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Research Note

The pattern of ocular dominance columns in flat-mounts of the cat visual cortex

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Summary. Ocular dominance (OD) columns in the cat visual cortex were visualized with autoradiography after intravitreal injection of (^{3}H)proline. Extending previous studies, a flat-mount technique was applied that enabled the analysis of the distribution of label throughout extensive regions of the visual cortex without requiring reconstructions from serial sections. OD-columns were confined to layer IV and consisted of isolated patches and short bands. The latter were parallel to each other and regularly spaced, the main trajectory being orthogonal to the 17/18 border. This pattern of the geniculo-cortical terminals was similar in the hemispheres ipsi- and contralateral to the injected eye. The mean periodicities of the OD-bands were virtually identical in the two hemispheres of the same animal: 850 μm and 830 μm in cat D1 and 770 μm and 800 μm in cat D2. However, the ipsilateral OD-columns appeared smaller, more heavily labeled and more sharply delineated than the contralateral columns.

Key words: Visual cortex – Ocular dominance columns – Flat-mount – Cat

Introduction

In the visual cortex of cats and monkeys, the geniculo-cortical afferents of the two eyes terminate in alternating regions known as ocular dominance (OD) columns (Hubel and Wiesel 1962, 1969). In the cat, reconstructions of OD-columns have so far been confined to relatively small areas of the visual cortex, leaving a number of questions regarding global features of the OD-columns unanswered (Shatz et al. 1977; Shatz and Stryker 1978; LeVay et al. 1978). Therefore, we combined a newly developed flat-mount technique (Freeman et al. 1987) with autoradiography of transneuronally labeled geniculo-cortical terminals. This allowed us to visualize the complete pattern of OD-columns in area 17 of the cat and to quantify its basic parameters.

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Methods

Two cats, age 10 weeks, were studied in the present experiment. For tracer injection, the animals were anaesthetized with a mixture of ketamine hydrochloride (15 mg/kg) and xylazine hydrochloride (10 mg/kg) i.m.. The right eye was injected intravitreally with 2.5 mCi (^{3}H)proline (injected volume 50 μl in cat D1 and 25 μl in cat D2). After a survival time of two weeks, the animals were sacrificed by an overdose of Nembutal, i.v., the occipital poles of the brain were removed, and the visual cortices were flat-mounted, prior to freezing the tissue on dry ice (technique described in detail in Freeman et al. 1987; see also Löwel et al. 1987). To provide landmarks for later superposition, three holes were melted in the flat-mounts with warm needles. Subsequently, 26 μm thick serial cryostat sections were cut parallel to the cortical surface. The sections were mounted on glass slides, immediately dried on a hot plate, and postfixed in 4% formaldehyde. They were then exposed to LKB-Ultrofilm for 8 weeks. The X-ray films were photographed and magnified (× 4.2) on photographic paper. Single autoradiographs were further analyzed with a digital image processing system (Imago II, Compulog). The density distributions of the films were coded in digital units, ranging from 1 (absolute white) to 256 (absolute black). The average columnar spacing was determined by one-dimensional Fourier analysis along vectors perpendicular to the main orientation of OD-bands (pixel distance was 50 μm at the magnification used; uneven illumination of the autoradiographs was corrected by high-pass filtering of each digitized image). In addition, the optical densities were measured along the same vectors (pixel distance was 20 μm; uneven illumination was corrected by calculating the ratios of the digitized images of the autoradiograph and of an empty piece of film respectively).

Results

After injection of (^{3}H)proline into one eye, area 17 and some extrastriate areas were labeled in both

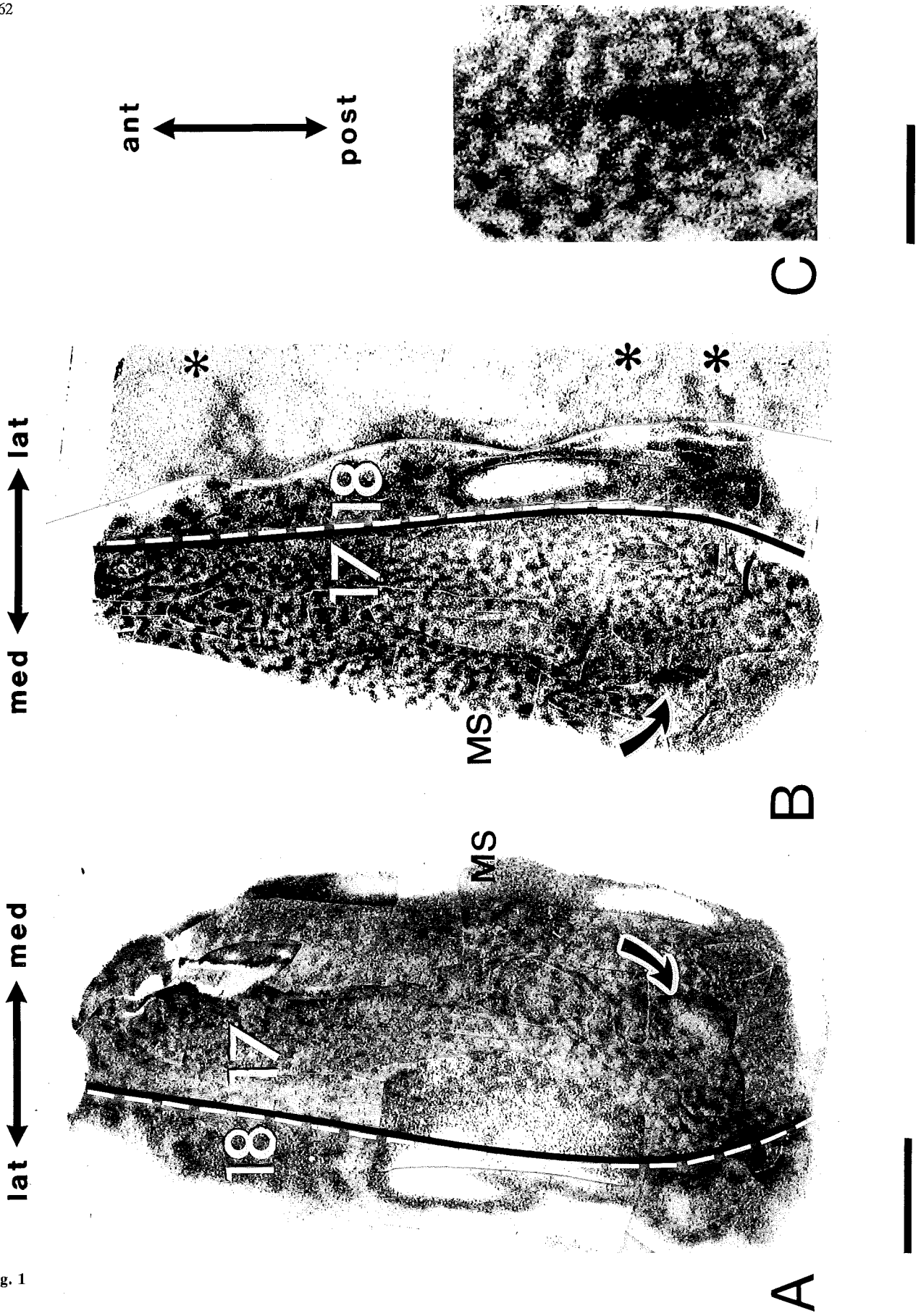

Fig. 1

hemispheres. The radioactive label was restricted to a tissue layer that was abut 350 μm thick (comprising 12–14 serial flat-mount sections) and located in a depth of 550 to 900 μm from the cortical surface, known from earlier work to represent layer IV. Despite the flat-mounting, single sections never covered the entire extent of the labeled layer. Therefore, photomontages were made from 10 to 15 adjacent sections in order to visualize the complete pattern of the terminal labeling. OD-columns of the right (ipsilateral) and left (contralateral) visual cortex of cat D1 are illustrated in Fig. 1. The labeled geniculo-cortical terminals appear as isolated patches, elongated slabs, and bands that extend over short distances. The latter are parallel to each other and roughly orthogonal to the 17/18 border. The optic disc representations are identifiable in both hemispheres as demarcated oval regions which consist of a solid patch of label on the ipsilateral and of a pale nearly label-free region on the contralateral side of the injection. They measure about 2.6 mm × 1.0 mm and have their long axis roughly parallel to the sagittal plane. OD-bands have the tendency to intersect the lateral and medial borders of the optic disc representation at right angles (see Fig. 1C). The representations of the areae centrales were distinguishable because the central 2–3° of area 17 (according to the cortical map of Tusa et al. 1978) were more lightly labeled than peripheral regions. The monocular segment is indicated by uniform labeling at the medial border of the contralateral area 17 and by the absence of labeling at comparable eccentricity on the ipsilateral side.

The comparison of the labeling in the two hemispheres reveals that the overall pattern is quite similar on both sides. In order to obtain a quantitative measure for the space constants of the OD-patterns, one-dimensional Fourier analyses were performed along vectors perpendicular to the orientation of the OD-bands. As indicated by the Fourier-spectra in Figs. 2A, B, the peak spatial frequencies of the bands are very similar in the two hemispheres of the same animal: 850 μm and 830 μm in cat D1 (Fig. 2A) and 770 μm and 800 μm in cat D2 (Fig. 2B).

However, the contrast of the pattern is higher on the ipsilateral than on the contralateral side and the ipsilateral OD-columns appear smaller and more sharply delineated than the contralateral columns. In order to quantify this difference, we measured the optical densities in the autoradiographs of both hemispheres along vectors perpendicular to the main orientation of the OD-bands (Figs. 2C, D). These measurements show that on the contralateral side intercolumnar spaces are slightly darker and columns lighter than on the ipsilateral side. The average difference in the grey level between regions of minimal and maximal labeling is 26 (s.d. = 7) in the ipsilateral and 17 (s.d. = 3) in the contralateral hemisphere of cat D1. The respective values in cat D2 are 28 (s.d. = 7) and 23 (s.d. = 7). In both cats, these interhemispheric differences in the optical densities are statistically significant (T-test, $p < 0.005$). The ratios of the contrast between ipsi- and contralateral hemispheres are 1.5 : 1 in cat D1 and 1.2 : 1 in cat D2.

The comparison of the waveform of the optical density distributions (Fig. 2C, D) implies that the ipsilateral eye dominance columns are smaller and more sharply delineated than their contralateral counterparts. This is in agreement with the finding that 40% of layer IV of the ipsilateral hemisphere was occupied by dense patches of label compared to 53% on the contralateral side (Shatz and Stryker 1978). These anatomical differences are likely to reflect the physiological dominance of the contralateral eye (Hubel and Wiesel 1962; Blakemore and Pettigrew 1970; Albus 1975; Shatz and Stryker 1978).

(^{3}H)proline labeling in non-striate areas

Radioactive label was also transported to area 18 and to a cortical strip about 4 mm lateral to the 17/18 border. In area 18, OD-columns appeared as isolated patches and bands that are more widely spaced than in area 17. However, because it is difficult to determine the exact location of the 17/18 border in flat-mounts, we did not quantify the mean periodicity of the bands in area 18.

Fig. 1. A, B Reconstruction of the (^{3}H)proline labeled OD-columns in layer IV of the visual cortices of cat D1, contralateral (left, **A**) and ipsilateral (right, **B**) to the eye injection. Exposure time of the autoradiographs: 8 weeks. The contrast of the labeling in the region around the contralateral area centralis (**A**) was enhanced photographically. The optic disc representations (indicated with arrows) appear in the posterior third of area 17 as oval regions. The approximate location of the 17/18 border is indicated by the white broken line. (^{3}H)proline labeling lateral to area 18 is illustrated in **B** (indicated with asterisks). Note that we again enhanced the contrast photographically in order to demonstrate the very faint labeling. MS = monocular segment. **C** Detail of the pattern of OD-bands in layer IV of the ipsilateral (right) hemisphere of cat D2. The solidly labeled patch is the representation of the contralateral eye's optic disc. Calibration bar for **A, B** = 5 mm, for **C** = 2.5 mm

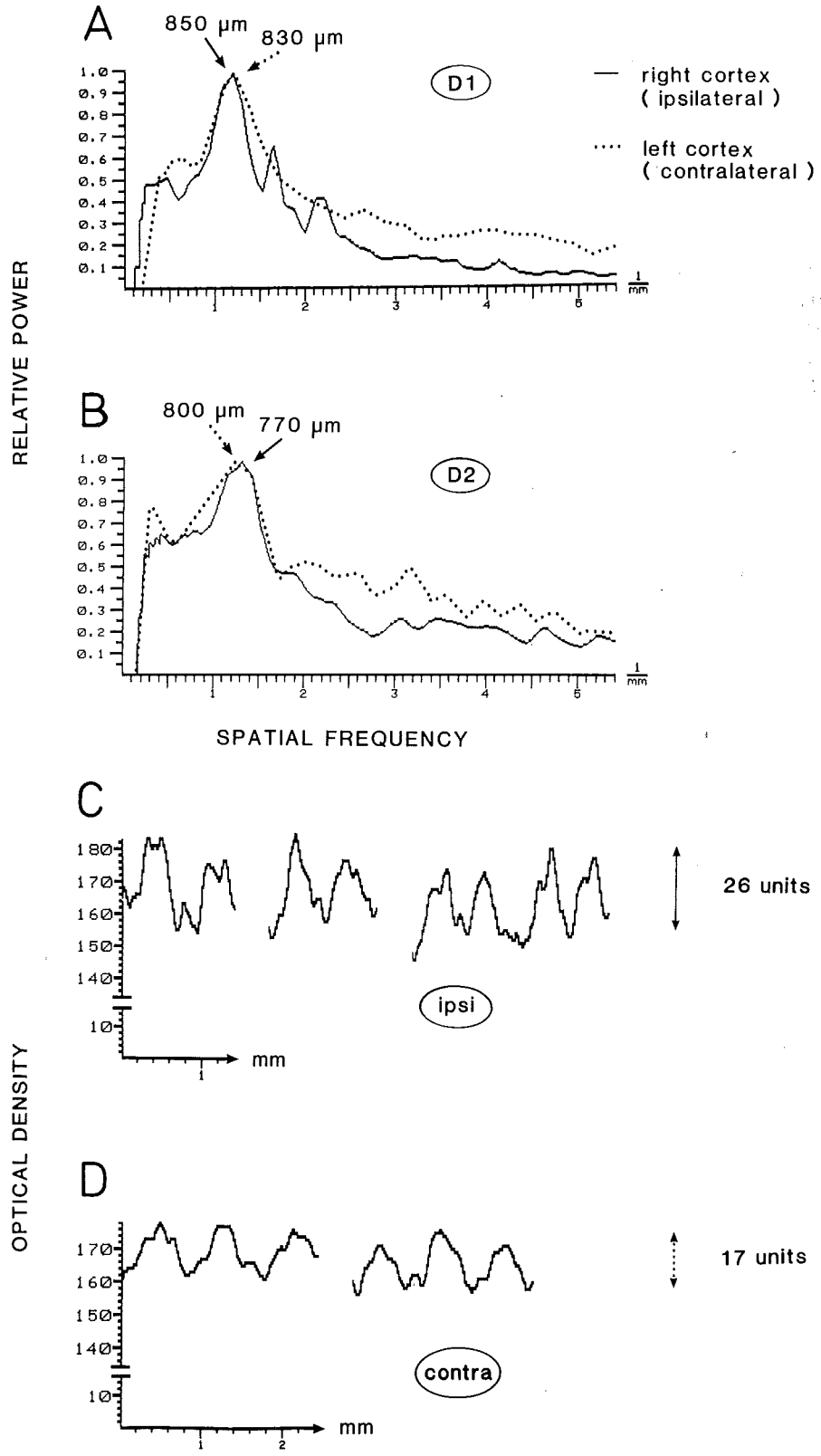

Fig. 2A–D. Computer-aided measurements of the spatial frequency spectrum and optical density distribution in the (^{3}H)proline autoradiographs. **A, B** One-dimensional Fourier analysis of the spatial organization of the OD-bands in cats D1 (**A**) and D2 (**B**). The x-axis represents the spatial frequency in cycles/mm, the y-axis the relative power of spectral components. Each graph represents an average of measurements along several parallel vectors perpendicular to the main trajectories of the OD-bands. The average spacing of adjacent bands in the right and left visual cortex is 850 µm and 830 µm in cat D1 and 770 µm and 800 µm in cat D2. **C, D** Measurements of the optical densities in the ipsilateral (**C**) and contralateral (**D**) hemisphere of cat D1. Higher optical densities represent higher (^{3}H)proline labeling. The average difference in the optical densities (difference between minimal and maximal labeling) is 26 units in the ipsilateral (**C**) and 17 units in the contralateral (**D**) hemisphere

The labeling lateral to area 18 was very faint and showed considerable interindividual and interhemispheric variability. In the ipsilateral visual cortex of cat D1, it consisted of a few bands running roughly parallel to the frontal plane (see Fig. 1B) and of several isolated patches.

Discussion

Our results confirm that OD-columns in the cat striate cortex are organized in a system of regularly spaced patches and bands that run roughly perpendicular to the 17/18 border (Shatz et al. 1977; Shatz and Stryker 1978; LeVay et al. 1978) and differ in optical density in the hemispheres ipsi- and contralateral to the injected eye (Shatz et al. 1977). By using a flat-mount technique, we were able to visualize the OD-pattern over the whole extent of striate cortex. This revealed that the overall pattern and the periodicity of the OD-bands in area 17 are very similar in the two hemispheres of the same animal. In contrast to the conditions in the monkey striate cortex (LeVay et al. 1985), we found no increase of band spacing with increasing eccentricity.

In all animals, there was a tendency of the areae centrales representations to be less heavily labeled than the periphery. We consider it unlikely that this is due to less dense innervation. While it is true that a disproportionally large surface area of visual cortex is devoted to the central portion of the visual field (Tusa et al. 1978), retinal ganglion cell density is also highest in that region (Stone 1965). Electrophysiological mapping studies suggest that the expansion of cortical representation actually reflects ganglion cell density (Tusa et al. 1978), implying that every ganglion cell innervates the same number of cortical neurons irrespective of its retinal position.

Another possibility is that retinal ganglion cells in the area centralis incorporated less (^{3}H)proline. If the amount of tracer available for a given retinal locus is constant, then proportionally less label would be accessible to the more densely packed ganglion cells in the central retina. Moreover, the ganglion cell layer in the area centralis consists of a double layer of cells (Ganser 1882; Stone 1965) and therefore is likely to hamper diffusion of the tracer to ganglion cells located in the 'deeper' layer. The size of this area is about 1.0 mm in diameter which corresponds to a visual angle of 2.5° (Peichl and Wässle 1979). This in turn fits well with the area of the less heavily labeled cortical region which extended up to an eccentricity of about 2–3°.

Interesting features of cortical organization are reflected in the shape and position of the optic disc representation. Whereas the blind spot in the retina is approximately circular, its cortical representation is elongated in an anterior-posterior direction. This suggests that the cortical representation of the retina is expanded perpendicular to the OD-bands. A recent 2-deoxyglucose mapping study in the cat visual cortex (Löwel et al. 1987) supports this notion. There it was demonstrated that relatively more cortical tissue is devoted to the representation of meridians parallel to the 17/18 border (one cortical representation of the vertical meridian). A similar anisotropic representation of the optic disc was observed in the primate striate cortex (LeVay et al. 1985).

The locations of the representations of the areae centrales and the optic discs point to a second anisotropy in the cortical map: as in the monkey striate cortex, relatively more cortical area seems to be devoted to the representation of the lower than to that of the upper quadrant (Tusa et al. 1978; Van Essen et al. 1984).

Furthermore, the present study also demonstrates for the first time that a band-like (^{3}H)proline labeling pattern exists in extrastriate areas lateral to area 18. On the basis of the cortical maps of Tusa and Palmer (1980) and Palmer et al. (1978) we refer to the observed labeling as being located primarily in area 19, possibly extending into areas 21a and/or the ventral lateral suprasylvian area (VLS). We consider it unlikely that the label resulted from further transneuronal transport from area 17 because it was restricted to layer IV and not visible in supragranular layers where cortico-cortical projections arise and terminate. Therefore the band-like labeling is likely to represent the termination pattern of afferents from the C-laminae or the medial intralaminar nucleus (MIN) of the lateral geniculate nucleus (LGN) (Heath and Jones 1971; LeVay and Gilbert 1976). Whether it represents OD-columns in extrastriate regions or is due to geniculate input alternating with extrageniculate input (Lee et al. 1985) can only be determined with further experimentation.

Finally, the comparison of the OD-columns in area 17 with the columnar system of orientation preferences reveals that the overall pattern of the former is much less regular than that of the latter: parallel bands do exist but they are visible only over short distances whereas iso-orientation bands are highly regular (Löwel et al. 1987). These conditions are reversed in the monkey striate cortex, where the OD-system shows a higher degree of order. In cats and monkeys, the average columnar spacing differs in the two functional systems. It is interesting to note that in both animals the system with larger space constants (the lower spatial frequency content) is the more regular one; the iso-orientation bands in the cat and the ocular dominance bands in the monkey.

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