braitenberg, *Biol. Cybern. 33:*179–186, '79).

Another characteristic feature, revealed by the flat-mount technique, was a periodic variation of 2-deoxyglucose uptake along the bands that gave them a beaded appearance. The mean center-to-center distance between adjacent beads on the same band was in the range of 0.9–1.2 mm and remained unchanged when horizontal and vertical gratings were presented in alternation. We propose that these beads reflect another columnar system whose features have yet to be determined.

Key words: orientation selectivity, area 17, functional organization

In their pioneering studies Hubel and Wiesel ('59) established that visual cortical neurons with similar orientation preference are clustered together. Cells recorded along electrode penetrations perpendicular to the cortical layers were found to prefer the same stimulus orientation whereas a gradual shift of orientation preference was observed along tangential penetrations (Hubel and Wiesel, '74; Albus, '75a,b; Humphrey and Norton, '80). Following the terminology of Mountcastle, Hubel and Wiesel referred to the cortical volume containing neurons with similar orientation

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preference as an orientation column. The development of the 2-deoxyglucose (2-DG) method by Sokoloff et al. ('77) made it possible to map the topographical arrangement of such orientation columns more comprehensively than with electrophysiological techniques (cat: Albus, '79; Schoppmann and Stryker, '81; Singer, '81; Singer et al., '81; Thompson et al., '83; Albus and Sieber, '84; monkey: Hubel et al., '77; tree shrew: Skeen et al., '78; Humphrey and Norton, '80; Humphrey et al., '80). This approach revealed that iso-orientation domains frequently have the shape of slabs and bands rather than of isolated columns. Hence a number of studies have been devoted specifically to an analysis of the geometry of iso-orientation domains, but the conclusions have remained somewhat controversial. On the basis of mathematical models of the development of orientation selectivity, Swindale ('82) and von der Malsburg and Cowan ('82) inferred that iso-orientation domains should be arranged as parallel and regularly spaced slabs with trajectories essentially orthogonal to area boundaries; 2-DG experiments in cats (Singer, '81; Singer et al., '81) and tree shrews (Skeen et al., '78; Humphrey and Norton, '80; Humphrey et al., '80) support this conjecture. An alternative geometrical concept has been put forward by Braitenberg and Braitenberg ('79) and Braitenberg ('83, '84, '85) in which it is proposed that iso-orientation domains are radially arranged: extending like spokes from centers that lack orientation selectivity. Hence, in their model the trajectories of iso-orientation bands encoding different orientations should differ from each other and should not show a fixed relation to area boundaries.

The goal of the present study was to test the validity of the two alternative concepts in the cat visual cortex by using the 2-DG method. We compared the 2-DG patterns induced by vertical, horizontal, or oblique gratings with those generated by presenting vertical and horizontal contours in alternation. If iso-orientation domains are arranged as parallel bands, the trajectories of the 2-DG bands must be invariant to changes in stimulus orientation. Hence, presentation of both horizontal and vertical contours in alternation should lead to patterns that resemble those induced by a single orientation except that in the former the spacing of activated bands should be reduced by a factor of 0.5. In contrast, according to the model of Braitenberg and Braitenberg ('79) the trajectories of activated iso-orientation domains should change with the orientation of the stimulus and alternating stimulation with orthogonally oriented contours should activate short slabs of cortical tissue that are orthogonal to each other and originate from regularly spaced zones of increased activity. We anticipated that a distinction between the two patterns would require very high spatial resolution and a complete representation of the 2-DG patterns in the visual cortex. Rather than relying on the commonly used reconstructions from serial sections we therefore developed a flat-mount technique that allows visualization, in a single autoradiograph, of the whole system of iso-orientation domains in both visual areas 17 and 18. Some of the results of this study have been published in abstract form (Löwel et al., '85).

MATERIALS AND METHODS

The 2-DG method

Since Sokoloff et al. ('77) developed the 2-deoxy-D-(^{14}C)-glucose (2-DG) method, a number of studies have demonstrated the general advantage of this technique for localizing stimulus-induced changes of neuronal activity in the vertebrate brain. The effects of visual (Hubel et al., '77), auditory (Scheich et al., '79), olfactory (Skeen, '77), and gustatory (Orlandi et al., '77) stimuli, and even of electrical self-stimulation (Gallistel et al., '77), have since been investigated. Radioactively labeled 2-DG metabolites such as 2-DG-6-P and 2-deoxy-glycogen accumulate in metabolically active brain regions in amounts proportional to the relative rates of glucose consumption. Hence, the optical density on autoradiographs of deoxyglucose-labeled sections is proportional to the net rate of deoxyglucose consumption during the period allowed for its uptake. The technique thus provides a "radioisotopic 'stain' for functional activity" (Plum et al., '76).

TABLE 1. Experimental Conditions[1]

Cat	Age	Weight (kg)	Dosage (μCi/kg)	Application	Orientation of visual stimuli: Left VF	Right VF
K14	11 weeks	0.85	294	i.v.	90°	0°
S2	3 months	0.90	278	i.v.	0°	135°
S3	3 months	1.80	139	i.v.	0°	45°
S4	8 weeks	0.90	139	i.v.	135°	0°
KF8	3 months	1.14	219	i.v.	90°	90° + 0°
KF9	5 months	1.60	156	i.v.	90°	90° + 0°
KF10	5 months	1.60	50	i.a.	90°	90° + 0°
KF11	5 months	1.60	50	i.a.	90°	90° + 0°
KF12	5 months	2.30	17.4	i.a.	90°	90° ("Maltese cross")

[1]Label: 2-deoxy-D-(U^{14}C)-glucose, Amersham, specific activity 310 mCi/mmol; i.v. = intravenous, i.a. = intraarterial, VF = visual hemifield, 0° = horizontal, 45° = oblique, 90° = vertical, 135° = oblique.

Surgical procedures

The data described in this study were obtained from nine cats the ages of which varied between 8 weeks and 5 months (Table 1). The cats were anesthetized initially with a mixture of ketamine hydrochloride (Ketanest 50 mg/ml, Parke-Davis; dose 0.2 ml/kg) and xylazine hydrochloride (Rompun 5%, Bayer; dose 0.15 ml/kg) injected intramuscularly. After tracheotomy, vascular access was assured either by a venous catheter or by bilateral lingual artery catheters (see Freeman et al., submitted). Subsequently, the cats were placed in a stereotactic head-holder, artificially respirated with a mixture of 70% N_2O/30% O_2, and paralyzed with a continuous infusion of hexcarbacholinbromide, i.v. (Imbretil, dose 5 ml in 45 ml of Ringer, 3ml/kg × hour). All wound edges were infiltrated with xylocaine. To eliminate as many potential sources of painful stimuli as possible we then cemented the animal's skull to a metal rod and removed the sterotactic ear and eye bars. As with standard electrophysiological recordings, the body temperature, ECG, pulmonary pressure, and CO_2-content of the expired air were continuously monitored. Body temperature was kept constant at 38°C and continuous infusion of a glucose-Ringer solution (Laevulose 5%, 4 ml/hour) through a gastric catheter compensated for fluid loss.

The nictitating membranes were retracted with Neo-Synephrine and the pupils were dilated with atropine. Black contact lenses containing artificial pupils of 2 mm diameter both guaranteed standard viewing conditions and protected the corneae. The refractive power of both eyes was determined with a Rodenstock refractometer and adjusted with lenses so that the eyes were focussed on a tangent screen (1.44 × 1.24 m) positioned 0.57 m in front of the eye plane of the cat. At this distance, 10 mm on the screen correspond

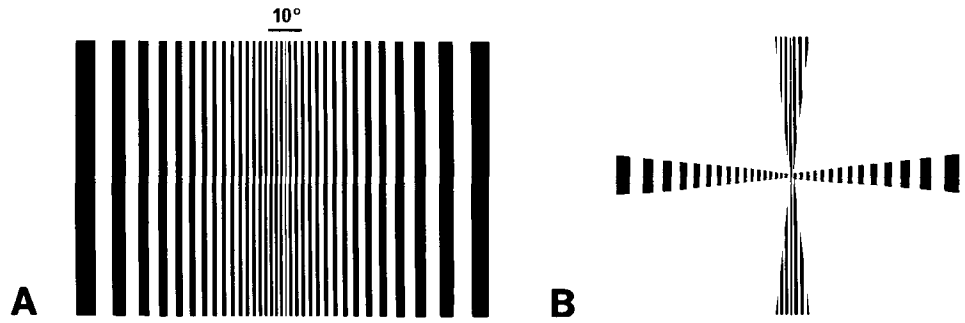


Fig. 1. Square wave gratings used for visual stimulation. A. Black and white pattern used for visual stimulation with horizontal, vertical, and oblique contours. The spatial frequencies of the grating vary logarithmically from 0.08 cycles/degree (c/deg) to 1.50 c/deg. B. "Maltese cross" stimulus used in experiment KF12. Stimulated visual angle was 1° in the center of gaze and increased to 10° in the periphery. The scale bar indicates 10° of visual angle. Note that the spatial frequency of the stimulus varies both along the vertical and the horizontal meridian, because the grating was continuously moved behind the cross-shaped templet on the tangent screen (see Materials and Methods).

to a visual angle of 1° in the center of gaze. Using a Zeiss fundus camera we mapped the major retinal landmarks (area centralis and optic disc) on the screen. The visual axes of both eyes were made convergent until the areae centrales were superimposed on the screen, either by using adjustable prisms or by deviating the eyeball with a hook attached to the insertion of the lateral rectus muscle. In some experiments we positioned flaps in front of the eyes to allow for alternating monocular stimulation.

Visual stimulation

The stimuli consisted of moving square wave gratings (luminance of bright and dark bars was 0.4 and 4.0 cd/m^2, respectively; contrast 80%) that subtended a visual angle of 114° (horizontal) × 106° (vertical) and were back-projected onto the tangent screen (see Fig. 1A). The stimuli thus covered the cats' entire binocular visual field. The spatial frequency of the grating varied logarithmically from 0.08 cycles/degree (c/deg) to 1.50 c/deg, the area with the highest spatial frequency being centered around the projection of the area centralis. The gratings were moved orthogonally to their orientation; the amplitude of the movement remained constant (39° visual angle in six experiments and 52° in three experiments) but the speed of movement changed continuously between 3°/second and 21°/second (2–28°/second in three experiments).

We applied three different stimulus combinations (summarized in Table 1).

1. In one animal, only the representations of the vertical meridian (VM) and the horizontal meridian (HM) were stimulated with vertical contours. For that purpose, we prepared a mask that covered the whole screen except for a cross-shaped area. The center of the cross was positioned on the area centralis. The width of the arms increased toward the periphery ("Maltese cross," see Fig. 1B) to compensate for the cortical magnification factor. In the center the aperture was 1° and increased to 10° in the periphery (see Fig. 4C).

2. Another four animals were stimulated with a horizontal grating in one visual hemifield and an oblique (three cats) or vertical (one cat) grating in the other.

3. Four cats were stimulated with one orientation in one hemifield and two orthogonal orientations in the other. To achieve this the entire visual field was stimulated with vertical contours. Every 16 seconds this stimulus was switched off for 16 seconds and replaced by a horizontal grating that was confined to the right visual hemifield. In all experiments in which two different stimuli were presented in the two visual hemifields, a 1–5°-wide strip along the VM was left unstimulated or was stimulated only with one orientation to avoid uncontrolled overlap.

We started the labeling with 2-DG between 5 and 26 hours after the initiation of anesthesia. Five minutes after the onset of light stimulation the 2-deoxy-D-(U^{14}C)-glucose (Amersham, specific activity 310 mCi/mmol) was administered i.v. or i.a. The protocols are summarized in Table 1. The animals were killed 55–70 minutes after the 2-DG injection with an overdose of Nembutal i.v. or with an intracardial injection of 5 ml 3 M potassium chloride.

In two experiments the deeply anesthetized cats were perfused transcardially with 600 ml of saline followed by 600 ml of 1.25% glutaraldehyde in 0.1 M phosphate buffer.

Preparation of flat-mounts and autoradiographs

After craniotomy three brain regions were removed: the left and right occipital poles and one block containing the lateral geniculate bodies and the superior colliculi. The latter was frozen directly onto precooled cryostat chucks (−70°C).

The cortex of both occipital poles was unfolded prior to freezing. The main steps in the flat-mount technique are illustrated in Figure 2. The arachnoid was dissected along the lateral, postlateral, suprasplenial, splenial, suprasylvian, and postsuprasylvian sulci to separate the gyri from each other. Next the white matter was incised to flatten the gyri. After this preparation the flat-mounts were frozen on dry ice (−70°C) between precooled glass slides. Adhesion of tissue to the slides was prevented by interposed parafilm (American Can Company).

To provide landmarks for later superposition three holes were melted in the flat-mounts with warm needles. Subsequently, forty to sixty 26–28-μm-thick serial sections were cut parallel to the cortical surface in a cryostat at −12 to −15°C. These sections were then mounted on glass slides and immediately dried on a hot plate at +80°C.

The labeled sections were exposed at +4°C on Agfa Mamoray M4 or T3 film. To allow for a comparison between the two hemispheres, sections with corresponding serial numbers (traversing approximately the same cortical depth) were exposed on the same film sheet. Optimal exposure

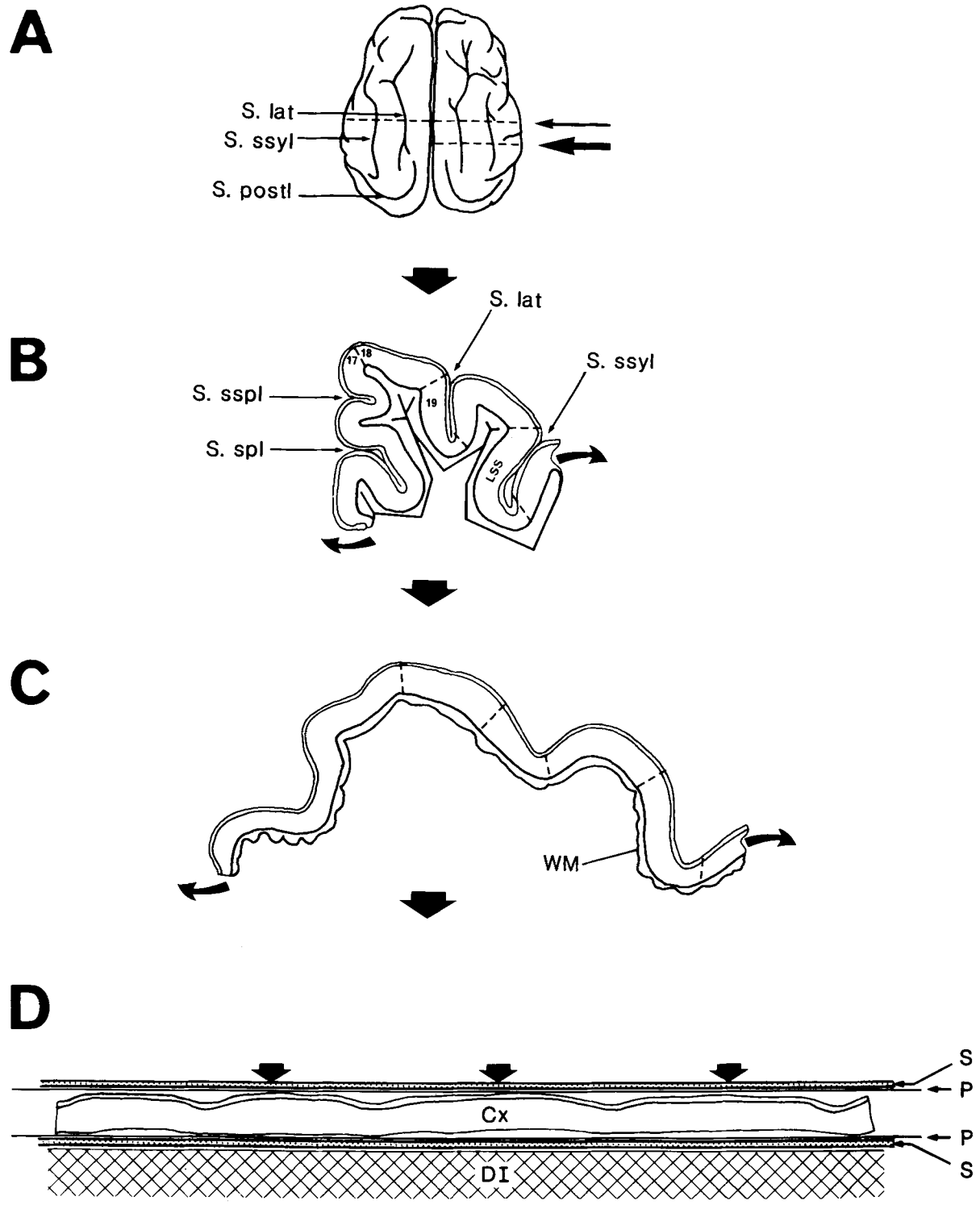


Fig. 2. Schematic summary diagram of the main steps in flat-mounting. A. Dorsal view of cat brain showing (thin arrow) the coronal blocking plane. S. lat = lateral sulcus, S. ssyl = suprasylvian sulcus, S. postl = posterolateral sulcus, S. sspl = suprasplenial sulcus, S. spl = splenial sulcus. B. Coronal section of right hemisphere, at level of thick arrow in A, showing incision planes for white matter and boundaries (dotted lines) for areas 17, 18, and 19 and lateral suprasylvian (LSS). The small curved arrows in B and C indicate the direction of flat-mounting. C. Coronal section after separation of white matter (WM) and unfolding of the cortex. D. Sandwich of cortex (Cx) between sheets of parafilm (P) and glass slides (S) on block of dry ice (DI). Thick arrows indicate even pressure to top of specimen during freezing.

time was assessed separately for each experiment from specially prepared samples and ranged between 2 and 9 weeks. A detailed description of the flat-mount technique and the intraarterial 2-DG application is intended to be published elsewhere (Freeman et al., submitted).

Image processing

Each single autoradiograph was copied with a magnification of 3.5 or 2.1 on film (Kodak Kodalith or Typon Typolith). To increase contrast and signal-to-noise ratio the magnified negatives of two to four serial sections were carefully superimposed by using the three needle holes as guidelines and contact copies were made from these montages.

Thereafter hand drawings were made on transparencies from each of these contact copies, and finally all drawings from one visual cortex were superimposed, using the needle holes as guidelines, and the resulting pattern was redrawn.

Single autoradiographs were further analysed by using a digital image processing system (Imago II Compulog). The density distributions on the X-ray films were coded in digital units ranging from 1 to 256. For visual examination the contrast of the pictures was increased by expanding the gray levels of the image over the dynamic range of the display unit and the autoradiographs were high-pass-filtered to eliminate inhomogeneities in the illumination of the X-ray films.

Line drawings were made at ×5 both from computer-contrasted single autoradiographs and from the hand drawings of the 2-DG pattern on overhead film. The representations of the HM, VM, and area centralis were marked according to our new cortical map (Fig. 4E).

We determined the average columnar spacing by two independent methods. Firstly, we measured the interband distance (center-to-center) by hand along a grid of parallel lines (0.75 mm apart, see Fig. 4F) whose orientation was adjusted perpendicular to the main orientation of the bands. Secondly, we made quantitative analyses on single autoradiographs with the image-processing system: (1) measurements proportional to the optical densities (gray distribution) along vectors perpendicular to the main orientation of the bands (without an additional correction for X-ray film nonlinearities) and (2) one-dimensional Fourier analyses along the same vectors to determine the main spatial frequencies of the 2-DG patterns. In addition, two-dimensional Fourier analyses were performed in two selected areas of striate cortex by using the computer facilities at the Technische Universität München, which were generously made available to us by Dr. Platzer and H. Glünder from the Lehrstuhl für Nachrichtentechnik.

RESULTS

Retinotopic mapping

Following visual stimulation with the "Maltese cross" stimulus (see Fig. 1B), increased deoxyglucose uptake in the striate cortex was restricted to the cortical representations of the horizontal and vertical meridians. Other parts of area 17 were only lightly and uniformly labeled (Fig. 3). Within the regions of increased 2-DG uptake, the active zones consisted of regularly spaced strips that probably correspond to iso-orientation bands matching the orientations contained in the stimulus. The total volume of activated cortex is strikingly different along the two meridians. Along the vertical meridian (VM) the width of the band of activated cortical tissue was narrow (2–3 mm) and independent of the elevation, while along the horizontal meridian (HM) it was wider and slightly increased in width (from 5 to 8 mm) with increasing eccentricity. Hence, there is an anisotropy in the representation of the two meridians in area 17 with relatively more cortical tissue devoted to the representation of meridians parallel to the VM. Moreover, it appears as if this anisotropy decreased with increasing horizontal eccentricity. Whether this latter distortion is confined to regions close to the horizontal meridian or whether it applies to the whole area cannot be decided from our material.

The comparison of the 2-DG pattern of the two meridians with the cortical map of Tusa et al. ('78, '79) revealed a difference in the curvature of the VM. This discrepancy is probably due to the different methods used to obtain a two-dimensional representation. In order to obtain a retinotopic map applicable to our flat-mounts, we modified Tusa's map, reducing the curvature of the VM but keeping the distances along the meridians unchanged (see Fig. 4). This modified retinotopic map made it possible to compare the spacing of columns between different visual field presentations. The location of the 17/18 border was inferred from the 2-DG representation of the VM and from the trajectory of the lateral sulcus and its relationship to area boundaries as demonstrated in the cortical maps of Otsuka and Hassler ('62), Sanides and Hoffmann ('69), Tusa et al. ('79), and Van Essen ('79).

2-DG patterns in flat-mounts of areas 17 and 18 after visual stimulation with a single orientation

Stimulation of the retinae with either vertical (see Fig. 5) or horizontal (see Fig. 6) or oblique (see Fig. 7) contours of logarithmically changing spatial frequency produced similar and highly ordered patterns of increased 2-DG uptake. In both areas 17 and 18 the activity patterns consisted of regularly spaced stripes that occasionally terminated in blind endings or fused with adjacent bands and ran perpendicular to the 17/18 border. In area 17, these bands sometimes extended without interruption from the top of the lateral gyrus to the monocular segment in the splenial sulcus, spanning distances of up to 10 mm. In the posterior part of area 17 the 2-DG patterns were consistently more patchy; the bands were shorter and tended to run in a more rostrocaudal direction. The bands in this particular area thus appear to extend radially from the representation of the area centralis, their trajectories remaining perpendicular also to the anterior, medial, and posterior boundaries of the zone of high 2-DG uptake in area 17. In all experiments the 2-DG patterns in the central part of area 17 were more diffuse than in the periphery.

Despite the irregularities of the 2-DG patterns the mean distance between the centers of adjacent bands was surprisingly constant. In the right hemisphere of cat KF10 (see Fig. 5), which was stimulated with vertical contours, the mean center-to-center band spacing was 1,060 μm (s.d. = 13 μm, n = 228) in area 17 and 1,220 μm (s.d. = 11 μm, n = 40) in area 18. In the left hemisphere of cat K14 (see Fig. 6), which was stimulated with horizontal contours, the corresponding values were 1,000 μm (s.d. = 14 μm, n = 125) and 1,290 μm (s.d. = 23 μm, n = 51), respectively. In all hemispheres the mean spacing of adjacent iso-orientation bands was significantly narrower in area 17 than in area 18 (t-test, $P < .001$). No significant differences in the mean band spacing were observed between the representations of

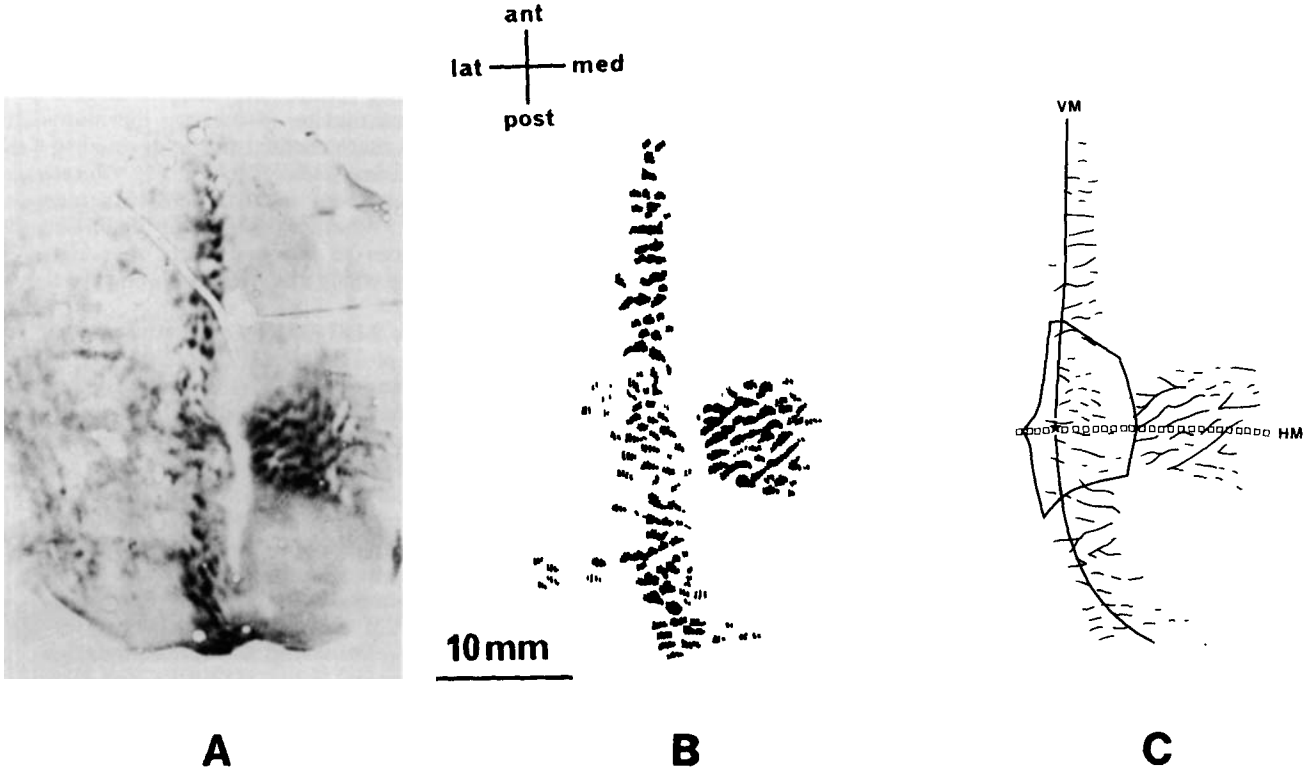


Fig. 3. Reconstruction of the topographical distribution of increased 2-deoxyglucose (2-DG) uptake in the left visual cortex of cat KF12. Visual stimulation of this experimental animal consisted of vertical logarithmic contours restricted to the horizontal and vertical meridian in a "Maltese cross" pattern (see Fig. 1B). The velocity of the grating varied between 3°/second and 20°/second. A. Autoradiograph of flat-mounted sections of the unfolded left visual cortex (three adjacent sections superimposed). B. Hand drawing of the 2-DG pattern, obtained from a reconstruction through all cortical layers. The discontinuity of the pattern along the HM is due to the fact that we omitted this region from reconstruction because incomplete unfolding caused an uncertainty in the correct alignment of adjacent sections. C. Line drawing from B. The representations of the horizontal (HM) and vertical meridian (VM) and of the central 0–5° in areas 17 and 18 are marked with open squares and lines, respectively. The star represents area centralis.

the upper or lower visual field, nor between area centralis and periphery, nor between oblique and horizontal or vertical contour stimulation.

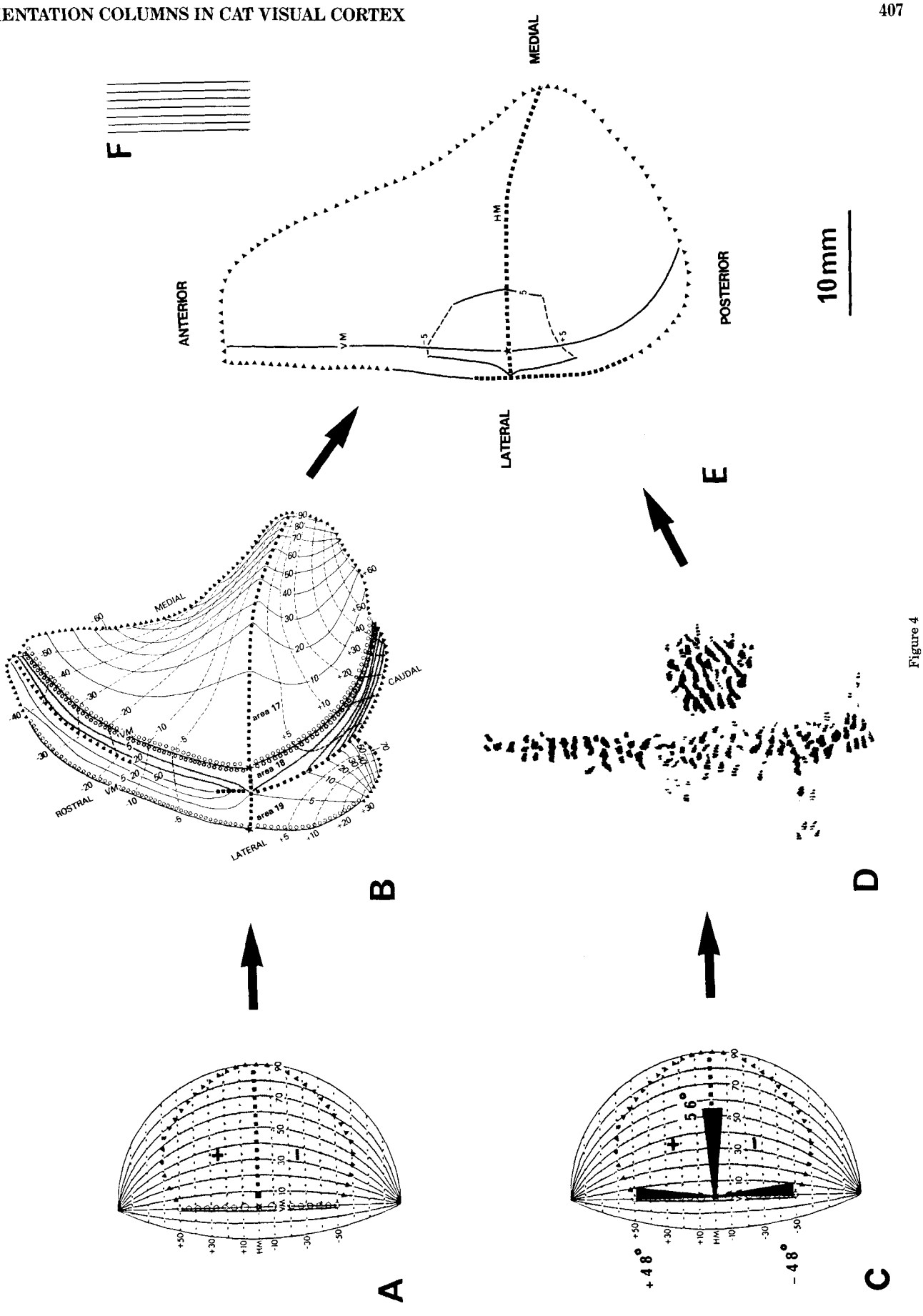
Despite substantial interindividual variability the principal spatial organization of the 2-DG patterns was remarkably similar in the visual cortices stimulated with horizontal, vertical, and oblique contours, respectively (compare Figs. 5–7). The principal trajectories of the bands were always orthogonal to the 17/18 border.

A characteristic feature of the bands in area 17 that has not been reported in previous 2-DG studies was their beaded appearance. This was represented by periodic variations in the optical density along the bands. To determine the center-to-center spacing of adjacent beads in a band we analyzed the optical density distribution parallel to the trajectories of the bands. One-dimensional Fourier analyses revealed that the distance between adjacent beads on the same band was of the order of 900–1,200 μm (see Fig. 9C). Hence the interbead spacing is of the same order as the mean interband spacing but seems to be more variable.

2-DG patterns after visual stimulation with two orientations

Alternating stimulation with horizontal and vertical contours induced rather complex activity patterns. Figure 8 shows an autoradiograph from such a double-stimulated visual cortex, in this case the left visual cortex of cat KF10. The contralateral single-stimulated hemisphere of the same animal was already shown in Figure 5. Note that the total duration of visual stimulation with vertical contours was equal in both hemispheres (see Materials and Methods). Thus, any absolute contrast differences between Figures 5A and 8A cannot be attributed to the amount of stimulation but most likely result from the superimposition (and subsequent photoreproduction) of a differing number of adjacent sections: two for the former and three for the latter. Because we were primarily interested in the qualitative comparison and demonstration of the 2-DG patterns and not in a quantitative analysis of 2-DG uptake, no correction was made for intensity differences. In the anterior part of

Fig. 4. Adjustment of the published retinotopic map of areas 17 and 18 to our flat-mount preparations. A. Perimeter chart showing the extent of the visual hemifield represented in area 17. B. Schematic representation of the visual hemifield in areas 17, 18, and 19. A and B reproduced from Tusa et al. ('78, '79). C. "Maltese cross" drawn in the perimeter chart of A to demonstrate the dimensions of the stimulus (+48°, −48°, lateral 56°). In the center of gaze the aperture was 1° and increased to 10° in the periphery. D. Hand drawing of the 2-DG pattern in the left visual cortex of cat KF12, stimulated with the "Maltese cross" (Fig. 3B). E. Corrected retinotopic map of areas 17 and 18 in flat-mounts of the visual cortex of cats. The main difference is the reduced curvature of the VM. Symbols according to the original retinotopic map of Tusa et al. ('78). F. Grid of parallel lines (0.75 mm apart) used to determine the average spacing of the iso-orientation bands.


F
MEDIAL
ANTERIOR
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VM
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E
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area 17
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+48°
-48°
56°

Figure 4

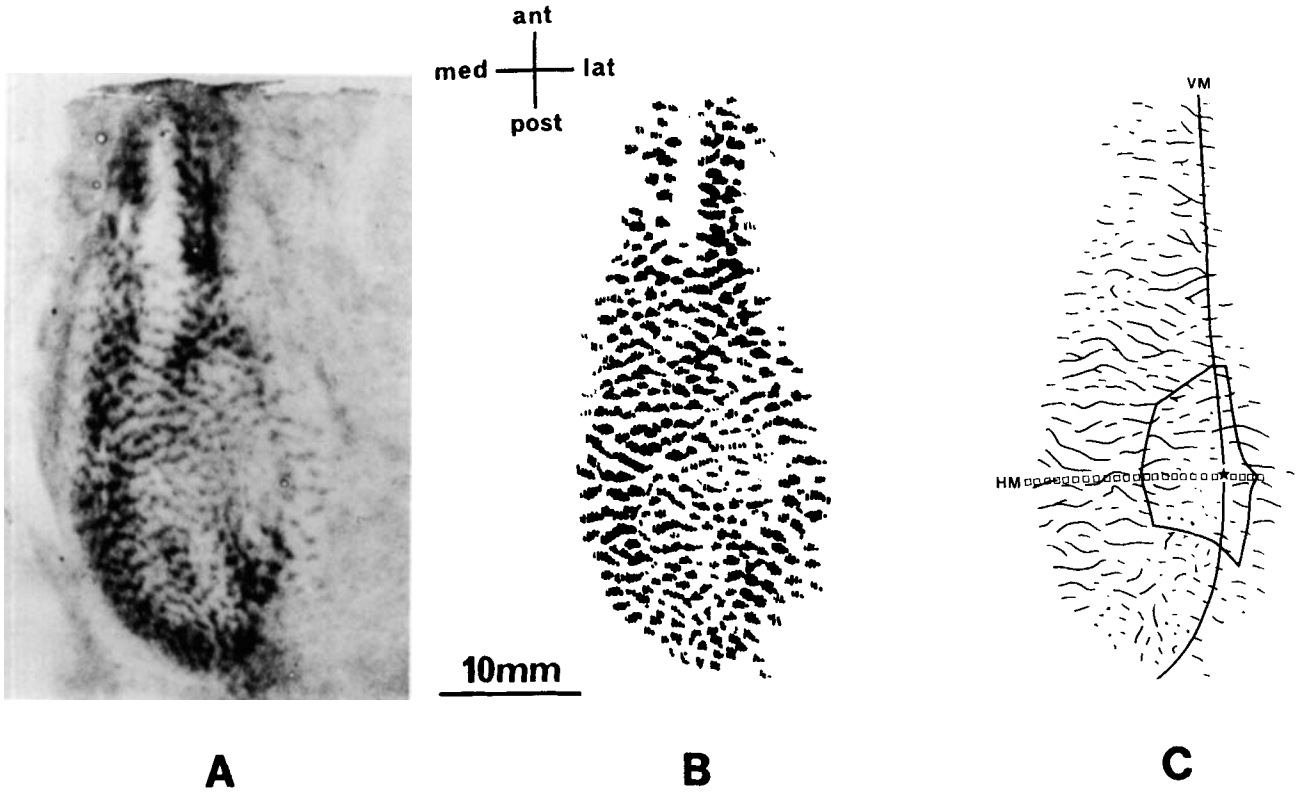

Fig. 5. Reconstruction of the topographical distribution of increased 2-DG uptake in the right visual cortex of cat KF10. The left visual hemifield of this animal was stimulated with vertical logarithmic contours. The velocity of the grating varied between 3°/second and 20°/second. A. Autoradiograph of flat-mounted sections through the unfolded right visual cortex (two adjacent sections superimposed). B. Hand drawing of the 2-DG pattern, obtained from a reconstruction through all cortical layers. The discontinuity of the pattern in the anterior part of area 17 is due to elimination of the sections from the reconstruction in this region, since incomplete unfolding resulted in an uncertainty of their correct alignment. C. Line drawing from B. The representations of the HM and VM, of the central 0–5° in areas 17 and 18 and of the area centralis are indicated as in Figure 3C.

the double-stimulated area 17 the 2-DG pattern consisted again of approximately parallel, beaded bands. In more posterior parts, however, the pattern was less regular and it was difficult to distinguish bands. However, when this was possible their trajectories were found to run perpendicular to the 17/18 border.

The most obvious difference between the patterns induced by one and two orientations, respectively, was in the center-to-center distance of adjacent bands. In area 17 of the double-stimulated hemisphere the mean band spacing was 570 μm (s.d. = 17 μm, n = 430) as compared to 1,060 μm on the side stimulated with only one orientation. Thus it appears that stimulation with two orthogonal orientations induces 2-DG patterns whose spatial frequency is nearly doubled.

To complement these measurements with an independent method, we analyzed the spatial frequency components of the 2-DG patterns with an image-processing system. The results of such one-dimensional Fourier analyses are summarized in Figure 9 and confirm the substantial difference in the band spacing in the two hemispheres of cat KF10. The spatial frequency spectrum of the cortex stimulated with a single orientation showed only one peak representing the center-to-center band spacing of about 1,100 μm while the spectrum from the double-stimulated cortex clearly contained an additional peak at 490 μm that corresponds to the doubled spatial frequency (Fig. 9A). The averaged spectra of one-dimensional Fourier analyses performed along numerous parallel vectors covering large regions of both the single- and double-stimulated striate cortices revealed the same features (Fig. 9B). The existence of a peak at the first harmonic (low spatial frequency peak at 1,050 μm), reflecting considerable spectral energy at this spatial frequency, may have a variety of causes. We consider it likely that as well as the beads, a somewhat noisier 2-DG pattern in the left visual cortex is responsible for the peak formation.

In the left visual cortex of cat KF10, which had been exposed to both vertical and horizontal contours, the beaded appearance of the bands was more prominent than in the right hemisphere, which had only been stimulated with vertical contours. One-dimensional Fourier analysis along vectors parallel to the trajectories of the bands gave similar results in both hemispheres. The dominant center-to-center spacing of the beads was about 1,180 μm in the right (Fig. 9C) and about 1,100 μm in the left visual cortex of cat KF10 (Fig. 9D). Hence stimulation with two orthogonal orientations did not seem to alter the bead spacing.

Additional two-dimensional Fourier analyses in topographically corresponding regions in the anterior part of both striate cortices showed clear differences in the spatial frequency components of the 2-DG patterns along different vectors. The spectrum of the single-stimulated area 17 was approximately circular. This indicates similar spatial frequencies in all directions, reflecting most likely the spacing of beads. However, the peak intensity values were in the

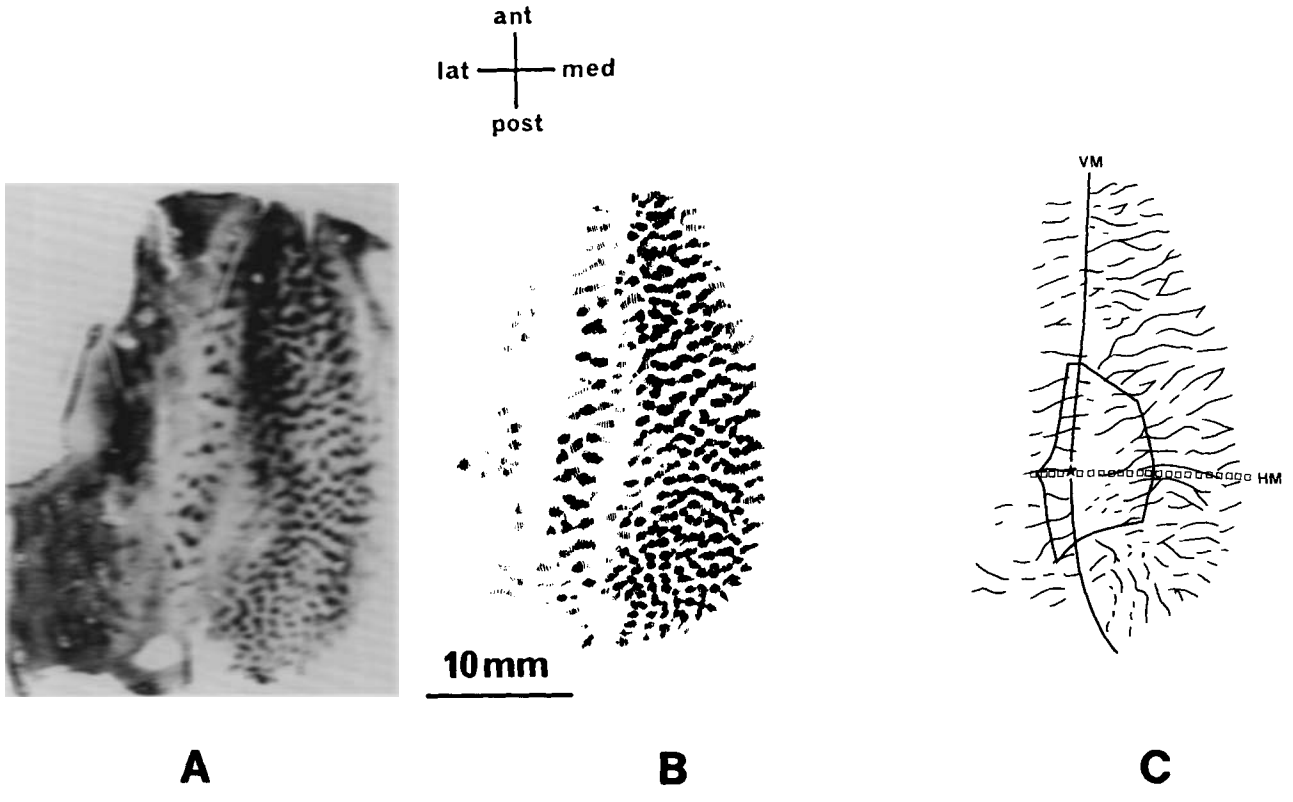

Fig. 6. Reconstruction of the topographical distribution of increased 2-DG uptake in the left visual cortex of cat K14. The right visual hemifield of this animal was stimulated with horizontal logarithmic contours. The velocity of the grating varied between 3.2°/second and 21°/second. A. Autoradiograph of flat-mount sections (three adjacent sections superimposed). The patterns in areas 17 and 18 are separated by a narrow, homogeneously labeled strip adjacent to the representation of the VM that was left unstimulated (see Materials and Methods). B. Hand drawing of the 2-DG pattern, obtained from a reconstruction through all cortical layers. C. Line drawing from B. The representations of the HM and VM, of the central 0–5° in areas 17 and 18 and of the area centralis are indicated as in Figure 3C.

axis perpendicular to the bands reflecting the band spacing. The spectrum of the double-stimulated left area 17 was elliptic (indicating different spatial frequencies along orthogonal vectors); the short axis had the same power as the circular spectrum from the single-stimulated hemisphere and was parallel to the bands while the long axis corresponded to the vector perpendicular to the bands. Hence, the two-dimensional Fourier analyses confirmed that stimulation with two rather than with one orientation increased the spatial frequency of the 2-DG pattern along vectors perpendicular to the bands but not along vectors parallel to the bands.

The translaminar organization of beads and bands

In order to investigate the vertical organization of the 2-DG pattern we made three-dimensional reconstructions from serial sections of the flat-mounts. These reconstructions revealed that irrespective of whether the inducing stimulus was vertical, horizontal, or oblique the bands and beads were in exact correspondence in the different cortical layers. The zones of increased 2-DG uptake extended without any tangential shift delineating slabs from layer I to layer VI, and there was no evidence for depth-dependent variations in the amplitude of 2-DG uptake.

DISCUSSION

Methodological considerations

The flat-mount technique. In previous 2-DG studies on the cat's orientation column system only relatively small regions of the visual cortex had been reconstructed from serial horizontal sections (Singer, '81; Singer et al., '81; Albus and Sieber, '84). Likewise, also the flat-mounts of cat striate cortex studied so far with the 2-DG method (Tootell et al., '81) did not cover the full extent of area 17. The flat-mount technique developed in this study allows us to visualize in single sections and without extensive reconstruction columnar arrangements in several cortical areas simultaneously. Our approach is—in several aspects—similar to two recently published flat-mount techniques (Tootell and Silverman, '85; Olavarria and Van Sluyters, '85), and its special advantages for 2-DG experiments are discussed in detail elsewhere (Freeman et al., submitted). While this method excludes the numerous sources of errors inherent in serial reconstructions it introduces the problem of uncontrolled distortions due to unfolding. In the anterior part of area 17, where the suprasylvian, lateral, suprasplenial, and splenial sulci run nearly parallel to each other, distortions are probably negligible. In the posterior part of area 17,

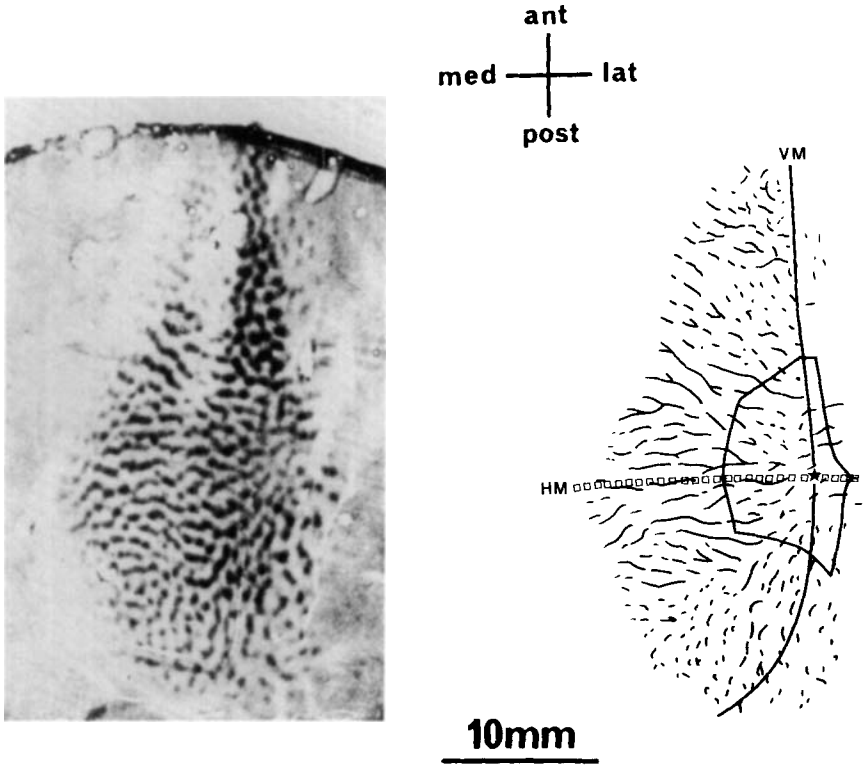

Fig. 7. The topographical distribution of increased 2-DG uptake in the unfolded right visual cortex of cat S4. The animal was stimulated with obliquely (135°) oriented square wave gratings projected to its left visual hemifield. The velocity of the pattern varied between 2.5°/second and 28°/second. A. Autoradiograph of flat-mount sections (photographic montage of three adjacent sections). B. Line drawing of the 2-DG pattern obtained from a reconstruction through the entire cortical thickness. The representations of the HM and VM, of the central 0–5° in areas 17 and 18 and of the area centralis are indicated as in Figure 3C.

however, where the lateral gyrus and the postlateral sulcus are strongly curved in situ, minor distortions are likely to occur with flat-mounting. Controls for these distortions would be difficult to provide. They are one likely cause for the finding that the curvature of the 17/18 border in our flat-mounts differed from the reconstructions of Tusa et al. ('78, '79). We wish to emphasize, however, that our map is in good agreement with that published by Van Essen and Maunsell ('80).

The 2-DG method. In the interpretation of 2-DG experiments it is crucial to consider the factors responsible for the accumulation of radioactive label. It is commonly assumed that glucose metabolism primarily reflects activity of the Na^+/K^+-pump (Mata et al., '80). This in turn is directly correlated with the electrical activity of excitable membranes. The relative contribution of the various excitable structures to the total glucose consumption may vary in different brain regions and remains to be determined in visual cortex. Auker et al. ('83) showed that there are discrepancies between spike activity and metabolic activity, suggesting the possibility that synaptic activity and transmitter release consume relatively more energy than postsynaptic spike generation and impulse conduction.

Schoppmann and Stryker ('81) demonstrated a clear correspondence between regions of maximal spike activity and maximal 2-DG labeling in the visual cortex of cats after stimulation with oriented contours. Similar results have been obtained in other sensory systems and were attributed to a tight coupling between neuronal input activity and actual postsynaptic spike responses (Theurich et al., '84). In view of all the above findings it appears admissible to interpret maximal radioactive labeling in the cat striate cortex as a reflection of both maximal spike activity and high levels of synaptic activity.

Another important question is whether the 2-DG patterns in our study were actually induced by the light stimuli or whether the structured activity patterns reflected stimulus-independent inhomogeneities of metabolic activity. The experiment in KF12 provides a clear answer. Visual stimulation with the "Maltese cross" induced a 2-DG pattern that was restricted to the cortical representations of the HM and VM. The other parts of area 17 were only lightly and uniformly labeled. Moreover, when the visual cortex of cats is stimulated with patterns containing a wide range of orientations and spatial frequencies, 2-DG labeling is homogeneous (Schoppmann and Stryker, '81; Albus and Sieber, '84).

This contrasts with the situation found in monkeys. Here stimulation with diffuse light alone (squirrel monkey) or parallel stripes of different orientations (squirrel and macaque monkey) produces inhomogeneous 2-DG distributions (Humphrey and Hendrickson, '80, '83; Horton and Hubel, '81). The sites of increased glucose consumption correspond to patches of enhanced cytochrome oxidase activity (Humphrey and Hendrickson, '83). Wong-Riley ('76, '79) has provided evidence that high cytochrome oxidase enzyme levels in the brain correspond to regions with high oxidative metabolism. In contrast to the findings in monkey visual cor-

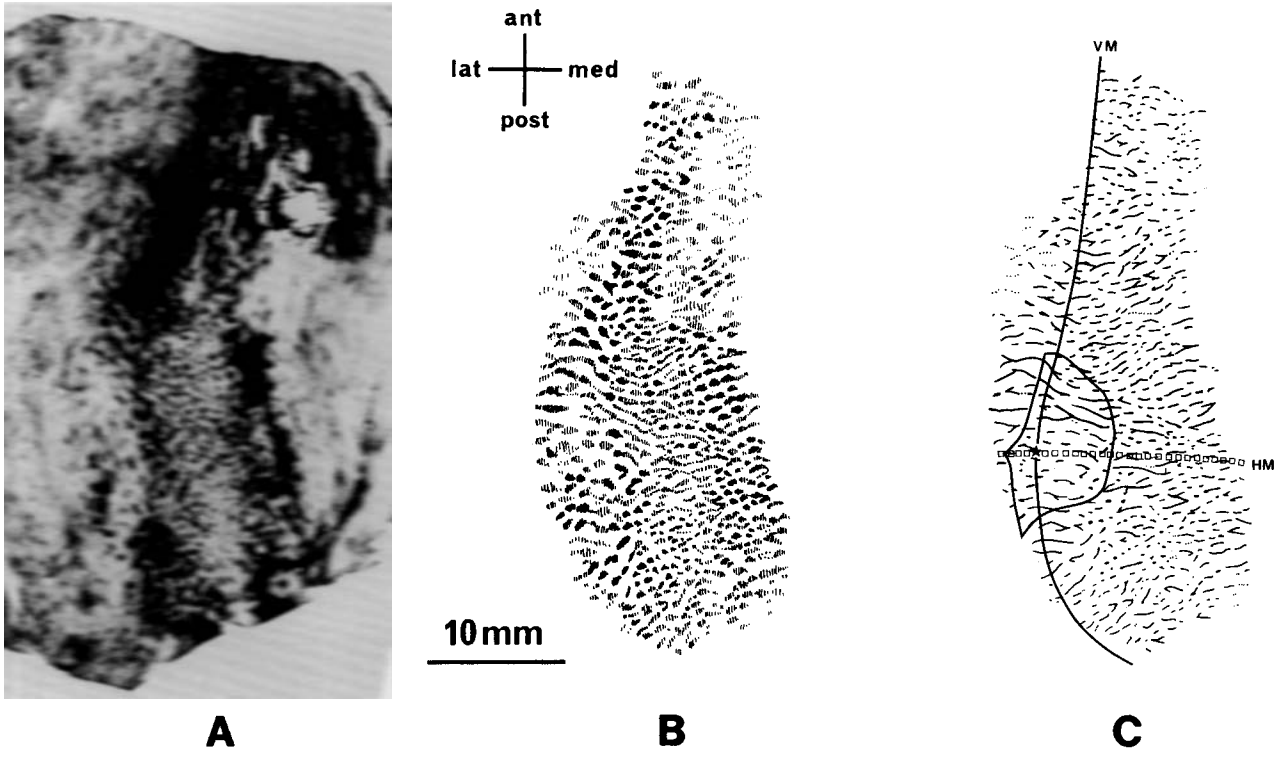

Fig. 8. Reconstruction of the topographical distribution of increased 2-DG uptake in the left visual cortex of cat KF10. The right visual hemifield of this experimental animal was stimulated with both vertical and horizontal logarithmic contours, whereas an approximately 5°-wide strip that borders the representation of the VM was stimulated only with one orientation (vertical contours) to avoid uncontrolled overlap (see Materials and Methods). A. Autoradiograph of flat-mount sections through the unfolded left visual cortex (three adjacent sections superimposed). B. Hand drawing of the 2-DG pattern, obtained from a reconstruction through all cortical layers. C. Line drawing from B. The representation of the HM and VM, of the central 0–5° in areas 17 and 18 and of the area centralis are indicated as in Figure 3C.

tex, no blobs or columns of increased cytochrome oxidase activity were found in cat (Price, '85). These findings taken together suggest that inhomogeneities of energy-supported background activity exist in the monkey but not in the cat visual cortex.

Topographic organization of iso-orientation bands in area 17

In agreement with previous 2-DG studies (Albus, '79; Singer, '81; Singer et al., '81; Schoppmann and Stryker, '81; Albus and Sieber, '84; Leutenecker et al., in preparation), our results indicate that neurons activated by patterns consisting of contours of a single orientation are clustered within elongated slabs of cortical tissue that appear to extend across all layers.

The vertical organization. Superposition of the serial sections of flat-mounts revealed that the 2-DG patterns were similar in the different layers and in exact correspondance. This agrees with previous 2-DG results (Albus, '79; Singer, '81; Singer et al., '81; Schoppmann and Stryker, '81; Albus and Sieber, '84), and most electrophysiological recordings devoted to this question, but is at odds with the electrophysiological findings of Bauer ('82, '83) and Bauer et al. ('80, '83). These authors found that orientation preference shifts by 45° to 90° at the transition between layers IV and V. At present there is no satisfactory explanation for this discrepancy. Layers V and VI receive a strong projection from supragranular layers (Lund et al., '79; Somogyi and Cowey, '81; Gilbert and Wiesel, '85), raising the possibility that the terminals of this descending projection rather than the neurons in infragranular layers contribute to the intensity of the 2-DG label in infragranular layers. However, since there is, in general, a good correlation between electrical activity and 2-DG labeling (Schoppmann and Stryker, '81) and since we never observed bands of label in infragranular layers that had no correspondence in more superficial layers, this interpretation is probably not very realistic.

Concerning the functional representation of oblique orientations in area 17, we could not confirm the "oblique effect" described by Silverman et al. ('80). In our experiments the 2-DG patterns were similar for all tested orientations both with respect to their vertical and horizontal organization. Thus, our data do not support the hypothesis that preferences for oblique orientations result from neuronal mechanisms that differ fundamentally from those responsible for vertical and horizontal preferences (Silverman et al., '80).

The horizontal organization. The present results confirm and extend previous analyses of the horizontal organization of iso-orientation domains in the cat visual cortex (Albus, '79; Singer, '81; Singer et al., '81; Albus and Sieber, '84; Leutenecker et al., in preparation; Schoppmann and Stryker, '81). In area 17 the iso-orientation domains form a rather regular pattern of parallel bands whose trajectories tend to be perpendicular to the borders of area 17. In the anterior part of area 17 the principal trajectories are mediolateral and in the posterior part they extend in a more rostrocaudal direction. Consistently, the iso-orientation bands were more continuous in the anterior than in the

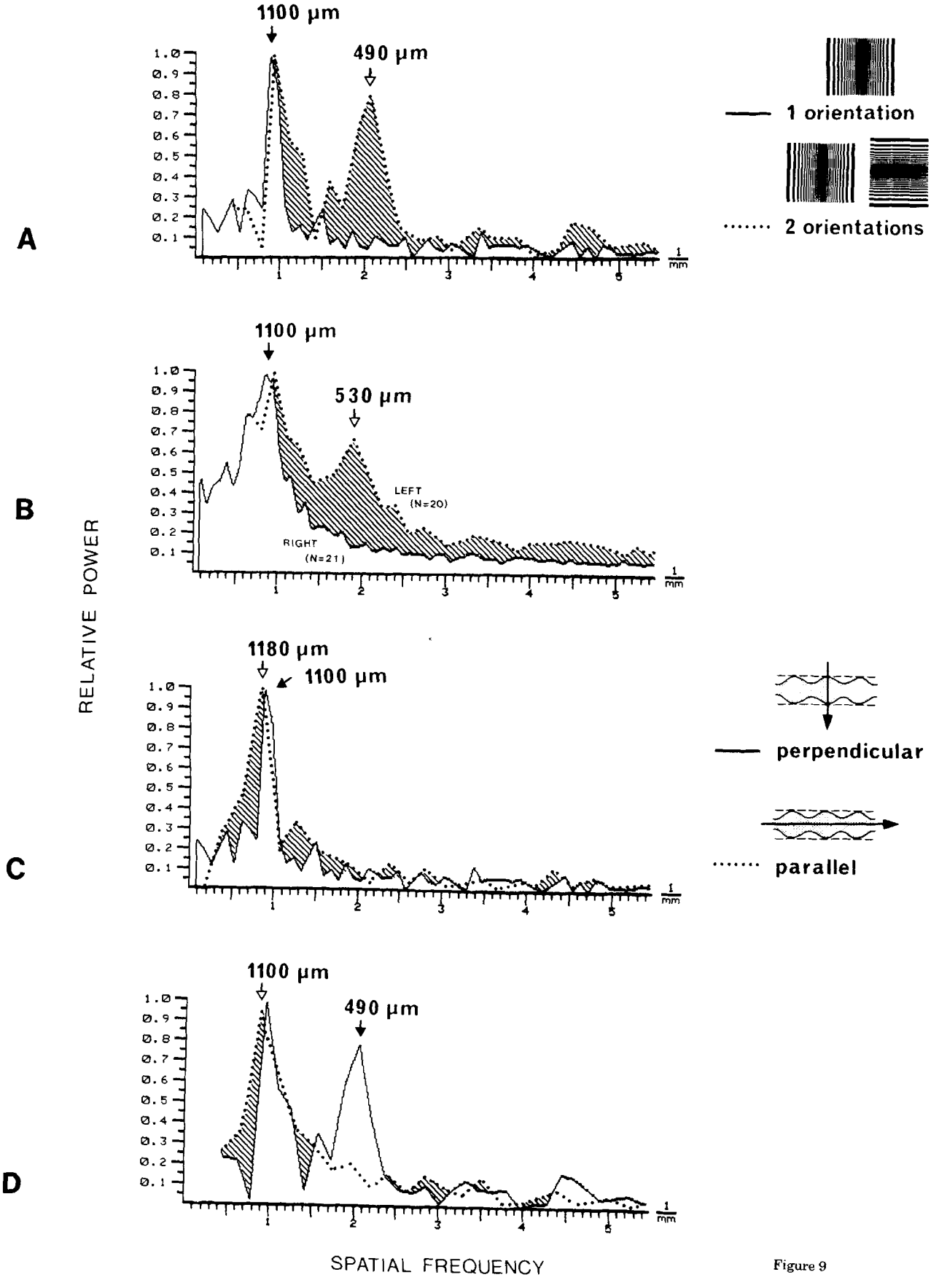
A
1100 µm
490 µm
1 orientation
2 orientations
B
1100 µm
530 µm
LEFT
(N=20)
RIGHT
(N=21)
C
1180 µm
1100 µm
perpendicular
parallel
D
1100 µm
490 µm
RELATIVE POWER
SPATIAL FREQUENCY

Figure 9

posterior parts of area 17. In the former region, the bands quite frequently extended without interruption from the representation of the vertical meridian on the dorsal crest of the lateral gyrus down to the monocular segment in the splenial sulcus. In the posterior part of area 17, while the trajectories of the bands remained orthogonal to the area boundaries, uninterrupted bands extending from boundary to boundary were not observed. We suggest that this is due to the strong curvature of the area 17 boundary in this region, leading to an intermingling of bands with different trajectories. In this context it is interesting to note that fixed relations between area boundaries and columnar trajectories have actually been predicted by theories about the self-organization of columnar systems (Swindale, '82; von der Malsburg and Cowan, '82).

In a recent 2-DG study of cat striate cortex, Albus and Sieber ('84) have argued that the pattern of iso-orientation columns is less regular than had been assumed previously, continuous bands being exceptional. We suggest that this discrepancy with our present results is due to the area-dependent variability in the regularity of the 2-DG patterns. Albus and Sieber ('84) have reconstructed only relatively small parts of the visual cortex and thereby may have underestimated the orderliness inherent in the global pattern.

Another consistent result of the present investigation was that the representation of the area centralis was more lightly labeled than the rest of area 17, iso-orientation bands being less clearly defined in this region. We presume this results from our stimulus configurations being inappropriate for optimal activation of the central area; however, we cannot exclude other factors since we did not vary our stimuli parametrically.

A striking property of the system of orientation columns is the constancy in the center-to-center spacing of adjacent bands. The mean spacing in area 17 was 1.0–1.1 mm and there were no differences between the representations of the upper or lower visual fields nor between area centralis or periphery. This is in agreement with related findings in the tree shrew (Humphrey et al., '80) and saimiri (Tootell et al., '82) and also seems to be the case for other columnar systems such as the ocular dominance columns (Hubel and Wiesel, '68, '69; Shatz et al., '77; Shatz and Stryker, '78), suggesting that the space constants of columnar systems are independent of the cortical magnification factor (Daniel and Whitteridge, '61).

Radial vs. parallel arrangement of iso-orientation bands. Stimulation with either horizontal or vertical or oblique square wave gratings produced 2-DG patterns with essentially the same organizational features. Combined stimulation with two orientations induced a similar pattern, the only major difference being the reduction of the mean distance between adjacent iso-orientation bands by a factor of 0.5. This result is compatible with the hypothesis that stimulation with two orientations activates twice as many parallel orientation bands as stimulation with a single orientation.

Our data thus indicate that cortical regions containing neurons that prefer the same stimulus orientation have the form of elongated slabs that run parallel to each other and that interdigitate with iso-orientation slabs that encode different orientations.

This is in agreement with the results of previous 2-DG studies in cats (Singer, '81) and tree shrews (Skeen et al., '78; Humphrey et al., '80) and with suggestions derived from physiological experiments (Albus, '75a,b) but is at odds with Braitenberg's assumption of a centric organization of orientation hypercolumns. Hence the Braitenberg theory cannot be generalized to cats and tree shrews without substantial modification. Whether it is applicable to the monkey striate cortex—for which it was originally developed—has not yet been tested. It is conceivable that in the monkey the existence of regularly spaced blobs with poorly "oriented" neurons imposes very different constraints on the development of iso-orientation slabs.

Local anisotropies of iso-orientation bands. An interesting feature of the orientation bands is their beaded appearance. In contrast to monkeys, where regularly spaced patches of increased metabolic activity are detectable even without specific visual stimulation (Horton and Hubel, '81; Humphrey and Hendrickson, '83), the beads in the cat visual cortex seem to depend on appropriate stimulation.

This beaded structure of the iso-orientation slabs may have a variety of causes. One possibility is that neurons within the beads are selectively activated by stimulus parameters other than orientation, reflecting the superposition of another columnar system. We consider it unlikely that the system of ocular dominance columns is responsible because all visual stimuli were presented binocularly. In addition the 2-DG patterns remained the same whether the eyes were stimulated alternatingly or simultaneously. Hence, neither eye dominance nor disparity sensitivity seems to be involved in the generation of the beads. Since our stimuli consisted of square wave gratings covering a large range of spatial frequencies, it is also unlikely that the beads reflect spatial frequency columns (Thompson and Tolhurst, '80; Tootell et al., '81). Another possibility is that the beads contain an increased number of bidirectional neurons while the "interbead" zones contain mainly direction-selective cells that would be activated only half as much by our bidirectional stimuli. According to Payne et al. ('80) neurons with the same direction preference are not randomly distributed in the visual cortex but are clustered together (see also Tolhurst et al., '81; Albright et al., '84), the mean tangential distance between such direction clusters being 574 μm. However, our measurements indicate

Fig. 9. One-dimensional Fourier analyses of the spatial organization of the 2-DG patterns in the right and left visual cortex of cat KF10. The x-axis represents spatial frequency in cycles/mm, the y-axis, the relative power of spectral components. A, B. Measurements along vectors perpendicular to the main trajectories of the iso-orientation bands to determine the average band spacing. Differences in the spatial frequencies of the patterns in the right and left visual cortex stimulated with one (solid lines) and two orientation(s) (dotted lines), respectively. The graphs in A represent the Fourier-spectra obtained from single vectors and in B the average of measurements along 21 (solid lines, right hemisphere) and 20 (dotted lines, left hemisphere) parallel vectors. The dominant spatial frequency in the right area 17, stimulated with vertical contours only, is 1,100 μm (solid arrows in A and B). The spatial frequencies in the contralateral hemisphere, stimulated with horizontal and vertical contours, are 1,050 μm (A and B) and 490 μm (open arrow in A) and 530 μm (open arrow in B). Note that the second peak at doubled spatial frequency occurs only after stimulation with two orientations and that there are no differences in the dominant spatial frequencies between single and averaged measurements. C, D. Comparison of band- and bead spacing as revealed by measurements along single vectors perpendicular (solid lines) and parallel (dotted lines) to the iso-orientation bands. C. The principal bead spacing in the single-stimulated right area 17 is 1,180 μm (open arrow) and is of the same order as the band spacing (1,100 μm, solid arrow). D. The principal bead spacing in the contralateral area 17, stimulated with two orientations, is 1,100 μm (open arrow). Note that there is no additional peak at higher spatial frequencies compared to measurements perpendicular to the bands (solid arrow).

that the mean bead spacing is 900–1,200 μm. This makes it unlikely that beads reflect direction selectivity columns.

A further possibility is that beads are related to neurons lacking end-zone inhibition, which constitute about 60% of all orientation-selective neurons in the central part of area 17 (Kato et al., '78; Orban, '84). In this case the less-labeled interbead regions would have to be attributed to orientation-selective end-stopped neurons, which can be expected to respond less well to the elongated contours of our gratings. However, we are not aware of any evidence for a tangential segregation of these two neuronal populations. So far there is only evidence for a differential laminar distribution of the two cell types (Gilbert, '77).

Finally, it is conceivable that the beads reflect cortical regions with an increased number of broadly tuned neurons where more neurons would be activated more strongly than in regions where cells have more elaborate demands for stimulus adequacy. However, so far there are no data supporting the idea of a clustered arrangement of neurons with a different range of selectivity in the cat visual cortex.

Injections of the enzyme horseradish peroxidase (HRP) into the primary visual cortex of tree shrews, cats, and monkeys (Rockland et al., '82; Rockland and Lund, '81, '82, '83; Luhmann et al., '85; Luhmann et al., '86) produces a patchy distribution of label adjacent to the injection site. Rockland and Lund ('83) attribute this pattern to a clustered distribution of neurons possessing reciprocal long-range connections. Recent studies in cats indicate that neurons with similar projection patterns are clustered together and Matsubara et al. ('84) speculate that cells giving rise to intrinsic connections are distributed in a columnar fashion. The beads could, thus, also be related to the patchy distribution of these intracortical connections. If beads were selectively coupled with each other through excitatory tangential connections—and there is recent evidence that these connections form excitatory synapses (Ts'o et al., '86)—then, due to reverberation, neurons within beads might become more active than the cells in interbead regions when a sufficient number of beads are driven by the respective optimal stimulus. Finally, it is also conceivable that intracortical inhibitory mechanisms prevent adjacent cortical regions from being uniformly active. In this case, the half-width of the average bead spacing would reflect the distance beyond which intracortical inhibition is no longer effective. Hess et al. ('75) estimated this distance to be in the range of 500 μm, which would be compatible with our conjecture.

Clearly, our present data do not allow us to draw any firm conclusions on the likely nature of the beads and further experimentation is required to test the predictions derived from the hypotheses formulated above.

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