Differential Imaging of Ocular Dominance and Orientation Selectivity in Monkey Striate Cortex

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Differential images of ocular dominance, acquired by comparing responses to the two eyes, reveal dark and light bands where cortical cells are dominated by the right and left eyes. These include most (but not all) histochemically stained cytochrome oxidase blobs in their centers. Differential images of orientation, acquired by comparing responses to orthogonal orientations, reveal dark and light bands that are reminiscent of the “orientation columns” reported earlier, on the basis of 2-deoxyglucose (2DG) autoradiograms (Hubel et al., 1978). However, they are shorter and more fragmented because they do not include regions lacking selectivity for orientation. Even though these “bands” derive from orientation-selective areas, comparisons with differential images of other orientations reveal that regions along their centers prefer different orientations. Hence, the orientation preferences inferred from “bands” in single differential images, or single 2DG autoradiograms, are not necessarily incorrect.

Interactions between ocular dominance and orientation were investigated by comparing differential images of orientation obtained with binocular and monocular stimulation, as well as by comparing differential images of ocular dominance obtained with different orientations. In both cases, the elicited interactions were minimal, indicating a remarkable and unexpected independence that subsequent experiments revealed arises, at least in part, from a lateral segregation of regions most selective for one eye and regions most selective for one orientation, in the centers and edges of ocular dominance columns. Since this can also be viewed as a lateral correlation between binocularity and orientation selectivity, it fits with the simultaneous emergence of these properties in layers receiving input from layer 4c, and suggests that each of these properties requires the other.

Visual perception proceeds at least in part through lateral rearrangements in the representation of visual space that take place, in many steps, as visual information passes sequentially through layers in the retina, the lateral geniculate nucleus (LGN),

and striate cortex. In the retina these transformations lead to center-surround receptive fields that accentuate responses to edges, while minimizing responses to diffuse light (Kuffler, 1953). In striate cortex they lead to binocularity and orientation selectivity—two response properties that are thought to be important for stereopsis and contour perception. Although neither binocularity nor orientation selectivity is understood entirely, both response properties are known to appear first in striate cortex, between laminae specialized for input from the LGN and laminae specialized for output to other cortical areas. Both properties are also known to be organized laterally in stereotyped and highly repetitive ways (Hubel and Wiesel, 1974a,b; Wiesel et al., 1974; LeVay et al., 1975; Rockland and Lund, 1983; Van Essen, 1984).

Binocularity results from the convergence of inputs from both eyes onto single cells. In old-world primates this occurs in striate cortex just after layer 4c, where most LGN afferents terminate. Even though this property emerges vertically, between layers, its most striking organization occurs laterally, in the emergence of strikingly regular ocular dominance domains. Superimposed on the binocularity of most cells is a tendency to prefer one eye, which Hubel and Wiesel (1962, 1965) referred to as “ocular dominance.” This preference is aligned vertically, overlapping in layer 4c with afferents from the favored eye. Since these afferents are arranged laterally in 0.4-mm-wide bands (see Fig. 1a), the regions dominated by each eye resemble slabs, as illustrated schematically in Figure 1b (Hubel and Wiesel, 1972; LeVay et al., 1975; Blasdel and Lund, 1983).

Orientation selectivity refers to the preference of most cortical cells for edges at certain orientations. Like ocular dominance, orientation selectivity appears for the first time in cells receiving input from layer 4c. It is characterized by two parameters, preference and selectivity, which refer to the orientation generating the strongest response, and to the range of effective orientations, respectively. Like ocular dominance, orientation preferences are aligned vertically and organized laterally. During lateral electrode penetrations, they rotate linearly with distance, as illustrated in Figure 2a, but only over short intervals because the linearity is broken intermittently by sudden reversals in direction and by discontinuous shifts. Hubel and Wiesel (1974a,b), who made these observations initially, reasoned that they implied an organization of rectilinear slabs, like those for ocular dominance (Hubel and Wiesel, 1972), and suggested a sheet of thin parallel slabs preferring slightly rotated orientations, as illustrated in Figure 2b. They were unable to demonstrate this conclusively however, with techniques available to them at the time (Hubel and Wiesel, 1963; Hubel et al., 1978; Mitzdorf and Singer, 1979).

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Figure 1. Organization of ocular dominance in monkey striate cortex. *a*, Ocular dominance bands from tangential sections of a monkey's right occipital lobe, visualized by the reduced silver method of Liesegang LeVay et al. (1975). Alternate bands have been inked in. From Hubel and Freeman (1977). *b*, Cells in layer 4c respond exclusively to the eye providing input. Since cells lying above one another tend to prefer the same eye, and since inputs from the two eyes are arranged as bands (see above), Hubel and Wiesel (1972) deduced that cells dominated by one eye or the other are organized as slabs, running perpendicularly between pia and white matter. *c*, While the illustration in *b* is valid, in the narrow sense of which eye is dominant, it ignores binocularity in the other layers. Because inputs from the two eyes converge outside layer 4c, and cells because the other layers respond well to either eye alone, it is more accurate to illustrate ocular dominance separately outside layer 4c as a sequence of slabs, where binocular responses shift gradually between

Figure 2. Organizational schemes for orientation preferences, deduced from microelectrode penetrations (Hubel and Wiesel, 1974a). *a*, Hubel and Wiesel's results for electrode penetrations parallel and perpendicular to the cortical surface. For perpendicular penetrations, the preferred orientation remains constant (except in layers 4a and 4c, where the selectivity for orientation is weak or absent). For lateral penetrations, the preferred orientation rotates linearly with distance in sequences of 0.5–1.0 mm. Linearity is broken at intervals longer than this by reversals in the direction of rotation. Linearity is also broken, along with continuity, at semiperiodic intervals by sudden abrupt shifts in orientation preference (not illustrated). *b*, Noting that different tangential penetrations yielded different rates of rotation for orientation preferences, which nevertheless remained linear functions of distance, Hubel and Wiesel (1974a) inferred that slightly rotated orientation preferences are most likely organized in stacks of parallel slabs, with a spacing of approximately 700 μm between slabs preferring the same orientation.

← extremes of left and right eye dominance that occur over centers of the corresponding bands of input in layer 4c. Note that this representation depicts zones between the centers of adjacent right and left eye slabs as regions of linear change, which are quite different from the sudden discontinuous shifts implied in the upper layers when binocularity is ignored, as it is in *b*.

Optical recordings

Because they can be obtained repeatedly, with little or no damage, optical measures of cortical activity have always held promise. Optical probes of membrane potential (voltage-sensitive dyes) have been known for some time (Cohen et al., 1972; Orbach et al., 1985), but they were not tried with television cameras earlier because dye-associated signals were considered too small and fleeting to be detected in this way. This turned out not to be a problem in striate cortex, however, where large signals can be induced easily with visual stimuli, and where they also can be sustained for many seconds (Blasdel and Salama, 1986).

The approach that proved optimal can best be described as "differential video imaging." It works by averaging two high-dynamic-range images of cortex responding alternately to complementary stimuli. One image is then subtracted from the other to show the distribution of relative preferences for each stimulus, which appear lighter or darker than background. Because this approach can be used repeatedly, with little or no harm to the cortex, it has enabled response comparisons to many different orientations in the same region of cortex and thereby made it possible to infer precise maps of orientation preference and selectivity. It also has made it possible to compare these maps with differential images of ocular dominance. And even though this approach has its own unique limitations, it offers the significant advantage that investigated animals remain alive and healthy, and available for further investigation.

This and the following companion article (Blasdel, 1992) document new observations that have been made with this technique. They also provide more details and theoretical considerations in the acquisition of differential images (Blasdel and Salama, 1986). This first article deals with the most basic patterns, differential images of ocular dominance obtained by comparing responses to the two eyes, and differential images of orientation obtained by comparing responses to orthogonal orientations. The following article deals with second- and third-order analyses that may be applied subsequently to explore distributions of orientation preference and selectivity and relate them to other structures.

Materials and Methods

Preparation. The results in this article are based on optical recordings from 10 macaque monkeys (*Macaca nemestrina*). The procedures used to prepare each animal for electrophysiological recording have been described extensively (Blasdel and Fitzpatrick, 1984; Blasdel and Salama, 1986; Blasdel, 1989a,b). Each animal was induced with a mixture of ketamine and xylazine, intubated, and provided with an intravenous catheter for the infusion of electrolytes and drugs. It was ventilated with a 2:1 mixture of nitrous oxide and oxygen, and as the ketamine wore off anesthesia was switched gradually to pentathol (0.2–2.0 mg/kg/hr, i.v.).

The animal was placed on a heated waterbed with its head in a stereotaxic frame. After the scalp had been reflected, a trephine was used to bore a 25 mm hole just behind the lunate sulcus, as close as possible to the midline. After this, a stainless steel chamber that could be used for either microelectrode or optical recordings was inserted and cemented to the cranium (Fig. 3a). For electrode recordings, this chamber was sealed by an O-ring supporting a large glass disk that was pressed against it by a micromanipulator. The latter contained a guide tube that allowed an electrode to be inserted and positioned anywhere on the cortex under visual guidance.

During the optical recordings, a threaded stainless steel plug, with an 18 mm glass window, was inserted into the chamber and adjusted until the glass rested a millimeter (or slightly less) above the cortical surface. A minimum separation of 0.5–1.0 mm was maintained between glass

and cortex to allow access to the dye solution (NK2367, 0.1% in saline), which was circulated through two 18 gauge stainless steel tubes attached to the plug.

At the time of physiological recording, an animal was paralyzed *partially* with pancuronium or vecuronium bromide, to stabilize the eyes. These were then fitted with hard, gas-permeable contact lenses whose curvatures were adjusted to bring the eyes into focus on the screen of a 19 inch monitor placed 1.5 m away. Anesthesia was maintained with pentathol (0.5–1.0 mg/kg/hr) and verified to be adequate by referring frequently to the electrocardiogram (EKG), blood pressure, and end-tidal carbon dioxide (CO₂), which were monitored continuously. The adequacy of anesthesia could also be verified frequently by the absence of reflexes (e.g., lateral canthal) since the level of neuromuscular blockade, which was assessed every 2 hr from muscle twitches induced by electrical stimulation of the median nerve, was never allowed to exceed 50%—a level that, even though it allows reflexes to be elicited, eliminates all but the smallest eye movements.

Sequential receptive field measurements, during the single-unit recordings, verified that any residual eye movements were minimal as long as anesthesia remained adequate. The movements that were observed were generally slow and transient, and limited to 0.25°. Larger movements of a degree or more were observed occasionally over extended periods of time (>12 hr), as noted previously (Pettigrew et al., 1979), but these are unlikely to have affected the optical recordings since the visual stimuli used were distributed through 11.4° of the animals visual field.

During physiological recordings, each animal was maintained in an anesthetized paralyzed state for 18 hr or less, after which the neuromuscular blockade was reversed and the animal allowed to recover. The pentathol was replaced by general, inhalation agents (halothane in nitrous oxide and oxygen), and the dural flap was sutured with 6-0 surgical silk. After the exposed dura had been treated with antibiotics (chloramphenicol) and steroids (Decadron), the chamber was sealed with a sterile Teflon plug. At this time, residual paralysis was assessed and, when necessary, counteracted with peridostigmine (i.m., after 0.2 mg atropine). After normal neuromuscular function had returned, anesthesia was discontinued and the animal was returned to its cage. Where the prolonged residual effects of barbiturates posed a problem to the animal's welfare, they were counteracted with methamphetamine (0.1 mg/kg, i.m.). After the animal had been returned to the vivarium, antibiotics (chloramphenicol, 50 mg/kg/d, and ampicillin, 50 mg/kg/d) and steroids (Decadron, 0.1 mg/kg/d) were given prophylactically until the next recording session, or until 3 d had elapsed without incident. No area of cortex was investigated more than three times, and comparisons of activity patterns were restricted to images obtained within a few hours of one another, from the same cortical locations.

Microelectrode recordings. For microelectrode recordings, the head chamber was sealed with a Pyrex disk 2 inches in diameter that allowed an unobstructed view of the cortical surface. A glass-insulated platinum-iridium electrode (Wolbarsht et al., 1960) was introduced through a pressure-sealed guide tube, embedded in the glass, until its tip became visible. It was then monitored through an operating microscope (Zeiss Opmi-1) while it was advanced until its tip lay just above the cortical surface. It could then be guided visually between blood vessels to any desired location in cortex. Once each site was selected, the tip was advanced rapidly to a depth of 200 μ m, where its position was recorded by taking a single video frame through the operating microscope and storing the image digitally on disk. The electrode was then advanced farther, in 5–10 μ m bursts, by a stepping motor microdrive (Central Engineering Services, California Institute of Technology), in a careful search for visually responsive units.

At each location, recordings were obtained from at least three visually responsive neurons in the upper layers, at separations of at least 100 μ m. For each unit receptive field position, orientation preference and selectivity were plotted separately for each eye, before ocular dominance was assessed. The receptive field positions for both eyes were then used to align them, with prisms, with the center of a monitor screen, 1.5 m away. The orientation preferences and ocular dominances determined at each location were used later on to verify results obtained optically. Areas where no visually responsive cells could be isolated from the upper 500 μ m were excluded from further study. This was not usually a problem, however.

Optical recording. A schematic illustration of the apparatus used to record optical signals appears in Figure 3a. It was assembled from commercially available components (Ealing, MA; Rolyn Optical, CA;

Figure 3. Schematic illustration of optical recording in monkey striate cortex. *a*, Experimental arrangement for monitoring optical signals. A 25 mm disk of bone was removed with a trephine and replaced with a threaded stainless steel ring, cemented to the skull. A round glass coverslip, secured to a second threaded ring, was screwed into this chamber to provide a clear view of the cortex (with dura reflected). Stainless steel inlet and outlet ports allowed dye solutions (NK2367 in $\sim 0.1\%$ in balanced salt solution) to be introduced and removed. Light from a 100 W tungsten halogen light was directed through a heat filter and an interference filter (720 ± 20 nm; Omega Optical, Brattleboro, VT), onto the end of a 0.25 inch fiber optic cable, which was used to eliminate vibrations. Light at the other end of this cable was collimated and directed through a diaphragm, which lay in a plane of point sources that was focused through the edge of a 50 mm objective onto the cortex. Reflected light was collected by the same objective lens and focused by a projection lens onto an image sensor that consisted either of a 2×2 photodiode array or a Newvicon-based television camera (COHU 5300). *b*, Averaged optical signal from a single photodiode that measured light from a $100 \mu\text{m}$ patch of cortex as it responded to 10 presentations of visual stimuli. Each presentation consisted of four episodes, lasting 2 sec apiece: (1) a blank screen, followed by (2) a vertical drifting grating, followed by (3) a horizontal drifting grating, followed by (4) a blank screen. As one can see from this signal, there is a sudden inflection less than 200 msec after the onset of the vertical grating, much faster than one would expect from intrinsic signals (Grinvald et al., 1986). The signal increases steadily during vertical stimulation, undergoes a small glitch with the transition from vertical to horizontal, and returns to baseline when the horizontal grating is removed. Sequential presentations were synchronized to both the electrocardiogram and the respiration to minimize movement artifacts, as suggested previously by Orbach et al. (1985). From Blasdel and Salama (1986).

Oriel, CT). Light from a 100 W quartz halogen lamp was directed through an interference filter and focused onto the end of a 0.25 inch fiber optic bundle by a parabolic reflector. Monochromatic light at 720 ± 20 nm was carried by this bundle to a condenser lens that generated distributed point sources of light at an image plane masked by a diaphragm. Light from these point sources was then collimated and deflected through the edge of a 50 mm focal length objective onto the cortical surface. Reflected light, captured by the same objective, was then relayed to a projection lens that focused it either on an array of photodiodes (United Detector Technology) or onto the faceplate of a Newvicon TV camera (COHU model 5300).

The optical system in Figure 3*a* delivered a light flux of approximately $3.9 \mu\text{W}/\text{mm}^2$ to the cortical surface, at wavelengths of approximately 720 ± 20 nm, a range selected to optimize the action spectrum of the voltage-sensitive dye NK2367 (Salzberg et al., 1977). Reflected light from the cortex irradiated the image sensor with an intensity of approximately $0.007 \mu\text{W}/\text{mm}^2$. In order to minimize photodynamic damage to the tissue, the numerical aperture, which varied between 0.2 and

0.6 with the working distance of the objective, was maximized and periods of illumination were restricted by a shutter to times immediately before and during frame collection. Since the techniques reported here rely on reflected and not fluorescent light, the required flux is much lower than that usually associated with photodynamic damage.

More detailed parameters are provided in other publications (Blasdel, 1989a,b; Blasdel and Salama, 1986; Haglund and Blasdel, 1991). But, one should realize that many of these are not critical and may not even be optimal since the approach is still new and in need of improvement. As long as minimal considerations are met in the design of optical and electronic components, many different approaches should work. The most important variable appears to be the physiological state of each animal, which must be optimal to support the large numbers of vigorously responsive cells needed to obtain differential images.

Frame collection. As one can see in Figure 3*b*, visual stimulation leads to a short-latency increase in optical absorption. The changes occurring before 1.5–2.0 sec derive from the voltage-sensitive dye (NK2367) that has been added. After this, they come from the dye as well as from

Figure 4. Visualization of ocular dominance bands. *a*, When striate cortex is viewed under green light, the vasculature appears prominent because hemoglobin absorbs strongly at this wavelength. *b*, Hemoglobin does not absorb strongly at the longer wavelength used to monitor activity, however, and consequently, the same vasculature is much less noticeable when viewed under monochromatic light at 720 ± 20 nm. From the image in *c*, moreover, where the ocular dominance of cortical tissue is visualized underneath most of the vasculature, it becomes clear that blood vessels smaller than $150 \mu\text{m}$ are virtually transparent to light at this wavelength. *c*, Differential image of ocular dominance bands obtained by averaging two images of cortex separately, while the right and left eyes were stimulated alternately, and subtracting the image acquired during left eye stimulation from that acquired during right eye stimulation. The degree of left and right eye preference is indicated by lightness in the image; regions lighter than neutral preferred the left eye while regions darker than neutral preferred the right. This image has been averaged laterally, by binning and convolution (to improve the signal : noise ratio), and normalized. Note the independence of this pattern from that formed by blood vessels in *a*. *d*, Image of a tangential section from the same cortical region after processing to reveal the mitochondrial enzyme cytochrome oxidase (Wong-Riley, 1979). Note the rows of blobs following trajectories similar to those of both light and dark ocular dominance bands, whose borders are indicated by white lines. From the work of Horton and Hubel (1980) and Tootell et al. (1988), it is known that blobs are correlated with the centers of ocular dominance columns.

intrinsic signals that may be monitored in the absence of dye, but which require 1.5 sec or more to begin (Grinvald et al., 1986). For reasons that still are not clear, these signals arise from a general, activity associated darkening of cortex (Blasdel and Salama, 1986; Grinvald et al., 1986). Optically speaking, the cortex shows some response everywhere, regardless of the eye, or orientation, used to drive activity. Images of the activity induced by visual stimulation are therefore best when images of cortex undergoing similar levels of activation are compared. Since we are interested primarily in preferences for one eye or for one orientation, the optimal approach is to modulate the eye or orientation while keeping general activity levels constant—an approach best referred to as “differential imaging.” Differential images of ocular dominance are obtained by comparing responses to the right and left eyes, while differential images of orientation are obtained by comparing responses to orthogonal orientations.

The temporal sequence for results reported here was as follows. The cortex was allowed to stabilize for 20–30 sec while the animal looked at a blank gray screen. Respiration was synchronized with the EKG by interrupting the respirator. After a delay of 0.5–1.0 sec, video frames were averaged for 1–3 sec. The respirator was then resynchronized and visual stimuli were presented—to one eye or at one orientation.

After a subsequent delay of 0.5–1.0 sec, frames were averaged again, for 1–3 sec, into a second frame buffer. The stimuli then were replaced by a blank screen until a similar sequence began 10–20 sec later, with complimentary stimuli—the other eye, or the orthogonal orientation—that were presented while two more sets of frames were added into a third and fourth frame buffer.

This double sequence, where frames were obtained (1) at rest, (2) for one stimulus, (3) at rest, and (4) for the complimentary stimulus, was repeated 3–10 times, depending on the size of optical signals available at the time (which were observed to depend on many factors, including depth of anesthesia and amount of dye) and the desired signal : noise ratio. The summed images in frames 3 and 4 were then subtracted from those in frames 1 and 2 to produce two difference images that indicated (1) spontaneous fluctuations and (2) the distribution of cortical regions preferring complementary stimuli. When everything proceeded optimally, the first frame was blank, indicating that spontaneous events were minor and that sufficient time had elapsed between trials for previous signals to have died down, and the second frame showed a differential image of activity induced by the two stimuli that corresponded closely to results obtained with single-unit recordings at corresponding sites. Pixels from both images were then grouped into 4×4 squares

Figure 5. Technique used to determine objectively the centers of left and right eye dominance bands. The differential ocular dominance image in *a* is treated with a gradient operator to obtain vectors that reverse direction in the centers of the ocular dominance bands (both light and dark). The angular components of these vectors are shown in *b*, where the reversals are evident as high-contrast edges. By taking another gradient of these angles, and recording magnitudes of the output vectors (ignoring direction), it is possible to obtain an impartial template of ocular dominance centers (*c*). This can be combined with information about ocularity, available in *a*, to produce templates for the centers of right and left eye bands, which are indicated in black and white in *d*. *e*, The edges of ocular dominance bands can be located by finding the neutral zones, between dark and light areas, where values cross. Because ocular dominance is changing consistently and linearly in these zones, a narrow line is produced. Wider areas, corresponding to the edges of ocular dominance bands, can be inferred by identifying values on either side of zero as well. *f*, Outline of the ocular dominance bands, inferred from transition zones, that is produced by this approach.

and averaged to improve the signal : noise ratio further, at the expense of spatial resolution, which was thereby reduced from 512×480 to 128×120 . Images were then stored on disk at this lower resolution. When recovered, they were convolved with a 4×4 Gaussian to smooth the square edges introduced by this procedure. Fluctuations over intervals larger than 1.5 mm were removed by convolving the image with

an appropriate sized Gaussian, subtracting the result from the original image, and normalizing.

The duration of frame collection was constrained by two parameters. Longer durations permitted greater improvements in the ratio of signal to noise because they allowed more averaging, and because the observed signals grew stronger over time. Shorter durations often proved superior,

Figure 6. This figure illustrates the algorithm used to extract and add orientation-dependent components from each differential image of orientation. Because the scalar values at each image location indicate preferences for one orientation or the other (orthogonal orientation) with positive or negative values, they can be converted into vectors by multiplying each one with a unit vector angled at twice the stimulus orientation. The orientation must be doubled because vectors (which are directed) and orientations (which are not) cycle back on themselves after rotating through 360° and 180°, respectively, and therefore can only be equated when orientation angles are doubled. This has the added benefit that *most different* orientations (those that are orthogonal) are represented by *most different* vectors (those rotated through 180°), which cancel. Different rows indicate responses to different pairs of orthogonal contours, and illustrate how the orientational dependency of each differential image is taken into account. The *right-hand drawings* illustrate the conversion to vectors, displayed as cosine and sine pairs, in Cartesian coordinates. Once differential image values have been converted in this way, they can be added at corresponding positions, to yield a field of output vectors that correspond, in size and direction, to orientation selectivity and orientation preference (times two) at each location. This is the format used in the following article (Blasdel, 1992). These vectors can also be displayed in Cartesian coordinates, in which case the cosine and sine components reflect preferences for vertical/horizontal, and left/right oblique—just like the differential images for vertical/horizontal and left/right oblique that were collected initially, and used partly for input.

however, because they avoided the noise associated with vascular events, many of which developed into severe problems after 2–3 sec or more. Since these vascular changes are regional and unpredictable, the ensuing artifacts cannot be removed easily, and are best avoided altogether. Practical intervals have been found to vary with many factors, especially depth of anesthesia, but usually are between 1.5 and 3.0 sec.

Visual stimulation. In all cases, visual stimuli consisted of four superimposed square-wave gratings at nonharmonic frequencies, moving back and forth at 1.5°/sec on the screen of a 20 inch Mitsubishi monitor (C-3910), placed 1.5–2.0 m away. The average screen luminance and contrast were set to 3 cd/mm² and 80%, respectively. Between periods of visual stimulation, grating patterns were replaced by a blank screen that also had a luminance of 3 cd/mm².

Differential images of ocular dominance. Figure 4a shows a region of striate cortex 4 mm across. The strong absorption of hemoglobin at 540 nm, the wavelength used to obtain this image, makes the blood vessels appear dark. Figure 4b depicts the same region under 720 nm light, the wavelength used to monitor activity, where the blood vessels appear less prominent because hemoglobin absorbs less light at this wavelength.

As one can see in Figure 3b, visual stimulation may cause the optical absorption of stained cortex to change by 0.1–0.2%—an amount too small to be seen by eye, but one large enough to be detected electronically. An example appears in Figure 4c, where bands of ocular dominance are apparent. Their correspondence with structures of ocular dominance reported earlier (Hubel and Wiesel, 1972; LeVay et al., 1975) can be verified in three ways: (1) from their qualitative, bandlike resemblance to these structures projected onto two dimensions, (2) from

the results of single-unit recordings used to check ocular dominance at strategic locations (Blasdel and Salama, 1986), and (3) by comparison with cytochrome oxidase blobs (see Fig. 4d), which are associated with the centers of ocular dominance bands (Horton and Hubel, 1980; Horton and Hedley-White, 1984; Tootell et al., 1988).

Algorithm for finding the edges and centers of ocular dominance bands. In order to implement many of the analyses in this and the following article (Blasdel, 1992), the edges and centers of ocular dominance bands must be identified. This can be accomplished most easily for the edges by finding the neutral zones, where ocular dominance values cross zero during transitions between light and dark extremes, as illustrated in Figures 5e and f. The centers are estimated most easily from the extremes themselves, where changes in value reverse direction. These can be located by taking the gradient of ocular dominance values and finding places where local gradient vectors reverse direction.

The procedure is illustrated in Figure 5. It begins with a best possible estimate of the underlying ocular dominance structure—one obtained by averaging all the best differential images of ocular dominance. As long as these are free from obvious artifacts (e.g., vascular signals, bleaching, etc.), this selection is somewhat arbitrary since the results are so repeatable (see below). The gradient is then taken. As one can see in Figure 5b, this produces a field of vectors whose directions reverse sharply in the centers of adjacent dark and light bands, where maxima and minima are achieved. Note that these reversals occur only in places where ocular dominance values are not changing consistently in any direction. They may be visualized by measuring the angles between adjacent vectors (see Fig. 5c). Large changes can then be combined with

Figure 7. *Top two rows.* Each frame in the upper rows reveals a differential image of orientation, obtained by comparing cortical images acquired during stimulation with one orientation and its orthogonal. Preferences for the orientations indicated by dark and light bars (*lower right corner*), appear as dark and light values in each image. Successive images were acquired with both contours rotated through 22.5° between frames. As one can easily verify, the images lying above one another, which are offset by 90° , are complimentary (see Results). In any particular frame, one can usually see short bands, running in particular directions. Nevertheless, when the stimulus orientations are rotated slightly, the apparent bands do not move laterally (as one might expect from the model in Fig. 2*b*), but break apart and form new patterns that run in different directions. *Bottom row.* Differential images of ocular dominance, obtained by subtracting frames of cortex responding to the left eye from frames of it responding to the right. Each of these images was obtained with visual stimuli at a different orientation (indicated in the *lower right corner*).

values from the original image to obtain separate templates for the centers of left (light) and right (dark) eye bands, as shown in Figure 5*d*.

In the results that follow, "signals" and "noise" are estimated from the differences between, and variances of, values in the centers of adjacent ocular dominance bands. Since ocular dominance values peak in the centers of bands, and fall to zero at the edges, the apparent size of these signals depends on the widths of designated "centers." Wider centers could lead to the measurement of smaller signals. Similarly, the amount of noise indicated by variance could be affected by averaging. Neither qualification poses a problem, however, since signal and noise measurements are made in arbitrary units, and comparisons are restricted to patterns that have been evaluated comparably. One might note that variations in the width of designated centers are minimized by the approach in Figure 5*a–d*, which excludes zones where ocular dominance values are changing consistently in any direction. One might also note that reductions in noise variance, achieved through averaging, are real and that the only penalties paid take the form of reduced temporal or spatial resolution.

Differential images of orientation. Differential images of orientation are obtained by stimulating the animal alternately with moving contours at one of two orthogonal orientations. Optical changes induced by one orientation are then compared with those induced by the other orientation through subtraction. Regions in the resulting difference image that are darker than background indicate preferences for the first orientation, while regions that are lighter than background indicate preferences for the orthogonal orientation. Regions that are neutral in tone either have no preference or did not respond.

When images are acquired with different pairs of orthogonal contours— $0^\circ/90^\circ$ and 15° and 105° , for example—the resulting difference images contain common information that cannot be combined without

taking a phase angle into account. This can be done most easily by multiplying the intensity at each location by a unit vector with an angle equal to twice the orientation used to generate positive (dark) images. This transforms each image into a field of vectors, as indicated in Figure 6. Intensity values for the images derived from vertical/horizontal ($0^\circ/90^\circ$), left/right oblique ($45^\circ/135^\circ$), horizontal/vertical ($90^\circ/0^\circ$), and right/left oblique ($135^\circ/45^\circ$), for example, are multiplied by unit vectors with angles of 0° , 90° , 180° , and 270° , respectively. Since negative values correspond to vector rotations through 180° , preferences for the orthogonal orientation, which is rotated through 90° by definition, are taken into account automatically by this scheme.

Once the differential images have been transformed, the resulting vectors can be added to reflect orientation weighted contributions from each image. Because stimulus orientations are multiplied by two, contributions generated by similar orientations reinforce one another, while those generated by orthogonal orientations cancel. The resulting vector sums can be displayed in Polar or Cartesian coordinates. When displayed in Polar coordinates, as they are in the following article (Blasdel, 1992), the angles and magnitudes of summed vectors correspond to orientation preferences (times two) and selectivities (and/or levels of responsiveness) in corresponding areas of cortex. When presented in Cartesian coordinates, as they are in Figure 6, cosine and sine components correspond to differential preferences for vertical/horizontal and left/right oblique, like the differential images of vertical/horizontal and left/right oblique used as input, but reflecting contributions from all the differential images used in making the calculation.

Histology. At the conclusion of each experiment, the animal was anesthetized deeply, given a lethal dose of Nembutal, and then perfused transcardially with a warm 0.5% solution of sodium nitrite in phosphate-buffered saline, followed by 2% paraformaldehyde and 1.25% glutar-

Figure 8. Top three rows, Differential images of orientation, similar to those in Figure 7, but for 12 different orientations (in increments of 15°). Bottom row, Differential images of ocular dominance, like those in Figure 7.

aldehyde in phosphate-buffered saline, followed by 10% sucrose in 0.01 M phosphate buffer. Relevant portions of striate cortex were removed and submerged in 20% and 30% solutions of sucrose, and after they sank they were flattened, frozen, and sectioned tangentially at 40 μ m on a sliding microtome (American Optical). They were then mounted and reacted for cytochrome oxidase according to the protocol of Wong-Riley (1979).

Once the slides had been coverslipped, optically investigated regions were identified with respect to superficial vascular profiles in the first sections. These and subsequent sections were then photographed with a video camera, using the same optical apparatus and magnifications that had been used initially to monitor activity. They were then aligned, magnified, and warped with respect to the small holes left by vertically running arterioles that also could be identified in video frames acquired at the time of physiological study.

Results

Figure 7 shows eight differential images of orientation, obtained by comparing responses to orthogonal contours (indicated by the crossed dark and light bars in the lower right corners), rotated through 180°, in 22.5° increments, and four differential images of ocular dominance, obtained by comparing responses to the

right and left eyes at four different orientations. Figure 8 shows 12 differential images of orientation, in increments of 15°, from a different animal along with four differential images of ocular dominance. As one can see, the differential images of orientation and ocular dominance in Figure 7 resemble those in Figure 8 in most important respects. They also resemble differential images of orientation and ocular dominance from eight additional animals, on which the conclusions in this and the following article are based. For simplicity and continuity of presentation, therefore, the results in this and the following article (Blasdel, 1992) will emphasize data from the cortical area represented in Figure 7, which were obtained from a 3 kg female *Macaca nemestrina*.

Signals, noise, averaging, and reproducibility

The differential images of ocular dominance in the bottom row of Figure 7 are reproduced in Figure 9, *a–d*, and averaged together in Figure 9*e*. Figure 9*f* was obtained by averaging the same four images with four additional images of ocular dominance that were obtained at a later time. In order to explore

Figure 9. *a–d*, Differential images of ocular dominance, obtained with contours at four different orientations (0°, 45°, 90°, and 135°), which are indicated in the lower right corner. These are the same frames that appear in the bottom row of Figure 7. The image in *e* reveals the improvement in signal to noise that results when all four images (*a–d*) are averaged together, and *f* shows the further improvement that occurs when four additional images (eight in all) are averaged. These improvements are also obvious statistically, as one can verify in Table 1. By using the template in Figure 5*d* to calculate the mean difference between values in the centers of right and left eye bands, and dividing this difference by the rms; one can show that the images in *a–d* have signal : noise ratios of approximately 4. This ratio improves to 6.1 in *e*, when all four images are averaged. Even greater signal : noise ratios are obtained when local contrasts are considered separately. The light and dark regions—like those outlined by the two rectangles in *f*—differ with a signal : noise ratio of 23.

Table 1. Signal : noise ratio ($\Delta X_m/\text{rms}$) for differential images of ocular dominance

# Trials	Orientation	Dark		Light		$\Delta X_m/\text{rms}$
		X_m	rms	X_m	rms	
1	0°	-40	14.9	24	15.9	4.2
1	45°	-40	16.3	28	15.8	4.2
1	90°	-32	13.8	28	13.4	4.4
1	135°	-40	17.9	24	16.4	3.7
4	All	-38	10.7	26	10.3	6.1
1	0°	-36	8.7	16	9.3	5.8
1	45°	-36	12.6	20	10.4	4.8
1	90°	-28	9.2	20	8.4	5.5
1	135°	-36	12.1	20	10.2	5.0
4	All	-34	9.2	19	7.8	6.2
1	0°	-45	7	23	4	11.9
1	45°	-53	7	24	5	12.6
1	90°	-38	5	45	3	20.1
1	135°	-47	11	24	5	8.3
8	All	-44	4	29	2	23.1

In each example, mean image densities and rms values were calculated separately for the centers of dark and light ocular dominance bands (corresponding respectively to the right and left eyes) defined by the white and black template elements in Figure 5*d*. From these, a "signal" value (ΔX_m) was estimated from the difference between the means calculated for the centers of the dark and light bands, and a "noise" value was estimated from normalized and quadratically averaged rms values in both (dark and light) zones. While both of these values—signal and noise—are specified in arbitrary units, their ratios are not, and hence give reasonable estimates of the signals giving rise to differential images. In all cases, X_m is derived from $(\Sigma X)/N$, where N equals the number of values in the sample area (in this case, specified by the template in Fig. 5*d*); rms is derived (by definition) from $[\Sigma_{i=1}^N (X_i - X_m)^2 / (N - 1)]^{0.5}$; rms_{ave} is derived from $[(N_{\text{light}} \text{rms}_{\text{light}}^2 + N_{\text{dark}} \text{rms}_{\text{dark}}^2) / (N_{\text{light}} + N_{\text{dark}})]^{0.5}$; and ΔX_m is derived from $X_{m(\text{light})} - X_{m(\text{dark})}$.

The first four trials are taken from the dark ($n = 913$) and light ($n = 1269$) elements in Figures 9*a-d*, indicated by the template in Figure 5*d*. Note that even though each of these is derived (from left and right eye comparisons) with contours at four different orientations, the resulting signal : noise ratios are approximately the same, ~ 4 . When all these images are averaged together (see Fig. 9*e*), the light and dark areas converge to stable values, while rms variations decrease, producing an improved signal : noise ratio of 6.1. This is also the result when separate differential images of ocular dominance are averaged 1 hr later, as one can see in the second four trials (and their sum). When all eight images are averaged (see Fig. 9*f*), the signal : noise ratio improves further, to 8.

The bottom four trials are similar to the first eight, but values for dark ($n = 77$) and light ($n = 99$) bands are taken selectively from the two rectangles indicated in Figure 9, *a-d* and *f*, thereby reducing the effect of global variations. Note that when this is done the signals in neighboring bands (averaged eight times) appear with a signal : noise ratio of 23.

statistical properties of these patterns, the centers of adjacent light and dark bands were located with the strategy illustrated in Figure 5, so that "signals" could be determined, in arbitrary units, from the difference between mean values in the centers of light and dark bands. A measure of "noise," corresponding to the root mean square (rms) of these values, was obtained (in the same arbitrary units) by calculating variances in the centers of light and dark bands separately, and taking the square root of their average.

From the first entry in Table 1, where results of these calculations appear for Figure 9*a*, one can see that mean values in the centers of dark and light bands differ by 64 ($40 + 24$). Since these values have a combined rms of 15.4, the bands that seem apparent have a signal : noise ratio of 4.2 ($64 \div 15.4$). When the four images in Figure 9, *a-d* are averaged (Fig. 9*e*), the signal : noise ratio rises to 6.1, indicating a 50% improvement. This is less than expected, for a fourfold increase in sample size, but reasonable considering that each of the four images averaged in Figure 9*e* was obtained with different stimulus orientations.

Better signal : noise ratios are observed locally. Values in the two small squares in Figure 9*a*, for example, differ by 68 with a combined "noise" of 5.7, which gives a signal : noise ratio of 11.9. Comparable measurements are apparent for these regions in Figure 9, *b-d*, as well (see Table 1, last 4 trials). In Figure 9*e*,

where all four images are averaged, the ratio is 19, and when eight images are averaged, it rises to 23. Obviously, then, the signal : noise ratio improves with averaging. Image values converge to stable offsets in the centers of light and dark bands, while random contributions converge to zero. Since this would not happen for patterns coalescing from random fluctuations, this behavior provides strong statistical support for the stimulus dependency of the patterns observed.

Since many findings in this and the following article (Blasdel, 1992) derive from measurements at the edges or in the centers of ocular dominance bands, the stability of these zones is important. As illustrated in Figure 5, the edges of ocular dominance bands can be identified by transition zones between the light and dark areas, where ocular dominance values pass through zero. These are zones where neither eye dominates strongly and responses are vigorously binocular. Figure 10*b* shows the borders that are determined when this procedure is applied to the differential ocular dominance image in Figure 10*a*. In Figure 10*c*, the same outline is applied to a differential image of ocular dominance that was obtained more than an hour after the one in Figure 10*a*, after the orientation patterns described below had been determined. As one can see from this comparison, as well as from that in Figure 10*d* where outlines of both differential images appear superimposed, these borders are extremely stable,

Figure 10. Reproducibility of differentially imaged ocular dominance bands. The image in Figure 9*e* is repeated in *a*. The transition zones between light and dark bands are then visualized with the procedure illustrated in Figure 5, *e* and *f*, and superimposed over a different ocular dominance image in *c*, that was acquired from an average of four more images 1 hr later. As one can see, the outline obtained from zero-crossings agrees well with the second pattern. Outlines of the two images *a* and *c* appear superimposed, in white and black, in *d*.

and show far less variation than would be necessary to affect conclusions in this article.

Differential images of orientation

This approach has proved particularly useful in the visualization of orientation preferences. Figure 7 shows eight images that were obtained by comparing responses to eight different pairs of orthogonal contours. Dark and light values indicate the degree of preference for orientations indicated by the dark and light bars in the lower right corner of each frame. As for the ocular dominance patterns in Figure 5, *e* and *f*, the neutral zones between the light and dark regions may be used to delineate regions with opposing preferences.

The first and third images in the top row of Figure 7, which reflect preferences for vertical/horizontal and left/right oblique, are replicated in Figures 11*a* and 12*a*. These can be verified in a number of ways. Single units can be recorded to determine preferences at a few locations (Blasdel and Salama, 1986), but these are too time consuming to provide a complete verification. A comprehensive proof must therefore be provided by optical images as well. This can be done by showing that patterns produced by similar orientations are similar, and that those produced by different orientations are related by a simple linear function to the angle of rotation. The simplest approach is to show that stimulus rotations through 90° produce inversions in contrast. This can be achieved by keeping all stimulus variables

Figure 11. *a*, Differential images of orientation derived by subtracting optical responses to horizontal stimuli from optical responses to vertical. The darker regions preferred vertical while the lighter ones preferred horizontal. Neutral zones (neither light nor dark), which expressed no preference for vertical or horizontal, could have derived from several different factors. They could have responded strongly but equally to both orientations (vertical and horizontal), as would have been the case if they preferred left or right oblique, and were sufficiently broad in their tuning to respond to vertical and horizontal, or if they lacked orientation selectivity altogether. It could also be, however, that these regions did not respond to the visual stimuli used at any orientation. *b*, Differential image of vertical/horizontal obtained from all eight differential images, using the technique illustrated in Figure 6, which works by extracting vertical/horizontal (as well as left/right oblique) components from each image and adding them

algebraically. The strong similarity between this pattern, and that in *a*, emphasizes the consistency of these results. The main difference between them derives from the greater signal: noise ratio for the pattern in *b*, which is attributable to the greater number of frames it represents. A more precise comparison is facilitated in *c*, where the outline of the pattern in *b* appears superimposed over the image in *a*. From *d*, where the same outline appears superimposed over an image of horizontal/vertical, one can see that the borders of the outline remain stationary, even though the contrast of the light and dark regions has inverted. Though it is expected that an inversion of stimulus orientations should lead to an inversion in contrast, one should stress that this is an orientation-dependent change; all other parameters remained the same. The stability of transition zones, indicated by zero-crossings, also underscores the repeatability of these patterns. These can be compared directly in *e*, where zero-crossings of the vertical/horizontal and horizontal/vertical patterns appear in white and black, respectively. The nonaccidental nature of this alignment is emphasized in *f*, where the same outline appears over a differential image of left/right oblique (from Fig. 12*b*). As one can see, there is no correspondence; the transition zones for vertical/horizontal (indicated by the *white outline*) do not correspond to the transition zones for left/right oblique. However, the two patterns are related in the sense that the white lines clearly bisect the darkest and lightest regions in the left/right oblique pattern. Since the white lines indicate places with no detectable preference for vertical or horizontal, their intersections with the darkest and lightest regions verify that the latter truly preferred left and right oblique.

Figure 12. This figure is similar to Figure 11, only for differential images of left/right oblique. *a*, Differential image of orientation derived by subtracting optical responses to right oblique stimuli from those to left oblique stimuli. The darker regions preferred left oblique while the lighter ones preferred right oblique. Neutral regions expressed no preference. As with the pattern in Figure 11, this could indicate that these regions responded strongly but equally to left and right oblique (if, e.g., they preferred another angle, but were broadly selective enough to respond to left and right oblique, or if they lacked orientation selectivity altogether). It also could indicate that they did not respond at all. *b*, Differential image of left/right oblique obtained by extracting left/right oblique components from the optical responses to eight different pairs of orthogonal contours and combining them algebraically. The strong similarity between the resulting pattern and that in *a* emphasizes the consistency of these components. The main difference between the patterns in *a* and *b* is that the signal: noise ratio is greater for *b* because more responses were averaged. *c*, A more precise comparison is facilitated when an outline of the pattern in *b* appears over the image in *a*. *d*, When the same outline appears over a differential image of right/left oblique (the reverse of that in *a*–*c*), one can see that the borders are stationary even though the light and dark regions invert. Though one should expect a contrast inversion when the order of presentation is inverted, one should stress once again that the only things that have changed are the orientations of stimuli; this is an orientation-dependent change. *e*, The neutral zones may be compared directly when the outlines of left/right and right/left oblique patterns are superimposed, as they are here, in white and black, respectively. *f*, The nonaccidental nature

constant (e.g., speed of movement, position in space, binocular alignment, contrast, timing of stimulus presentation and frame collection, etc.), except for orientation. In Figure 11 one can see that a 90° rotation of both stimulus orientations has the required effect by noting that dark and light regions in Figure 11c appear light and dark, respectively, in Figure 11d. This is also true for Figure 12, c and d, which means that these images are repeatable and dependent on orientation since this was the only parameter varied. The outlines may be compared directly in Figures 11e and 12e, where those from Figures 11c and 12c appear in white, and those from 11d and 12d appear in black.

The linear orientation dependency of these patterns can be illustrated by extracting vertical/horizontal and left/right oblique components from each image and showing that they add constructively. This is done in Figures 11b and 12b, which show the averages of vertical/horizontal and left/right oblique components from all the orientation images in Figure 7. When these are compared to differential images of vertical/horizontal and left/right oblique that were acquired directly, in Figures 11a and 12a, it is clear that the patterns are virtually identical even though the signal: noise ratios are higher in Figures 11b and 12b, as one would expect from greater averaging. The similarity is emphasized in Figures 11c and 12c, where outlines from Figures 11b and 12b have been added to the images in Figures 11a and 12a to facilitate comparison.

The matches indicated in Figures 11, c–e, and 12, c–e, their indication of real and not illusory alignments, are emphasized in Figures 11f and 12f, where the same outlines appear over differential images of orientations rotated through 45°. The image in Figure 11f was taken from 12b, while that in Figure 12f was taken from 11b. As one can see, the mismatches are striking, with the thin white lines that segregate light and dark regions in Figures 11c and 12c, bisecting the darkest and lightest zones in Figures 11f and 12f. Since the lines in Figure 11f are derived from neutral zones in Figure 11b, where there are no preferences for vertical or horizontal, their bisections of dark and light peaks are consistent with their indicated preferences for left and right oblique. And the converse can be seen in Figure 12f, where lines reflecting no preference for left or right oblique intersect the dark and light zones preferring vertical and horizontal.

Orientation columns

The differential images in Figure 7 are strongly periodic. In the vertical/horizontal one, replicated in Figure 11a for example, one can easily connect light and dark regions together into flanking parallel bands resembling the “orientation columns” seen earlier with 2DG (Hubel et al., 1978). The delineation of these bands becomes clearer when neutral zones between light and dark regions are highlighted, as they are in Figure 11, c and d. From the lateral shifts that seem to result from 90° rotations in stimulus orientations (e.g., Fig. 11c,d) it seems likely that smaller movements might occur incrementally for smaller rotations, in between. This is not the case, however. As one can see from successive images in the top row of Figure 7, where stimulus orientations have been rotated through 22.5°, the strings of light and dark regions making up these bands break up, and for

Figure 13. When the ocular dominance slabs illustrated in Figure 1b are combined with the orientation slabs illustrated in Figure 2b, one arrives at Hubel and Wiesel's “ice-cube” model of cortical organization. This is only an illustration, but it nevertheless makes clear what should happen if one set of orientation slabs is activated through a single eye. Even if the two sets of slabs do not intersect at consistent angles, the stimulated orientation slabs should be interrupted at intersections with the closed eye. Conversely, one could say that the stimulated ocular dominance slabs should be interrupted where they intersect with non-stimulated orientations. After Hubel and Wiesel (1977).

rotations through 45° they form new bands. This can be seen clearly by comparing Figures 11c and 12c, where differential images of orientations offset by 45° are outlined (see Figures 11f and 12f). The bands that seem apparent certainly do not run parallel.

One reason for this is that these bands do not represent *preferences* for any particular orientation. They may indicate a relative preference for one of two tested orientations, which were orthogonal. But responses to the angles in between are ambiguous in any particular image. The problem can be seen clearly in Figure 12f, where zero-crossings from the left/right oblique pattern in Figure 12b are highlighted over the vertical/horizontal image from Figure 11b. Since regions that actually *prefer* vertical (or horizontal) should be neutral with respect to left or right oblique, they should correspond to zero-crossings, delineated by lines, in this image. This is true for the darkest (and lightest) parts of the “bands” in Figure 12f—regions that clearly prefer vertical (or horizontal)—but not for the less intense parts of these bands in between. These regions may respond better to vertical than horizontal, but if they are not bisected by lines they cannot *prefer* vertical; they must prefer some other angle in between.

Actual orientation preferences are clearly more complicated than any single differential image, or single 2DG autoradiogram, can reveal. Interactions between patterns obtained with different pairs of orientations are explored further in the following article. From results in Figures 7, 11f, and 12f, however, it is clear that

of this alignment is emphasized when the same outline appears over a differential image of vertical/horizontal (from Fig. 11b). As one can see from this overlay, the transition zones for left/right oblique (indicated by white lines) do not lie over the transition zones for vertical/horizontal, but bisect the darkest and lightest parts of the pattern. And since these lines indicate places with no detectable preference for left or right oblique, their intersections with the darkest and lightest regions verify that the latter truly preferred vertical and horizontal.

Figure 14. Interactions between ocular dominance and orientation. *Top row*, Four differential images of orientation (for four different pairs of orthogonal contours, $0^\circ/90^\circ$, $45^\circ/135^\circ$, $90^\circ/0^\circ$, and $135^\circ/45^\circ$) that were obtained with both eyes open. *Middle row*, Four differential images of orientation, obtained with one (the left) eye closed. As one can see, there is an obvious effect on the apparent signal : noise ratio—the patterns in the *middle row* appear noisier—but there is little or no indication of the ocular dominance bands evident in the bottom row. There is, for example, no obvious fading in regions dominated by the closed eye. *Bottom row*, The result of a converse experiment where four differential images of ocular dominance were obtained with contours at different orientations (0° , 45° , 90° , and 135° , respectively). As one can see, the resulting patterns look approximately the same; there are no obvious gaps in any of them. This suggests a surprising independence between mechanisms responsible for ocular dominance and orientation.

actual iso-orientation bands do not extend farther than 0.5 mm, and that they cannot run parallel over distances longer than this.

Interactions between ocular dominance and orientation

The differential imaging strategy used here relies on small changes in optical absorption induced by competing visual stimuli. The modulation of ocularity, achieved by alternately stimulating the two eyes, produces patterns associated with ocular dominance, while the modulation of orientation, achieved by stimulating alternately with orthogonal contours, produces images associated with orientation. But what happens when aspects of these two paradigms are mixed? When differential orientation images are obtained with one eye closed, for example? Or when ocular dominance images are obtained with one orientation? The simplest interpretation of the models in Figures 1*b* and 2*b* is illustrated in Figure 13, and suggests that ocular dominance and orientation should interact. This should be true whether bands associated with them intersect at consistent or random angles (Hubel et al., 1978)—there should be nodes in regions of overlap.

These possibilities are tested explicitly in Figure 14, where the upper and middle rows depict differential images of orientation obtained with binocular and monocular stimulation, respectively. Signals in the upper row are stronger, as one would

expect from binocular facilitation. But this is true everywhere, and differences other than this are minor. There are no shadows of closed eye dominance bands (bottom row), and there are no nodes. This conclusion is emphasized in Figure 15*a*, where the vertical/horizontal pattern obtained with the left eye closed has been subtracted from the one obtained with both eyes open.

Quantitative measures can be applied to find some effect. If responses in the binocular regions are screened out, and those in the centers of right and left eye bands are compared directly, it can be shown (Table 2) that the ratio of left to right eye responses is greater when both eyes are stimulated than when the right eye is stimulated alone, but only by 3%—an effect far smaller than one reasonably would expect from the simplified model in Figure 13, or from previous observations (Hubel et al., 1978).

A comparable, but converse, example appears in the bottom row of Figure 14, where four differential images of ocular dominance were acquired with contours at different orientations. As one can see, there are no nodal points in these images either—no obvious holes or blank or pale spots to suggest inactivated regions. Even though they were acquired with different orientations (offset by at least 45°), all patterns look remarkably similar, suggesting that they derive from mechanisms insensitive to orientation. This conclusion receives further support from

Figure 15. The lack of interaction between mechanisms responsible for ocular dominance and orientation is emphasized in *a* and *b*. *a*, The result of subtracting the differential image of vertical/horizontal obtained with the left eye closed from the one obtained with both eyes open. If there were any tendency for this image to be fainter in regions dominated by the left eye, this should be apparent in the result of this subtraction. There should be something reminiscent of the ocular dominance bands in *c*, or at least some combination of ocular dominance and orientation. But, as one can see, this is not the case. *b*, The result of subtracting a differential ocular dominance image obtained with horizontal contours from one obtained with vertical contours. If mechanisms of ocular dominance and orientation interacted linearly, this should (in theory) produce orientation-dependent patterns reminiscent of those in *d*, in regions dominated by each eye. But, as one can see, it does not. *c*, Ocular dominance bands, obtained with vertical from the same region of cortex, provided for comparison. *d*, Differential image of orientation for vertical/horizontal, from the same region of cortex, provided for comparison.

Figure 15*b*, where ocular dominance bands produced with horizontal contours have been subtracted from ones produced with vertical contours, in order to emulate more closely the comparisons made with differential images of orientation. Although the resulting difference image is not exactly blank, there is no suggestion of the localized orientation preferences that clearly are apparent in Figure 15*d*, where a differential image of vertical/horizontal appears.

This apparent contradiction arises from a somewhat naive expectation that orientation selectivities remain constant across the cortical surface, an expectation implied by Figure 13. This is known to be incorrect, however, since orientation selectivity decreases periodically near blobs (Horton and Hubel, 1980; Horton and Hedley-White, 1984; Livingstone and Hubel, 1984a). And since selectivity can vary, the absence of interaction between orientation and ocular dominance can be explained by a covariance between the selectivity for orientation and binocularity. If regions most selective for orientation are also the most binocular, stimulation through one eye should have little effect

other than to decrease the apparent signal : noise ratio, as observed in Figure 14. And if the regions most selective for one eye are less selective for orientation, differential images of ocular dominance should not depend greatly on this variable.

A possible covariance of orientation selectivity with binocularity suggests that differential images of orientation should be more pronounced at the edges of ocular dominance bands than at the centers, and this possibility can be tested directly. This is done in Figure 16, where an outline of the ocular dominance bands appears over differential images of vertical/horizontal and left/right oblique. The latter are modulated more strongly at the edges (indicated by lines) than they are at the centers of ocular dominance bands. The difference becomes even more striking when orientation patterns are viewed separately through windows corresponding to the centers (Fig. 16*c,d*) and edges (Fig. 16*e,f*) of ocular dominance bands. This can be quantitated by calculating rms values through each window, which show 24% and 38% greater modulation at the edges for vertical/horizontal and left/right oblique patterns (see Table 3).

Table 2. Interactions between ocular dominance and orientation

Pattern	Left eye (<i>N</i> = 4228)		Right eye (<i>N</i> = 4620)		$\Delta R/R$
	X_m	rms	X_m	rms	
Binocular					
0°	−1	21.0	−1	19.2	−9%
45°	−1	22.6	0	21.6	−4%
90°	0	21.2	−1	20.2	−5%
135°	−1	20.0	−1	19.4	−3%
Σ		21.6		20.1	−7%
Right Eye					
0°	−1	19.4	−1	20.4	5%
45°	−1	16.6	−1	12.6	−24%
90°	0	16.4	−2	14.0	−15%
135°	−2	19.2	−2	16.8	−13%
Σ		18.0		16.2	−10%

The calculations used to derive X_m and rms are explained in Table 1 notes. The first four rows under the heading “Binocular” refer to the modulation of cortical responses by orthogonal contours in the centers of right and left eye dominance bands (derived from the template in Fig. 5*d*). $\Delta R/R$ is derived from the difference between right and left eye rms values, divided by normalized and quadratically averaged rms values for both eyes. As one can see, rms values resulting from binocular stimulation show a consistent bias in favor of the right eye, which for all values averaged, is approximately 7%. The rows under the heading “Right Eye” were calculated in the same way, but from the middle row of images in Figure 14, which were obtained with the left eye closed. Consistent with expectations from binocular facilitation and a simple comparison of images in the top two rows of Figure 14, the degree of modulation (reflected by rms) is reduced in each zone by ~18%, but the decrease in regions dominated by the left eye is only slightly less than that in regions dominated by the right, and the bias in favor of regions dominated by the right eye only increases to 10% (a 3% difference)—less of an effect than one might have expected from the complete interruption of contributions from the left eye.

Evaluation of vascular contributions

Blood vessels figure prominently in most views of cortex—so much so that it is difficult to believe they do not contribute substantially to the observed images (Orbach and Van Essen, 1986). One must keep in mind, however, that the strategy employed here—differential imaging—does not detect features that are prominent so much as ones that change in rhythm with visual stimulation. Static features are removed, as are ones that change slowly. And while blood vessels may change their absorption over time (Grinvald et al., 1986; G. G. Blasdel, unpublished observations), they do so with different dynamics than neurons, whose contributions may therefore be isolated.

The absence of vascular artifacts can be verified in a number of ways, the simplest of which is to compare sites of apparent activity with the vasculature. If any of these derive from blood vessels, they should resemble blood vessels in shape, size, and direction of propagation. As one can see in Figure 17, *c* and *d*, however, this is not the case. The ocular dominance bands in Figure 17*a* are all straight and fat, and they all run in the same direction with a definite periodicity. Blood vessels, on the other hand, are sinewy and narrow. They run in every direction with no obvious regularity.

Vascular artifacts do occur from time to time, especially near blood vessels larger than 200 μm in diameter, but when they do they are obvious and easily avoided. Of greater concern, therefore, are any subtle influences that may not look like blood vessels but nevertheless may cause problems later on. One way of exploring this possibility is to examine the intersections of blood vessels with ocular dominance boundaries. Any blood

Table 3. Regional variation in the modulation of differential orientation images

Pattern	Image intensities				
	X_m	rms	<i>N</i>	$\Delta X/X$	$\Delta R/R$
Vertical/horizontal					
Centers	−2	34	2,648	—	—
Boundaries	0	42	3,198	—	24%
Left/right oblique					
Centers	0	32	2,648	—	—
Boundaries	0	44	3,198	—	38%
Vasculature					
Centers	1	46	2,648	—	—
Boundaries	0	45	3,198	—	2%

The calculations used to derive X_m , rms, and *N* are the same as those described in Table 1 notes. $\Delta R/R$ reflects the difference between rms values calculated over the boundaries and centers of ocular dominance bands (indicated by the template in Fig. 5*d*), divided by normalized and quadratically averaged values in both zones, and indicates modulation differences (by orthogonal contours) in these two zones. Vertical/horizontal values are derived from the images in Figure 16, *c* and *e*, while left/right oblique values are derived from images in Figure 16, *d* and *f*. As one can see, these signals are stronger near the edges of ocular dominance bands, in the zones of binocular overlap, than they are in the centers—regardless of orientation. The negligible values for X_m in all cases indicates there are no orientation preferences associated with any of these structures. The last two rows, under the heading “Vasculature,” provide the same analysis for blood vessels [where X_m and rms both provide rough estimates of blood vessel density (from the degree of darkness as well as from induced fluctuations)]. As one can see, vascular distribution differences between the centers and edges of ocular dominance bands are minimal to nonexistent, and accordingly are unlikely to serve as a basis for differential images of ocular dominance bands or any other differences between their centers and edges.

vessel causing a subtle bias should do consistently, along its entire trajectory, which means that the zero-crossings indicated by white lines in Figure 16*c* should be affected. These should bow inward or outward if intersecting blood vessels are creating a bias. There are a few places, near the largest blood vessels, where this cannot be ruled out (Fig. 17*c*), but given the extreme sensitivity of this test, it is far more striking how infrequently this occurs. Many prominent blood vessels intersect the borders of ocular dominance bands with impunity, as though they were utterly transparent.

Similar conclusions can be reached quantitatively, by calculating the mean and standard deviations of pixel values at strategic locations in the vascular image to see if the density of vascularization (indicated by darkness) correlates with the centers or edges of ocular dominance bands. The results appear in Table 3, where there clearly is no bias.

From these and other tests, it is clear that blood vessels do not contribute dynamically to the images observed. They could contribute statically, however, if they attenuate light used to monitor activity. This possibility can be evaluated from the contrast of differential images near blood vessels. Blood vessels that block light should leave shadows of reduced contrast. Obvious examples are difficult to find, however, even when a vascular outline is provided for comparison. In Figure 17*d* one can see that some of the strongest responses were recorded from regions covered by blood vessels, which suggests they were not a significant barrier to light at the wavelengths used to monitor activity.

Since superficial blood vessels play so little a role in generating ocular dominance images, there is no a priori reason to assume they contribute to orientation patterns either. One should realize nevertheless that similar arguments apply: (1) differential images

Figure 16. The absence of interaction between ocular dominance and orientation, apparent in the differential images in Figures 14 and 15, could be explained if mechanisms responsible for these properties were segregated laterally. One can see suggestions of this in *a* and *b*, where outlines of the ocular dominance bands appear superimposed over differential images of vertical/horizontal and left/right oblique. There is a subtle, but clear, tendency for the modulation intensity to peak along these lines and, conversely, to diminish between them. The tendencies can be seen more clearly by viewing the differential images through windows corresponding to the centers (*c*, *d*) and edges (*e*, *f*) of ocular dominance bands. As one can see, these images are much more strongly modulated at the edges (*e*, *f*) than they are in their centers (*c*, *d*). This difference can also be shown quantitatively by measuring the rms modulations through each window. The results of this measure appear in Table 3, where they indicate a 24%–38% greater modulation in the edges than in the centers of the ocular dominance bands.

of orientation do not resemble blood vessels even slightly, and (2) blood vessels have no obvious effect on zero-crossings (see Fig. 17*e,f*). In the case of orientation, however, one has the additional observation that light and dark peaks, reflecting pref-

erences for orthogonal orientations, move laterally with rotations in stimulus orientation, in linear proportion to the angle of rotation. This is difficult or impossible to achieve with vascular artifacts.

Figure 17. Comparisons between outlines of differentially imaged ocular dominance bands and blood vessels. *a* and *b* show how objective outlines of ocular dominance bands and blood vessels can be obtained by labeling regions where these images pass through neutral zones (which in normalized images correspond to zero). A simple comparison of the images in *a* and *b* reveals that the differential image of ocular dominance bands could not possibly derive from blood vessels (as suggested by Grinvald et al., 1986). In contrast to the ocular dominance bands, which are thick and fat, highly periodic, and which run predominantly from top to bottom, the blood vessels are sinewy and narrow (with a fractal self-similarity across scales) and nonperiodic and they run in many different directions. This obvious lack of correspondence can be emphasized by superimposing an outline of the ocular dominance bands on an image of the vasculature directly. From this superposition (*c*), it is apparent that most blood vessels are transparent to the differential imaging strategy used. Even subtle influences can be ruled out by reference to regions where blood vessels intersect the outlines of ocular dominance bands. There is no detectable effect (indicated by a net inward or outward bowing) of these zero-crossings in most places where blood vessels cross. As one can see in *d*, the presence of overlying blood vessels does not even produce a detectable attenuation in the strength of the optic signals used to obtain the differential images. This is consistent with the weak absorption, by hemoglobin, of light at 720 nm, and suggests that most small blood vessels are virtually transparent to light at this wavelength. *e* and *f*, Comparison between differential images of orientation and the vasculature. The outlines in *e* and *f* show outlines of the differential images of vertical/horizontal and left/right oblique that appear in Figures 11*b* and 12*b*, superimposed on an image of the vasculature. As one can see, there is no obvious relation. The outlines are just

Discussion

These and earlier studies (Blasdel and Salama, 1986; Blasdel, 1989a,b) show that differential imaging may be used to visualize patterns of cortical organization. By comparing images of cortex stimulated by each eye separately, one can visualize ocular dominance, and by comparing images of cortex stimulated by orthogonal orientations, one can explore preferences for orientation. The resulting images clearly derive from robust, repeatable signals since they improve steadily with averaging.

Optical signals, vasculature, and artifacts

The signals producing these images are still not understood precisely. The most likely sources include (1) vascular artifacts, (2) intrinsic signals, (3) dye-stained neurons, and (4) dye-stained glia, each of which is considered in turn.

Blood vessels. Grinvald et al. (1986) noted that some blood vessels change their absorption in response to visual stimulation, and suggested that such changes might play a role in patterns reported earlier (Blasdel and Salama, 1986). When this possibility was tested explicitly in this study, however, no influences were found. This should not be surprising since the entire procedure was engineered to minimize vascular artifacts (which are obvious when they occur), and since the evidence for them was never strong.

Intrinsic signals. These derive from a general activity-associated darkening of cortex (Blasdel and Salama, 1986), which was studied in detail by Grinvald et al. (1986) and T'so et al. (1990), who more recently showed that these "intrinsic signals" can be used to visualize patterns of cortical activation. Even though the mechanisms responsible for producing them are not understood at present—they could derive from a number of different events, including the dilation of capillary beds, increased light scattering of released potassium, and so on—they offer an attractive alternative to optical probes of membrane potential, which often can be quite toxic. The major drawback to intrinsic signals appears to be their speed. They require 1–1.5 sec to begin, in monkey striate cortex, and several more to mature (Grinvald et al., 1986). As one can see from the photodiode trace in Figure 3b, signals from dye-stained cortex are faster than this, appearing with an onset latency of 200 msec and maturing within 1.5 sec, which makes them easier to record and average before vascular events become a problem.

Neurons. Fast, dye-dependent signals probably derive from the depolarization of neural membranes, most of which belong to fine axons and dendrites in the neuropil. Even though these depolarizations occur quickly, within a millisecond or less, they can be detected by slow devices (e.g., television cameras) because they also affect the time integrated potential.

Glia. Slower but larger signals are also expected from glial cells, responding secondarily to the accumulation of potassium. Intracellular recordings in cat striate cortex (Kelly and Van Essen, 1974) have shown that glial cells depolarize by 5 mV or more in response to visual stimulation, and that they can be

just as orientation selective as neurons. Optical recordings from presumed glial cells, moreover (Levran and Grinvald, 1986), show a continuous activity-driven change in absorption, similar to that shown in Figure 3b.

Geometric constraints. Since mammalian cortex is transparent to longer wavelengths of light, optical signals are likely to be recorded through depths of a millimeter or more (unless specific steps are taken), and this can lead to problems with parallax if the optical axis is not aligned exactly with the response properties under investigation. The consequences can be severe, since a divergence of only 15° over 1 mm generates a registration error of 260 μm —half the width of an ocular dominance column. An exact alignment is impossible, however, since response properties are not organized by Euclidean rules. Even those known to extend "vertically" through layers (e.g., ocular dominance, orientation preference) extend along biological axes, defined by radial glia and axon bundles, that are difficult to judge *in vivo*, and that vary (Lund, 1973; Lund and Boothe, 1975; Fitzpatrick et al., 1985). Alignment properties are unavoidable, therefore. They are minimized nevertheless by light-absorbing dyes, which restrict light penetration to the upper layers. Even the intrinsic signals, monitored in the presence of light-absorbing dyes, are more likely to arise from the upper layers.

Comparison with 2-deoxyglucose

While differential images of orientation bear some resemblance to the "orientation columns" visualized previously with 2DG (Hubel et al., 1978), they are also shorter, and less bandlike, for reasons that should be considered. In the case of 2DG, the accumulation of label indicates metabolic activity. Where natural variations can be ignored (Humphrey and Hendrickson, 1983), local labeling differences can be taken to indicate activity differences that were induced by a particular orientation of stimulation. The assay is for *responses*, however; preference and selectivity are not addressed. If an animal is stimulated with vertical, for example, the regions labeled with 2DG include (1) those preferring vertical over all other orientations, (2) those *responding* to vertical even though they *prefer* another orientation, as well as (3) those simply *responding* to all orientations.

Because responses to orthogonal orientations are always compared, the regions identified through differential imaging are different. When vertical/horizontal responses are compared, for example, regions registering darker than background include those preferring vertical to horizontal, while those registering lighter than background include those preferring horizontal to vertical. Regions responding the same to vertical and horizontal do not register.

The main difference between these approaches, therefore, is that 2DG labels all regions that *respond* to a particular orientation while differential imaging indicates only the ones that *prefer* it. The distinction would not matter if orientation selectivity remained constant and orientation preferences rotated linearly over the entire cortical surface, as suggested in Figure 2b. Bands of cells preferring a particular orientation and bands

as likely to run parallel to blood vessels as they are to intersect them at right angles. From this independence, one can be confident of two things: (1) that blood vessels play no role in the generation of these images, and (2) that blood vessels smaller than 150 μm in diameter do not even interfere with the monitoring of optical signals from cortical cells beneath them. Points where blood vessels intersect the outlines at right angles offer a particularly sensitive measure of vascular influence. If blood vessels in *e* contributed significantly to the apparent preference for vertical or horizontal, the *white lines* should bow outward or inward at points of intersection. Since few if any such distortions are apparent, however, one can conclude that small superficial blood vessels play little or no role in the generation of these patterns.

Figure 18. Three hypothetical examples of lateral changes in orientation preference. In *a*, one sees the result expected from Figure 2*b*, where orientation is represented as a continuous sheet of parallel slabs. Lateral displacements through cortex are accompanied by linear rotations in orientation preference. This is highly unrealistic, however, as the linearity of these sequences is known to be disrupted semiperiodically by sudden reversals in the direction of rotation (*b*), as well as by discontinuous shifts (*c*). The notion that all bands of 2DG labeling, reflecting responses to one orientation, indicate preferences for that orientation, is based on the assumption illustrated in *a*, that preferences are represented in an unbroken sequence of linear rotations. When orientation preferences are represented more accurately, however, as they are in *b* and *c*, it is clear that nonlinearities can generate bands as well in regions where cells may respond to a particular orientation without preferring it. This possibility that is particularly disturbing on account of the frequency of nonlinearities, which is about the same as that for “orientation columns” visualized with 2DG. Accordingly, it is unreasonable to conclude that the centers of 2DG bands indicate preferences for orientations used to drive them, especially when these bands are narrower, or labeled more faintly, than others nearby.

of cells responding to it would then be the same. But this is not the case; orientation selectivity varies just as regularly as orientation preference (Livingstone and Hubel, 1984*a*; Blasdel, 1989*a*), and linearity is disrupted at regular intervals (Hubel and Wiesel, 1974*a*; Blasdel and Salama, 1986). Accordingly, regions identified with 2DG and differential imaging must differ at many locations. Those lacking orientation selectivity are always *included* in 2DG images and *excluded* from differential images. The most reasonable interpretation of 2DG “orientation columns,” therefore, is that they appear longer because they consist of numerous shorter segments, that actually are selective for orientation, strung together by regions that are not (e.g., blobs).

Iso-orientation bands

Since 2DG labels all the regions that *respond* to a particular orientation, it labels more than those that actually *prefer* it. The nonspecific labeling in blobs is one example, but even if we exclude these regions, the remaining labeled segments do not necessarily represent the stimulus orientation either. The problem arises from the assumption, illustrated in Figure 18*a*, that orientation preferences rotate linearly over large distances. If this were true, slabs preferring the stimulus orientation would clearly take up more label than their neighbors, and be indicated (in tangential sections) by bands of label (Hubel et al., 1978). This is an oversimplification, however, since the linearity and continuity of orientation preferences are known to undergo frequent disruptions (Hubel and Wiesel, 1974*a*; Blasdel and Salama, 1986).

One nonlinear disruption is illustrated by the reversal in Figure 18*b*, where orientation preferences rotate from horizontal to left oblique, but then back to horizontal again. Since many striate cells respond over ranges of 90° or more, it is clear that the middle, oblique-prefering zone will respond more strongly to vertical than those on either side, causing it to accumulate more 2DG and label as a band (in tangential sections) even though none of the cells within it prefer vertical. Bands may also rise artifactually from the discontinuous changes associated with fractures, as illustrated in Figure 18*c*.

The possibility of confusing these disruptions with actual orientation columns would not be of much concern if they were infrequent, but they clearly are not. As Hubel and Wiesel (1974*a*) found initially, and Blasdel and Salama (1986) verified later, lines of nonlinear change (reversals and fractures) occur at approximately the same frequency as the “orientation columns” visualized with 2DG, every 0.5–1.0 mm. They are therefore a major concern.

The problem is illustrated more graphically in Figure 19*a*, where the “horizontal iso-orientation slabs” that might be identified (between blobs) by 2DG are simulated by the outlined white parts of a vertical/horizontal differential image. The assumption prevailing in previous studies (Hubel et al., 1978; Schoppman and Stryker, 1981) would have been that regions preferring horizontal are aligned with the centers of the light bands that seem apparent. When actual orientation preferences are calculated, however (see Blasdel, 1992), and preferences for horizontal are indicated in white (Fig. 19*b*), it becomes apparent

that this is not always the case. Horizontal iso-orientation bands always intersect the lightest portions of the white bands, as they should, and a few even continue down their centers, but many then run off to one side. One cannot assume, therefore, that bands that seem apparent in single differential images, or in 2DG autoradiograms, are aligned with the iso-orientation slabs for any orientation. Studies based on such assumptions (e.g., Gilbert and Wiesel, 1989) should therefore be reconsidered.

Interactions between ocular dominance and orientation

It is surprising to find that unilateral eye closure has so little effect on differential images of orientation. One might have expected attenuation in regions dominated by the closed eye, but such effects are subtle. The main consequence of eye closure appears to be a reduction in the overall signal : noise ratio, which is virtually as pronounced in regions dominated by the open eye as it is in regions dominated by the closed eye. This is gratifying in some respects, since it implies that the similar perceptions induced by scenes viewed through one or both eyes are mirrored by similar patterns in cortex, albeit weaker or stronger. It disagrees, however, with previous observations that closure of one eye fragments the orientation "columns" seen with 2DG (Hubel et al., 1978). The disagreement is only superficial, however, and can be explained easily with the non-selective labeling of blobs. Because blobs are dominated strongly by one eye, alternate rows of them should be affected by eye closure. Whether this is apparent depends on whether they are seen. Since they form a regular component of the 'orientation columns' seen with 2DG, the closure of one eye should interrupt these patterns periodically where alternate blobs fall silent. Since blobs are excluded from differential images, on the other hand, the closure of one eye should not produce an effect.

The apparent segregation of cortex into regions strongly dominated by one eye and regions strongly selective for orientation requires further exploration. One explanation is that optical signals include strong contributions from layer 4c, where binocularity and orientation selectivity are absent, that are stronger in the centers of ocular dominance bands. Such a contribution is made unlikely, however, by recent experiments showing that differential images of ocular dominance are prevented by small doses of the GABA-blocker bicuculline, which disrupt ocular dominance in the upper layers (Blasdel and Haglund, 1989; Haglund and Blasdel, 1991). Signals percolating up from layer 4c should not be affected by bicuculline since the ocular dominance bands in layer 4c receive input only from one eye. Contributions from layer 4c are also unlikely in the presence of the light-absorbing dye used (NK2367), which blocks light penetration to the deeper layers.

On account of the ambiguity between selectivity and responsiveness that limits this approach (see Materials and Methods), it is also conceivable that cells in the centers of ocular dominance bands do not respond well to the black-and-white contours used as visual stimuli. This would be true for color-selective blob cells, for example. It is also conceivable that binocular facilitation plays a role, augmenting responses more at the edges of ocular dominance columns than at their centers. Yet another possibility is that the lateral mixing of optical signals through light scatter or lateral averaging (used to improve the signal : noise ratio) reduces the apparent selectivity for orientation near zones of discontinuous change, which are particularly abundant in the centers of ocular dominance bands (Blasdel and Salama, 1986; Blasdel, 1992).

Figure 19. This figure illustrates ambiguities complicating the interpretation of a single differential image. The top figure (*a*) depicts a differential image for vertical/horizontal, with the borders between light and dark areas highlighted in black. A simple assumption, for this image, might be that the adjacent light and dark bands that are apparent are iso-orientation bands for horizontal and vertical, in which case regions preferring horizontal should project down the centers of the light bands.

This assumption is tested in *b*, where white areas indicate regions that actually prefer horizontal, as deduced from all differential images collectively (Blasdel, 1992). As one can see in this overlay, these lines intersect the lightest zones consistently, as one would expect for regions preferring horizontal. Their relation to the white bands is complicated, however, since they frequently do not run down the centers. One can conclude from the overlay in *b*, therefore, that iso-orientation bands preferring horizontal are not always aligned with the centers of the white bands apparent in *a*. And one can also conclude that orientation preferences cannot be inferred from any single image. Since the information conveyed by a single 2DG autoradiogram is even less than that conveyed by a single differential image (where two responses have been compared), the resulting ambiguities are likely to be greater in patterns produced by this technique.

None of these possibilities provide a complete explanation, however. The possibility of reduced responsiveness is inconsistent with the strong responses generated in the centers of ocular dominance bands by the same stimuli when ocular dominance is imaged. And the underlying assumption in all these suggestions—that orientation selectivity remains high in the centers of ocular dominance columns, and only *appears* reduced—is inconsistent with the observed insensitivity of differentially imaged ocular dominance patterns to orientation (bottom row of

Fig. 14). While each of these factors (and others) might contribute slightly to these observations, the most appealing explanation is also the simplest, that binocularity and orientation selectivity are correlated.

Conclusions

Apart from the technical achievement of visualizing cortical activity *in vivo*, and showing that differential images are free of obvious artifacts, this work has advanced the understanding of cortical organization in four ways. It has shown (1) that groups of cells preferring a single orientation can no longer be described, and should not be represented schematically, as slabs extending farther than 0.5 mm laterally; (2) that even short iso-orientation slabs are not aligned with the bands seen in single differential images (or with 2DG); (3) that regions strongly dominated by one eye and regions strongly selective for orientation are partially segregated in the lateral domain; and (4) that orientation selectivity correlates highly with binocularity. The functional implications of these findings are developed further in the following article (Blasdel, 1992), where the topographies of orientation preference, selectivity, and continuity are calculated from images presented here, and explored in greater depth.

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