Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 2621–2623, March 1998
Neurobiology

Unequal representation of cardinal and oblique contours in ferret visual cortex

DAVID M. COPPOLA, LEONARD E. WHITE, DAVID FITZPATRICK, AND DALE PURVES*

Department of Neurobiology, Box 3209, Duke University Medical Center, Durham, NC 27710

Contributed by Dale Purves, December 19, 1997

ABSTRACT **We have measured the amount of cortical space activated by differently oriented gratings in 25 adult ferrets by optical imaging of intrinsic signal. On average, 7% more area of the exposed visual cortex was preferentially activated by vertical and horizontal contours than by contours at oblique angles. This anisotropy may reflect the real-world prevalence of contours in the cardinal axes and could explain the greater sensitivity of many animals to vertical and horizontal stimuli.**

When the influence of orientation is tested behaviorally in humans and a variety of other species, visual performance is typically better in response to vertical and horizontal stimuli than to stimuli presented obliquely (1, 2). In humans, grating acuity, the perception of Landolt-C test objects, and fine-line acuity are all improved when measured with vertical and horizontal contours compared with oblique ones. Other aspects of visual performance that are enhanced when measured with vertical and horizontal stimuli include judgments of line orientation, estimation of stimulus position, reaction time, perceptual group matching and learning tasks, stimulus discrimination and generalization, and the perception of a variety of optical illusions.

Although the neurobiological basis of this bias (usually referred to as the "oblique effect") is not clear, one possibility is that more neural machinery is devoted to processing vertical and horizontal contours than to processing oblique ones. Because processing oriented information involves orientation-selective cells in the visual cortex that are clustered in domains called orientation columns (3–6), this possibility can be assessed by both physiological and anatomical means. For instance, it has been reported that somewhat more neurons in the lateral geniculate nucleus and visual cortex of both cats and monkeys respond preferentially to vertical and horizontal orientations than to oblique stimuli (7–12). Moreover, stimuli in the cardinal axes evoke larger cortical potentials measured with surface or depth electrodes than do oblique stimuli (13–16). Given the columnar arrangement of orientation-selective cells in the visual cortex, these findings imply that more cortical space is allocated to processing vertical and horizontal stimuli than to processing oblique contours.

To test this idea, we examined the visual cortex of 25 adult ferrets by optical imaging of intrinsic signal, a technique that has been used to demonstrate the map of orientation preference in the visual cortex of a variety of species (17–21).

MATERIALS AND METHODS

Twenty-five ferrets (all older than postnatal day 45) were anesthetized and secured in a modified stereotaxic frame that left the animal's field of view unobstructed. During surgery, anesthesia was maintained with 2–3% isoflurane in a 2:1 mixture of nitrous oxide and oxygen delivered via a tracheotomy; expired carbon dioxide and the electrocardiogram were monitored continuously. Expired carbon dioxide levels were kept near 4.0% by adjusting ventilation parameters, and body temperature was maintained at 37.5°C by a thermostatically controlled heating blanket. A craniotomy was performed over the left occipital region and, after making an aperture in the dura, a stainless steel chamber with a glass window was cemented to the skull and filled with normal saline or 50 cs silicone fluid (Boss Products, Elizabethtown, KY). After iris dilation with ophthalmic atropine, contact lenses were placed on the corneas to prevent drying and to focus the eyes on the computer monitor used for stimulus presentation.

When combined with digital image analysis, optical imaging provides a means of measuring the amount of visual cortex devoted to the analysis of different stimulus orientations (Fig. 1). To acquire such images, the isoflurane concentration was reduced to 0.75–1.0% to facilitate the activation of cortical circuits by visual stimulation, and a paralytic agent (vecuronium bromide; 0.2 mg/kg per h, i.v.) was administered to prevent eye movements. The imaging methods were similar to those described by Bonhoeffer and Grinvald (19, 22) and are more fully characterized elsewhere (21, 23). In brief, optical imaging of intrinsic signals was accomplished by using an enhanced video acquisition system (Optical Imaging, Germantown, NY) consisting of a tandem lens macroscope attached to a low-noise video camera. The cortical surface was illuminated with red light (≈700 nm), and the recorded signal passed through an analog video-enhancement amplifier and was digitized and stored for further processing by software provided by the manufacturer. A separate stimulus computer (386 PC with SGT+ graphics board and STIM software provided by Kaare Christian, Optical Imaging, Germantown, NY) was used to present high-contrast rectangular wave gratings (6.25° dark phase, 1.25° light phase); the animal viewed the stimulus monitor binocularly at a distance of 29 cm. Four gratings oriented at 0°, 45°, 90°, and 135° with respect to horizontal were used; each panned back and forth at a constant rate (15–30 degree/sec) along the axis orthogonal to its orientation. Typically, data were acquired during two sets of 20 consecutive stimulus trials: one set of trials consisted of a randomized presentation of a pair of orthogonal gratings (i.e., 0°/90° or 45°/135°); the other set consisted of a similar presentation of the remaining pair. The summed images acquired during the presentation of one grating were then subtracted from the summed images acquired during the presentation of the orthogonal grating to create differential maps of orientation preference (i.e., difference images) for each set of trials. Thus, two difference images, which measured 655 × 480 pixels at a scale of 75 pixels per millimeter, were generated from each stimulus pair, making a total of four difference images (i.e., 0°-90°, 45°-135°, 90°-0°, and 135°-45°) per experiment. The difference images were smoothed by using an 11 × 11 pixel

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*To whom reprint requests should be addressed. e-mail: purves@neuro.duke.edu.

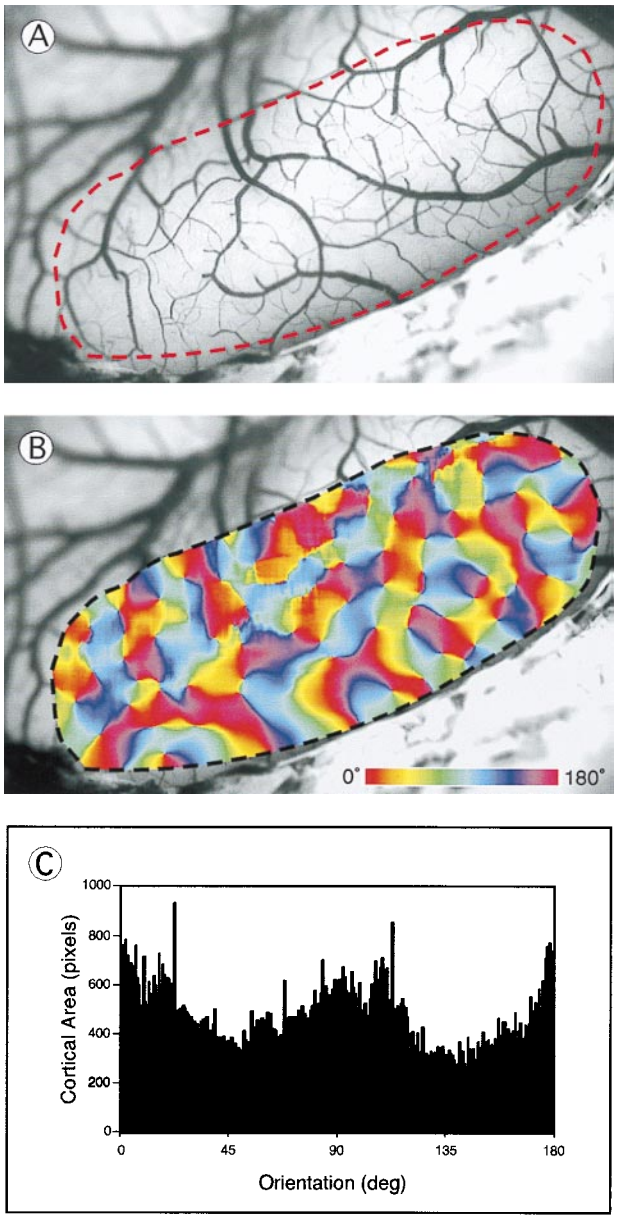

FIG. 1. Optical imaging and analysis of orientation preference maps in the ferret visual cortex. (*A*) Image of the exposed region of the visual cortex in ferret 22 (see Fig. 2), showing typical appearance of the cortical surface (dotted line indicates the region analyzed in *B* and *C*). (*B*) Orientation preference map of the cortical regions responding best to different stimulus orientations (bar shows the orientation color code; bar length = 3 mm). (*C*) Histogram of the region of interest (see dotted line in *A*), showing the number of pixels (i.e., the area of cortex) that responded best to each orientation. Note the peaks near the vertical and horizontal meridians, which indicate that more cortex responded best to contours in the cardinal axes compared with those at oblique angles.

mean filter kernel; the low-frequency noise was reduced by convoluting the image with an 80×80 pixel mean filter kernel and subtracting the result from the original image. Finally, vector summation of the four difference images was done on a pixel-by-pixel basis to create a color-coded orientation preference map. For some animals, four additional angles (22.5°, 67.5°, 112.5°, and 157.5°) were also employed, and difference images were generated by dividing the summed images acquired during the presentation of one grating by the summed images acquired during the presentation of all eight gratings (termed an "orientation cocktail"). The resulting difference images (eight in number) were processed as described above; orientation preference maps were generated in the same manner. Before collapsing the data for statistical comparison of near-cardinal and near-oblique angles, we assessed the overall relationship between preferred orientations and amount of related cortical space (see Figs. 1*C* and 2*A*).

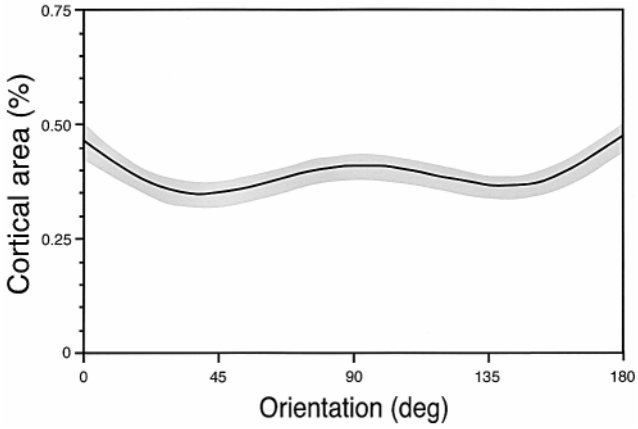

FIG. 2. Amount of cortical space devoted to the analysis of different stimulus orientations. The overall profile of orientation versus cortical area: the black line is a polynomial fit to the raw pixel data combined from 25 animals; the gray band represents a similar fit to the mean ± SEM. The smooth profile indicates that we were not undersampling the cortex because of the small number of stimulus angles used.

RESULTS

A polynomial fit to the mean orientation profile versus cortical area of all 25 ferrets showed smooth peaks around the cardinal axes, with troughs centered near the oblique angles (Fig. 2). This profile of the entire sample of ferrets suggested that more cortex is devoted to processing cardinal than oblique orientations. To confirm this point, we pooled the angles within ±22.5° of the cardinal and oblique axes (Fig. 3). On average, 7.2% more cortical area responded preferentially to cardinal compared with oblique angles (Table 1). Not only did this average difference reach a high level of statistical difference, but all 10 animals with the greatest disparity in cortical area responding to the different angles (>10%) favored the cardinal axes. These differences are consistent with the results of a recent study by Chapman and Bonhoeffer (24). Finally, the

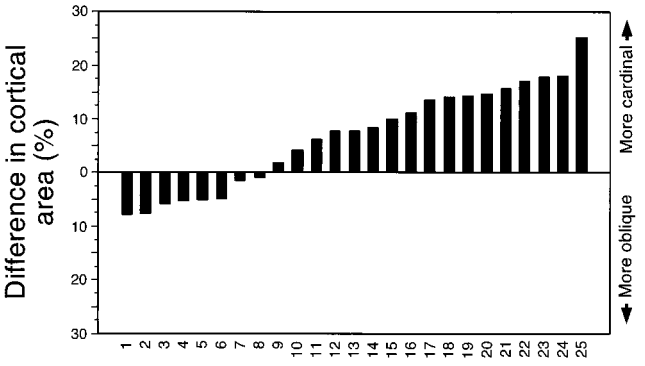

FIG. 3. Percentage difference in the amount of cortical area responding best to cardinal (0° and 90°) and oblique (45° and 135°) angles for each of the 25 animals examined. Data from the angle plot histograms (Fig. 1*C*) were collapsed around each orientation (±22.5°). Bars show the deviation from the zero line, which represents an equal distribution of cortical space to all orientations. Seventeen of the 25 ferrets examined showed a greater amount of cortex responding best to the cardinal axes.

Table 1. Combined results (means ± SEMs) from the orientation histograms obtained in 25 ferrets (see Fig. 1*C*), collapsed around each of the four axes of interest (±22.5°)

Axes	Absolute cortical area, mm²	% of cortical area	*t* value; *P* value*
Cardinal (0° + 90°)	9.7 ± 0.35	53.6 ± 0.86	3.64; $P < 0.002$
Oblique (135° + 45°)	8.5 ± 0.38	46.4 ± 0.86	
Horizontal (0°)	5.0 ± 0.18	27.7 ± 0.51	4.15; $P < 0.003$†
Vertical (90°)	4.7 ± 0.17	25.9 ± 0.50	
Left oblique (135°)	4.3 ± 0.19	23.7 ± 0.48	2.76; $P > 0.05$†
Right oblique (45°)	4.1 ± 0.19	22.7 ± 0.51	

*Determined by paired *t*-test.
†Because these are posthoc tests, the *P* values have been multiplied by the total number of statistical tests undertaken.

amount of cortex responding preferentially to horizontal and vertical gratings was not equal. On average, 2% more cortex preferred horizontal than vertical orientations, with 20 of the 25 ferrets showing a bias in this direction; this posthoc comparison also reached a high level of statistical significance (see Table 1).

The validity of the cortical anisotropy we report depends on ruling out alternative (artifactual) sources of bias in the orientation preference maps. One concern was that the characteristics of the monitor and/or the aspect ratio of the rectangular screen could have produced differences in the strength of cardinal and oblique stimuli; any such differences would have induced a spurious disparity in the cortical response. Five experiments in four different ferrets therefore were done to determine the cortical response when the stimulus monitor was in an upright position and when it was rotated 135° to interchange cardinal and oblique orientations with respect to the position of the animal. The variation in cortical area preferring cardinal and oblique stimuli in these two conditions was not significantly different from that seen in identical experiments repeated at different times in the same animal with the monitor upright. In two further experiments, a circular aperture was placed over the computer screen to eliminate orientation-specific differences in bar length; again, the variation with this reconfiguration of the stimulus was not different from that seen with repeated observations in the same animal. Taken together, these controls indicate that the bias we found in the representation of cardinal and oblique orientations in the visual cortex cannot be attributed to differences in the efficacy of the different stimulus gratings. Finally, because the intrinsic signal in optical imaging derives, in part, from the ratio of deoxygenated to oxygenated hemoglobin, the subarachnoid blood vessels themselves can generate a strong artifact. As a further control, therefore, we masked out the major blood vessels in three experiments by an image-thresholding technique to subtract any contribution they might make. Again, there was no evidence for a systematic bias in the optical signal attributable to the major blood vessels.

DISCUSSION

These observations indicate that appreciably more circuitry in the ferret visual cortex is devoted to processing contours oriented in the cardinal axes than to oblique contours, a bias in the mature visual cortex that accords with the early emergence of orientation domains that respond preferentially to cardinal stimuli (25).

This finding provides another example of visual system anisotropy that apparently has its origins in the properties of the world itself—in this case the preponderance of cardinal contours dictated by gravity and other ubiquitous influences (ref. 26; D.M.C., H. R. Purves, A. McCoy, and D.P., unpublished data). A bias favoring vertical and horizontal orientations is also evident in the astigmatic optics of the eye of some species, which ensures that contours in the cardinal axes are in sharper focus than oblique contours (27, 28), and in the distribution of retinal ganglion cells and their primary dendrites in carnivores and other mammals, which are more densely arrayed along the vertical and horizontal meridians (29–31). By extending this bias favoring the cardinal contours to the modular organization of visual cortex, our results suggest that the oblique effect may be the consequence of a greater devotion of visual system circuitry to the analysis of contour information in the cardinal axes.

1. Appelle, S. (1972) *Psychol. Bull.* **78,** 266–278.
2. Howard, I. P. (1982) *Human Visual Orientation* (Wiley, New York).
3. Hubel, D. H. & Wiesel, T. N. (1968) *J. Physiol. (London)* **195,** 215–243.
4. Hubel, D. H. & Wiesel, T. N. (1974) *J. Comp. Neurol.* **158,** 267–294.
5. Hubel, D. H. & Wiesel, T. N. (1977) *Proc. R. Soc. Lond. B* **198,** 1–59.
6. Hubel, D. H. (1988) *Eye, Brain, and Vision* (Freeman, New York), Scientific American Library Series.
7. Pettigrew, J. D., Nikara, T. & Bishop, P. O. (1968) *Exp. Brain. Res.* **6,** 373–390.
8. Mansfield, R. J. W. (1974) *Science* **136,** 1133–1134.
9. Leventhal, A. G. & Hirsch, H. V. B. (1980) *J. Neurophysiol.* **43,** 1111–1132.
10. Blakemore, C. B., Garey, L. J. & Vital-Durand, F. (1981) *J. Physiol. (London)* **319,** 78P.
11. Berman, N., Payne, B. R., Garcia-Kennedy, R. & Murphy, E. H. (1981) *Invest. Ophthalmol. Visual Sci.* **20,** 147 (abstr.).
12. Orban, G. A. & Kennedy, H. (1981) *Brain Res.* **208,** 203–208.
13. Campbell, F. W. & Maffei, L. (1970) *J. Physiol. (London)* **207,** 635–652.
14. Mansfield, R. J. W. & Ronner, S. F. (1978) *Brain Res.* **149,** 229–234.
15. Bonds, A. B. (1982) *Exp. Brain Res.* **46,** 151–154.
16. Bonds, A., Casagrande, V. A., Norton, T. T. & DeBruyn, E. J. (1987) *Visual Res.* **27,** 845–857.
17. Grinvald, A., Lieke, E., Frostig, R. D., Gilbert, C. D. & Wiesel, T. N. (1986) *Nature (London)* **324,** 361–364.
18. Grinvald, A. (1991) *Brain Topography* **5,** 71–75.
19. Bonhoeffer, T. & Grinvald, A. (1991) *Nature (London)* **353,** 429–431.
20. Obermayer, K. & Blasdel, G. G. (1993) *J. Neurosci.* **13,** 4114–4129.
21. Bosking, W. H., Zhang, Y., Schofield, B. & Fitzpatrick, D. (1997) *J. Neurosci.* **17,** 2112–2127.
22. Bonhoeffer, T. & Grinvald, A. (1996) in *Brain Mapping: The Methods*, eds. Toga, A. W. & Mazziotta, J. C. (Academic, New York), pp. 55–97.
23. Weliky, M., Bosking, W. H. & Fitzpatrick, D. (1996) *Nature (London)* **379,** 725–728.
24. Chapman, B. & Bonhoeffer, T. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 2609–2614.
25. Chapman, B., Stryker, M. P. & Bonhoeffer, T. (1996) *J. Neurosci.* **16,** 6443–6453.
26. Switkes, E., Mayer, M. J. & Sloan, J. A. (1978) *Vision Res.* **18,** 1393–1399.
27. Shilo, W. (1977) *Documenta Ophthalmologica* **44,** 403–419.
28. Charman, W. N. & Voisin, L. (1993) *Ophthal. Physiol. Opt.* **13,** 3–11.
29. Wassle, H., Levick, W. R. & Cleland, B. G. (1975) *Exp. Brain Res.* **37,** 475–494.
30. Hughes, A. (1977) in *The Visual System in Vertebrates*, ed. Crescitelli, F. (Springer, Heidelberg), pp. 613–756.
31. DeBruyn, E. J., Wise, V. L. & Casagrande, V. A. (1980) *Vision Res.* **20,** 315–327.