Optical Imaging of Functional Organization of V1 and V2 in Marmoset Visual Cortex

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ABSTRACT

Using optical imaging of intrinsic cortical signals, we examined the functional organization of visual cortical areas V1 and V2 of the marmoset (Callithrix jacchus). Previous studies have reported that adult marmosets do not have ocular dominance columns (ODCs); however, recent studies have called this into question. Using optical imaging methods, we examined whether ODCs could be detected in adult marmosets. We found evidence for functional ODCs in some marmosets but not in others. The activation patterns, when present, were relatively weak and appeared as a mosaic of irregular bands or islands. Consistent with studies in other New World monkeys, these data suggest the presence of ODC variability within the marmoset population. Orientation maps in V1 revealed iso-orientation domains organized in semicontinuous bands oriented orthogonal to the V1/V2 border, a pattern unlike that in Macaque monkey. The presence of directional preference maps in V1 was also suggested. In V2, similar to V2 in Macaque monkeys, stripe-like regions of orientation selectivity overlay the pale cytochrome oxidase regions of V2; zones not selective for orientation overlay the cytochrome thin stripes. However, unlike Macaques, we did not observe clear evidence for orientation maps overlaying thick cytochrome oxidase stripes. In sum, our data suggest that significant organizational differences exist between the organization of V1 and V2 in the marmoset and that of Old World primates. Implications for the establishment of functional ocular dominance columns, the coestablishment of multiple featural maps, and cortical magnification factors are discussed.

Key words: ocular dominance columns; orientation; V2 stripes; cortical magnification; New World primate

In the present investigation, we have used optical imaging methods to study visual cortical organization in the marmoset, Callithrix jacchus, a New World anthropoid whose lissencephaly is so complete in the occipitoparietal region that it allows unobstructed viewing of all the visual cortical areas in both dorsal and ventral streams. The marmoset may have unusual variants of some features of visual cortical organization. These variants might provide natural experiments illuminating the functional significance of these features of functional organization. For example, a number of studies report that marmosets lack segregation of geniculate afferents to layer IV of V1 (termed ocular dominance columns, or ODCs) (Spatz, 1979; DeBruyn and Casagrande, 1981) and yet have substantive binocular vision and stereopsis, raising puzzling questions regarding the possible function of ODCs. Closer examination reveals that marmosets have clear ODC ar-

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rays at birth, but that these mostly disappear as the young marmoset matures and gains visual experience (Spatz, 1989). Could this postnatal fusion of ODC arrays in marmosets be connected with the fact that marmosets are the dwarfs of New World anthropoids, with small heads and even smaller pupillary separations and thereby a marked reduction in binocular parallax? Other studies indicate that ODCs can be found in some adult marmosets (Chapport-Piquemal et al., 2001) and that ODCs can be induced both in young (Sengpiel et al., 1996) and even in adult animals (Markstahler et al., 1998), making marmoset visual cortex fertile ground for pursuing issues of cortical functional organization and plasticity.

In the mammalian cerebral cortex, each cortical area must represent multiple sensory parameters. How multiple sensory maps are simultaneously represented in a single two-dimensional sheet has challenged both biologists and modelers (Swindale, 2000). How does the organization of ocular dominance columns or the lack thereof affect the organization of orientation domains in V1? Iso-orientation domains appear as a regular mosaic and form pinwheels as in Macaque V1 (Blasdel and Salama, 1986; Bonhoeffer and Grinvald, 1993), but in the ferret, where ODCs are more patchy and irregular in appearance, iso-orientation domains are more elongated or stripe-like (Chapman et al., 1996). With respect to direction, there appears to be no columnar organization for direction in V1 or V2 of the Macaque monkey, but in the cat area 18 and ferret area 17 directional columns have a consistent relationship to orientation columns (Shmuel and Grinvald, 1996; Welisky et al., 1996). It is also unknown how organization of ocular information might affect functional organization in V2, the second visual area, where most cells are binocularly driven. The three thin, pale, and thick cytochrome oxidase stripes in V2 of Macaque monkey are characterized by a predominance of cells that are selective for color, contour orientation, and disparity, respectively (Hubel and Livingstone, 1987; Roe and Ts'o, 1995; Ts'o et al., 2001). Whether in area V2 the dark and light cytochrome oxidase stripes in marmosets (Rosa et al., 1997; Lyon and Kaas, 2001; Collins et al., 2003) are functionally similar to those in the Macaque monkey is also unknown. Using optical imaging methods, we find both similarities and differences in functional organization between the marmoset and the Macaque monkey.

**MATERIALS AND METHODS**

**Surgical Preparation**

Four adult marmosets (Callithrix jaccus) were used for these experiments. Following an initial anesthetic dose of ketamine hydrochloride (40 mg/kg), animals were tracheotomized and a catheter was implanted in the femoral vein for drug delivery. Anesthesia was maintained throughout the experiment by a constant infusion of sodium thiopental (2 mg/kg/hr) or sufentanil (8 mg/kg/hr). Animals were paralyzed (pancuronium bromide, 100 μg/kg/hr) and respirated; following paralysis, the level of anesthetic sufficient during surgical procedures was maintained. Heart rate was continuously monitored, rectal temperature was maintained at 38°C, and expired CO2 was maintained at 4%. After dilation of the pupils (atropine sulfate 1%), eyes were refracted and fitted with appropriate contact lens to focus on a tangent screen 28 inches in front of the animal. A craniotomy and a durotomy, roughly 1 cm in size, were made over a region overlying visual cortex, exposing a visual cortical area near the V1/V2 border representing 2–10° eccentricity.

**Optical Imaging**

Optical imaging of intrinsic cortical signals was used to localize functional compartments within visual cortex. The details of imaging procedures have been described elsewhere (Grinvald et al., 1986; Ts'o et al., 1990; Roe and Ts'o, 1995, 1999) and will only be described briefly here. For increased cortical stabilization during optical recording, an optical chamber was cemented over the craniotomy, filled with lightweight silicone oil, and sealed with a coverglass. The cortical surface was illuminated through the chamber window with 630 nm wavelength light provided by optic fiber light guides. Images of reflectance change (intrinsic hemodynamic signals) corresponding to local cortical activity were acquired using an Imager 2001 (Optical Imaging, Germantown, NY; for details, see Grinvald (1988), Bonhoeffer and Grinvald (1995), Ts'o et al. (1990)). Signal-to-noise ratio was enhanced by trial averaging (10–20 trials per stimulus condition), but without synchronization of acquisition to heart rate or respiration. Animals were positioned on a floating bench to minimize motion artifacts.

Images of the cortical surface were collected during presentation of visual stimuli to the eyes. Stimuli were generated using STIM (Kaare Christian) controlled by an IBM PC/AT with a Sargent Pepper Number Nine graphics card and were projected onto a rear projection screen at 114 cm using a Panasonic video projector. Sinusoidal luminance and chromatic contrast (red/green isoluminant) drifting gratings (2–15°/sec) of different spatial frequencies (0.5–2 cycle/°) and orientations (0, 45, 90, 135°) were presented in a pseudorandom fashion. Blank condition consisted of an even-gray screen equal to the average luminance of the sinusoidal luminance grating condition. A mechanical shutter in front of each eye allowed for independent stimulation of each eye. For each stimulus, 10–20 trials were presented in random order (3-sec duration per stimulus; 10- to 15-sec interstimulus interval). Images were acquired without synchronization to respiration and heartbeat.

Images were digitized, collected, and processed. All frames acquired for each stimulus condition were summed and divided by the sum of blank stimulus trials; this procedure maximizes signal-to-noise ratios and minimizes effects of uneven illumination. Blood vessel artifact was at times substantial, leading to low signal-to-noise ratios. To compare two functional properties (e.g., ocular dominance), the sum of images obtained under one stimulus condition (e.g., left eye) was subtracted from that obtained under another (e.g., right eye). For orientation maps, we used a “cocktail blank” reference, constructed by summing the responses to all four cardinal orientations (Bartfeld and Grinvald, 1992; Bonhoeffer and Grinvald, 1993). Such cocktail blanks are activated blanks that serve to average out biological artifacts such as those due to blood vessels (Bartfeld and Grinvald, 1992). Difference images were then scaled, clipped, smoothed, and displayed on a color monitor and printed out for further inspection and comparison.

**Image Analysis**

Two types of image analyses were conducted: single-condition analyses and two-condition subtraction analy-
ses. Single-condition maps were obtained by subtracting the blank image from those images obtained from each given stimulus. Single-condition maps indicate the response magnitude at each location in the image for a particular stimulus condition. Such blank subtractions not only measure change from baseline, but also reduce blood vessel artifact and minimize effects of uneven illumination. Dark pixels in single-condition maps indicate a response to that stimulus condition greater than that of the blank condition image; gray pixels indicate a response not different from blank; and lighter pixels could indicate some level of activation less than that in blank.

Whereas single-condition maps reveal the presence of response to a particular stimulus, stimulus subtraction reveals preference for one stimulus over another. The sum of images obtained for one stimulus condition (e.g., left eye) is subtracted from that obtained under another (e.g., right eye). In this case, dark pixels indicate preference for one condition, light pixels a preference for the other condition, and gray pixels equal preference for both. Single-condition maps provide the most reliable indication of stimulus-specific activations, but this may occur at the expense of signal-to-noise ratio. Difference maps yield better overall signal quality by accentuating the preference for one condition over another, but activations common to both stimulus conditions are eliminated. In this article, because signal strength in the marmoset visual cortex was weak in general, subtractions yielded much clearer maps than single-condition analysis. The images shown in the article are thus all difference maps.

Histology
At the end of data collection, animals were then deeply anesthetized with an intravenous dose of sodium pentobarbital (85 mg/kg) and perfused through the heart with 4% paraformaldehyde. Following extraction of the brain, the desired cortical region was removed, flattened, and immersed in 30% sucrose solution. The cortical tissue was then sectioned tangentially at 30 μm and sections were reacted for cytochrome oxidase histochemistry (Wong-Riley, 1979). Cytochromeoxidase and cytochrome oxidase sections were aligned with optical images by aligning imaged surface vasculature patterns with locations and sizes of vascular lumen in superficial sections of cortical tissue.

RESULTS
Four adult male marmosets were available and used for these studies. Because interpretation of the functional images were greatly aided by subsequent cytochrome oxidase histology, we first present our method of tissue alignment with the tissue sections.

Alignment of Images With Cytochrome Oxidase Histology
To align the optical images with the cytochrome-stained sections, we first aligned the cortical vessel map with a cytochrome oxidase section from the superficial layers (200–300 μm depth; Fig. 1A–C). The largest blood vessel lumen in cytochrome sections were marked with dots (Fig. 2A; dots placed to the right of vessel lumen; red dots in V2, green dots in V1). This dot pattern was then overlaid onto the vascular image (Fig. 1D) and shifted around until a reasonable alignment was obtained between locations of dots and locations where blood vessels dive into the cortical surface (compare dots in Fig. 2A and B; Fig. 2B, dots placed to the right of locations where vessels dive into cortex). Since the cytochrome section is obtained from a cortical depth of 300 μm, it is expected that the blood vessel terminations will have small shifts (<50 μm) from the surface locations. Despite these small shifts, the vessel alignments are quite reliable as only an appropriate alignment will give a large number of match points. Furthermore, because of the density and distribution of match points across the entire imaged region, this method produces an alignment with a degree of precision greater than the typical alignment obtained using a small number of electrolytic lesions. We thus have reasonable confidence in the alignment of functionally and anatomically demarcated domains and used this method to aide in our interpretation of visual activation patterns.

Functional Organization in V1 and V2
Orientation in V1. Optical imaging revealed orientation maps that delineated areas V1 and V2 in the marmoset. Figure 3A illustrates a map obtained by subtracting the sum of all horizontal conditions (all left eye horizontal and all right eye horizontal, dark pixels) and the sum of all vertical conditions (all left eye vertical and all right eye vertical, light pixels). Similarly, Figure 2B illustrates the difference between all acute (all left eye acute and all right eye acute, dark pixels) and oblique (all left eye oblique and all right eye oblique, light pixels) conditions. In area V1, clear interdigitating horizontal and vertical orientation domains with a periodicity of about 400 μm were observed (Fig. 3A, indicated by red arrows). Obtuse and acute orientation domains with a similar spatial periodicity, though less regular, were also obtained (Fig. 3B, indicated by red arrows). This periodicity is consistent with physiological recordings that found that “a 180-deg range of orientations (an orientation hypercolumn) was covered by a block of V1 some 400–800 μm wide” (Sengpiel et al., 1996).

Interestingly, unlike in the macaque monkey or in the cat, horizontal and vertical orientation domains appeared to be organized in an elongated fashion orthogonal to the V1/V2 border (particularly apparent in Fig. 3A), somewhat reminiscent of the organization of ocular dominance columns in the macaque monkey and quite distinct from the more lattice-like organization for orientation found in macaque monkeys (cf. Ts’o et al. (1990), Blasdel (1992), and Bartfeld and Grinvald (1992); for similar methods in Macaque, compare, e.g., Ramsden et al. (2001), Fig. 4). The apparent orthogonality of iso-orientation columns to the V1/V2 border has also been described in the ferret (Chapman et al., 1996), the cat (Shmuel and Grinvald, 2000), and owl monkey (Xu et al., 2004).

To examine the organization of this map, we generated a color-coded orientation vector map, in which the orientation of the strongest response (0, 45, 90, or 135) is indicated by a color. However, in contrast to the standard color-coded orientation maps with characteristic pinwheels [e.g., see same analysis used in Macaque, Ramsden et al. (2001), Fig. 3, Roe (2003), Fig. 6C], this analysis revealed no structure at all and appeared relatively uniform across the image (not shown). Although it is possible that this was due to weakness of signal, it may suggest broad orientation tuning of marmoset visual cortical cells or, alternatively, a significantly more mixed population of orientation preferences at each pixel location. In another
Fig. 1. Cytochrome oxidase section and cortical vascular pattern. A: Low-power image of cytochrome oxidase section taken from about 300 μm depth. Scale bar = 1 mm. B: Same as A. V1/V2 border indicated by black arrows. Region of cytochrome section shown in C indicated by white box. C: Higher-power view of region indicated in B. V1/V2 border indicated by downward arrow at top of image. D: Image of cortical vasculature corresponding to C (Fig. 2). Scale bar = 1 mm (C and D).
study, a pinwheel-like organization was found in the marmoset V1 (Liu and Pettigrew, 2003), possibly suggesting variability in functional organization within the species.

**Orientation in V2.** The location of the V1 orientation map border colocalizes with the V1/V2 border (Fig. 4D and F) as determined by alignment with the cytochrome oxidase sections. Consistent with V2 of other Old World and New World monkeys, the thin and thick cytochrome oxidase stripes alternate in what can be described as a thin/pale/thick/pale cycle (Fig. 4A, alternating black and red arrows). As has been previously described, cytochrome oxidase stripes in the marmoset can be irregular and patchy in appearance and thin and thick stripes cannot always be distinguished (Rosa et al., 1997). In this case, the thick stripes appear less distinct and more irregular than the thin stripes in the cytochrome section.

In contrast to the Macaque monkey, optical imaging of orientation response elicits much weaker activation in V2 than in V1 (Fig. 3). It is possible that extrastriate areas are less easily activated in the marmoset and/or that the stimuli used were more effective for activation of V1. Under our recording conditions in response to the limited spatial frequencies presented, the optical signal in V2 was roughly 30% the magnitude of the V1 signal. To accentuate the V2 signal, we clipped and rescaled the gray values of the image shown in Figure 3B and reillustrate this image in Figure 4C (because of the rescaling V1 appears quite saturated).

However weak, this activation did reveal some orientation structure consistent in some ways with what has been demonstrated in Macaque V2. As shown in Figure 4C, there are narrow regions of either alternating orientation domains in V2 (indicated by alternating white and black dots in Fig. 4D). These regions of semiregular alternation appear within presumed pale stripe zones (approximate locations indicated by white circles at left of Fig. 4C and E). Orientation domains in the central region that overlie the thick stripe appear less distinct. This apparent lack of orientation structure in the thick stripes differs from that in the Macaque and could be due to a true difference between these species. However, further studies need to be done to explore this issue. The imaged region overlying...
Thus, the V2 activation pattern in the marmoset is at least in part consistent with the functional organization of V2 in the macaque monkey.

**Ocular dominance in V1.** To examine whether marmosets exhibit ocular dominance, we obtained optical images of V1 during monocular right eye and left eye stimulation. Ocular dominance maps were generated by subtracting the sum of all orientation images obtained through one eye from those through the other eye. Since these same images produced consistent orientation maps (Fig. 3), they are likely to reflect true stimulus-related response and not artifact.

Given the variation in reported strength and pattern of ocular dominance domains in marmosets, we expected to obtain clearer maps in some marmosets than others. We present two cases, one with stronger (case 1) and one with weaker (case 2) ocular dominance columns. In both cases, subtraction of summed left and right eye stimulation conditions produced images suggestive of some segregation of ocular activation. In case 1 (Fig. 5B; same case as in Fig. 6), in which only V1 was in the field of view, the ocular dominance map reveals a pattern of irregular alternating light and dark bands, roughly 200–400 μm wide (dark regions outlined below in white). In case 2 (Fig. 5A; same case as in Figs. 3 and 4), eye-specific activation is weak. However, there is some fluctuation of activity that suggests eye preference. In the upper part of the image, there is a hint of a dark band of activation (left eye, outlined in white below) adjacent to a lighter band (right eye). In the lower part of this image, potential ocular dominance columns (outside of blood vessel artifact region indicated by white arrow) are punctuated by irregular dark patches (suggested patches encircled below by white outlines). Area V2, in contrast, appears as relatively even gray with no structure for ocular dominance. The imaged V1 patterns in these two cases are reminiscent of the irregular ocular dominance columns seen in adult marmosets [cf. Chappert-Piquemal et al. (2001), Fig. 2] or in monocularly deprived marmosets with stabilized ocular dominance columns (Sengpiel et al., 1996). Ocular dominance columns in marmosets are reported to have an anatomical and physiological periodicity of roughly 700 μm and often appear in discontinuous patches rather than long continuous columns. Consistent with this architecture, the images in Figure 5 suggest a left/right cycle of about 600–700 μm (scale bar = 1 mm). Thus, these data support the view that at least some adult marmosets have ocular dominance columns.

**Direction.** Previous studies in the ferret and the cat have demonstrated clear organization for direction preference in primary visual cortex (Weliky et al., 1996; however, no tangential organization for directionality has been reported in V1 of macaque monkeys. To examine whether the marmoset is more similar to carnivores or to Old World primates in this respect, we obtained optical images to moving gratings moving in eight different directions (0, 45, 90, 135, 180, 225, 270, 315°). All images were...
Fig. 4. Functional organization of area V2 in the marmoset. A: Cytochrome oxidase-stained section. Same as in Figure 1A. Dark stripe in V2 are indicated by arrows. Thin stripes (indicated by red arrows) appear most clearly. Thick stripes (indicated by black arrows) are somewhat less distinct. White box indicates region shown in B–F. B: Image of cortical vasculature, same as Figure 1D. C: Imaged V2 stripes. Same image as Figure 3B except analysis was done with different clipping values to accentuate weaker orientation activation in V2. Locations of cytochrome oxidase stripes (seen in E) are indicated at left by black arrows (thick stripes), red arrows (thin stripes), and circles (pale stripes). D: Same as C. Cytochromes oxidase thin stripe outlines (red) and V1/V2 border (white) superimposed on image. Note alternating dark and light patches (indicated by black and white dots) within presumed pale stripe zones. The orientation map in center thick stripe zone is less distinct. Thin stripe zones are relatively even gray and do not contain structure for orientation. E: Cytochrome oxidase of region within white box in A. Same field of view as C. Stripes indicated by arrows and circles at left as in C. F: Same image as E. Outline of thin stripes suggested by red outlines. V1/V2 border indicated by white line. Scale bar = 1 mm (B–F).
obtained monocularly. The images shown in Figure 6 are all obtained by appropriate summing of the identical set of images used in Figure 5B. Figure 6A illustrates an orientation map obtained in this case. Darker regions are sum of activations by 135° (up and to the right, monocular presentation to left eye and right eye) and 315° (down and to the left, monocular presentation to left eye and right eye) gratings. Lighter regions are sum of activations by 45° (up and to the left, monocular presentation to left eye and right eyes) and 225° (down and to the right, monocular presentation to left eye and right eyes) gratings. As in Figure 3, notice the elongation of the iso-orientation domains (extending roughly from lower left to upper right of image).

To examine possible direction-specific activations, we used the same images collected for the orientation maps, but subtracted images obtained during presentation of moving gratings in opposing directions. Since the orientation of the stimuli is common to both conditions, any orientation-specific contribution to the signal should be eliminated in the subtraction. This result is illustrated in Figure 6B. Darker regions are more responsive to 45° gratings (moving up and to the left); light regions are more responsive to 225° gratings (moving down and to the right). Due to the small number of trials collected (12 trials), this subtracted map is relatively weak. However, its structure appears distinctly different from that of the orientation map, lacking the elongated appearance of the orientation domains in Figure 6A and exhibiting a more patchy appearance.

To examine qualitatively whether there is any relationship between the presumed direction activation zones and the orientation domains, we circled the darker (45° preferring) direction domains in red and the lighter (225° preferring) direction domains in blue. When this red/blue pattern is overlaid on the orientation map in Figure 6A, on inspection there is no obvious relationship between the two maps, although the periodicities appear similar. Red circles fall in dark zones, light zones, and on the borders of light and dark zones of Figure 6A. Similarly, blue circles fall in dark, light, and intermediate zones of Figure 6A. This is not dissimilar to the qualitative overlays of direction and orientation maps seen in Weliky et al. (1996).

The relationship of directional map to ocular dominance organization is shown for comparison in Figure 6C (same image as shown in Fig. 5A). Clearly, the periodicity of this map differs from those of the orientation and direction maps. As shown by the overlay in Figure 6C (below), there are putative directional columns of both types in each of the left eye and right eye ocular dominance domains (each of the dark and light ocular dominance domains have both red and blue circles). In sum, our data are suggestive of directional domains in V1 of the marmoset and suggest possible organization for direction merits further study in the marmoset.

**Blobs.** In the macaque monkey, blobs are reported to have a high concentration of nonoriented color cells (Livingstone and Hubel, 1984) and typically have either a red-green or blue-yellow preference (Ts'o and Gilbert, 1988; Landisman and Ts'o, 2002). In the marmoset, blue cone inputs project to the blobs via the interlaminar layers of the lateral geniculate nucleus (LGN) (Hendry and Yoshioka, 1994; Martin et al., 1997). Furthermore, there is a sex-linked dichotomy for color vision. Whereas both males and females have blue cones, only females are true
trichromats and males are dichromats (lack red-green pigment). As all the marmosets we studied were males and thus are presumed dichromats (Yeh et al., 1995), so we did not expect any red-green vs. blue-yellow activation. In two marmosets, we used isoluminant red-green and blue-yellow gratings that have been used in imaging color blobs and stripes in Macaque monkeys (Ts’o et al., 1990; Roe and Ts’o, 1995, 1999). As expected, optical images revealed no preferential red-green vs. blue-yellow activation. Neither did we observe any preferential activation when luminance was compared with red-green or with blue-yellow activation (cf. Roe and Ts’o, 1995).

**Functional architecture.** To examine possible alignment of blob distribution and ocular dominance, we overlaid the blob map on the ocular dominance map. At least in the several potential ocular dominance domains, there was no obvious relationship. The centers of the ocular dominance patches did not overlie the cytochrome blobs; neither did the patch centers fall consistently between blobs. Examination of the relationship between blobs and orientation domains also revealed no obvious alignment.

**DISCUSSION**

**Optical Imaging of Marmoset Visual Cortex**

Because of the fragile nature of these small animals (weight 400–600 g), marmosets were often difficult to maintain under anesthesia for long periods of time (Bourne and Rosa, 2003; Schiessl and McLoughlin, 2003). Data collection periods were therefore often limited. As a result, typically 10–20 trials were collected per condition. Furthermore, due either to species-specific differences in
cortical response or to anesthesia-related differences, optical signal strength in the marmoset in our hands was weaker than that in the macaque (Ramsden et al., 2001). The images presented in this article demonstrate clearly discernible maps, although the signal-to-noise ratio would likely have been improved with additional trials. Confidence in the imaged signal was bolstered by obtaining multiple types of functional maps from single cortical locations (e.g., Figs. 3–6). Signals due purely to artifact or to generalized changes in optical response are unlikely to produce maps that are specifically structured and distinct from one another and are likely to be due to stimulus-related responses. In addition, the clear correlation of these images with anatomical landmarks revealed in cytochrome oxidase makes it even less likely that the imaged responses are artifactual.

**Ocular Dominance Columns in Marmosets**

The presence of ODCs in marmosets has been controversial. As in squirrel monkeys and owl monkeys (Kaas et al., 1976; Hendrickson et al., 1978; Livingstone, 1986; although see Rowe et al., 1978), it was previously believed that adult marmosets lack anatomical ODCs (Spatz, 1979, 1989; DeBruyn and Casagrande, 1981) and that these columns could be stabilized with certain visual experience, such as monocular lid suture, during development [Sengpiel et al. (1996); cf. Livingstone (1996) for similar result by inducing strabismus in squirrel and owl monkeys]. Despite the reported lack of ODCs, physiological examination of normal marmoset V1 revealed some weak fluctuation of ocular dominance that extended outside layer 4 to layers 2/3 and 5 (Hubel and Wiesel, 1978; Rowe et al., 1978; Sengpiel et al., 1996; Livingstone, 1996, for owl monkey and squirrel monkey). However, a recent study demonstrated the clear presence of anatomical ODCs in layer 4C of two normal adult marmosets (Chappert-Piquemal et al., 2001). Furthermore, functional ODCs can be induced in adult marmosets, but appear only transiently: following monocular silencing by intravitreal TTX injections in adult marmosets, ODCs are rapidly induced (within 24 hr, as revealed by staining for the immediate early gene protein zif268), but surprisingly disappear within 10–20 days (Markstahler et al., 1998; cf. Silveira et al., 1996 in *Cebus apella*). Thus, ODCs in marmosets, either those in normal juveniles or induced in adults, do not appear to remain stable. Given the differing reports in adult animals and the apparent influence of experience on the appearance and disappearance of ODCs in marmosets, it is likely that there is a significant degree of variability in the presence of ODCs within the adult marmoset population. Indeed, this would be consistent with ODC variability found in other New World monkeys, in which patterns range from strong and distinct, to weak or indistinct, to absent and where periodicity and pattern of the columns vary from individual to individual (Adams and Horton, 2003; cf. Kaas et al., 1976 vs. Rowe et al., 1978; Livingstone, 1996). Such variability could arise from normal genetic variation or from individual visual experience during development.

The optical imaging data in this study is consistent with previous studies. Consistent with hypothesized variability in ODC presence within the marmoset population, ocular dominance columns were found in some marmosets and not others (two out of four). In parallel with reported weak physiological fluctuation (Sengpiel et al., 1996), optical images exhibited some degree of ocular segregation, although weak. The patterns of imaged ODCs are consistent with the “irregular islands” or “mosaic of irregular columns” previously shown and the widths were within the range previously reported: 250–400 μm/column width (Sengpiel et al., 1996; Markstahler et al., 1998; Chappert-Piquemal et al., 2001). These data provide the first imaging evidence of ocular dominance columns in New World monkeys and are consistent with the view of ocular dominance variability within the marmoset monkey.

**Different Cortical Magnification Factors?**

The periodicity of a single thin/pale/thick/pale stripe cycle in marmosets is roughly 2 mm (see also Rosa et al., 1997). This is roughly two-thirds the period of that in the squirrel monkey (Tootell et al., 1983; Malach et al., 1994) and owl monkey (Tootell et al., 1985; Xu et al., 2004), half that in the Macaque monkey (Tootell and Hamilton, 1989), and 40–50% that in humans (Horton et al., 1990; Horton, personal communication). Given that the same amount of visual space is represented in V2, if the cortical magnification factor of a single stripe cycle (thin/pale/thick/pale cycle) were constant across species, then there should be the same number of stripe cycles across species despite differences in total area. However, published figures do not support this. It is estimated that the number of stripe cycles per dorsal hemisphere in the marmoset is 6–8, in the owl monkey 8–10, in the macaque 12–13, and in the human 22–24. While some increase in the size of stripes could be attributed to factors such as increase in neuropil, glia, and neuron size, the fact that there are more stripe cycles in larger brains means that each stripe cycle represents proportionately less visual space [i.e., the cortical magnification factor increases; cf. Roe and Ts’o (1995)]. The implied changes in magnification factor are not likely to be due simply to differences in the numbers of sensory inputs [e.g., in both macaques and humans there are roughly 1 million retinal ganglion cells (Rakic and Riley, 1983; cf. Windrem and Finlay, 1991)]. These data, therefore, support the theory that increase in cortical size, while due in some part to increased modular size, is attributed largely to increase in the number of cortical modules (Rakic, 1988; Purves et al., 1992; Huffman et al., 1999). Thus, if a stripe cycle is thought of as a functional unit, then in primates with larger cortical area, each unit is devoted to a smaller amount of visual space; in corollary, more units are devoted to each degree of visual space (Roe and Ts’o, 1995; Rosa et al., 1997; Lyon et al., 2002; Schiessl and McLaughlin, 2003; Shmuel et al., 2005).

What do increased number of functional units offer? More units may be needed to process additional visual features. Let us consider adding the representation of another visual feature, such as color, to the processing unit. The additional circuitry needed for such a representation may exceed the limits of that unit size. That is, the required increase in the number of neurons would also have increasing pressures on the number of synapses and extents of dendrite/axon arbor size. Potential limits on synaptic number or arbor size may destroy the functional unity of the module and may in turn lead to an anatomical and thereby functional fractionation of the module. What may result is a regrouping that then leads to increased number of modules. Alternatively, increased module number may be needed to accommodate increased number of connections with other cortical loci. Even if there is no
increase in the numbers of features represented, given a constant number of connections per neuron, an increase in the number of neurons is accompanied by a disproportionately large increase in volume due to maintaining connections (Ringo, 1981). This growth in connectional volume may itself limit the growth of the functional unit size, thereby leading to the addition of units.

Cortical Architecture: Relationships Between Relatively Fixed and Highly Malleable Maps

In the adult macaque, orientation and ocular dominance have a predictable relationship. That is, orientation singularities tend to fall in the centers of ocular dominance columns and iso-orientation domains tend to cross ocular dominance borders at right angles (Bartfeld and Grinvald, 1992; Blasdel, 1992). The structure of orientation columns is present from a very early age and appears highly resistant to dramatic changes in visual experience and to the concomitant changes in geniculocortical inputs during altered visual experience (Antonini and Stryker, 1993; Kim and Bonhoeffer, 1994; Chapman et al., 1996). In contrast, ocular dominance columns exhibit varying degrees of segregation (ranging from sharp segregation, such as that following strabismus, to lack of segregation altogether, such as in the normal marmoset), varying degrees of bias in contralateral/ipsilateral distribution in layer 4, and differing patterns of columnar continuity or patchiness. These differences are not only seen from across species comparisons, but also following different visual experience during development. Thus, whereas ocular dominance columns are malleable and susceptible to visual deprivation paradigms, orientation columns appear much less so.

We consider two possible views of this dichotomy. One view suggests that orientation columns are a scaffold on which other features, such as ocular dominance columns, hang. In developmental terms, this could mean that specification of orientation occurs before that of ocular specification during development. Precedence for this developmental sequence has previously been shown in ferrets (Chapman et al., 1996). Another view is more functional. It is possible that orientation maps appear more constant because orientation is a continuous parameter, whereas ocular dominance is more bimodal in nature. The competitive forces between multiple iso-orientation populations may be more computationally constraining than those between two populations of ocular afferents. This difference may then lead to the perceived constancy of orientation maps.

The question remains as to how orientation and ocular dominance maps may be established independent of one another. A key difference between these two featural domains is that ocularity is determined by individual geniculate afferents arising from either left eye layers or right eye layers in the geniculate. Orientation selectivity, however, is not only determined by appropriate convergence of geniculate afferents (Hubel and Wiesel, 1977; Chapman et al., 1991; Ferster et al., 1996) but also highly influenced by intracortical mechanisms (Sillito, 1975; Fregnac et al., 1988; Greuel et al., 1988). Thus, during the period of ocular dominance plasticity when individual geniculocortical arbors are being remodeled, they must as a population still seek to maintain continuity of orientation representation and minimize total number of singularities or fractures. This constraint for continuity must be stronger than the need to maintain a constant relationship with overall ocular dominance pattern. Alternatively, as suggested by one recent study (Adams and Horton, 2003), the presence or pattern of ocular dominance may be more strongly determined by genetic constraints than by experience or other functional constraints. In this case, one would not expect to find a consistent relationship with orientation maps.

Whether cytochrome oxidase blobs are a fixed architectural feature of visual cortex is also a question. Blob patterns are present even after early binocular enucleation during development (Kuljis and Rakic, 1990) and remain relatively constant in number throughout development (Purves and LaMantia, 1993). As observed in other New World monkeys, no consistent relationship between identified ocular dominance domains and cytochrome oxidase blobs was discerned in these data. Whether alignment between ocular dominance and blobs is present in juvenile marmosets is unknown. In normal macaque monkeys, cytochrome oxidase blobs are centers of high monocular dominance and align along the centers of ocular dominance columns (Te'o et al., 1990). Following monocular enucleation or monocular deprivation, blobs of the deprived eye shrink in size but retain their position along ocular dominance column centers (Horton, 1984). However, disruption of the alignment has been demonstrated following anisotropic amblyopia induced with lens rearing (Roe et al., 1995) and following reduction of parvocellular geniculate inputs as a result of fetal X-irradiation (Roe et al., 1999). Thus, the blob/ocular dominance association is not a fixed architectural feature of primate visual cortex (Horton and Hocking, 1996). Although this issue needs further investigation, we suggest, given the relative persistence of blob patterns across primate species and with different visual experience paradigms, that any change in blob/ocular dominance relationship is again primarily due to ocular dominance malleability.

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