THE CORTICAL REPRESENTATION OF SHADOWS CAST BY RETINAL BLOOD VESSELS

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ABSTRACT

Purpose: We inquired whether the representation of angioscotomas could be detected in the primary (striate) visual cortex.

Methods: In 12 normal squirrel monkeys, the ocular fundi were photographed and retinal vascular landmarks were projected onto a tangent screen for calibration. Each animal then underwent monocular enucleation under general anesthesia. Animals were perfused after 8 to 10 days, and flat-mounted sections of striate cortex were processed for the metabolic enzyme cytochrome oxidase (CO).

Results: In each animal, the cortical region corresponding to the blind spot appeared as a 3 x 2 mm oval in the CO staining pattern. It stood out because it received input from only 1 eye. In 9 of 12 animals, the representation of the major retinal vessels was also visible, for the same reason. In our best examples, CO sections showed about 10 thin lines radiating from the blind spot representation. Some could be traced for 15 mm, all the way to the vertical meridian. Vessels only 12 minutes of arc in diameter were represented in the cortex. Each angioscotoma representation in the cortex could be matched with its corresponding retinal vessel in the fundus.

Conclusions: Our findings show that (1) the visual field map in layer IVc is more precise than indicated by physiological studies, and (2) visual experience must refine the final pattern of geniculocortical projections, given that the retinal vessels can produce a shadow only after birth.


INTRODUCTION

In the primary visual cortex of many mammalian species, the inputs serving each eye are segregated into discrete zones called ocular dominance columns. These columns are present throughout the striate cortex, except in the representation of 2 monocular regions: the temporal crescent and the blind spot. Many techniques have been used successfully to map the cortical representation of the temporal crescent and the blind spot. Perhaps the simplest approach is to stain the cortex for a mitochondrial enzyme, cytochrome oxidase (CO), after enucleation of one eye. Because CO activity is regulated by metabolic demands, a prompt reduction in staining occurs within ocular dominance columns serving the missing eye (Fig. 1). The temporal crescent and the blind spot representations are outlined as monocular zones embedded in a mosaic of alternating light (enucleated eye) and dark (remaining eye) ocular dominance columns.

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A third monocular compartment in the visual field, usually ignored by ophthalmologists, arises from the shadows of retinal blood vessels. Mapping his blind spot, Helmholtz was able to trace the proximal portions of the major blood vessels emanating from the optic disc (Fig. 2). He correctly inferred that these blood vessels cre-
Angioscotomas were discovered by Helmholtz, shown in this drawing of his right eye's blind spot with stumps of three large retinal vessels.

Angioscotomas were previously described in the visual field because the opaque erythrocytes prevent light from reaching the photoreceptors. In 1926, Evans coined the term “angioscotoma” to refer to parts of the visual field obscured by retinal blood vessels. He published an extensive plot of the angioscotomas in the visual field (Fig 3) obtained with a stereocampimeter. Provided the subject can maintain steady fixation, angioscotomas can be demonstrated easily with use of a wide variety of perimetric techniques.

In the present study, we report that representations of angioscotomas are revealed in monkey visual cortex when 1 eye is removed and the tissue is stained for CO activity. Discovery of the representation of the angioscotomas indicates that the retinotopic map in the cortex is more finely detailed than realized previously. It also provides direct evidence that patterns of connections to the cortex are remodeled early in life by visual experience, since the retinal vessels cannot begin to cast a shadow until after birth.

**METHODS**

Twelve normal adult squirrel monkeys (Saimiri sciureus) were used from colonies at the California Regional Primate Research Center, Davis. All experimental procedures were approved by the Committee on Animal Research at the University of California, San Francisco. Animals were placed in a stereotaxic frame after induction of general endotracheal anesthesia with 2% isoflurane. They were paralyzed with succinylcholine to abolish eye movements and artificially respirated. The pupils were dilated with 1% cyclopentolate hydrochloride and 10% phenylephrine hydrochloride. The retinas were photographed with a Topcon fundus camera. The fundus camera was then used as a reversing ophthalmoscope by placing a mirror over the objective lens to project the positions of retinal land marks onto a tangent screen for calibration. Finally, 1 eye was enucleated to differentiate monocular and binocular zones in striate cortex with CO histochemistry. After the experiment, animals were given buprenorphine hydrochloride (0.02 mg/kg every 8 hours), a potent analgesic, until they had recovered from surgery.

After a survival time of 8 to 10 days, monkeys were euthanized by injection of pentobarbital (150 mg/kg) and perfused through the heart with 1 l of saline followed by 0.5 l of 2% paraformaldehyde in 0.1 M phosphate buffer. Visual cortex was dissected from each occipital lobe in a single piece and flattened gently by squeezing it between a glass slide and a sponge. After postfixation overnight in 1.33% paraformaldehyde plus 30% sucrose, the tissue was sectioned tangential to the pial surface at 35 μm with a freezing microtome. Sections were mounted on glass slides, air-dried, and reacted for CO activity. In some cases, the eyes were postfixed with osmium tetroxide, embedded in epon-araldite, sectioned at 1 μm, and examined in the light microscope.

Flat-mounted cortical sections were imaged at 600 dots per inch on an Agfa Arcus II scanner fitted with a transparency adapter. Images of individual sections were imported into Photoshop 5.0 and montaged to provide a complete reconstruction of CO activity in layer IVc, the principal site of geniculate input to striate cortex.

**RESULTS**

The pattern of CO activity in squirrel monkeys with normal vision in both eyes appears completely homogeneous in layer IVc of striate cortex. Results from such control animals have been described previously. In the present study, we report that following monocular enucleation, the representation of angioscotomas became visible in 9 of 12 animals. A typical example is illustrated in Figs 4 through 11. Figures 4 and 5 show the right and left retinas, respectively, in this animal. The left eye was enucleated.

In the macaque, a sharply demarcated pattern of columns resembling zebra stripes always emerges after monocular enucleation (Fig 1). By contrast, in the squirrel...
rel monkey, the ocular dominance columns are often quite rudimentary.\textsuperscript{16-21} In Figs 6 and 7, the level of CO activity appears nearly uniform, reflecting diffuse, poorly segregated input from the remaining right eye. At higher power, hints of ocular dominance columns could be seen in some regions of the cortex, but they are not visible in this low-magnification picture.

In Fig 6, a pale oval is visible in the left striate cortex, corresponding to the representation of the blind spot of the right eye. Parenthetically, we point out that it is misleading to refer to the "representation of the blind spot," because a scotoma does not need to be represented. Rather, the pale oval is a region receiving input exclusively from the ipsilateral eye, because the contralateral eye contributes nothing. We refer colloquially to this region as the blind spot "representation" simply because it corresponds to the retinotopic coordinates of the blind spot of the contralateral eye. It is not actually representing the blind spot. The same caveat applies to our use of the term angioscotoma "representation."

CO levels declined throughout most of the left striate cortex during the 8 to 10 days following enucleation of the left eye, but they remained at an intermediate level because of innervation provided by the remaining right eye. In the blind spot representation of the right eye, however, all innervation was lost after removal of the left eye. Consequently, CO activity fell more than in surrounding binocular cortex, as evinced by the emergence of a pale oval silhouetted against a darker background of enzyme staining.

Looking carefully at Fig 6, one can see thin, pale, threadlike structures emanating from the blind spot representation. Their optical density is similar to that of the blind spot representation, suggesting that they also correspond to regions of cortex innervated exclusively by the left eye. They represent the angioscotomas of the major blood vessels exiting the right optic disc.

Figure 7 shows the right cortex from the same animal.
On this side, the blind spot representation is dark because it has remained normally innervated by the intact right eye. In the far periphery, a pale triangular region is visible. This is the monocular crescent representation. It appears pale because all input has been cut off after enucleation of the left eye. The rest of the cortex has an intermediate optical density, reflecting partial loss of innervation after removal of one eye.

As in the other hemisphere, thin lines radiate from the blind spot representation in Fig 7. They are dark, like the blind spot representation, because only the intact right eye projects to them. They represent the angioscotomas of the missing left eye.

Figures 8 through 11 show the match between the retinal vessels in each eye and their representation in the contralateral cortex. The perimeter of striate cortex corresponds to the vertical meridian, providing a fiduciary point as each major vessel crosses the vertical meridian in the retina. In addition, occasional branch points are visible, which aid in assigning each retinal vessel to its cortical image.

The largest veins in Figs 4 and 5 are 80 μm wide, corresponding to about 0.50 degrees in this squirrel monkey. Figure 12 shows the inferior temporal arteriole in Fig 4 in an epon section cut parallel to the horizontal meridian. The vessel is situated in the nerve fiber layer, which is greatly thickened because of its proximity to the optic disc. It has an internal diameter of 40 μm. In this part of the retina, the vessel casts a shadow approximately 4 cones wide. Some of the smallest vessels represented in the cortex—for example, those coded pink and yellow in Fig 9—are only 30 μm wide, corresponding to 12 minutes of arc. In epon sections, the pink vessel was found to cover about 4 to 5 cones. Although it is much smaller than the vessel in Fig 12, it approaches the macula, where the concentration of cones is higher. By contrast, the yellow vessel heading toward the periphery covers only a few cones.

**FIGURE 8**
Drawing of retina in Fig 4, indicating in color vessels whose shadow is visible in left cortex. Star indicates fovea.

**FIGURE 9**
Drawing of retina in Fig 5, indicating in color vessels whose shadow is visible in right cortex. Star indicates fovea.

**FIGURE 10**
Drawing of cortex in Fig 6, showing represented angioscotomas in color, corresponding to colored vessels in figure above. MC, monocular crescent; V2, second visual area.

**FIGURE 11**
Drawing of cortex in Fig 7, showing color-coded angioscotomas from retina pictured above. MC, monocular crescent; V2, second visual area.
The Cortical Representation of Shadow Cast by Retinal Blood Vessels

FIGURE 12
Plastic 1-µm section showing inferotemporal arteriole in right retina (Fig 4). Lumen is empty of red cells because the animal was perfused. Vessel's shadow deprives the cones located 250 µm underneath. Arrows denote junction between cone inner and outer segments.

DISCUSSION

To the ophthalmologist, the primate retina is one of the most beautiful and important structures ever devised by nature. It is pleasing to see its vascular pattern mirrored in the brain. That angioscotomas are represented in the visual cortex makes it obvious why blood vessels are excluded from the fovea, the point of greatest resolution in the visual field.

It has been known since the pioneering work of Japanese ophthalmologist Tatsui Inouye\(^2\) that primate striate cortex contains a retinotopic map. The precision of the retinotopic map has been difficult to ascertain in monkeys. Microelectrode recordings at any point in striate cortex demonstrate a certain amount of random scatter in the position of individual receptive fields.\(^3\) This scatter reduces the exactness of the retinotopic map. Neurophysiologists have overestimated the amount of scatter in layer IVc because its cells are extremely small and therefore difficult to isolate for single-cell recordings. Multunit recordings inevitably blur the precision of the retinotopic map because responses are combined from cells with different receptive field positions.

Our findings indicate that the retinotopic map in layer IVc is extraordinarily precise. Blood vessels as small as 12 minutes of arc in diameter are represented clearly at an eccentricity of 7 to 8 degrees. Vessels that cast a shadow only a few cones wide can be detected in the cortex. It is eminently logical that the exquisite spatial resolution of the retina should be preserved in the cortical map. Otherwise, much of the information about position encoded by the tightly packed photoreceptor array would be lost in the transfer of visual signals from the eye to the brain.

These results also provide a clue to the role of visual experience in the refinement of connections to the visual cortex. During development, axon terminals serving each eye intermingle in layer IVc when they first reach the primary visual cortex.\(^4\) In macaques, geniculocortical afferents begin to segregate into ocular dominance columns by late fetal life. Ocular dominance columns are already present at birth, indicating that their formation does not require visual experience.\(^5\) After birth, however, visual experience can alter drastically the anatomy of the ocular dominance columns. Eyelid suture leads to remodeling of geniculocortical projections.\(^6\)\(^,\)\(^7\) The ocular dominance columns serving the normal eye expand at the expense of those belonging to the deprived eye. Shrinkage of the deprived eye's columns is thought to underlie the profound amblyopia that develops from early form deprivation.

A similar process explains why the representation of retinal vessels becomes visible in the cortex. We assume that after birth the larger retinal vessels cast a shadow that is sufficiently dense to deprive the cones situated underneath, just as eyelid suture deprives the whole retina. This highly localized pattern of visual deprivation causes retraction or impaired development of the eye's geniculocortical arbors in corresponding regions of the retinotopic map. These regions of the cortex are abandoned to the other eye. After the critical period is finished, this remodeling of cortical inputs cannot be reversed. If 1 eye is subsequently removed during adulthood, angioscotomas are revealed for the same reason that the monococular crescent and blind spot representations become visible: CO levels are affected differentially in monocular and binocular regions of the cortex.

The representation of angioscotomas in the cortex illustrates that the eyes compete for cortical connections during normal development, not just in the occasional subject with an ocular anomaly (e.g., congenital cataract). It also shows that this competition is waged on a local scale and need not involve the whole eye.

In macaques and humans, the cortical representation of angioscotomas is not visible. In these species, geniculocortical afferents are always highly segregated into ocular dominance columns. Therefore, ocular dominance segregation takes precedence over retinotopy, and the pattern of angioscotomas is obscured by ocular dominance columns. In most squirrel monkeys, the opposite prevails. Ocular dominance segregation is weak, so that the retinotopic course of angioscotomas through the cortex is preserved. However, in our study, 3 squirrel monkeys had well-segregated ocular dominance columns, like those of macaques and humans. In these animals, as one might expect, angioscotomas were not visible in the cortex.
REFERENCES


DISCUSSION

Dr. Alfredo A. Sadun. The primate visual cortex is organized in several respects. First, there is a retinotopic map so that each point in the visual field via the retina projects a map onto the primary visual cortex (V-1 cortex). Jonathon Horton has previously helped elucidate our understanding that this retinotopic map is far from linear, as the macula is vastly over-represented. A second organization of V-1 cortex is the segregation of ocular inputs into alternate bands from each eye, which are termed ocular dominance columns.

Dr. Horton and Adams have revisited the technique of cytochrome oxidase (CO) staining of ocular dominance columns to investigate features in the V-1 area of visual cortex. In particular, they have demonstrated the manifestation of regions of vascular patterns in layer IVc that represent the retinal vasculature. Or, more precisely, they have demonstrated those areas in the visual cortex that, by receiving afferents of input by those photoreceptors lying in the shadow beneath retinal blood vessels, are not represented in the visual cortex. They refer to these areas as angioscories.

Similarly, when the authors show us a pale oval (their Fig 10) in the striate cortex, it is a negative representation of the blind spot. Negative, in the sense that this region only receives input from the (right) eye whose blind spot is not being illustrated. This monocular dependent area, reflecting the right blind spot, shows up in relief when the authors removed the fellow left eye and then, 8-10 days later, sacrificed the animal for CO staining. The CO only stains the areas that the intact right eye inputs, which, of course, does not include the blind spot and angioscories of the right eye.

Horton and Adams have done much more than show us the power of the CO technique and the beauty of the retinal vasculature invertedly reflected on the visual cortex. They demonstrated angioscories that radiated and branched from the representation of the blind spot as far as 15 mm. Some of the angioscories were only 12 minutes of arc in diameter! This is extraordinary if one
considers that 20/20 vision means the resolution of 60 seconds, or 1 minute of arc. This is certainly a more precise retinotopic map in IVc of V-1 than was previously considered.

How can we explain the resolution of 12 arc minutes diameter so far eccentric? Twelve arc minutes is about 20/200 but our vision at 8 degrees is a bit worse.

One explanation may lie in the fact that vernier acuity is much better than Snellen acuity. Indeed, notwithstanding the 30 arc second packing of cones (which translates to a limitation of 60 arc seconds resolution = 20/20) and the wavelength of green light (which creates diffraction problems limiting vision to about 20/20), hyperacuity has indeed been demonstrated to exist to the point of 7 arc seconds (=20/3). This probably depends on cortical processing which may even be analogous to a Fourier Transform.

An important take-home message provided by the present work is that visual experience must refine the pattern of these projections to V-1 cortex. It is known that in monkeys the geniculate input to IVc begins as a course and largely branching tree. Over time, this gets refined. While the ocular dominance column organization is present by birth, only post-natal visual experience could, by further refinement, create the inverse reflection of angioscotomas whose existence in the retina depends on light. This is also consistent with the results of many other studies of experimental amblyopia.

It is interesting that in macaque and humans, such angioscotomas are not visible on the cortex. The authors explain that this is because in these species ocular dominance takes precedence. Retinotopy is lost in favor of ocular dominance. Yet, in squirrel monkeys, retinotopy is more clearly seen, as the ocular dominance segregation is much weaker. Are the trade-offs between retinotopy and ocular dominance of a fundamental significance, or are they just epiphenomena of different developmental patterns? I eagerly await further work in this area to provide us a better insight on the meaning of the precise and beautiful organization of the visual cortex.

REFERENCES


Dr Jonathan C. Horton. Thank you Dr Sadun for those valuable remarks.

It would be nice to correlate visual acuity with the resolution of the cortical retinotopic map. We are hampered in our efforts by the fact that within the central 3° we do not see the angioscotomas in the cortex. You may have noticed that the cortical angioscotomas peter out as they approach the foveal representation. We believe that when the retinal vessels drop below the size and thickness required to deprive the photoreceptors, they become invisible in the cortex.

Amblyopia is a major clinical problem of interest to a large segment of the membership of this society. Why is the cortex vulnerable to this disease? Why not simply freeze things before birth so that the visual system is impervious to deprivation of 1 eye during early childhood? Our studies demonstrate that visual stimulation early in life is critical for the maturation of the cortex. The development of the angioscotoma representations in the cortex provides direct evidence for the role for visual experience in the formation of the cortical architecture.

Finally, I would like to address the issue of why angioscotomas have never been seen previously in the cortex. It’s true that they’re not present in the human or in the macaque. The reason is that these species have highly developed ocular dominance columns. This means that the angioscotomas are unable to connect across the columns to create a continuous line that your eye can see as an angioscotoma. Instead, the angioscotomas are fractured into short segments, camouflaged if you will, by the pattern of the ocular dominance stripes. It is a whim of nature that squirrel monkeys have rather rudimentary columns, so visuotopy triumphs over ocular dominance column segregation, and one can see the cortical representation of the angioscotomas. Why one primate species should organize its ocular inputs in a strictly segregated fashion, whereas another animal allows them to interdigitate rather promiscuously, I cannot yet say. Thank you.