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Anatomical evidence of subcortical contributions to the orientation selectivity and columns of the cat's primary visual cortex

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Abstract

Physiological studies have demonstrated a subcortical origin for orientation selectivity and the orientation columns of the primary visual cortex. However, there are no anatomical data showing how subcortical cells contribute to this important property. Optical imaging, combined with 1,1′-dioctadecyl-3,3,3,3′-tetramethylin-docarbocyanine perchlolate (DiI) and biocytin retrograde tracing, reveals that relay cells projecting to a single orientation column representing the horizontal meridian were clustered within 300 μm in the dorsal lateral geniculate nucleus (LGN). Interestingly, some labeled cells were located on a line parallel to an iso-elevation line in the LGN. Thus, according to the quantitative projection of the visual field to the LGN (J. Comp. Neurol. 143 (1971) 101), their receptive fields must distribute horizontally in alignment in the visual field providing the first anatomical evidence for Hubel and Wiesel's model of simple cell receptive fields (J. Physiol. 160 (1962) 106).

Keywords: Orientation selectivity; Visual cortex; Lateral geniculate nucleus; Column; Optical imaging; Biocytin; 1,1′-Dioctadecyl-3,3,3,3′-tetramethylin-docarbocyanine perchlolate

Orientation selectivity is a fundamental property of most neurons in the primary visual cortex and underlies the basis of form perception. Cortical cells with a similar preferred orientation are organized in a columnar manner from the pial surface to the white matter in the cortex. Initially, Hubel and Wiesel hypothesized that the receptive fields of simple cells in the primary visual cortex were formed by the convergence of many geniculate neurons with concentric receptive fields. These fields must be arranged in a straight line on the retina according to the axis orientation of simple cell receptive fields [5,6]. This model is simple and reasonable, and has been supported by physiological studies [2,3,7]. However, there has been no direct anatomical evidence to support it. In contrast, many studies have demonstrated that about 70% of ganglion cells in the retina and relay cells in the dorsal lateral geniculate nucleus (LGN) of cats are orientation sensitive [8,10,11,13,14,17]. The orientation sensitivity of LGN cells is not influenced by visual experience, but decided by genetic factors [17]. Their preferred orientations are parallel to a line jointing the receptive field center and the area centralis of the cat's retina [10,11].

The purpose of our study was to probe anatomical evidence of geniculate contributions to the orientation selectivity and columns of the visual cortex using an intrinsic signal revealed through optical imaging combined with 1,1′-dioctadecyl-3,3,3,3′-tetramethylin-docarbocyanine perchlolate (DiI) and biocytin retrograde tracing methods. The results suggest that for most cells in the primary visual cortex, the orientation-biased subcortical inputs help generate their orientation selectivity. The convergent geniculocortical connection may possibly therefore contribute to the orientation selectivity in some simple cells.

Ten adult cats were used for the intrinsic signal optical imaging on the primary visual cortex. All procedures were similar to those for single unit recording as previously described [12]. A mixture of gallamine triethiodide (20 mg/kg per h) and sodium pentobarbital (3 mg/kg per h) was intravenously injected to maintain anesthesia and paralysis. The animals' physiological conditions were kept in normal ranges throughout the experiment. The visual cortex corresponding to ~5–15° projecting elevation

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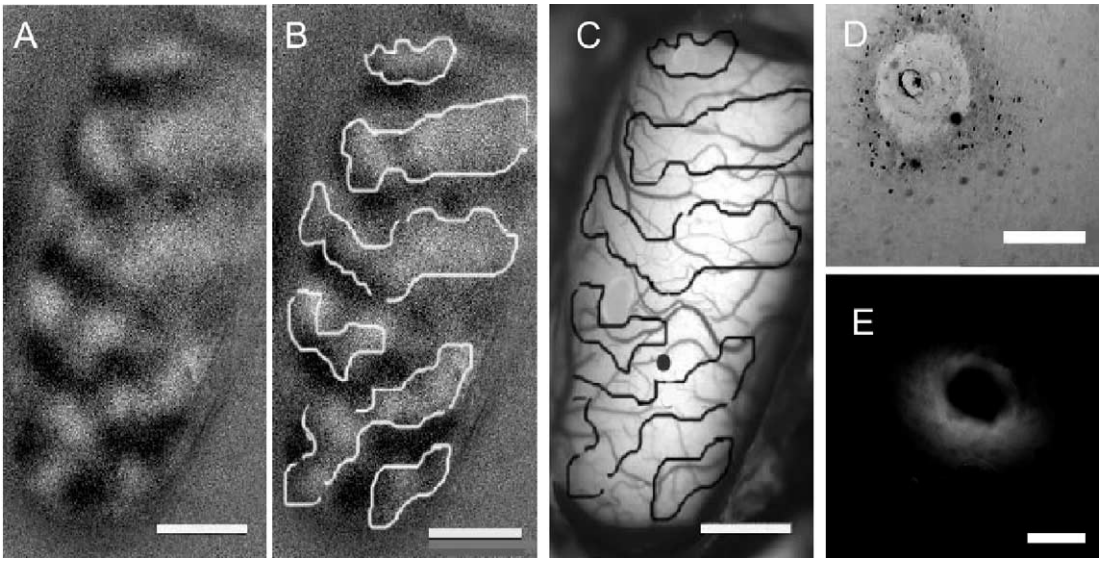

Fig. 1. Photographs showing the procedures for application of biocytin or DiI into a single orientation column center revealed by the optical imaging based on intrinsic signals (A–C). The contour of orientation columns that prefer a horizontal grating stimulus (dark areas in A) was highlighted (B), and then superimposed on the vessel map of the identical cortex (C). Accordingly, the injection site indicated by a black point in (C) was selected for biocytin or DiI application. The injection areas of biocytin and DiI are shown in (D) and (E), respectively. Horizontal stimulus gratings were used in all the experiments in the study. The mean luminance, spatial frequency, temporal frequency and contrast of the drifting grating were 19 Cd/m^2, 0.5 cycles/degree, 2 Hz and 0.9, respectively. Scale bars: (A–C), 1.5 mm; (D), 500 μm; (E), 100 μm.

in the visual field was exposed and a chamber was cemented onto the skull. The chamber was filled with artificial cerebrospinal fluid and sealed with a cover glass. All investigations involving animals conformed to the policy of the Society for Neuroscience on the Use of Animals in Neuroscience Research. All efforts were made to minimize the number of animals used and their suffering.

An area-scan charge-coupled device (CCD) camera (DALSA, Canada) was used to record the optical images of intrinsic signals from the exposed cortex as previously reported [16]. The vessel map on the cortical surface was obtained by green light (546 nm) shining on the cortex and the functional orientation maps of the cortical cells responding to horizontal grating stimuli (Fig. 1A) were obtained with red light (640 nm). The cats were stimulated binocularly with drifting square-wave gratings horizontally oriented with a spatial frequency of 0.2 or 0.5 cycles/degree, contrast of 0.9 and a temporal frequency of 2 Hz.

The contour of a highlighted orientation map (Fig. 1B) was superimposed onto the vessel map of the same cortex as shown in Fig. 1C. Under guidance of the overlapped map, the center of a horizontal grating-activated column (dark stripe) was selected as the injection site for biocytin or DiI. The 1.5–5% of biocytin–lysine (Sigma, USA) in 0.5 M NaCl and 5% of DiI (Sigma, USA) in DMSO were injected perpendicularly into the cortex at the depth of 0.5–1.2 mm, respectively. The size of biocytin injection area was about 300–500 μm in diameter (Fig. 1D,E). For three cats, immediately after removal of the visual cortex, the tissue was cut in tangential sections of 90 μm. The biocytin-labeled neurons were detected though the avidin–biotin–peroxidase reaction as described by Chang et al. [1].

The DiI injected cortical tissues from seven cats were treated conventionally [4]. After the injection, the animal was sacrificed by an overdose of sodium pentobarbital. Two blocks of the cortex and thalamus of interest were dissected, and stored in a fixative of 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, for 4–6 months. Then, the brain tissues were cut in serial coronal (for LGN) or tangential (for cortex) sections of 60 μm. The images of the labeled neurons in the LGN were captured with a fluorescence microscope and a CCD camera. All the LGN sections were divided into two alternative groups: one for Nissl staining; and the other for detection of DiI labeling.

Fig. 2 shows the visual cortical cells and their neuronal connections in an orientation column, within which the cortical cells responded preferentially to horizontal oriented gratings during the previous optical imaging. The biocytin-labeled columnar area was roughly, but not exactly orthogonal to the pial surface due to the highly curved cortex and the Vibratome cutting. In addition to a cluster of cells in layer III, another cluster of retrograde labeled cells in layer IV is clearly observed, indicating a feedforward neuronal connection from layer IV to layer III. The width of the labeled columnar area was about 300–400 μm. A tract of axons orthogonal to the pial surface appeared in layers II and III.

The geniculo-cortical projection was observed in the DiI retrograde labeled sections (Fig. 3A–D). In most cases, the labeled relay cells were scattered apart in different coronal sections from 100 to 1500 μm (A and B) within the LGN.

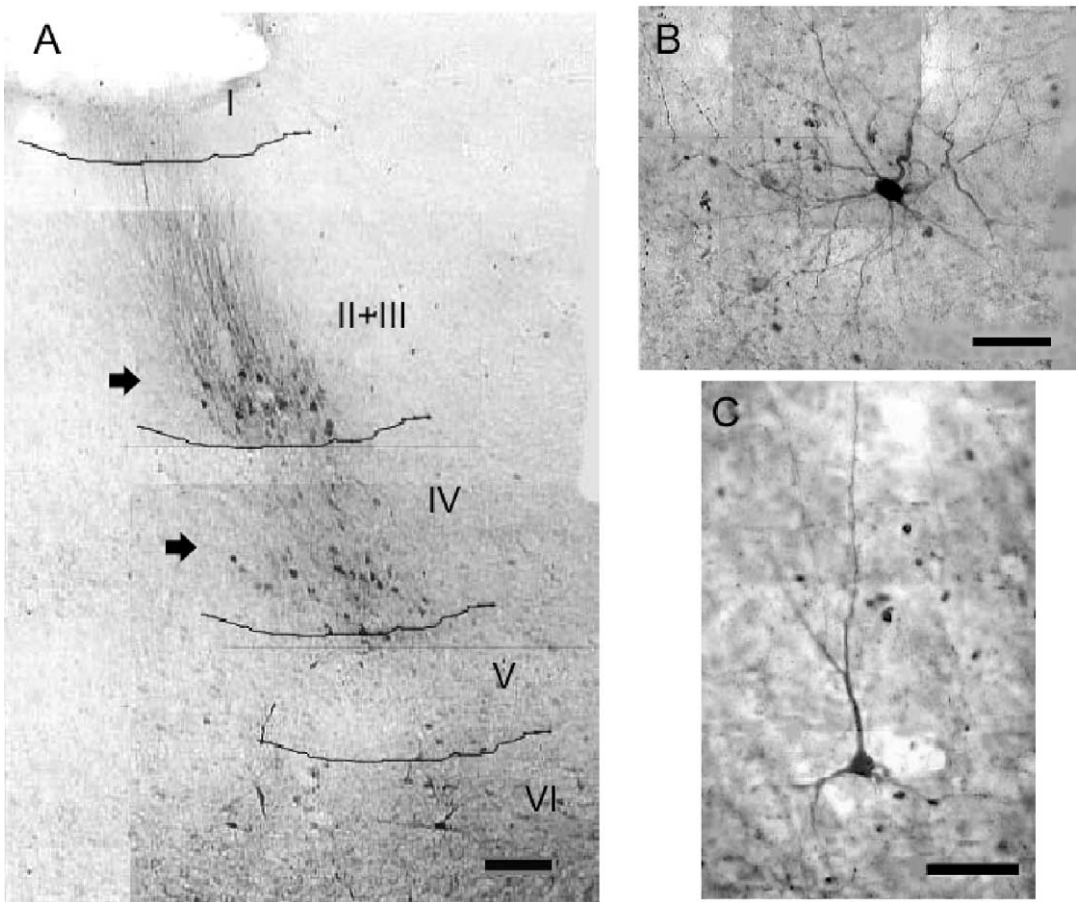

Fig. 2. Neurons and their connections within an orientation column in the primary visual cortex revealed by biocytin labeling. The injection site of biocytin was located in layer III of the cortex. (A) Two clusters of labeled cells denoted by arrows are clearly shown in layers III and IV, respectively. (B) A stellate cell of layer IV in a tangential section. (C) A pyramidal cell of layer III in a coronal section. Scale bars, 100 μm.

However, in some cases, many labeled neurons were clustered together within 300 μm (C), suggesting that those relay cells project into a single orientation column in agreement with the physiological finding that relay cells with a similar preferred orientation are clustered within 150–200 μm in the LGN [10]. Interestingly, we found that there were three relay cells located on a straight line of 300 μm in layer A, which was parallel to the boundary of layers A and A1 (D). The locations of the three cells in the LGN were marked in the Nissl stained neighboring section next to the DiI-labeled one (E), and were shown in a dotted contour diagram (F). According to Sanderson's classical measure of the quantitative projection of the visual field to the LGN in the cat [9], the receptive fields of these relay cells must be located on a line of approximate 1.4 mm (equivalent to about 5°) on the retina, which is roughly parallel to the horizontal iso-elevation line of −10° with a lateral eccentricity of 5–10° in the inferior visual field. These cells were labeled retrogradely by the injection of DiI into the center of a single orientation column, within which cortical cells preferred to respond to a horizontal grating stimulus. As these geniculate cells were very close to each other, about 150 μm in between, they may presumably converge onto a simple cell in layer IV in the primary visual cortex.

To the best of our knowledge, this may be the first anatomical evidence supporting Hubel and Wiesel's model [5,6]. However, this result seems unusual, even rare in our observation. It is well documented that about 70% of relay cells have already exhibited orientation sensitivity in the LGN [10,14,17], and these cells with a similar preferred orientation are clustered together within the LGN providing the possibility of forming orientation columns in the visual cortex [10]. On the other hand, there are many mechanisms for further sharpening the orientation selectivity of cortical neurons, including excitatory and inhibitory intracortical interactions [15]. Therefore, the orientation-biased geniculate input to the cortex would be enhanced greatly through those multiple mechanisms.

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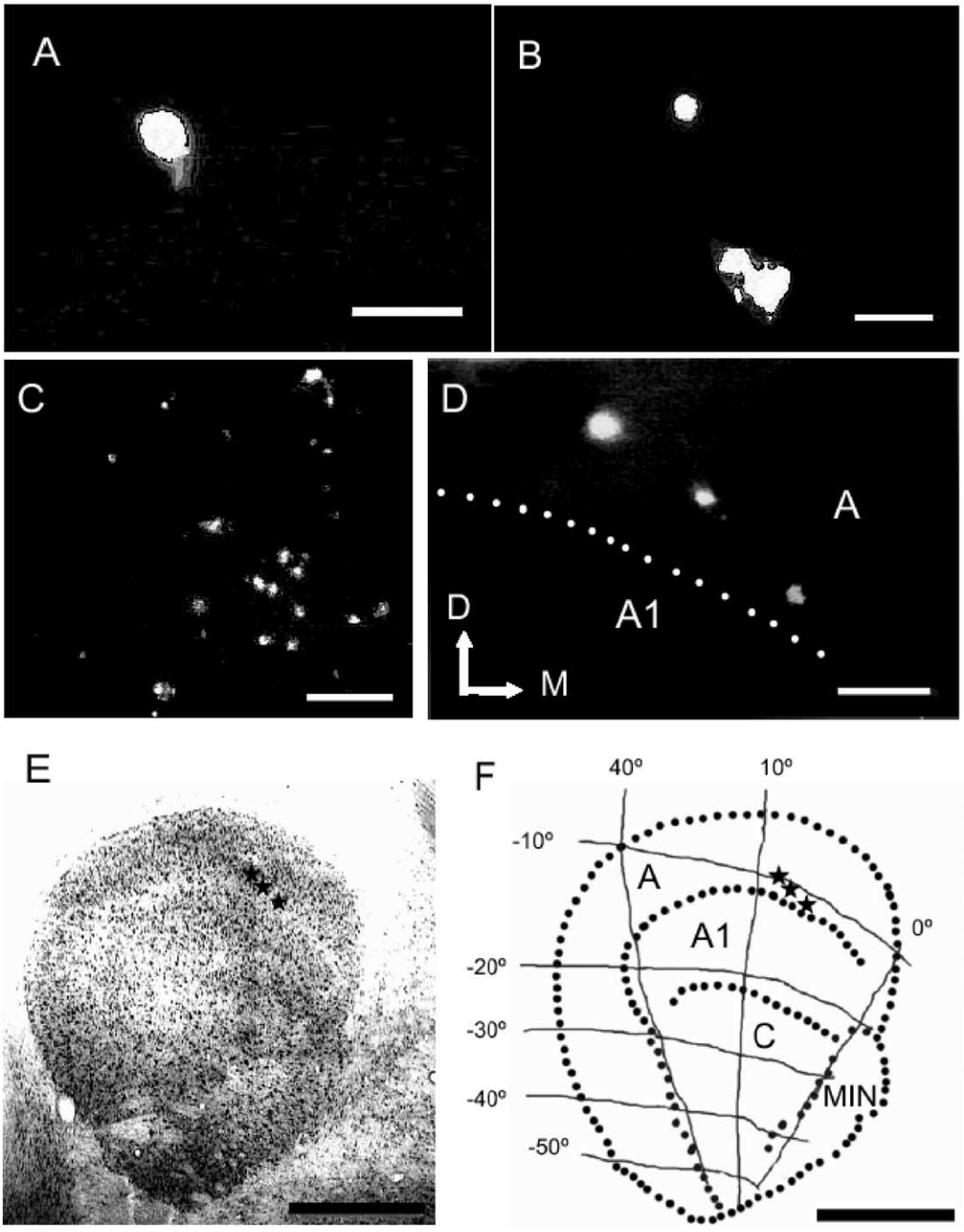

Fig. 3. Photographs of coronal sections of the DiI-labeled relay cells in the LGN revealed by fluorescence microscopy. (A,B) Several separated relay cells projecting from the LGN to a single orientation column in the visual cortex. The two sections are about 1300 μm apart from each other in the LGN. (C) Clustered distribution of the relay cells projecting to a single orientation column preferring a horizontal visual stimulus grating. (D) Three DiI-labeled neurons were positioned on a line roughly parallel to the border between layers A and A1 (dotted line) in the LGN. (E) The coronal section of the Nissl stained LGN, neighboring the section of (D), in which the three neurons shown in (D) were detected and marked by stars. The section was positioned about 8.3 mm anterior from the ear bars in the Horsley–Clarke coordinate. (F) The contour diagram of the section of (E). According to Sanderson's classical work, the numbered thin solid lines were used to indicate the iso-elevation (horizontal) and iso-azimuth (vertical) lines, which were projected from the visual field to the LGN. A, A1 and C indicate the layers of the dorsal LGN. MIN, medial interlaminar nucleus. Scale bars: (A–D), 100 μm; (E,F), 1100 μm.

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