Functional Organization of the Cat Visual Cortex in Relation to the Representation of a Uniform Surface

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INTRODUCTION

A visual image of an object consists of two sorts of complementary features: a contour and a surface surrounded by the contour. Neurons in the early stages of the visual cortex are known to respond to contour stimuli with a specific orientation preference so that individual cells detect only part of the contour within their small receptive fields (Hubel and Wiesel 1962). The neural mechanisms underlying representation of the interior of surfaces are much less well understood. It is known, however, that in the primary visual cortex (V1), some neurons respond to large spot stimuli that cover their classical receptive fields or to luminance changes of the entire visual field (Bartlett and Doty 1974; DeYoe and Bartlett 1980; Kayama et al. 1979; Maguire and Baizer 1982). Moreover, two groups recently reported that some striate neurons respond to changes in the luminance of a uniform plane and that these neurons are even affected by luminance changes outside their classical receptive fields (Kinoshita and Komatsu 2001; Rossi and Paradiso 1999; Rossi et al. 1996). These findings indicate that the early stages of the visual cortex may be involved in surface representation as well as contour representation. How neurons responsive to a uniform surface interior are distributed in the visual cortex remains unknown, however.

It is well documented that in V1 neurons with similar orientation preferences and overlapping receptive fields form orientation columns and that those with the same ocular preference form ocular-dominance columns (Hubel and Wiesel 1962, 1963; Shatz et al. 1977). Optical-imaging techniques enabling visualization of the spatial arrangement of the orientation-preference map have revealed the presence of both linear zones, where orientation preference gradually changes, and singular points and fractures, where orientation preference rapidly changes (Blasdel 1992; Bonhoeffer and Grinvald 1991, 1993; Bonhoeffer et al. 1995; Rao et al. 1997). Our aim in the present study was to examine how cortical activity elicited by presentation of a uniform surface is distributed within the visual cortex and how this distribution relates to the spatial arrangement of the orientation-preference map. To accomplish this, we used two complementary techniques: optical imaging, which is well suited for investigating the distribution of neuronal activity within a wide cortical region and the accumulation of neurons responsive to similar stimuli (Blasdel and Salama 1986; Bonhoeffer and Grinvald 1996; Grinvald et al. 1993; Bonhoeffer et al. 1995; Rao et al. 1997). Our primary findings were that there are a series of patchy regions in area 18 where uniform plane stimuli evoke strong activation and that these regions seem to have a close relationship with the singular points in the orientation-preference map. Preliminary results were presented in abstract form (Tani et al. 2001).

METHODS

Preparations

Three young cats (2–9 mo old, 1–3 kg) were surgically prepared under sterile conditions. After inducing anesthesia with ketamine hydrochloride (7.5 mg/kg im) and medetomidine hydrochloride (0.06–0.08 mg/kg im), the cats were intubated and artificially ventilated, and anesthesia was maintained with 1.0–1.5% of isoflurane in a 1:1 mixture with N2O-O2. Electrocardiograms, end-tidal CO2, and rectal temperatures were continuously monitored and maintained.

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within normal limits throughout the surgical procedure. After placing each animal in a stereotaxic frame, a craniotomy was made over the lateral gyrus of both hemispheres to expose a large portion of areas 17 and 18, and a custom-made stainless steel chamber was cemented onto the skull. The dura was then separately removed from each hemisphere so that the central sinus was left intact, after which the margin of the dura was coagulated to suppress tissue growth on the cortical surface. The cortical surface was then covered with a cellulose sheet (Seamless Cellulose Tubing, Sankyoujunyaku, Tokyo), and the recording chamber was filled with 2% agar that was mixed with gentamicin sulfate and dexamethasone sodium phosphate. The recording chamber was then sealed up with a polyvinylidene chloride sheet, chloramphenicol-fradiomycin sulfate ointment, and a plastic plate. Antibiotics (cefoxidine sodium, 33–100 mg/kg or cefazolin sodium hydrate, 17–50 mg/kg) were given after the surgery. The animals were allowed to recover for 7 days before experimentation.

Optical-imaging experiments were conducted once a week. For these experiments, the animals were anesthetized as described in the preceding text and paralyzed with pancuronium bromide (Mioblock, Sankyou, Tokyo, 0.05–0.1 mg · kg$^{-1}$ · h$^{-1}$ iv). The agar was then removed from the recording chamber, and the cortical surface was flushed with saline. After carefully removing any tissue that had grown on the cortical surface, the chamber was refilled with agar, and a coverslip was placed over it. During the recording period, the isoflurane level in the anesthetic was reduced to 0.5–1.0%, although if any signs of distress appeared, the level was increased until the signs disappeared. The pupils were then dilated and the eye lenses relaxed by topical application of tropicamide-phenylephrine hydrochloride. Contact lenses (Danker Laboratories, Sarasota, FL) with appropriate curvature were used to prevent the eyes from drying. Optical disk of each eye was projected onto a tangent screen placed in front of the animal, and sharp projection of the retinal vasculature indicated that the eyes were focusing on the visual stimuli. The location of fovea was determined to be 14.6° medial to and 6.5° below the location of the optic disk (Bishop et al. 1962). During electrophysiological studies, the location of the optic disk was checked every 1–2 h. In all cases, apparent shifts in eye position were found to be smaller than the receptive field sizes. After the imaging, the chamber was flushed with saline and then closed using the same procedure described for surgery.

All procedures related to animal care and experimentation were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1996) and the Guiding Principles for Research Involving Animals and Human Beings and were approved by our institutional animal experimentation committee.

### Visual stimuli

Visual stimuli were generated using a PC computer with a VSG 2/3 graphics board (Cambridge Research Systems, Rochester, UK) controlled by custom software and displayed on a CRT display (Iiyama MT08621E or Nanao FlexScan E67Ts) placed 30 or 57 cm in front of the subject. All stimuli presented were full-screen size, extending over $80 \times 60$ or $36 \times 27^\circ$ of visual angle. The refresh rate of the CRT was 120 frames/s. Several kinds of visual stimuli were used in the optical imaging experiments. To selectively activate neurons responsive to a uniform surface, we changed the luminance of the entire display between 1 (black) and 90 cd/m$^2$ (white) in a square-wave fashion at temporal frequencies of 0.5, 1, 2, and 4 Hz, although in most cases, we used a frequency of 2 Hz (uniform plane stimulus). To stimulate neurons sensitive to local luminance contrast, we used several stimuli, including a checkerboard pattern, square-wave gratings, and sine-wave gratings. The checkerboard pattern was stationary, and the black and white areas (lattice size, 3.3°) were reversed at temporal frequencies of 0.5, 1, 2, and 4 Hz; again in most cases we used a frequency of 2 Hz. We used both stationary and moving gratings with spatial frequencies of either 0.5 or 0.15 cycles°. The reversal of the checkerboard pattern and the stationary gratings was done in a square-wave fashion as for the uniform plane stimulus. The maximum and minimum luminances of the gratings were 90 and 1 cd/m$^2$, respectively. For the moving gratings, the direction of motion reversed every 1.5 s, and the velocity was changed depending on the spatial frequency so that temporal frequency of the stimulus was always 2 Hz.

For the extracellular recording experiments, we used a uniform plane, checkerboard pattern, and moving square-wave grating as the basic stimulus set. The uniform plane and checkerboard pattern were the same as used for optical imaging. The gratings were moving square-wave gratings (0.15 cycle°, 2 Hz), and the direction of motion reversed every 0.5 s. We also tested the spatial frequency selectivity of neurons using a set of stationary sine-wave gratings (0.08, 0.15, 0.5, and 1.0 cycle°) together with uniform plane stimuli. In this test, the luminance of the gratings and uniform plane changed between 1 and 90 cd/m$^2$ over a sinusoidal time course (2 Hz).

### Optical imaging

Intrinsic signals were measured using standard techniques (Bonhoefer and Grinvald 1996). Data acquisition was controlled using VDAQ software (Optical Imaging, Germantown, NY) running on a PC computer. The cortical surface was illuminated at a wavelength of 630 or 700 nm, and the focal plane was adjusted to 500–600 μm below the surface using a tandem-lens macroscope arrangement (Ratcliff and Grinvald 1991). Images were obtained with a CCD video camera (768 × 480 pixels, Bischke CCD-5024N) and digitized using a differential video-enhancement system (Imager 2001, Optical Imaging). Recorded areas were about 10 × 15 mm in size, but we analyzed a smaller region of the lateral gyrus in each hemisphere, where the images were in focus. The acquired data were then input to another PC computer for further analysis. We also imaged the blood vessel pattern on the cortical surface using 545 nm light. The duration of the visual stimuli was 5 s, and each stimulus was repeated 26–95 times in a pseudo-random sequence. Images were acquired between 2 and 5 s after stimulus onset.

### Analysis of optical images

Optical images were analyzed using TVMIX (Optical Imaging) and custom software based on IDL (Research System, Boulder, CO). To examine responses to uniform plane stimuli, differential optical images were computed by dividing images obtained during presentation of the uniform plane by images obtained during presentation of the checkerboard pattern, gratings, or a blank gray screen. Optical images obtained during presentation of gratings were computed as the average of the responses to gratings in four orientations separated by 45°. Subtraction of a second-order polynomial function was used to eliminate low spatial frequency noise that may have resulted from unstable, nonuniform illumination on the curved cortical surface (Tanaka and Mogami 2000). A band-pass filter with a Gaussian kernel (50–1,000 μm radius) was also used to smooth the optical images. For each differential image, the range of response magnitudes within ±3 SD of the mean response magnitude for the entire imaged area was normalized to a grayscale value. A new scale was used to represent the response magnitude in subsequent analyses.

To evaluate the significance of the responses to the uniform plane stimuli, we conducted one-sample t-test in which response to the plane stimulus was compared with that to the control stimulus on a pixel-by-pixel basis across recording trials; active regions were indicated by a flock of statistically significant pixels (with negative values, 2-tailed t-test, $P < 0.01$). We discarded regions of less than 100 × 100 μm because the limit of resolution of the optical imaging system was estimated to be 100 μm (Bonhoefer and Grinvald 1996). The size of the active regions ($L$) was defined as $L = (a \times b)^{1/2}$, where $a$ is the length of long axis and $b$ is that of short axis.

We used the difference in their spatial frequency preferences to...
determine the functional border between areas 17 and 18. Images elicited by high spatial frequency gratings (0.5 cycle/°) were divided by images elicited by low spatial frequency gratings (0.15 cycle/°). The resultant differential images contained a sharp transition between a large dark region occupying the postero medial part of the imaged area and a remaining brighter region (Fig. 3A, top). The darker region was regarded as area 17, the brighter region as area 18 (Bonhoeffer et al. 1995; Hung et al. 2001; Ohki et al. 2000).

To obtain an orientation-preference map, responses to gratings with four different orientations separated by 45° were obtained separately, after which differential optical images were computed as dividing them by the average response to all four orientations. Thereafter vector summation of all four single-condition maps was computed on a pixel-by-pixel basis. The angle of the summed vector corresponded to the preferred orientation at individual pixels, and this value was represented using a color scale to form an orientation-preference map. The length of the summed vector corresponded to the degree of orientation specificity, and this value was represented using a gray scale to form an orientation-magnitude map (Rao et al. 1997).

Electrophysiological recording

Extracellular recordings were conducted after the final optical imaging session. Recording sites were determined using the cortical blood vessel patterns as a reference, and glass-coated platinum-iridium microelectrodes (1–2 MΩ at 1 kHz) were placed using a hydraulic microdrive (MO-95, Narishige, Tokyo). Because optical imaging is thought to reflect neuronal activities in the superficial layer of the cortex (Bonhoeffer and Grinvald 1996; Kisvárday et al. 1994), we recorded multiple units containing a few neurons in the superficial layer of area 18. All recording sites were restricted to within 800 μm of the surface of the cortex, above the depth where the high spontaneous activities and brisk on-off responses associated with layer 4 were obtained (Gilbert 1977; Snodderly and Gur 1995). Neuronal signals were amplified (×10,000, 150 Hz to 10 kHz, MEG-6116, Nihon Kohden, Tokyo) and converted to pulse signals using a window discriminator (DDIS-1, BAK, Germantown, MD), after which the unit pulses were fed to a PC-computer at a sampling rate of 1 kHz.

Once the neuronal activity was isolated, we examined the basic response properties using a hand-held stimulator and an audio monitor of spike discharges. After determining the optimal orientation and size of a bar stimulus, it was used to plot the receptive fields of the superial microdrive (MO-95, Narishige, Tokyo). Because optical imaging is thought to reflect neuronal activities in the superficial layer of the cortex (Bonhoeffer and Grinvald 1996; Kisvárday et al. 1994), we recorded multiple units containing a few neurons in the superficial layer of area 18. All recording sites were restricted to within 800 μm of the surface of the cortex, above the depth where the high spontaneous activities and brisk on-off responses associated with layer 4 were obtained (Gilbert 1977; Snodderly and Gur 1995). Neuronal signals were amplified (×10,000, 150 Hz to 10 kHz, MEG-6116, Nihon Kohden, Tokyo) and converted to pulse signals using a window discriminator (DDIS-1, BAK, Germantown, MD), after which the unit pulses were fed to a PC-computer at a sampling rate of 1 kHz.

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Histology

In one animal, we confirmed the positions of the recording sites histologically. After all the experiments were complete, we used Elgiloy electrodes to mark several positions along the functional border between areas 17 and 18, which was previously determined by optical imaging. This cat was then deeply anesthetized with pentobarbital sodium (Somnopenyl, Kyoouritsu, Tokyo, 97.2 mg/kg) and perfused transcardially with 4% paraformaldehyde solution containing potassium ferricyanide. The brain was then removed from the skull, sectioned (50 μm thickness) in the coronal plane, and stained with cresyl violet and cytochrome oxidase. All markings, which were easily identified under microscopic examination, were located in the middle of the lateral gyrus, where the cortical layers had the anatomical characteristics of the transition zone between areas 17 and 18 (Payne 1990).

Results

Surface-responsive regions

In this study, we examined the distribution of activity elicited by large uniform surfaces and compared it with that elicited by stimuli having local luminance contrast (checkerboard pattern and gratings). Figure 1A shows a top view of the cortical blood vessel pattern in an imaged area from one cat (cat 1). This area corresponds to the lateral gyrus containing areas 17 and 18, which are situated between the midline and the lateral sulcus (inset). Optical signals elicited by uniform plane stimuli and recorded from this region are shown in Fig. 1, B–D. In the differential optical image obtained by dividing the response to a uniform plane by the response to a checkerboard pattern (Fig. 1B), the darker regions correspond to locations where the uniform plane caused stronger activation than the checkerboard pattern. These regions formed a group of spots aligned in an anterior-posterior direction in both hemispheres (arrowheads). When the response to a uniform plane stimulus was divided by the response to a blank gray screen (Fig. 1C), dark regions were observed in the same locations as in B, clearly indicating that it was the luminance change in the uniform plane stimulus that caused activation in these regions. Finally, when variously oriented moving gratings were used as yet another control stimulus, the same dark regions were again clearly observed (Fig. 1D).

That the dark regions were more clearly visible in B and D than in C indicates that employment of control stimuli with local luminance contrast enhanced the visualization of selective activation by a uniform stimulus. We therefore used the checkerboard pattern as the control stimulus in subsequent analyses. In addition, while the luminance of the uniform plane stimulus and the checkerboard pattern was reversed at four temporal frequencies (0.5, 1, 2, and 4 Hz) for the data summarized in Fig. 1, the temporal frequency had little if any effect on the results. In subsequent analyses, therefore we fixed the temporal frequency of the stimuli at 2 Hz.

To determine the extent of regions in which the uniform plane stimulus elicited strong activation, we evaluated the statistical significance of the activation on a pixel-by-pixel basis (t-test, P < 0.01, see METHODS). Regions exhibiting significant activation in response to a uniform plane stimulus were designated “surface-responsive regions” (Fig. 2). Figure 2, A and B, shows data obtained from the same hemisphere shown in Fig. 1 (cat 1), whereas C and D show data obtained from
another cat (cat 3). Figure 2, A and C, shows the differential optical image, whereas in B and D, surface-responsive regions determined by applying a statistical criterion are indicated by their red color.

**Location of surface-responsive regions**

The region recorded in our optical imaging included both areas 17 and 18. To determine which of these two areas contain the surface-responsive regions, we visualized the border between the two in differential optical images as between regions responsive to a higher spatial frequency stimulus (area 17) and those responsive to a lower spatial frequency stimulus (area 18; Fig. 3A, top; see METHODS). Figure 3A (bottom) shows the differential optical image calculated from the responses to a uniform plane and a checkerboard pattern recorded from the same cortical area. Superimposition of the images showing the border between areas 17 and 18 (---) and the contour of the surface-responsive regions (white open areas) indicated the surface-responsive regions to be located in area 18 but not in area 17. In Fig. 3A (top), there appeared several dark regions in area 18 that seemed to overlap with surface-responsive regions. However, judging from the results of the unit recording experiments described later, it is highly unlikely that dark shade of these spots reflect the neural sensitivity to high spatial frequency stimuli. We interpret that lack of responses to either stimuli used for the differential optical image as shown in Fig. 3 (0.15 or 0.5 cycle/° gratings) resulted in the shade of these regions darker than the surrounding area in area 18.

The spatial relationship between the surface-responsive regions and the border between areas 17 and 18 was largely consistent across hemispheres in all three cats (Fig. 3, B–D). Although the size of the surface-responsive regions differed considerably from one another, five of six hemispheres (except for Fig. 3D, bottom) contained a group of more than one large spot (455–1464 µm; mean, 954 ± 255 µm, n = 17) aligned along the area 17/18 border. According to the retinotopical map of area 18 of the cat (Payne 1990; Tusa et al. 1979)—and confirmed by our extracellular recordings (see following text)—these surface-responsive regions are in the region representing the lower visual field, around the vertical meridian, and include some areas in the ipsilateral visual field. We could not examine the region representing the upper visual field because it is located more posteriorly and is mostly hidden in the lateral sulcus.

**Localization of surface-responsive regions in the orientation-preference map**

The results described so far suggest that there is a functional organization related to the representation of a uniform plane in the visual cortex. If so, how are these structures related to the functional organization already known to exist in the same cortical areas, namely the orientation-preference map. To obtain an orientation-preference map, we evaluated the responses to gratings (0.15 cycle/°) in four orientations separated by 45°. Figure 4A shows single-condition maps for each orientation recorded from the same hemisphere shown in Fig. 3, A and B (bottom), whereas Fig. 4B shows an orientation-preference map computed on a pixel-by-pixel basis as a vector summation.
Indeed, the averaged orientation magnitude inside the surface-responsive regions with that in the surrounding vicinity in area 18 as the averaged orientation magnitude in the surface-responsive regions tended to be low (dark areas). Although dark spots also appear outside the surface-responsive regions and showed no response to a uniform plane stimulus. On the other hand, cells 3 and 4 were recorded outside the surface-responsive regions and showed no response to a uniform plane stimulus and some response to a checkerboard pattern.

**Comparison of the properties of neurons inside and outside the surface-responsive regions**

Our optical imaging experiments suggested that there are surface-responsive regions in area 18 and that they overlap the singular points in the orientation-preference map. To test whether the results of the optical imaging reflect the physiological properties of the neurons in these regions, we next conducted extracellular recordings from the same cortical areas in four hemispheres, placing an electrode in 83 sites within area 18; 30 sites were inside and 53 were outside the surface-responsive regions. One or two multi-neuron signals were recorded from the superficial layers with each penetration, and a total of 121 multi-neurons were recorded. Hereafter, we will refer to multi-neurons simply as neurons or cells.

Figure 6 shows some typical neuronal responses to a basic set of stimuli recorded inside and outside the surface-responsive regions. Cells 1 and 2 were recorded inside and showed strong responses to a uniform plane stimulus and rather weak responses to a checkerboard pattern (Fig. 6B); these cells responded to both the dark and bright phases of the uniform plane stimulus. On the other hand, cells 3 and 4 were recorded outside the surface-responsive regions and showed no response to a uniform plane stimulus and some response to a checkerboard pattern. All four cells were activated by the optimal grating, and had orientation selectivity (Fig. 6B, orientation selectivity indexes are shown in parentheses). Both inside and outside the surface-responsive regions, a large majority of all four single-condition maps, as well as the contours of the surface-responsive regions recorded in the same hemisphere. This example, which is typical of all six hemispheres studied, shows that the surface-responsive regions tended to spread around the singular points of the orientation-preference map.

We examined the coincidence of the surface-responsive regions and the singular points by comparing the magnitudes of the orientation preferences inside and outside the surface-responsive regions. It was previously demonstrated that regions around the singular points have lower orientation magnitudes than those within linear zones (Blasdel 1992; Bonhoeffer and Grinvald 1993; Bonhoeffer et al. 1995; Rao et al. 1997). This may be due to neurons having various preferred orientations coexisting around the singular points (Maldonado et al. 1997), making the orientation-specificity of the region low. If surface-responsive regions coincide with singular points, we would expect that orientation preference would be lower inside these regions than outside them. In Fig. 5A, the orientation-magnitude map of the same cortical area shown in Fig. 4B is coded with a gray scale and clearly shows that orientation magnitude in the surface-responsive regions tended to be low (dark areas). Although dark spots also appear outside the surface-responsive regions, they are wider inside than outside the regions. We confirmed this tendency by comparing the averaged orientation magnitude inside the surface-responsive regions with that in the surrounding vicinity in area 18 as indicated by the rectangle in Fig. 5A. Indeed, the averaged orientation magnitude inside the surface-responsive regions was significantly lower than that outside the regions (Fig. 5B; cat 3 LH; t-test, P < 0.001), and this difference in orientation magnitude was observed in all six hemispheres studied (Fig. 5B; t-test, P < 0.001).
neurons exhibited orientation selectivity: variations in the responses to stimuli with different orientations were statistically significant (ANOVA, *P* < 0.05) in 36 of 44 neurons (81.8%) inside the surface-responsive regions and in 74 of 76 neurons (97.4%) outside the regions. 

The receptive fields of cells 1 and 2 were located in the ipsilateral visual field (Fig. 6C), suggesting that the surface-responsive regions existed in the transition zone of area 18 (Diao et al. 1990; Ohki et al. 2000; Payne 1990; Tusa et al. 1979; Whitteride and Clarke 1982). Moreover, the sizes of the receptive fields of these neurons (root of area was 4.1° ± 0.8°, *n* = 27) were consistent with those previously reported in area 18 (Diao et al. 1990; Payne 1990). In one hemisphere of one cat (cat 3), we made two series of electrode penetrations in a medial-lateral direction across the area 17/18 border and sampled neurons from both inside and outside the surface-responsive regions. In addition, we did a series of electrode penetrations in each hemisphere of another cat (cat 2) to examine the shift in the size and location of the receptive fields. We found that the direction of the shift of the receptive field positions reversed and the size changed at positions corresponding to the area 17/18 border determined by the optical imaging (data not shown). These results support the idea that surface-responsive regions are located in the transition zone within area 18.

We examined the responses to a uniform plane stimulus in 121 neurons. Figure 7 summarizes the results obtained from three hemispheres. Each circle denotes a recording site, and colors indicate whether responses were statistically significant (*t*-test, *P* < 0.01): red circles indicate sites of significant responses, whereas green circles indicate nonsignificant ones. For cat 2, the proportion of neurons responding significantly to a uniform plane stimulus was 81.8% (18/22) inside the surface-responsive regions and 36.1% (13/36) outside the regions, and for cat 3, the proportion was 81.8% (18/22) and 29.3% (12/41). It thus appears that a uniform plane stimulus causes stronger activation at the cellular level inside surface-responsive regions than outside of them.

We compared the responses to a uniform plane and to a checkerboard pattern in 70 neurons (Fig. 8A) and found the ranges of their amplitudes to be comparable. As exemplified in Fig. 6, a majority of neurons recorded inside the surface-responsive regions showed stronger responses to the uniform plane than to the checkerboard pattern, whereas neurons recorded outside the regions showed the opposite tendency. The preference for the uniform plane and the checkerboard pattern seemed to be complimentary between inside and outside the surface-responsive regions, which is consistent with the optical imaging showing that the regions activated by the uniform plane were emphasized when the checkerboard pattern was used as the control stimulus.

We also compared the responses to a uniform plane and optimal gratings in 120 neurons (Fig. 8B). Both inside and outside the surface-responsive regions, responses to optimal
gratings tended to be stronger than those to a uniform plane, although this tendency was less obvious for neurons recorded inside the regions. Indeed, several neurons even showed stronger responses to a uniform plane than to the gratings; this confirmed that the unique property of the surface-responsive regions is their sensitivity to uniform plane stimuli, not their lack of sensitivity to oriented contours.

Neuronal responses to a uniform plane stimulus at the singular points

The optical imaging showed that surface-responsive regions tended to contain the singular points of the orientation-preference map. Still, many singular points (Fig. 4) and neurons responsive to a uniform plane stimulus (Fig. 7) were located outside the surface-responsive regions. One explanation for this may be that neurons responsive to a uniform plane stimulus exist nearby the singular points even when they are outside the surface-responsive regions. As low orientation magnitude is a good indicator of a singular point, we tested this possibility by examining the relationship between the amplitudes of the responses of individual neurons to a uniform plane stimulus and the magnitude of the orientation preference at each recording site. Figure 9A shows an example in which the data from Fig. 7 (bottom) are superimposed on the orientation-magnitude map, which is represented as a gray scale. It is notable that the red points representing surface-responsive neurons tend to overlap the dark regions, regardless of whether they are inside or outside the surface-responsive regions. Data summarizing the responses of 121 neurons indicate there to be a negative correlation between the two sets of values (Fig. 9B); this was statistically significant for neurons inside (filled circles, \( r = -0.50, P < 0.001 \)) and outside (open circles, \( r = -0.39, P < 0.001 \)) the regions. Apparently, neurons located near singular points are more responsive to a uniform plane stimulus whether or not they are within a surface-responsive region.

Why then were surface-responsive regions visible only at
certain positions near the area 17/18 border when optically imaged? One possibility is that accumulation of such neurons might be peculiar to surface-responsive regions, making these regions detectable by optical imaging. The whole story, however. It can be seen from Fig. 9 regions detectable by optical imaging. This does not seem to be another factor that makes surface-responsive regions detectable by optical imaging.
less, in 18 of the 61 neurons recorded (29.5%), responses to the two phases differed by more than a factor of 2 (LS > 0.33 or LS < −0.33). These cells may carry information about the luminance of a uniform plane stimulus or the direction of the luminance changes.

**DISCUSSION**

**Visual stimuli, surface-responsive regions, and potential artifacts**

We will first consider the nature of the visual stimuli responsible for the activation of the surface-responsive regions and potential artifacts. Our electrophysiological findings as well as earlier studies of the retinotopic map (Tusa et al. 1979) indicate that the cortical areas imaged in the present study extended from the vertical meridian to about 20° in both the left and right visual fields and between about 5 and 30° from the horizontal meridian in the lower visual field. The extent of the uniform plane stimulus was 36 × 27 or 80 × 60° and was centered on this part of the visual field so the stimulus covered the entire visual field represented by the imaged area or nearly so. Furthermore, the orientation-preference map was clearly observed over the entire imaged area, indicating that our display effectively stimulated these regions. The receptive field
because we studied only restricted cortical areas where the medial-lateral axes. This is an unlikely explanation, however, nonuniform signal strength along the anterior-posterior and signals. First, curvature of the cortical surface may result in spatial contrast at the border of a uniform plane might be transmitted to the surface-responsive regions by means of horizontal connections within area 18 or feedback connections from higher visual stages, its contribution to neuronal activity should be canceled by computation of the differential images. Actually, the signal amplitudes were rather uniform within the orientation-preference map; moreover, the surface-responsive regions appeared as a group of spots rather than as a continuous band. It is therefore unlikely that curvature of the cortical surface was a major contributor to the responses.

Second, optical images may reflect signals from the surface vasculature. In our study, the CCD-camera was focused 500–600 μm below the cortical surface; consequently, the surface vasculature was sufficiently blurred to eliminate such artifacts. When a large blood vessel produced artifacts in the images, the shape of the surface-responsive regions was easily differentiated from the pattern of the surface vasculature.

Third, a potential contribution of binocular stereopsis as a source of artifact due to misalignment of two eyes can be disregarded because we also observed surface-responsive regions when using a blank gray screen as a control. To summarize, we conclude that visual stimulation by the interior of a uniform surface was responsible for activating surface-responsive regions.

We identified the areas 17/18 border based on the difference in spatial frequency preference between these two areas. For stationary grating stimuli, spatial phase of each stimulus differs at each point in the visual field, and this may cause differential activation of neurons sensitive to the spatial phase of the stimulus. However, as moving grating stimuli yielded similar results, such a possibility can be denied.

Previous studies have shown that there are neurons in V1 that are responsive to a uniform plane stimulus covering their entire receptive field. Response magnitudes of many such surface-responsive neurons correlated monotonically with the luminance of the uniform plane stimuli (Kinoshita and Komatsu 2001; Maguire and Baizer 1982). In the present study, roughly one-third of the surface-responsive neurons clearly distinguished the black and white phases of a uniform plane stimulus. Although these neurons may have luminance sensitivity similar to that reported in V1 neurons and participate in representation of the light intensity, because our stimulus had only two levels of luminance, it is not clear whether they carried information about the luminance of the uniform plane stimulus or the direction of the luminance changes.

Localization of the surface-responsive neurons

Our findings suggest that surface-responsive neurons have close relationships with the singular points in the orientation-preference map. Because neurons with widely varying preferred orientations intermingle in the regions near singular points (Maldonado et al. 1997), these regions are not suitable for representing contours with a specific orientation. Blasdel and his colleagues proposed that regions near singular points participate in the representation of surfaces containing textured patterns (Blasdel 1992; Blasdel and Obermayer 1994). The present findings suggest that some of the singular points and their neighboring regions participate in representing the interiors of uniform surfaces rather than textured surfaces. A recent study has shown that regions near the singular points have
uniform connections with all orientation domains (Yousef et al., 2001). This clearly contrasts with the lateral connections of orientation domains that are known to connect regions representing similar orientations. As the contour enclosing a surface region necessarily contains all orientations, anatomical connections of singular points may be suited to integrate information from various contour elements as well as from the interior to form the representation of the surface region. Orientation magnitude is low not only near pinwheel centers but also near fractures. So it is interesting to know whether there is difference in response properties of cells sampled from pinwheel centers and those sampled from fractures. However, as is seen in Fig. 4, orientation maps in our study had only short fractures near pinwheel centers, and it is hard to distinguish samples from fractures and those from pinwheel centers. The lack of long fractures is consistent with previous studies of the orientation map in the cat area 18 (Bonhoeffer and Grinvald, 1993).

We also found that surface-responsive neurons were densely accumulated in specific locations, thereby forming surface-responsive regions that were detectable by optical imaging. These regions were localized in area 18 near the border between areas 17 and 18. Inside these regions, the classical receptive fields of the surface-responsive neurons were mostly centered around the vertical meridian from about 10° in the ipsilateral visual field to a few degrees in the contralateral visual field. Areas 17 and 18 in each hemisphere represent mainly a contralateral visual hemifield, but a small part of the ipsilateral visual hemifield, along the vertical meridian, is also represented (Diao et al., 1990; Ohki et al., 2000; Payne, 1990; Tusa et al., 1978, 1979; Whitteride and Clarke, 1982). The cortical regions representing the ipsilateral visual field are referred to as the “transition zone” in which retinotopic maps of the right and left hemispheres overlap. Transition zones of both hemispheres are mutually linked through callosal connections (Olavarria, 1996; Payne, 1991; Payne and Siwek, 1991), and the specific localization of the surface-responsive regions suggests that such callosal linkages may contribute to their formation. As such, one possible function of the surface-
responsive regions may be to link visual information about large surfaces extending across both the right and left hemifields.

One could argue that surface-responsive neurons might not be necessarily clustered in functional domains; instead they could be interspersed with contour neurons homogeneously across the cortex to convey surface information throughout the visual field. We indeed observed surface-responsive neurons outside the regions near the singular points. On the other hand, specific localization of surface-responsive regions may suggest that these regions have special function in surface representation. One possibility is that these regions represent groups of neurons that pool surface responses from interspersed neurons to transmit signals about surface interior to the opposite hemisphere or to higher cortical areas. Another possibility is that neurons located inside surface-responsive regions prefer especially large stimulus such as an illumination of ganzfeld. If this is the case, such neurons need not be distributed wide-spread in the entire retinotopic map of area 18; instead, they may be concentrated in some restricted regions. Detailed examination of the response properties of surface-responsive neurons both inside and outside surface-responsive regions will be necessary to obtain better perspective on the functional significance of surface-responsive regions.

Surface-responsive regions were found only near the transition zone of area 18 and not in the corresponding part of area 17. Previous electrophysiological studies have shown that, in both cats and monkeys, some striate neurons respond to a uniform surface stimulus (Kinoshita and Komatsu 2001; Rossi and Paradiso 1999; Rossi et al. 1996). However, the density of this population of neurons may not be sufficient to enable detection by optical imaging. A similar situation was observed in area 18, where surface-responsive neurons were scattered outside the region. In addition, we cannot exclude the possibility that the surface-responsive regions exist within area 17 in the medial wall of the hemisphere, where optical imaging cannot record neural signals.

If surface-responsive neurons are densely accumulated in area 18 but not in area 17, what factors might account for this difference? In the cat visual cortex, area 18 receives direct projections from the lateral geniculate nucleus (LGN) as well as from area 17 (Humphrey et al. 1985; LeVay and Gilbert 1976; Niimi and Sprague 1970), so that areas 17 and 18 can be regarded more as parallel processors than as hierarchical processing steps (Hendrickson et al. 1978; Hubel and Wiesel 1972). This raises a possibility that areas 17 and 18 are specialized for different aspects of visual processing. For example, area 18 mainly receives input from Y cells in the LGN, whereas area 17 receives inputs from both X and Y cells (Humphrey et al. 1985; LeVay and Gilbert 1976). In addition, area 18 neurons prefer lower spatial frequency stimuli than do area 17 neurons (Bonhoeffer et al. 1995; Hung et al. 2001; Issa et al. 2000; Movshon et al. 1978; Ohki et al. 2000). Accumulation of surface-responsive neurons in area 18 may thus be another example of the functional specialization of this cortical area.

**FIG. 10.** Comparison of the spatial frequency properties of neurons recorded inside and outside the surface-responsive regions. A: spatial frequency tuning curves of individual neurons (••••) and the averages (—) recorded inside (top) and outside (bottom) the regions. Responses were examined using sine-wave gratings having 4 spatial frequencies (0.08, 0.15, 0.5, and 1 cycle/°) and a uniform plane stimulus (P) and then normalized to the maximum response of each neuron. B: distribution of the peak spatial frequency of neurons recorded inside (top) and outside (bottom) the surface-responsive regions.

**FIG. 11.** Distribution of the luminance selectivity indexes representing the preference for either a black or white surface (see METHODS). □ and ■, neurons recorded inside and outside the surface-responsive regions, respectively.
Surface-responsive regions and the representation of surface

In addition to uniform plane stimuli, a majority of surface-responsive neurons showed rather strong responses to gratings. Such dual responsiveness is not surprising, given what has been observed previously in V1. For example, area 17 neurons previously found to be responsive to a uniform plane stimulus also responded to a small spot and a bar stimulus (Kinoshita and Komatsu 2001; Rossi and Paradiso 1999), suggesting that the surface-responsive neurons participate in the representation of two sorts of complementary features contained within the visual image of an object: a contour and a surface surrounded by a contour. These neurons may exhibit orientation-selective responses when a contour is presented to the receptive field, but the same neurons respond to a uniform plane when the receptive field is contained within the interior of a uniform surface.

An alternative though not exclusive idea is that responses to these two sorts of stimuli are opposite extremes of a continuous response spectrum. Visual cortical neurons exhibit a range of spatial frequency selectivities (Maffei and Fiorentini 1973; Movshon et al. 1978; Tolhurst and Thompson 1981), and a single neuron is usually tuned to a wide range of stimuli with different spatial frequencies. Surface-responsive neurons can thus be regarded as a group of cells that are sensitive to stimuli with extremely low spatial frequencies but that nonetheless respond to stimuli with higher spatial frequencies as long as the stimuli are within the range of their tuning.

The focus of the present study was on the representation of a uniformly painted surface. In natural scenes, however, object surfaces often contain many local luminance contrast components with various orientations that combine to form a textured pattern. Although we did not examine responses to textured patterns, the results obtained with the checkerboard pattern are suggestive. Our extracellular recordings revealed that neurons inside the surface-responsive regions were more strongly activated by a uniform plane than by a checkerboard pattern and that the opposite tendency existed outside the surface-responsive regions. We therefore suggest that different populations of neurons represent the interior of a uniform surface and a textured surface, the former being represented by neurons in the surface-responsive regions and the latter by neurons outside those regions.

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