inally published in 1948). These geometries cannot support the inferences we have studied, however, for they preserve no metric properties [R. Courant and H. Robbins, *What is Mathematics?* (Oxford Univ. Press, New York, 1941)].

8. We thank C. R. Gallistel, for focusing our attention on formal geometric analysis of our experiment, for suggesting important controls and variations on the basic experimental paradigm, and for suggesting a statistical analysis derived from the avian navigation literature; L. R. Gleitman for extensive overall conceptual guidance; U. Neisser for comments on a previous draft of this paper; and K. Feldman for running sighted control subjects and transcribing the videotaped sessions. This work was aided by a social and behavioral sciences research grant from the National Foundation–March of Dimes and a William T. Carter Foundation grant, both to L. R. Gleitman.

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Staining of Blue-Sensitive Cones of the Macaque Retina by a Fluorescent Dye

Abstract. *Intravitreal injection of a fluorescent dye, Procion yellow, results in the complete and systematic staining of a cone population in the monkey retina. These cones form an approximately regular array whose separation varies with retinal eccentricity. They are absent in the very center of the fovea, and their density peaks at 1°. The distribution of stained cones resembles that reported for blue-sensitive cones of other primates and, consistent with such an identification, they are found with less incidence in species having lower concentrations of blue cones.*

The neural retina is a highly organized structure with a crystalline-like array of tightly packed cones and rods in a two-dimensional matrix. Microspectrophotometric work has provided evidence of three cone types in the primate retina, each having peak sensitivity at a different part of the spectrum (*1*)—"blue-," "green-," and "red-sensitive" cones. To our knowledge, morphological differences among these cone types have not been reported in primates. Functionally, however, blue cones have unique properties. In humans and (Old World) macaque monkeys, the green and red cone systems have similar electrophysiological and psychophysical properties, whereas those of the blue cone system are different (*2*). There are also differences between dysfunctions of these cone systems leading to color vision disorders. Congenital disorders of the green and red cone systems correspond to sex-linked inheritance, whereas those of the blue cone system correspond to autosomal inheritance (*3*). In addition, blue cones are often involved in acquired color vision disorders secondary to retinal disease [Köllner's rule (*4*)], indicating an undue vulnerability to retinal insult.

In 1970, Laties and Liebman (*5*) reported that the intravitreal injection of a tissue-reactive fluorescent dye, Procion yellow, stained the outer segments of cones, but not of rods, in the amphibian retina. Using greater amounts of the dye, we have obtained a striking result. In the retina of the monkey, not only are all cone outer segments stained with Procion yellow, but the entire soma of some cones is completely stained by the dye, producing a Golgi-like silhouette. Such cones are organized in a rather regular array and have a characteristic retinal distribution.

Procion yellow M4RAN (Polysciences), 5 to 7 percent in deionized water, was injected (0.15 ml) intravitreally into the eye of anesthetized rhesus and cynomolgus monkeys. Leakage of dye was reduced by the slow removal of the needle. In some animals we also injected Lucifer yellow (Polysciences) (*6*) simultaneously with or subsequently to the Procion dye in a weight ratio of 1:50 to 1:100 of Lucifer to Procion yellow. Except for one monkey, which was kept in the dark during and after the injection, the animals were kept in a normal light (200 trolands): dark cycle for 18 to 30 hours (*7*). The animals were then killed with an overdose of pentobarbital. The eyes were fixed, often by arterial perfu-

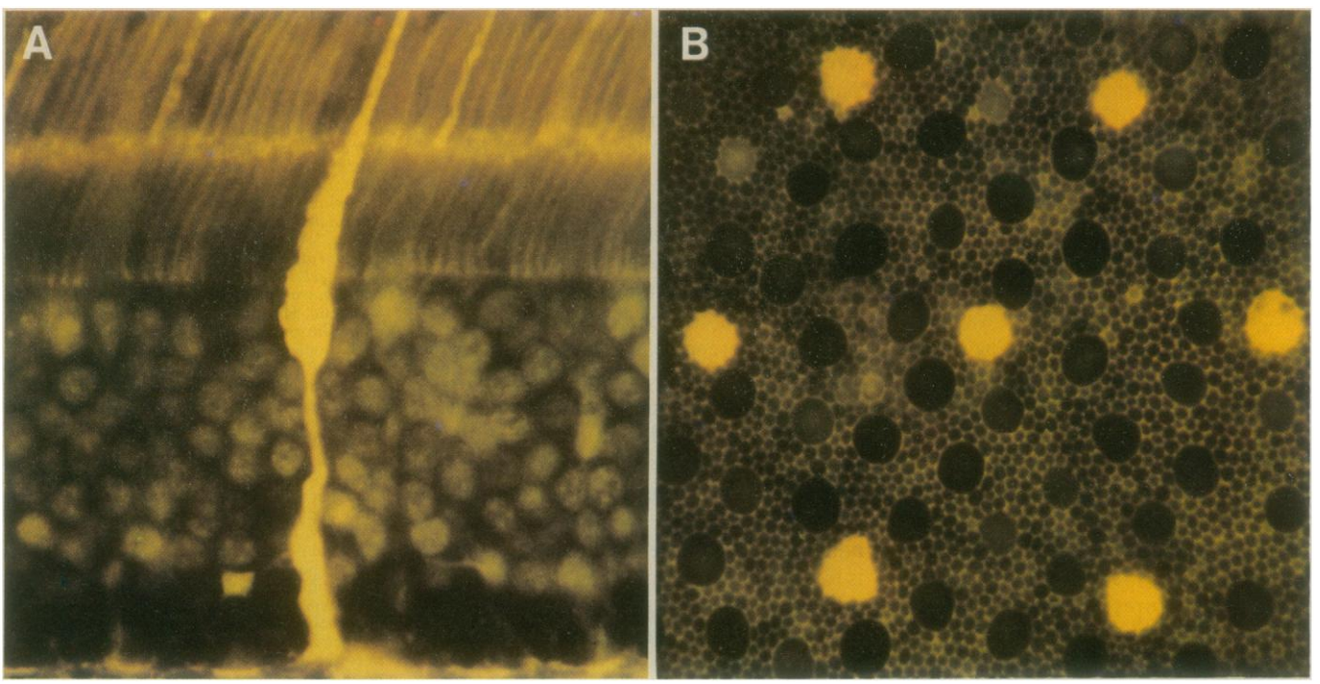

Fig. 1. (A) Radial section of rhesus monkey retina (~ 20° eccentricity) showing a cone completely stained by Procion yellow among other cones unstained except for their outer segments. (B) Tangential section passing through the outer limiting membrane showing a regular array of stained cones; unstained cones and rods appear as holes in the stained mesh of the outer limiting membrane.

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sion, with a formalin fixative. Whole retinas, stripped of pigment epithelium, were used for density counts. Retinal pieces were dehydrated through a graded series of acetone, embedded in Epon, and cut (10 to 15 μm) in the radial or tangential plane. The material was observed in episcopic or (dark-field) diascopic fluorescence microscopy; measurements were not corrected for shrinkage.

Figure 1A shows a radial section of a cone located at an eccentricity of ~ 20°. All parts of the cone are completely stained by Procion yellow. Figure 1B shows a tangential section of the same peripheral retina cut at the plane of the outer limiting membrane. The inner segments of Procion-stained cones form a nearly perfect hexagon around a centrally positioned cone, indicating a triangular packing of stained cones among unstained ones. The latter cones can be seen as darker round structures smaller than the cones completely stained by the dye. The finer cobblestone pattern is produced by unstained rod inner segments protruding through the stained outer limiting membrane.

Completely stained cones forming an approximately regular array interspersed among unstained cones were found throughout the retina of all examined monkey eyes. Figure 2A shows a radial section illustrating the regularity of distribution of Procion-stained cones (arrows); both limiting membranes are also stained, especially the inner one. Figure 2B is a tangential section at the plane of the outer limiting membrane. Procion-stained cones form a rather regular but imperfect array, which in some retinal areas appears to correspond to triangular packing (*8*). In order to better visualize those cones that were not completely stained by Procion yellow, we counterstained the retina with Lucifer yellow, which under the conditions we used stains all photoreceptors and other retinal cells (Fig. 2, C–G). Although the outer segments of all cones are stained by Procion yellow (*5*), the inner segments and remaining parts of the soma of the majority of cones were stained by Lucifer yellow (bluish-green fluorescence in Fig. 2, E–G).

Cones completely stained by Procion yellow showed a characteristic distribution across the retina. Figure 2H is a tangential section of the central retina;

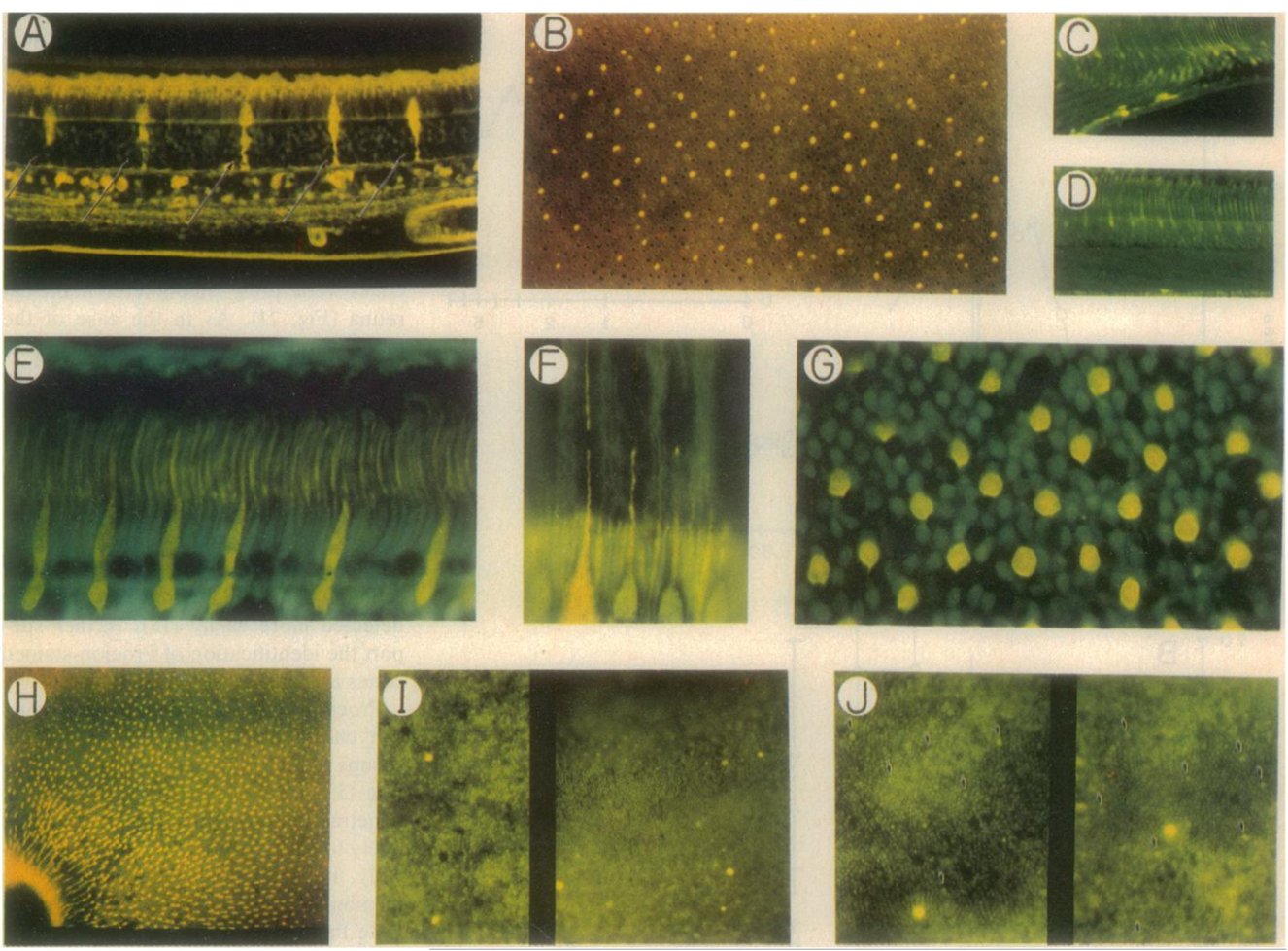

Fig. 2. (A) Cynomolgus monkey peripheral retina; arrows point to cones completely stained by Procion yellow. (B) Rhesus monkey peripheral retina; inner segments of Procion-stained cones are seen as yellow dots. (C and D) Cynomolgus monkey foveola and fovea counterstained with Lucifer yellow; all receptors are stained. (E) Cynomolgus monkey parafoveal retina with double fluorescent staining; outer segments of all cones are stained by Procion yellow, as well as the entire soma of some cones; the inner segment, nucleus and pedicle of most cones are stained by Lucifer yellow. (F) Cynomolgus monkey perifoveal retina. (G) Rhesus monkey fovea; tangential section at the plane of cone inner segments. Procion-stained cones are larger than Lucifer-stained cones. (H) Rhesus monkey foveola-fovea region at the level of cone inner segments. Foveolar center is at bottom left corner; its high concentration of stained structures is due to cone outer segments. Procion-stained cones are most concentrated about 1° from foveola. (I) Tangential (left) and oblique (right) sections of rabbit retina with double fluorescence staining. (J) Tangential sections of cat retina with double fluorescence staining. Small arrows point to inner segments of Lucifer-stained cones. Calibration bar: 40 μm (A, C, and D), 30 μm (B), 20 μm (E), 10 μm (F, I, and J), 12 μm (G), and 50 μm (H).

the bottom left corner shows the superior half of the foveola. The stained elements in the foveola are the outer segments of foveolar cones whose somata were not stained by Procion yellow, while the stained elements in the extrafoveolar area are the inner segments of cones completely stained by the dye. Cones stained with Procion yellow were absent in the foveola but present elsewhere in the retina. Procion-stained cones of rhesus and cynomolgus monkeys show a peak density in the foveal region (Fig. 3A) and are absent in the central-most 20 to 30 minutes of arc. A tangential section of the temporal 1° retina is shown in Fig. 2G. The mean (± standard deviation) separation between Procion-stained cones was smallest at the region of maximal density (32.37 ± 7.3 μm at 1°; $N = 82$), and it increased toward the periphery (64.69 ± 12.11 μm at 30°; $N = 92$) (Fig. 3B). The averaged percentage of Procion-stained cones relative to the total cone population showed a narrow range of variation and a slope of 0.14 percent per degree between 1° and 30° (Fig. 3C).

We believe that this select population of regularly arrayed cones that are completely stained by Procion yellow are blue-sensitive cones. (i) In the macaque retina, we have observed a distribution of Procion-stained cones resembling that of histochemically identified blue cones of the baboon (*9*); the percentage of such baboon cones also shows a narrow range of variation with retinal eccentricity (slope of about 0.13 percent per degree). (ii) The distribution of Procion-stained cones closely follows that of cones that degenerate after exposure to intense 463-nm light in the macaque (*10*). (iii) The comparatively large intercone distance between Procion-stained cones in the fovea (average of 32 μm, which corresponds to about 8 minutes of arc) predicts a visual resolution close to that mediated by human blue-sensitive cones (*11, 12*). (iv) Procion-stained cones are absent in the central-most foveal region, as are blue cones in subhuman primates (*9, 10*) and humans (*12*).

Behavioral and electrophysiological studies have shown that blue cones are present in the rabbit (*13*) and, to a much lesser extent, the cat retina (*14*). In these species, Procion-stained cones were seen less frequently than in the monkey retina, especially in the cat, and these cones did not show any obviously regular array (Fig. 2, I and J). Procion-stained cones of the rabbit were sometimes seen in clusters; the right side of Fig. 2I shows an oblique section through one of such clusters in the region of the visual streak, passing through outer segments (top) and inner segments (bottom) stained by Procion yellow. Many outer segments but only a few inner segments appear stained by Procion yellow. The percentage of Procion-stained cones was about 4 to 7 percent in many clusters, but it was markedly reduced in noncluster regions (Fig. 2I, left). Procion-stained cones were rarely encountered in the cat retina (Fig. 2J). As in the case of the monkey retina (Fig. 2, F and G), Procion-stained cones of the cat and the rabbit had larger inner segments than Lucifer-stained (or unstained) cones (Fig. 2, I and J). The scarcity of Procion-stained cones in the cat retina, where a blue cone system can be grudgingly detected (*14*), and the somewhat higher incidence of such cones in the rabbit retina, where a blue cone system can be detected more easily (*13*), further support the identification of Procion-stained cones as blue-sensitive cones.

Procion yellow is tissue-reactive and can chemically bind to protein amino groups of the cell membrane before fixation (*5, 6*). Although the dye does not penetrate into healthy cells (*15*), it is likely to alter membrane function chemically, kill those cells most sensitive to membrane dysfunction, and, thus, penetrate them. The most parsimonious hypothesis at present is that the penetration of Procion yellow into blue cones is due to their marked vulnerability to metabolic insult, a characteristic probably responsible for Köllner's rule (*4*). This possibility is supported by preliminary results of intravitreal injections of trypan blue (a dye that penetrates into "leaky" cells) and Procion yellow (*16*), and it is

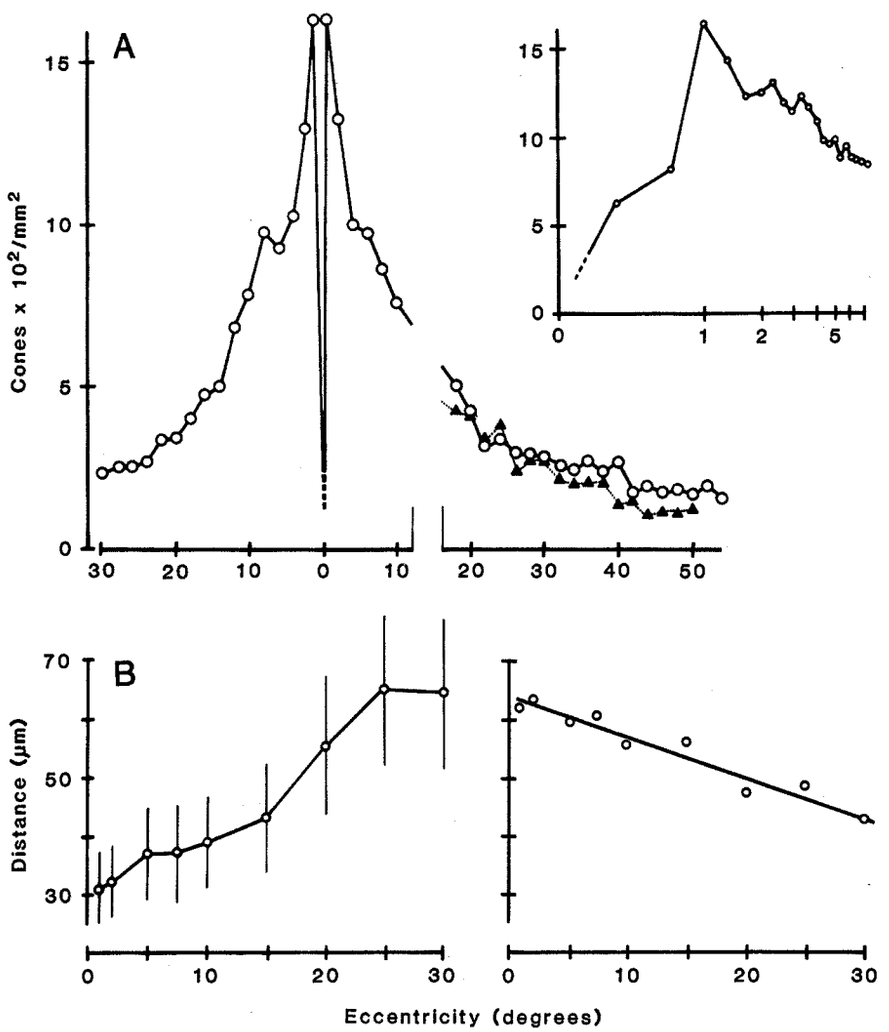

Fig. 3. (A) Density of Procion-stained cones in 2° steps; interruption of abscissa indicates optic disk. Circles, density along the horizontal meridian; triangles, density along a line parallel to the vertical meridian from the optic disk to 50° superior. Inset shows in more detail the distribution in the central temporal 10° in 0.3° steps; because of the geometry of this region, measurements at < 0.3° (dashed line) are somewhat less precise than those at higher eccentricities. (B) Mean intercone distance from 0° to 30° temporal along the horizontal meridian; bars show ± 1 standard deviation. (C) Percentage of Procion-stained cones relative to total cone population from 0° to 30° temporal along the horizontal meridian.

consistent with recently reported metabolic differences between cones and rods, and among cone types in the cyprinid retina (*17*).

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Altruism in an Antarctic Fish

Daniels (*1*) concludes that males who occupied the nests of experimentally removed females in an Antarctic fish species were behaving altruistically as replacement nest guards. He rejects the hypotheses that the replacement fish were behaving selfishly or as parents. We disagree with his conclusions for the following reasons.

First, Daniels argues that his failure to observe displacement of females from nests is evidence that there was no competition for nest sites, even though such sites were also used by the fish as protection against predators when there was no ice cover (*1*, *2*). More importantly, he reports a probability value of less than .01 for physical measurement differences between nest guards and nonguards [Mann-Whitney *U*-test; reference 18, in (*1*)], but he concludes that this statistic indicates similarity between them. A small probability allows rejection of the null hypothesis of "no difference." If guards are larger or in better condition than nonguards, it implies that occupying a nest site is selfish behavior that benefits the guard.

Daniels also rejects the hypothesis that the replacement fish (all males) were parents, since he did not see them in the vicinity of their nests before the females were removed. However, it was reported that the fish "roved," and one should therefore not assume that fathers would be frequently near their nests. Furthermore, in the majority of teleost families, males are the nest builders and guards of eggs (*3*). This suggests that the males who occupied the nest sites were the fathers of those eggs.

In light of the above, we feel that there is insufficient evidence for rejection of the hypotheses that replacement fish were behaving selfishly or as parents. We applaud the use of multiple hypothesis testing, but caution that more parsimonious explanations (*4*) of complex behavioral phenomena should be thoroughly investigated before acceptance of more complicated hypotheses.

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I conclude that altruistic behavior is a reasonable explanation of nest guard replacement in *Harpagifer bispinis* (*1*). However, selfish and misdirected behavior hypotheses are not discredited by the available data, but these hypotheses are less able to explain the entire range of observed behaviors. I argue only that the available data do not lead to the rejection of the altruistic behavior hypothesis.

Meikle *et al.* note a mistake in the original report (*1*); reference 18 is in error, and I apologize. I compare characteristics of guards and nonguards, using the Mann-Whitney *U* statistic and, as stated in the text, the measurements indicate no significant differences between the two groups. The probability value is incorrect and should read $P \simeq .22$ for standard length, $P \simeq .15$ for condition factor, and $P \simeq .33$ for fullness index.

The remaining arguments are simplified and incomplete. If individuals benefit from nesting, intraspecific competition for nests can be expected (*2*). I make no such claim for nest sites as stated in this comment. In fact, I state that the protected sites, that is, overhanging rocks and stacked rubble identical to nest sites, are abundant in the rubble bottom coves where *H. bispinis* is found. For competition to occur some resource, in this case the site, must be limiting (*3*); this does not appear to be the case.

Even if I had observed replacement guards near nest sites in the field, I would reject the parental behavior hypothesis for several reasons. I grant that fish rove and I do not assume that fathers remain near the nest. I argue that, for the father to be the first replacement guard, he should be found near the nest. If he is not, other fish can be expected to find the nest first and assume guard responsibilities, as they did in the laboratory. It is possible that the nest is readily identifiable to the father by the topography of the site (*4*) or a peculiar scent. This needs to be established; now it merely leads to the increased complexity of the speculation. That males build and tend nests in most teleost families where nesting has been reported hardly supports the contention that this is true in *H. bispinis*, nor does it suggest, as Meikle *et al.* say, that the male replacements fathered the eggs over which they assume guardianship. I agree that complex behaviors must be thoroughly investigated before hypotheses are rejected.

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