Monocular Cells Without Ocular Dominance Columns

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INTRODUCTION

Recording in cats and monkeys, Hubel and Wiesel (1962, 1968) discovered that cells in the primary visual cortex vary in their responsiveness to stimulation of each eye. This property is thought to arise because the axonal projections from the lateral geniculate nucleus (LGN) serving each eye are distributed into alternating zones called ocular dominance columns. In macaques, ocular dominance columns are always well segregated, and virtually all cells in layer 4C respond exclusively to either the right eye or the left eye (Blasdel and Fitzpatrick 1984; Hubel and Wiesel 1968, 1977; LeVay et al. 1980; Livingstone and Hubel 1984). It remains unknown why monocular cells in the macaque cortex are segregated into distinct columns or why monocular cells exist in the first place.

The layout of ocular dominance columns can be shown easily by processing V1 for cytochrome oxidase (CO) due to monocular enucleation (Wong-Riley 1979). In the macaque or human, loss of metabolic activity results in paler CO staining in the missing eye’s columns, yielding a zebra-stripe pattern in flattened tissue (Horton and Hocking 1996c). In the squirrel monkey, this approach has revealed marked variation in the expression of ocular dominance columns (Adams and Horton 2003a). Some squirrel monkeys lack ocular dominance columns altogether, whereas others have well-developed columns resembling those found in macaques and humans. The reason for such structural heterogeneity among squirrel monkeys is obscure, but it has established the principle that among individual members of a single species, radical variation can occur in the functional architecture of a given cortical area. This variation in columnar anatomy may be under genetic control (Kaschube et al. 2002).

Our main goal in this present study was to determine if monocular cells are present in layer 4C of squirrel monkeys that lack ocular dominance columns. If monocular cells were present, it would mean that ocular dominance columns are not required for their existence. On the other hand, if only binocular cells were detected, it would imply that ocular dominance columns are requisite for monocular cells to occur. To distinguish between these possibilities, we have recorded the responses of cells in layer 4C to stimulation of each eye.

In the macaque, cells outside layer 4C are usually binocular because they receive convergent input from one or more synapses from monocular cells within layer 4C (Hubel and Wiesel 1977). However, the presence of ocular dominance columns in 4C biases their responsiveness toward one eye. In squirrel monkeys without ocular dominance columns, cells outside layer 4C should be more likely to respond equally to both eyes. To test this prediction, we have also recorded from cells outside layer 4C.

In the macaque, each CO patch (blob) receives a direct, monocular geniculate input from the same eye that supplies the underlying column (Horton and Hocking 1996a). In squirrel monkeys with weak or absent columns, the patches are known to receive binocular koniocellular input (Fitzpatrick et al. 1983). We examined squirrel monkeys with well-segregated ocular dominance columns in layer 4C to determine if the konio input to patches remains binocular or becomes monocular as in the macaque.

Finally, in the macaque, the CO patches are organized like pearls on a string with each strand aligned in perfect register with an ocular dominance column in layer 4C (Horton 1984). In contrast, CO patches in squirrel monkeys with weak or absent columns are not organized into monocular rows (Horton and Hocking 1996b). We inquired whether patches become “macaque-like” in squirrel monkeys with well-defined columns, i.e., do they line up with the ocular dominance columns? These questions are posed to probe the impact of ocular
dominance columns (or their absence) on the functional architecture and physiology of striate cortex.

METHODS

Physiology

Single-unit recordings were made in nine normal, adult squirrel monkeys. Animals were intubated and ventilated with 2% isoflurane in a 50/50 mixture of O₂-N₂O. After placement in a stereotaxic frame, a 2 × 3-mm exposure was made in the skull 3.5 mm from the midline and 9 mm anterior to the lambdoid suture, at a point overlying the V1/V2 border. Succinylcholine HCl (45 mg · kg⁻¹ · h⁻¹ iv) was infused continuously to eliminate eye movements. The inspired and expired concentrations of isoflurane, O₂, and N₂O were monitored continuously as was end-tidal CO₂, rectal temperature, urine out-put, and electrocardiogram. A tungsten in-glass electrode (typically 5 MΩ impedance) (Merrill and Ainsworth 1972) was advanced through a slit in the dura in a dorsosventral direction, aimed parallel to the cortical layers in opercular V1. In later experiments, we used tetrodes (Thomas Recording, Giessen, Germany) to improve spike isolation. At least two electrolytic lesions were made per electrode penetration to correlate recording sites with cortical layers defined by their characteristic pattern of CO staining.

Ocular landmarks were plotted on the tangent screen using a reversing ophthalmoscope or a modified fundus camera. The receptive fields of single cells were hand-mapped and then tested quantitatively by presenting oriented bars or stationary flashed spots generated using custom software written for a visual stimulus generator (Cambridge Research Systems, Rochester, UK). Stimuli were back-projected onto a translucent screen using a 60-Hz digital light projector (Model HP-XB31, Hewlett-Packard, Palo Alto, CA). Electrical signals were digitized at 25 kHz and stored electronically for off-line spike isolation (Spike 2, Cambridge Electronic Designs, Cambridge, UK). In addition, tuning curves were generated on-line to assess orientation selectivity. Each eye was stimulated separately while the fellow eye was occluded with a tightly fitting black rubber eye cup that blocked all light.

For each cell, we calculated an ocular dominance index as follows: OD = (I – S)/(I – C) + (C – S), where I = response to the ipsilateral eye, C = response to contralateral eye, and S = background firing level (LeVay and Voigt 1988). Rarely, the index can take a value <0 or >1 because S can exceed C or I. In these instances, we rounded up to 0 or down to 1 to yield an index ranging from 0 (contralateral eye only) to 1 (ipsilateral eye only). The ocular dominance index was adapted to the traditional Hubel and Wiesel (1962) scale by subdividing it into seven equal bins numbered 1 (contra-eye dominated) through 7 (ipsi-eye dominated); e.g., 1 = 0.00–0.14, 2 = 0.15–0.29, etc.

Eye injection and histology

After about 24 h the physiological recordings were concluded. While the animal was still under general anesthesia with isoflurane, a retrobulbar injection was made of 2% lidocaine with epinephrine 1:100,000 to supply additional orbital anesthesia. It was necessary to enucleate one eye to reveal the ocular dominance columns. When present, they become visible because enzyme levels fall in regions of layer 4C supplied by geniculocortical afferents serving the missing eye (Horton 1984; Wong-Riley and Carroll 1984). In three animals, the ocular dominance columns were also labeled by injecting the remaining eye with 2 mCi of [¹⁴C]proline. The results obtained by labeling the columns with CO and [¹⁴C]proline were identical.

After the enucleation was completed, animals were revived to allow time for [¹⁴C]proline transport or a change in cortical CO activity. After regaining consciousness, they were given an opiate analgesic (buprenorphine HCl, 0.03 mg/kg im) at least three times per day until fully recovered. After a period of 7–10 days, animals received a lethal dose of sodium pentobarbital (150 mg/kg iv) followed by transcardial perfusion with normal saline (1 l) and 1% paraformaldehyde (1 l). All procedures were approved by the UCSF Committee on Animal Research.

Brains were removed and a flatmount was prepared containing V1 and V2. After further fixation and cryoprotection, the flatmount was cut at 30–40 μm on a freezing microtome parallel to the pial surface. In animals that received a [¹²⁵I]proline eye injection, alternate 20-μm sections were dipped in photographic emulsion (Kodak NTB2) and exposed in a dark room for 6 weeks for autoradiography. Otherwise, all sections were processed for CO to prepare montages of layer 4C (Adams and Horton 2003b).

Data analysis

Cortical CO flatmounts and autoradiographs were imaged with a Diagnostic Instruments Spot RT Slider camera mounted on an Olympus SZH10 stereomicroscope. Image files were imported into Illustrator 9.0 or Photoshop 6.0 to prepare figures. A spatial correlation was performed to quantify the relationship between patches and ocular dominance column. To define the patches, the CO image was high-pass Fourier-filtered to correct for global changes in section density (due to small differences in layer 3 depth across the section). The resulting image was then blurred with a Gaussian filter (σ = 35 μm) to eliminate blood vessels. It was thresholded by assigning to black (gray scale value = 0) all pixels darker than the modal value. All remaining pixels were assigned to white (gray scale value = 1). In this binary map, the patches corresponded to the dark pixels (Figs. 8, B and D, and 9, B and D).

The borders of the ocular dominance columns in montages of layer 4C from the identical region were defined by using the Canny edge detector (Canny 1986) implemented in Matlab. A spatial correlation was then computed between patches and column borders for a region of cortex measuring 13.5 × 13.5 mm. This was done by averaging images of the borders (1 pixel thick) of the ocular dominance columns (Figs. 8E and 9E) centered on each pixel in every patch (Boyde and Casagrande 1999; Lia and Olavarria 1996). We used a region of the ocular dominance column border image measuring 1.4 mm per side, which corresponds to almost two column pairs. This process produced an average border image that showed the relationship between each patch pixel and each ocular dominance column border pixel. As a control, the same spatial correlation was performed with the ocular dominance column border pattern rotated 90° with respect to the patch image. The distribution of pixel values in the spatial correlations reflects the presence or absence of a consistent relationship between patches and column borders. Normal and rotated spatial correlations were normalized and their pixel values compared statistically with Levene’s test to detect equal variance. To illustrate the spatial correlations, each image was shifted to a mean pixel value of 0.5 and normalized to the highest contrast image (Fig. 9F).

RESULTS

Monocular cells without ocular dominance columns

The principal aim of these recordings was to learn if the absence of ocular dominance was correlated with an absence of monocular neurons in layer 4C. We hypothesized that animals with columns would have many monocular cells, whereas monkeys without columns would have few if any. Our approach was to record from an animal for 24 h to characterize the ocular preference of as many single cells as possible. Afterward, the animal was allowed to recover so that it could be tested for any evidence of ocular dominance columns, using CO histology and/or [¹¹C]proline autoradiography. We never
injected $[^3]H$proline into an eye prior to recording, for fear that it might damage the eye and thereby influence the ocularity of cells in the cortex.

For these studies, it would have been ideal to compare a group of animals lacking columns with another group possessing highly segregated columns. Unfortunately, there is no practical way to select animals in advance based on ocular dominance columns. Consequently, we encountered a spectrum in column expression: four animals had no columns, two animals had intermediate columns ($\sim250 \mu m$ wide), and three animals had coarse columns ($\sim450 \mu m$ wide). The animals reported here showed variability in column expression with eccentricity (Adams and Horton 2003a). This animal fell into the coarse group.

In all nine monkeys, we found that the overwhelming majority of cells (excluding those in layer 4C) responded to stimulation of either eye (Fig. 1). Thus we confirmed reports by previous investigators that monocular units in the squirrel monkey are rare outside layer 4C (Hubel and Wiesel 1978; Livingstone and Hubel 1984). To determine if the ocular dominance profiles obtained from squirrel monkeys with varying degrees of ocular dominance column expression could each have been sampled from the same underlying distribution of cells, we performed two-sample Kolmogorov-Smirnov tests on the histogram data in Fig. 1. This test compares two samples and returns a $P$ value corresponding to the likelihood that each could have been drawn from the same distribution. The test was applied pair-wise to the histograms for squirrel monkeys with coarse, intermediate, or no ocular dominance columns. All $P$ values were $>0.05$, indicating that all three histograms could have been sampled from the same data distribution. However, when compared individually and collectively with the macaque ocular dominance histogram (Fig. 1, inset) all four $P$ values were $<0.05$, demonstrating that the squirrel monkey and macaque data are significantly different. Thus cells outside layer 4C are more binocular in the squirrel monkey than in the macaque.

As one would expect from the histograms in Fig. 1, it proved impossible for us to guess accurately while recording from an animal whether it would later turn out to have ocular dominance columns. Midway through this series of recording experiments, it became apparent that there was little correlation between the ocular dominance profile and the degree of column segregation in any individual monkey. Any effort to find such a correlation was stymied by the fact that most units were binocular, regardless of the animal’s column anatomy (Fig. 1). Therefore we decided to shift our focus to recordings in layer 4C, where monocular cells, if present, would be most abundant.

In recordings from the first six monkeys, analysis of physiological data from layer 4C was hampered by difficulty isolating single units. Cells in this layer are small and densely packed, making them hard to resolve with a single microelectrode. If spikes are not adequately isolated, two neighboring monocular cells, each driven by a different eye, can be mistaken for a single binocular cell. Consequently, to improve isolation of single units (Gray et al. 1995), we used glass-insulated tetrodes to record from the last three monkeys. The tip of the glass fiber was pulled and ground to increase the impedance of each channel to 1.8–2.0 MΩ at 1 kHz.

During recording sessions, layer 4C could be recognized by noting an increase in background activity and a loss of orientation tuning (Hubel and Wiesel 1968; Livingstone and Hubel 1984; Schiller et al. 1976; Snodderly and Gur 1995). At each suspected layer 4C recording site, moving bars were presented at every 30° to compile tuning curves. If orientation tuning was absent, we then stimulated the field center with stationary spots (78 cd/m2), 0.5–2° in diameter, against a dark background (0.06 cd/m2). After recording responses to spots at each site, electrolytic lesions (50 kHz, 20 μA for 20 s) were made for later histological verification of layer 4C. All lesions were recovered later in layer 4C, attesting to the reliability of identifying this layer on the basis of its characteristic physiology.

Figures 2 and 3 show examples of recording sites in layer 4C from two different squirrel monkeys. CO staining is homogeneous in layer 4C despite removal of the right eye after the recording session, indicating that ocular dominance columns were absent in these monkeys. Because tetrodes leave no visible tissue tracks and cortex becomes distorted in the flattening process, only data from recording sites in the immediate vicinity of a lesion could be reliably assigned to layer 4C. The tetrode enabled us to isolate numerous cells at a single recording site. Frequently, a mixture of left-eye, right-eye, and binocular cells were recorded simultaneously (Figs. 2, C and E, and 3, C and F). This fine interspersing of cells with differing ocular dominance profiles at a single recording site makes it unlikely that there exist “mini” ocular dominance columns.

Figure 4 shows an example of three cells recorded at a single site in layer 4Cβ. Cell isolation was accomplished off-line by first thresholding each tetrode channel at 0.3 mV. Then principal components analysis was performed on the ratios of the peak amplitudes from each channel to the mean peak amplitude, or on a composite of the four spike waveforms. At this recording site, plotting the first three principal components of each spike yielded 3 clusters, designated as cells 1, 2, and 3. It is worth emphasizing that if we had recorded at this site with a standard microelectrode we might have concluded that a single, binocular cell was present.

To confirm that each cluster contained spikes from a single cell, we plotted interspike interval histograms (Fig. 5). In every

![Figure 1](http://jn.physiology.org/doi/10.1152/jn.02003.2006.supp.2255)
case, there was an absolute refractory period no less than 1.5 ms, suggesting that each cluster was comprised of action potentials from a single cell.

As mentioned in the preceding text, spike isolation using a single microelectrode was inadequate in layer 4C. This forced us to discard the layer 4C data acquired from the first six monkeys, including data from an animal without ocular dominance columns. Recordings were made in the last three monkeys using tetrodes. By chance, all the animals in this latter group lacked ocular dominance columns. In these three animals, we recorded 118 cells from 44 recording sites, each in the immediate vicinity of an electrolytic lesion later recovered and confirmed to be in layer 4C.

The ocularity of single cells was determined by presenting to each eye 50–100 cycles of stimulus on (0.5 s), stimulus off (0.5 s), followed by an intertrial interval (1 s). Cells were classified into seven categories using a quantitative index of ocular dominance (LeVay and Voigt 1988) (see METHODS). Sixty two of 118 units in layer 4C fell into categories 1 or 7 (Fig. 6).

Hubel and Wiesel’s (1962) qualitative method for classifying cells reserved categories 1 and 7 for cells judged subjectively to be absolutely monocular. Our ocular dominance scheme allowed some cells with very weak responses in the nondominant eye to enter bins 1 and 7. It is quite likely that any listener he hearing these units over an audio monitor would have placed them in category 1 or 7. However, to identify strictly monocular cells, we applied two statistical tests to the cells. First we used the Mann-Whitney rank sum test to compare the mean firing rate for each eye during stimulus periods and intertrial periods. For the 118 cells in layer 4C, 40 (34%) showed no significant difference between the mean background firing rate and the firing rate during stimulation of one of the eyes.

![Fig. 2](image2.png) Absence of ocular dominance columns and verification of recording sites in layer 4C. A: left V1 showing 2 lesions (arrows) in layer 4Cβ in a single, flatmount cytochrome oxidase (CO) section. The tetrode was advanced tangentially from the V2 border for a distance of 8.5 mm through V1. B: magnified view of the upper lesion. C: drawing of the lesion in B showing its center (black circle) and the relative position of 4 recording sites (rows of circles). Each circle represents a single cell, color-coded according to its ocular dominance index (0, contralateral eye only; 1, ipsilateral eye only). D: magnified view of the lower lesion. E: schematic drawing of the lesion in D. The asterisk denotes the recording site illustrated in Fig. 4.

![Fig. 3](image3.png) Electrode lesions and recording sites in layer 4C. A: CO section from the left V1 of another squirrel monkey showing no ocular dominance columns in layer 4C after monocular enucleation. B: magnified view of the 4Cβ electrode lesion shown in A. C: recording sites, relative to the electrode lesion, showing the ocular dominance index of each isolated cell according to the color-coded scale below. D: right V1 from the same monkey, showing a lesion in 4Cβ (arrow). E: higher power view of the lesion in D. F: recording sites, with ocular preference of each isolated cell.

![Fig. 4](image4.png) Single-unit data from a recording site in layer 4C (see asterisk, Fig. 2E). A: traces are mean spike shapes for each channel of a tetrode, shown for three isolated cells. SDs of the mean are depicted with transparent shading. Responses were elicited by 80 cycles of on-off stimulation for each eye. B: poststimulus time histogram for the left and right eyes for each cell above with ocular dominance indices. Cell 1 gave a tonic on-response to the right eye but responded weakly to the left eye. Cell 2 gave transient on- and off-responses to stimulation of either eye. Cell 3 gave a tonic on-response to the left eye it was the only monocular cell. The background rate is indicated by the thin black line.
visual inspection of poststimulus time histograms, we noticed that some of these cells had very transient on or off responses from stimulation of the nondominant eye that failed to raise the mean firing rate significantly above background. To eliminate these cells from the monocular population, we examined the distribution of spike times during the response period using the two-sample Kolmogorov-Smirnov test. This test compared spikes times during the response period to a random distribution of events over the same period. Presumably, spikes from a monocular cell occurring during stimulation of the nondominant eye should be randomly distributed with respect to the stimulus. If the distribution of spike times were significantly different from a random distribution, the test would return a $P$ value $< 0.05$, indicating that a response could not be ruled out. Of the 118 cells, 34 (29%) were monocular according to this statistical test. Only cells that showed no significant response ($P > 0.05$) in one eye with both statistical tests were classified as monocular: 24/118 cells met this criterion.

The preceding method for classifying cells as monocular is quite stringent. It is not possible to compare this measure directly with the qualitative methods used in most prior studies, because they were based on monitoring spike rates by ear. However, our method would be less likely than subjective classification schemes to overestimate the number of truly monocular cells. Our data indicate that $\sim 20\%$ of cells in layer 4C were absolutely monocular, even in the absence of ocular dominance columns (Fig. 6, black shading). The ocular dominance profile for layer 4C in squirrel monkeys without columns (Fig. 6) was not significantly different ($P > 0.05$, 2-sample Kolmogorov-Smirnov test) from the qualitative profile in macaques from Hubel and Wiesel’s (1977) data (Fig. 6, inset). The validity of this comparison is obviously limited, due to methodological differences.

To conclude that the monocular units encountered in layer 4C were single cells, one must eliminate the possibility that we recorded from geniculocortical afferent fibers. It is difficult to recognize geniculate afferents while recording from striate cortex. When cortical cells are silenced by muscimol, however, one can identify isolated geniculocortical afferents. Their amplitude is in the range of 20–100 $\mu$V, and they have a characteristic triphasic waveform (Chapman et al. 1991; Chatterjee and Callaway 2003). All our units were thresholded at 0.3 mV to eliminate contamination from geniculocortical fibers. In addition, the units that we recorded in 4C had a biphasic waveform. For these reasons, it is very likely that they were cortical cells, not geniculate fibers.

**Koniocellular input to patches is not segregated by eye**

In the macaque, CO patches receive a direct input from konio cells in the lateral geniculate nucleus (Hendry and Yoshioka 1994). After $[^{3}H]$proline injection into one eye, every other row of patches is labeled, coinciding with the labeled ocular dominance columns in 4C (Horton and Hocking 1996a). Therefore the direct konio input to CO patches in layer 2/3 is segregated by eye, as is the parvo and magno input to layer 4C.

In squirrel monkey, the situation is different. After tracer injection into one eye, every CO patch in the supragranular layers is labeled (Fitzpatrick et al. 1983; Horton 1984; Horton and Hocking 1996b; Itaya et al. 1984; Livingstone and Hubel 1982; Weber et al. 1983). This input is probably koniocellular, because it comes from the intercalated layers of the LGN (Fitzpatrick et al. 1983). Although these cells have not been stained for type II calmodulin-dependent protein kinase in the

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squirrel monkey, it would appear that the konio input to CO patches is not segregated by eye in this species. However, previous experiments were performed in animals without ocular dominance columns or in animals with only rudimentary columns. We sought to determine if the koniocellular input to patches is segregated by eye, as in the macaque, when the ocular dominance columns in layer 4C are robust.

Figure 7 shows the ocular dominance columns from the left V1 of a normal, adult squirrel monkey, labeled by CO and transneuronal autoradiography. The columns appear nearly as well formed as those present in the macaque. The patches in the upper layers are arrayed with a typical spacing of 280 μm (Fig. 7C), measured by determining their peak in a spatial Fourier spectrum. The measurement for ocular dominance column width, determined by the same method, was 344 μm. An adjacent autoradiograph shows patches of \(^{3}H\) proline, representing transneuronal retinal input via the koniocellular pathway (Fig. 7D). Comparison of the CO section and the autoradiograph reveals that every single CO patch is labeled (Fig. 7G). Therefore even in squirrel monkeys with highly segregated ocular dominance columns in layer 4C, the koniocellular input to the CO patches remains unsegregated. This result was confirmed in an additional animal.

**No relationship between patches and columns**

In the macaque, each row of CO patches in the upper layers is aligned with an ocular dominance column in layer 4C. Squirrel monkeys with well-developed ocular dominance columns were examined for evidence of a similar relationship between patches and columns (Fig. 7H). The patches were located indiscriminately with respect to ocular dominance columns.

To quantify the relationship between patches and ocular dominance column borders, a spatial correlation was performed (Fig. 8). This produced a low-contrast image with little structure (Fig. 8F). A spatial correlation was also performed after a 90° rotation between the columns and patches. This produced a similar low-contrast spatial average (Fig. 8G). There was no significant difference in variance \((P > 0.1, \text{Levene’s test of equal variance})\) between normalized images produced by the correlation between aligned and rotated border and patch images, indicating that patches are distributed randomly with respect to ocular dominance columns. This result was confirmed by performing the same analysis in another squirrel monkey with clearly defined ocular dominance columns.

For comparison, the spatial correlation between patches and column borders was analyzed in a normal macaque (Fig. 9). The center of the spatial average appears bright because very few column borders infringe on patches (Fig. 9F). The dark contours represent the average border location with respect to the patch pixels. They are \(\approx 500 \mu m\) apart, corresponding to the mean column width for this particular macaque. This result illustrates that when columns and patches are organized in register, a spatial correlation shows clearly their relationship. Introduction of a 90° rotation between the patch and column images generated a low-contrast spatial average with little structure (Fig. 9G). Comparison of the spatial averages before and after rotation showed a significant difference in the pixel variances \((P < 0.01, \text{Levene’s test})\).

**DISCUSSION**

These recordings show that, even in squirrel monkeys without ocular dominance columns, cortical cells that receive direct geniculate input often show a strong monocular basis. More than half the cells in layer 4C fell into ocular dominance category 1 or 7. In terms of “absolute” monocularity, \(\sim 20\%\) of the cells in layer 4C showed no statistically significant response to stimulation of the other eye. We often encountered monocular cells that responded to different eyes at the same microelectrode recording site (Figs. 2 and 3). Thus intermingled cells driven exclusively by the left or right eye were finely scattered among a population of binocular cells. We found no physiologic evidence for “minicolumns” of left or right eye cells, which might have been too small to resolve with CO histochemistry.

The occurrence of monocular cells in animals without ocular dominance columns could not be a matter of chance. Assuming that the input from a single geniculate cell is sufficient to make a cortical cell respond, the probability of a cell being monocular in a random system is \(2/n^n\), where \(n\) is the number of geniculate cell inputs. Thus for \(20\%\) of the cells in layer 4C to be monocular by chance, one would predict that each cortical cell receives input from an average of four fibers. In macaque layer 4Cβ, each stellate cell is estimated to receive input from 20 to 40 parvocellular afferents (Blasdel and Fitzpatrick 1984). Assuming a similar situation in the squirrel monkey, it is apparent that geniculate afferents serving the same eye must selectively target the same cell to account for the proportion of monocular cells in layer 4C. The circuitry responsible for monocular cells may develop by activity-dependent refinement of synaptic connections or via molecular cues (Katz and Shatz 1996). Whatever the mechanism, our data cast doubt on the idea that it is the function of ocular dominance columns to generate monocular cells by producing wholesale physical segregation of geniculocortical afferents serving the left and the right eyes. Instead, our results show that geniculate afferents, interspersed on a microscopic scale, can synapse selectively onto stellate cells to produce monocular cells in layer 4C.

Monocular cells have been found in the binocular cortex of the mouse as defined by the qualitative method of Hubel and Wiesel (1962). All members of this species lack ocular dominance columns (Dräger 1974). The percentage of monocular cells ranges between 20 and 30% (Dräger 1978; Gordon and Stryker 1996; Hensch et al. 1998; Metin et al. 1988). In almost every instance, monocular cells were reported to be driven only by the contralateral eye. The mouse (unlike the primate) does not receive a balanced input from each eye in binocular cortex. Dräger and Olsen (1980) found a 10:1 ratio of contra/ipsi projecting cells in corresponding binocular regions of the two eyes. In a sense, the binocular cortex in the mouse is tanta-amount to a single contralateral eye dominance column. Under these circumstances, it is not surprising that many monocular, contralateral-eye cells are present. If each cell received an average of 11 geniculate afferents, by chance 30% of cells would respond only to the contralateral eye \((0.9^{11} = 0.30)\). In the primates, 11 geniculate afferents per cortical cell would produce only 0.1% \((2^{11} = 0.30)\) monocular cells. These calculations suggest that the percentage of monocular cells could be a matter of chance in the mouse but not the squirrel monkey.
FIG. 7. Koniocellular input to patches is binocular, even in animals with ocular dominance columns. **A**: single CO section from the left striate cortex of a normal squirrel monkey, showing coarse, high-contrast ocular dominance columns in layer 4CB following enucleation of the right eye. The large pale region to the bottom right is the representation of the blind spot. Three blood vessels used as fiduciary marks to align sections are indicated with arrows. **B**: autoradiograph 70 μm more superficial to the CO section in A, showing ocular dominance columns labeled by [3H]proline injection into the remaining left eye. The columns appear bright in darkfield. Note that the columns in A and B correspond well, although they appear less sharp in B because the section passes through layer 4Ca. **C**: CO section from the upper layers, showing patches. **D**: autoradiograph adjacent to the CO section in C. Patches of [3H]proline representing konio input from the LGN are visible. **E**: patches in C were outlined by high-pass filtering the image (cut-off 350 μm), blurring with a Gaussian kernel (diameter: 35 μm) and thresholding at the modal gray level. Red outlines denote the borders of thresholded patches. **F**: [3H]proline-labeled regions from the image in D, thresholded as in E. Green contours represent the borders of thresholded proline patches. **G**: red and green contours from E and F have been filled and overlaid. Overlapping regions in the red and green images are displayed in yellow. Note the high level of correspondence between the 2 patterns, indicating that every patch receives a direct konio input from the LGN. **H**: ocular dominance column borders from A defined by the Canny edge detector algorithm superimposed on the patches (gray), defined as the yellow regions in G. Unlike in the macaque, patches are not located in the centers of ocular dominance columns.
One prior study has addressed the ocularity of units in layer 4C of the squirrel monkey (Livingstone 1996). Cells were isolated at 19/41 recording sites; there were 29 monocular cells and a single binocular cell. With the exception of this latter unit, no binocular cells were reported in layer 4C. It was concluded that virtually all cells in layer 4C of the squirrel monkey are monocular and that they are segregated in a “salt-and-pepper” fashion. However, in the single animal in which recordings were made, the anatomy of the ocular dominance columns was not examined. It was assumed that the animal had no columns, because it had been reported previously in the literature that squirrel monkeys

![Image of ocular dominance columns and patches in the squirrel monkey.](http://jn.physiology.org/)

FIG. 8. No alignment of ocular dominance columns and patches in the squirrel monkey. A: CO section showing ocular dominance columns surrounding the blind spot representation in the left V1 of a normal squirrel monkey after enucleation of the left eye. B: CO patches from the same region, in layer 2/3. C: borders of ocular dominance columns in A, defined by the Canny edge detector algorithm, drawn 1 pixel wide (14 μm). D: binary map of the CO patches shown in black (see METHODS), superimposed on the image in B. E: overlay of patches and column borders. There is no obvious relationship between them. F: average of the column borders relative to every patch pixel, compiled from over a hundred thousand individual spatial correlations. There is only slight modulation in density, indicating that CO patches in the squirrel monkey are not aligned with ocular dominance columns. G: spatial average as in F, generated after rotating the column borders through 90° with respect to the patches. The rotation produces a spatial average with similar contrast to that seen in F. The spatial average has been scaled to the same range of grayscale values used for the macaque spatial average (Fig. 9F). Scale bar A–E = 5 mm, F and G = 500 μm.
lack ocular dominance columns (Hendrickson and Tigges 1985; Hendrickson et al. 1978; Hubel et al. 1976; Tigges et al. 1977). Subsequently, it was demonstrated that the expression of ocular dominance columns in the squirrel monkey is variable (Adams and Horton 2003a) with some animals having columns. One suspects, given that Livingstone (1996) encountered virtually no binocular cells in layer 4C, that she recorded from an animal with macaque-like ocular dominance columns. We did not manage to make successful layer 4C recordings in such animals, but taken

FIG. 9. Alignment of ocular dominance columns and patches in the macaque. A: autoradiographic montage of 4Cβ showing brightly labeled ocular dominance columns after monocular [3H]proline eye injection. B: CO patches from the same region in layer 2/3. C: borders of the ocular dominance columns superimposed on A. D: thresholded CO patches, (see METHODS), superimposed on B. E: comparison of patches and column borders, showing tendency for patches to align with column centers. F: spatial average of the column borders relative to every pixel assigned to a patch. This patch-centered spatial average of the border map has a bright center, indicating that a column border rarely passes through a patch. The dark contours, ~500 µm apart, corresponds to the average ocular dominance column border location, which happens to run vertically in this portion of striate cortex. G: spatial average as in F, generated after rotating the column borders through 90° with respect to the patches. The rotation eliminates the structure seen in F by randomizing the relationship between column borders and patches. Scale bar A–E = 5 mm, F and G = 500 µm.
together, the results from our labs suggest that squirrel monkeys show a range in the proportion of monocular cells in 4C, from 20% in animals without columns to nearly 100% in animals with columns.

What is the impact of ocular dominance columns on the ocularity of cells outside layer 4C? Our recordings outside layer 4C did not show a greater prevalence of monocular cells in squirrel monkeys with ocular dominance columns than in those without (Fig. 1). As our experiments unfolded, it became apparent that any difference, if present, was subtle and would be revealed only by recording in a much larger number of animals. Our data were sufficient to confirm, however, that binocular cells in striate cortex outside layer 4C are more common in the squirrel monkey than in the macaque (Hubel and Wiesel 1978; Livingstone and Hubel 1984). This was true even in those squirrel monkeys that had ocular dominance columns (Fig. 1).

The abundance of binocular cells, even in squirrel monkeys with ocular dominance columns, was consistent with our anatomical findings. The koniocellular input to patches in layer 2/3 was derived from both eyes despite the presence of well-segregated ocular dominance columns in layer 4C (Fig. 7). Furthermore, the patches were not aligned with the ocular dominance columns (Fig. 8), demonstrating the lack of a spatial relationship. Thus ocular dominance columns in the squirrel monkey do not extend their influence as strongly into the layers outside 4C to “capture” the patches and shift the ocular bias of cells toward one eye. This observation may explain why cells outside layer 4C are more binocular in squirrel monkeys (even with sharp columns) than in macaques.

There is no spatial relationship between patches and ocular dominance columns in the squirrel monkey even in those animals with well-developed ocular dominance columns. In the cat, which has nebulous patches but quite sharp ocular dominance columns, alignment has been reported (Hübener et al. 1997; Murphy et al. 1995; but see Boyd and Matsubara 1996). The bush baby, a prosimian, has more distinct CO patches than the cat (Horton 1984). A recent optical imaging study has demonstrated a lack of alignment between CO patches and ocular dominance columns in this species (Xu et al. 2005).

In squirrel monkeys with columns, it is hard to explain why parvo and magno projections should be segregated by eye, but konio inputs are not. It is also difficult to explain why the ocular dominance columns and CO patches remain independent, given that they are spatially aligned in the macaque. Collectively, these observations undermine the notion that ocular dominance columns are functionally important, at least when they occur in the squirrel monkey.

In macaque V1, most units are disparity-tuned (Poggio 1995). They often appear to have a strong monocular bias but show facilitation or suppression with binocular stimulation, depending on the disparity of the stimulus (Poggio and Fischer 1977; Read and Cumming 2004; Smith et al. 1997). These binocular interactions are not apparent when the cell is tested monocularly. Our data simply show that cells in the squirrel monkey are more likely to respond equally to stimulation of either eye than cells in the macaque. However, we did not assess disparity tuning. It is conceivable that a classification of neurons based on responses under conditions of binocular stimulation at different disparities might yield a more similar ocularity profile for the macaque and squirrel monkey. No prior study has evaluated disparity tuning in the squirrel monkey.

We have shown that monocular cells exist in squirrel monkeys without ocular dominance columns. The fact that they are preserved, even in the absence of columns, suggests that they serve a special role in visual processing. They may encode the position of images on each retina so that disparity information can be extracted by binocular cells (Ohzawa et al. 1990). It would be worthwhile to test a group of squirrel monkeys to see if the stereocuity of individual animals correlates with the degree of segregation of geniculocortical afferents in layer 4C and, by implication, with the percentage of monocular cells. Livingstone et al. (1995) showed that one can drive evoked responses in the squirrel monkey by shifting disparity in dynamic random-dot stereograms. This finding implies the ability to detect stereoscopic depth, but in this study, the animal tested was not examined for the presence or absence of ocular dominance columns.

It remains to be demonstrated that squirrel monkeys without ocular dominance columns have stereopsis. It is possible that animals without ocular dominance columns have too few monocular cells to possess high-grade stereopsis. We think this idea is unlikely, because stereopsis facilitates efficient foraging and predation. If columnless monkeys lacked stereopsis, they would be placed at a major competitive disadvantage. Approximately one in three squirrel monkeys is without ocular dominance columns (Adams and Horton 2003a). This fact would seem impossible if ocular dominance columns are important for stereopsis.

Our recordings confirm that a major difference exists between macaques and squirrel monkeys in the binocularity of cells in striate cortex. This difference brings us back to our main question: what are ocular dominance columns for? In principle, one could perform a range of psychophysical tests in a large group of squirrel monkeys to identify a visual function that is deficient in animals with poor or absent columns. This approach would be worthwhile, but if no deficiency was found, even after an exhaustive battery of tests, one would always suspect that the pertinent visual parameter had simply been overlooked.

As an alternative strategy, we have examined animals with and without columns to define how the presence of columns affects the physiology and anatomy of striate cortex. Our data show that when columns are absent, a sizeable complement of monocular cells still exists in layer 4C. When columns are present, the patches in the upper layers remain binocular in their geniculate innervation and unaffiliated with the ocular dominance column situated below in layer 4C. These findings suggest that there is no major, qualitative difference between squirrel monkeys with ocular dominance columns, and those without them, except perhaps in the percentage of monocular cells in layer 4C.


**Itaya SK, Itaya PW, and Van Hoesen GW.** Intracortical termination of the retino-geniculo-striate pathway studied with transynaptic tracer (wheat germ agglutinin-horseradish peroxidase) and cytochrome oxidase staining in the macaque monkey. Brain Res 304: 303–310, 1984.


