Structure of columns in monkey striate cortex induced by luminant-contrast and color-contrast stimulation

(visual neurons/2-deoxy[14C]glucose/organization of cortex/primate brain)

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ABSTRACT Color and black and white line stimulation produce qualitatively and quantitatively different 2-deoxy[14C]glucose column patterns in the striate cortex of macaque monkeys. Micradensityometry of these stimulus-dependent columns reveals interlaminar density differences that serve as a signature for the stimulus conditions which produced them. Such columns are individually distinct and form a three-dimensional mosaic throughout striate cortex.

How neurons of the visual cortex analyze information about the outside visual world has been the quest of neuroscientists for decades. Gross electrotentials evoked by visual stimulation have given us some insight into the mapping of visual space in cortex, whereas use of the microelectrode has provided a more detailed spatial mapping and information about the preferences of individual neurons for various sorts of visual stimulation. Moreover, the microelectrode has suggested ways in which particular stimulus-sensitive neurons are grouped or aggregated within the six layers of cortex. The well-known works of Mountcastle (1) and those of Hubel and Wiesel (2), among others, have provided clear evidence for a "columnar" organization of neurons in cortex. Finally, Michael (3) has shown that color-sensitive neurons of the monkey cortex appear grouped into a columnar organization.

With the introduction of the isotope-labeled 2-deoxyglucose (2-dGlc) method for marking excited neurons (4, 5), the prospect for marking and revealing for study the mosaic of columns throughout visual cortex became a reality. Indeed, this technique has been used on several occasions to demonstrate columns of neurons and neuronal processes within areas of visual cortex induced by monocular stimulation (eye dominance columns) or stimulation by lines of a single orientation (orientation columns) (6–12). What has been missing in many of these reports has been specification of stimulus conditions, or a subsequent detailed analysis of the column structure and distribution, or both. It is the purpose of this report to demonstrate (i) that the functional structure of the stimulus-dependent 2-dGlucolumn in the striate cortex of monkey serves as a signature for the stimulus conditions that produced the column and (ii) that considerable insight can be gained by detailed microdensitometry as to the interlaminar form as well as the mosaic and the distribution of these columns throughout visual cortex.

MATERIALS AND METHODS

Macaque monkeys (Macaca fascicularis) were lightly anesthetized and paralyzed, and their two eyes were centered and focused on a visual display at 1-m distance. Square wave grating patterns were generated on the face of a color television tube. A Sony Trinitron color television set had been modified to gain independent control of the color guns, which in turn were driven by laboratory-built logic function generators. Such generators are controlled through a selection panel giving independent control over spatial frequency of square-wave gratings and of the colors, luminances, and direction and rate of drift of such gratings. The spectral compositions and luminances of the gratings were measured by using EG & G models 580 and 585 spectroradiometers. Luminance matching in these experiments was done by using a photometric filter (EG & G 580-00-31) that alters the model 583 photodiode to approximate the human luminosity function. As the middle and long wavelength spectral sensitivity of monkey and man are quite similar, the red and green (R/G) stripes were individually brought to an arbitrary luminance of 8.4 × 10^{-4} lumens/cm². Where colorless gratings were used, the overall luminance of the black and white (B/W) stripe pattern was brought to the same value.

In one experiment, vertical B/W grating patterns of 1 cycle per degree (cpd) were drifted in a single direction at 1 deg/sec. A comparison experiment was the same except that luminance-matched R/G lines were drifted to preferentially stimulate the visual color mechanisms over the luminance mechanisms. An intravenous pulse of 2-[14C]dGlc (100 μCi/kg of body weight; 1 Ci = 3.7 × 10^{10} becquerels) was given; the visual stimulation continued for the next 45 min. A tungsten microelectrode recorded stimulus-controlled single unit responses before and during the 45-min stimulation period after the injection, thus assuring us that the brain was visually responsive throughout.

The brains were removed from monkeys under deep anesthesia and processed for autoradiography to reveal the pattern of differential uptake by neurons of the visual cortex. The brains were quickly frozen in liquid-nitrogen-cooled freon, sectioned at 7- to 10-μm thickness, dried quickly on coverslips, and placed against SB5 x-ray film for 30 days. Care was taken that the exposure time was the same for all experiments.

All microdensitometry of the resulting autoradiographs was done in the Biomatics Department of the University of Texas M. D. Anderson Hospital and Cancer Research Institute. The image analysis system consisted of a Cohu (model 7120) video camera as input to a Colorado Video (model 270) digitizer having 512 × 480 pixels with eight bits of gray per pixel. A Modcomp II/220 computer (96,000 bytes of memory) and a wide variety of peripherals completed the system. The translaminar density and the horizontal spacing between columns was achieved by scanning the image with a narrow slit. For the point-by-point large field scans (1.75 × 5.5 mm; 41,245 points), each point represented the density of a spot of brain approximately 15 μm in diameter. A 63-gray-level relative density scale

Abbreviations: 2-dGlc, 2-deoxyglucose; B/W, black and white; R/G, red and green.
was used but was reduced to a more convenient 0–9 print scale. All density profiles are relative and ordinal only. Averaging of the column profiles and all area and distance measurements were made with a HP85 graphics tablet and computer.

RESULTS AND DISCUSSION

Fig. 1A illustrates the pattern of stimulus-dependent columns induced in striate cortex by color stimulation (a luminance-matched R/G grating) as compared to that produced by stimulation by an equal-luminance high-contrast B/W grating (Fig. 1B). Casual examination of these two sample patterns suggests that the R/G and B/W gratings excite different subpopulations of neurons in the striate cortex of monkey. The two neuronal subpopulations appear to have different distributions within the six layers of cortex. When compared to adjacent Nissl-stained brain sections, it is clear that the R/G color stimulation preferentially excited neurons and neuronal processes in layers V, IVc, IVa, and III, with horizontal extensions interconnecting high densities in IVa and IVc. This interlaminar density distribution matches remarkably that reported by Michael (13) for

![Figure 1](image_url)
the distribution of the color-sensitive simple cells of monkey cortex.

In comparison, B/W gratings produced distinct high densities of uptake in layers VI and upper IVc, with a relatively thin column extending across the superficial layers to the cortex surface. Hubel et al. (6, 7) reported columns induced by oscillating B/W, vertically oriented stripes which differed from these columns by showing a continuous interconnecting band in layer IVc. In four binocular experiments with monkeys under panchromonium bromide paralysis and with a single spatial-frequency grating pattern drifting at a constant rate, an uninterrupted band in layer IVc has been obtained only once and is shown here in the R/G experiment. In the present experiments, although the two eyes were focused on the center of the screen, exact superimposition of the foveae and, consequently, binocular receptive fields was not done. Thinking that the interrupted band in layer IVc might reflect some “pericolumnar inhibition” (ref. 1, p. 39) consequent to asynchronous stimulation of the two eyes by a drifting grating of single spatial frequency, we repeated the experiment taking care to superimpose the receptive fields for a binocular neuron of the striate cortex by use of a variable prism. Once again, the pattern was interrupted in layer IVc, replicating the result shown here in the B/W experiment.

**Fig. 2.** Average column density contour \((n = 10\) each) for R/G stimulation \((A)\) and B/W stimulation \((B)\). The average contour is shown superimposed on a point-by-point density scan for one autoradiograph from which the average column density contour was derived. Only the three highest density contours are shown. \((\text{Bar} = 0.1\ \text{mm})\)
By scanning microdensitometry, we measured the density profiles across the cortical laminae of a large number of these columns so as to arrive at an average density profile of a column produced by R/G stimulation as compared to that of columns produced by B/W stimulation. Fig. 1 C and D shows such average profiles. Clear interlaminar differences exist between columns produced by color and by B/W stimulation. These patterns support what has been shown by microelectrode studies—that neurons of striate cortex are grouped together into functional units so as to process information about specific stimulus features (e.g., color, orientation, direction of movement, etc.). Moreover, these results demonstrate that the neuronal aggregation is specific for different laminae and that they form a mosaic of columns throughout stimulated visual cortex. An interesting exercise is to superimpose the density outlines of a string of columns generated by B/W stimulation with similar columns produced by R/G stimulation. It becomes evident that when the two patterns are shifted 180° in phase, there is a remarkable interdigitation of the two profiles—i.e., the dark column of one pattern fits the light spaces between columns in the other pattern. This idea of the interdigitation of functional columns and their actual intercolumn spacing are in agreement with the conclusions of Michael (3) based upon tangential microelectrode penetrations of striate cortex.

Finally, that these columns are determined by the visual stimulus and do not merely reflect some endogenous structural pattern [such as that revealed by cytochrome oxidase staining (14)] is supported by the fact that the pattern is restricted to the spatial representation of the stimulus field (22° visual angle) in striate cortex.

To determine the structure of the columnar mosaic within striate cortex, point-by-point density measurements were made for many of the cortical columns as shown in these autoradiographs from horizontally sectioned brains. Isodensity contours of the three highest densities (0-9 print scale) were then drawn to outline the column structure. By tracing and superimposing such contours for about a dozen columns, average column contours were made for each experiment and are shown in Fig. 2 A and B along with samples of individual column density contours. Area and distance measurements showed the average column produced by the B/W grating pattern to be approximately 2/3 the size of the average column produced by R/G grating stimulation, most of the difference in high density areas being attributable to the lateral bands in layers IVa and IVc of the R/G column. The area of centralmost high density is approximately the same in each type of column. The B/W column widths are more easily measured, being approximately 0.200 mm wide in layer VI.

It has been claimed that eye-dominance columns revealed by intracocular injection of [3H]proline are slabs or bands coursing throughout the cortex and are not modulated in density (except for laminar differences) within these bands (15). Tangential sections through these brains marked by 2-dGlC suggested to us that 2-dGlC patterns are indeed arranged in rows, but are distinctly modulated in density within these rows or bands. Such patterns have been observed by others (6, 7, 9), and under low magnification they appear as beads on a string—i.e., periodic densities along a row.

To illustrate the feature of column spacing within the cortical mosaic, point-by-point density scans were done of seven serial autoradiographs in tangential section through the superficial layers of the lateral calcarine sulcus of striate cortex of a monkey stimulated by B/W gratings. Again, density profiles were drawn for the three highest densities, the density profiles of all seven serial sections were superimposed, and average density profiles were derived. Shown in Fig. 3, the reconstructed and average form of the field of columns appears to consist of parallel rows that branch and converge occasionally but clearly contain periodic density modulations. By measuring the distances between the columns within rows and comparing these with distances between columns between rows we could test the hypothesis that the mosaic of columns is a square array. Such measurements show the array to be other than square, in that the average distance between columns within rows is only 0.285 mm (SD = 0.08, n = 38), whereas the average distance between rows is 0.565 mm (SD = 0.05, n = 83). This between-row spacing is virtually identical to that reported by Hubel et al. (6, 7).

Moreover, the clusters of color-sensitive neurons found by Michael (3) had the same spacing (calculated from Fig. 9; n = 16, \( \bar{x} = 0.567 \), SEM = 0.08) being alternated with neuronal clusters not responsive to color. Therefore, the intercolumn distance appears to be the same for color-contrast and for luminance-contrast columns. The degree of spatial interleaving of these two sets of columns requires a double-label experiment, and the results remain to be determined. Although the cross-sectional areas of these highest densities vary considerably between the striate layers, an estimate of the average column area was determined from these composite drawings to approximate that of a circle having a diameter of 0.075 mm for the central highest density (n = 22) and a diameter of 0.160 mm (n = 28) for the next highest density.

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