Intrinsic projections within visual cortex: Evidence for orientation-specific local connections

(cortical organization/iso-orientation bands/ocular dominance/horseradish peroxidase/topography)

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ABSTRACT The functional organization of intrinsic connections within area 18 of cat visual cortex was studied using combined electrophysiological and anatomical techniques. Physiological recordings were first used to map the distribution of orientation preference, ocular dominance, and receptive-field location relative to the cortical surface. Next, localized injections of lectin-conjugated horseradish peroxidase were made into physiologically identified regions within area 18. We found that (i) the local cortical interconnections are made preferentially between cell populations with orthogonal preferred orientations and are independent of the ocular dominance of the cortical cells, (ii) the map of visual space in the cortex is anisotropic with the magnification factor for vertical at least twice that for horizontal visual space, and (iii) the pattern of cortical projections compensates for the functional asymmetry so that a population of interconnected cells represents a roughly circular region of visual space.

A variety of anatomical methods have revealed that projections of cortical neurons to other cortical areas are often clustered and that these clusters are periodic in their distribution across the surface of visual cortex (1–3), other sensory cortical areas (4–7), and non-sensory cortical structures (8, 9). More recently, periodically clustered projections have been shown to occur within a single cortical area (10–13). What role might these clustered projections play in cortical processing? In visual cortex, it is possible that such projections play a functional role related to feature extraction properties of cortical neurons. This conjecture receives support from studies showing that neurons with particular physiological characteristics are arranged in clusters on the cortical surface (14–19), and it has been the subject of theoretical discussion (20). However, to date there is no direct evidence for any relationship between local anatomical connections and physiological response properties.

In an attempt to understand the rules governing the intrinsic projections of visual cortex we combined physiological recording and anatomical tracing techniques in a study of cortical area 18 of the cat. We report here evidence for the roles of orientation selectivity and topographic representation on the specificity of local intracortical connections. Our findings indicate that (i) cortical cells receive a projection from nearby cortical cells whose averaged best orientation is approximately orthogonal to their own, (ii) the cortical magnification factor is anisotropic, and (iii) the local projections compensate for the anisotropy in the cortical magnification factor so the overall area of visual space represented by a population of interconnected cells is roughly circular.

MATERIALS AND METHODS

Our studies were conducted in cortical area 18 of nine normal cats. The naturally flattened surface of area 18 was ideal for mapping the surface distribution of response properties. After removal of ≈1 cm² of skull and dura, we photographed the exposed brain surface. The stereotaxic position of the exposed brain surface ranged from −2.0 to +6.0 mm (posterior to anterior) and from 0.0 to 5.0 mm (lateral). The cortex was viewed through a dissecting microscope and the electrode was positioned above areas free of blood vessels. A multiple-unit recording was made at each penetration at a depth of 200–400 μm from the surface using extremely fine glass-coated, platinum/iridium microelectrodes (21, 22). Penetrations were spaced at ≈300–500 μm intervals in a grid-like array using the micrometer settings on a Narashige electrode holder. Each penetration was numbered and labeled on an enlarged photograph of the exposed cortical surface using the pattern of blood vessels and the micrometer readings to determine its position accurately. At each recording locus, we examined ocular dominance, orientation preference, and receptive field location. Small areas of cortex (≈9 mm²) were mapped in detail, and the spatial organization of these physiological response properties was studied in relation to the cortical surface. Usually, we accumulated between 80 and 125 data points over a period of 2 days. Orientation preference, ocular dominance, and cortical positional coordinates were then entered into a computer program. The power spectra for preferred orientation and ocular dominance were obtained from a Fourier transform and the position of the peak in the spectrum was used to determine the periodicity of the response properties.

Approximately 24–36 hr before the end of the recording session, horseradish peroxidase (HRP; 20% in sterile saline, cases JMM1, JMM4, 4SP) or wheat-germ agglutinin (WGA) conjugated to HRP (WGA–HRP, 1% in sterile saline, cases 6SP, 12SP, 13SP, 19SP, 35SP) was pressure-injected via glass micropipettes (tip inner diameter, 8–10 μm). Injections were made into cortical areas with similar preferred orientations or into areas with similar eye preferences. Opportunities to place an injection into a single area that possessed both similar preferred orientations and similar eye preferences were rare. Since the physiological recordings were from neurons above layer 4 (as determined by the micrometer depth readings and the absence of neural background responses associated with the inputs from the lateral geniculate nucleus), we focused the injection of tracer into the superficial cortical layers and analyzed only those labeled cells found in horizontal sections corresponding to cortical layers I–IV. The micropipette was placed initially at a depth of 700–

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Abbreviations: HRP, horseradish peroxidase; WGA, wheat germ agglutinin.

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FIG. 1. (Legend appears at the bottom of the next page.)
800 μm from the cortical surface. At this depth, 10–15 nl (measured as a calibrated drop in the meniscus level of the micropipette) of HRP or WGA–HRP was injected by pressure. The micropipette was then immediately brought up to a depth of 300–400 μm from the surface and left in situ for 5 min. This procedure resulted in a conical-shaped injection site: the diameter of the injection site was largest in the upper cortical layers and gradually tapered toward white matter. Fluorescent dyes were injected into other nearby cortical areas and served as landmarks for histological reconstructions. Twenty-four to 36 hr later, the animals were deeply anaesthetized and perfused with phosphate buffer followed by either 4% buffered paraformaldehyde, or a mixture of 2.5% buffered paraformaldehyde and 0.5% buffered glutaraldehyde. This was then rinsed with a solution of 10% buffered sucrose. Cortical blocks were sectioned horizontal- ly, tangential to the surface of area 18. The ipsilateral lateral geniculate nucleus was sectioned in the coronal plane. Sections were cut at a thickness of 40–60 μm and were pro- cessed by the tetramethylbenzidine procedure (27).

RESULTS

Our physiological data revealed the following topographic features of neuronal responses in area 18:

(i) The vertical (elevational) and horizontal (azimuthal) components of receptive-field location are mapped in the cortical anteroposterior and mediolateral planes, respectively (23). We observed a marked anisotropy in the retinotopic map with the magnification factor for vertical being at least twice that for the horizontal (24).

(ii) Neurons with similar eye dominance properties were clustered. In six animals, the periodicity of these clusters as determined by spectral analysis ranged between 1.2 and 1.8 mm. We did not observe a consistent direction of elongation of these clusters (25).

(iii) In the same six animals, the cells responding best to a given range of stimulus orientations were located within elongated, branching bands running approximately from postero- medial to anterolateral across the cortical surface (Figs. 1 A and B and 2 A and B). The period of these iso-orientation bands, as determined by spectral analysis, was remarkably constant and averaged 1.23 ± 0.03 mm (25).

Injections of HRP and WGA–HRP produced several aggregates of labeled cell bodies within 2 mm of the injection site. In addition, the WGA–HRP-labeled cells were sur- rounded by a granular type of reaction product characteristic of anterogradely and/or collateral transport. An example of the number and size of the clusters of labeled cells is shown in Fig. 1 C and D.

Charts of the injection sites, marker sites, and labeled cell bodies were drawn at both low and high magnification using drawing tubes attached to a Wild dissecting microscope and a Leitz compound microscope. The charts were enlarged or reduced to the same magnification as the photograph of the cortical surface and then aligned with it using the injection site and marker lesions as references. Thus, we were able to make a direct comparison of the location of the labeled cells bodies in the upper two-thirds of the cortex and the topo- graphic maps describing the physiological responses (Figs. 1 A and B and 2 A and B). This comparison provided the fol- lowing correlations:

(i) Injections into iso-orientation domains with a range of 45° or less labeled cell bodies in regions where orientation preferences were roughly orthogonal to those of the injection site cells. We observed this for four cases in which the injection site was confined to an iso-orientation domain of 45° or less (Figs. 1 E and 3 A). The angular differences between the mean preferred orientation (α) of the injection site (α) and the projection areas (β) in these four cases are 85° (6SP), 76° (4SP), 86° (19SP), and 76° (12SP, not shown).

In two other cases, the injection site spanned a greater range of preferred orientations (45°–90°), and the projection areas also spanned greater orientation ranges. Nevertheless, the angular differences between averaged orientation values for injection and projection areas were 73° (35SP) and 86° (13SP).

(ii) The cortical region in which labeled cells were found extended further in the anteroposterior than the mediolateral direction (Figs. 1 and 2). This was observed for all six WGA– HRP injections regardless of the best orientation of the cells within the injection site and is consistent with earlier studies on silver-stained material, indicating that the majority of fibers run along the anteroposterior axis in cat visual cortex (28). The anisotropy in the cortical magnification factor is complemented by the anisotropy in the local anatomical con- nections such that the accumulated receptive field for a pop- ulation of labeled cells (arising from a single injection) corre- sponds to a roughly circular area of visual space (Fig. 4).

(iii) No correlation between the ocular dominance distri-
Fig. 2. Distribution of physiological responses in relation to the cortical surface of case 19SP. As in Fig. 1A and B, preferred orientation (A) and ocular dominance (B) are labeled over the recording sites in the surface diagrams of the cortical tissue. In A, the cortical areas with preferred orientations centered around horizontal (range, ±45°) are shaded. A microinjection of WGA-HRP was placed in a group of cells with orientation preferences centered slightly off the horizontal (hatched circle). Labeled cell bodies were found in distinct clusters surrounding the injection site, and two of the three clusters were within the physiologically mapped area. Both of these clusters were in areas previously shown to have orientation preferences centered near vertical (±45°). Each stippled square represents 20 labeled cell bodies. The population of labeled cells are distributed further in the anteroposterior rather than in the mediolateral direction. The ocular dominance distribution of the cells within the injection site is shown in B. The site of the injection was chosen for its homogeneous orientation preferences. Ocular dominance distribution includes ipsilateral-, binocular-, and contralateral-eye-dominated points. Data are graphed in Fig. 3.

Distributions of the injected and labeled cells was evident even when the injections were centered on contralateral or ipsilateral eye clusters (Figs. 1B and F, 2B and 3B). In two cases, the injections were centered on cortical sites at which visual responses were evoked primarily through the contralateral eye. In one of these (6SP), the labeled cell bodies were found in areas in which visual responses were evoked through the contralateral eye as well as in other areas in which the responses were evoked primarily through the ipsilateral eye. In the other case (JMM4), the labeling was found in areas in which visual responses were evoked primarily through the contralateral eye.

In 19SP and JMM1, the injection sites were centered on
cortical areas influenced mostly by stimuli viewed through the ipsilateral eye, and in both cases the majority of labeled cells was found in areas influenced primarily by stimuli viewed through the contralateral eye. However, because stimuli viewed through the contralateral eye influenced the majority of cortical responses surrounding these injection sites, the specificity may be more apparent than real (Fig. 3B, Control).

**DISCUSSION**

Our results show that injections of a retrograde tracer into iso-orientation domains fill cell bodies in surrounding cortical areas that were shown by physiological recordings to have orientation preferences, on average, orthogonal to those of the cells within the injection site. It seems likely that such a connection (e.g., horizontally oriented cells projecting to vertically oriented cells) is $\gamma$-aminobutyric acid (GABA)-mediated. Such inhibitory connections would enhance orientation selectivity by narrowing the range of stimulus orientations that excite a given cortical cell. Indeed, previous physiological studies have shown that the orientation specificity of cortical cells decreases and is often eliminated completely after the application of bicuculline, a GABA antagonist (29–31). An alternative hypothesis (20), that of cells connecting to other cells with similar (i.e., parallel not orthogonal) orientation preferences is inconsistent with our findings.

There exists another relationship between the intrinsic anatomical connections and the physiological response properties. Cells projecting to a given region in cortex have receptive fields that, when taken as a population, evenly surround the receptive fields of the injected cells (Fig. 4). However, this circular surround in visual space is achieved only because the anatomical connections extend further in the anteroposterior than in the mediolateral direction. The anisotropy in the cortical projections compensates for the anisotropy in the cortical magnification factor, which causes a given circular area of cortex to map onto a horizontally elongated elliptical zone in visual space. Hence, the intrinsic connections restore a functional symmetry to receptive field location in visual space at the expense of anatomical symmetry.

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