Nerve Fiber Layer of the Owl Monkey Retina: Retinotopic Organization

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The organization of nerve fiber bundles, stained by microelectrode injection of horseradish peroxidase, was examined in the retina of the owl monkey. Fibers were traced across the retina in whole mounts and serial sections to evaluate their retinotopic order. Long nasal fibers showed little tendency to wander among bundles but were distributed randomly within the bundles near the disc. Long arcuate and papillomacular fibers, in sharp contrast, spread laterally among many bundles as they approached the disc, but were segregated within the bundles in a scleral position. These results indicate that horizontal retinotopy among bundles is well developed in nasal but not temporal retina; vertical retinotopy within bundles is well developed in temporal but not nasal retina of the owl monkey. These findings are significantly different from those of a recent study of the macaque monkey. Invest Ophthalmol Vis Sci 24:265-269, 1983

The axons of retinal ganglion cells cross the retina in bundles or fascicles. Each nerve fiber bundle contains axons that originate in a wedge- or arcuate-shaped retinal sector that extends out from the disc. Thus, each bundle contains some fibers whose intraretinal path is short and some whose intraretinal path is relatively long.

Horizontal retinotopy, based on the collection of fibers from neighboring retinal areas in neighboring bundles, is obvious in nerve fiber bundle organization. But the purity of this horizontal retinotopy would be degraded if there were lateral dispersion or interchange of fibers among bundles as described by Polyak. He also noted that the fibers of midget ganglion cells meander considerably in the fiber layer. A different view is expressed by Radius and Anderson, who state that there is little if any lateral dispersion of fibers among bundles in the retina of the owl and macaque monkeys.

Vertical retinotopic organization of fibers within the bundles implies that long nerve fibers are separated from short fibers of peripapillary origin by fibers of intermediate length. According to Polyak and recently confirmed by Ogden, long fibers cross the retina on the vitreal surface of the bundles in the macaque. This retinotopic distribution of nerve fibers was challenged by Radius and Anderson and Minckler, who concluded that long fibers were segregated in the scleral half of the nerve fiber bundles.

Disagreement as to the nature of nerve fiber layer retinotopy in the primate may be the result of the use of different species and techniques. Much of Polyak's work involved the use of the Golgi procedure in chimpanzees; the results of Radius and Anderson were obtained from owl and rhesus monkeys and involved identification of degenerating fibers following retinal photocoagulation; Minckler examined the retinas of rhesus monkeys after injections of horseradish peroxidase (HRP) into the optic disc; and Ogden traced fibers across the retinas of cynomolgus and bonnet macaques following intraretinal microinjections of HRP. Only the last of these experimental procedures permits selective visualization of long fibers and thus provides evidence of their position in the nerve fiber layer that is free of ambiguity. In the present study, nerve fiber layer retinotopy in the owl monkey was studied following intraretinal injections of HRP. The results, which complement those of a previous study in the macaque, reveal significant differences between these species.
Materials and Methods

Horseradish peroxidase was injected into each eye of two adult male owl monkeys (Aotes trivirgatus). In the four eyes, a total of eight injections were placed in nasal retina about 3 mm from the optic disc, four injections were paramacular, and seven involved the superior or inferior arcuate bundles, 3–6 mm from the disc. All injections were made with fine beveled micropipettes, positioned in the nerve fiber layer by physiologic criteria during recording of nerve fiber action potentials.5 The animals were killed about 24 hours after the injections, and the retinas were prepared as flat whole mounts, fixed in 2% glutaraldehyde. After standard reaction with diaminobenzidine,6 the whole mounts were embedded flat in epon, a sliding microtome, and the sections were mounted on Teflon-coated glass slides. Selected sections were subsequently remounted and resectioned at 2 microns for detailed microscopic examination.

Results

Lateral dispersion of fibers was seen among bundles of the temporal retina, but was not seen among bundles of the nasal retina. Figure 1A illustrates a typical retinal whole mount from an experiment in which four injections (labeled 1–4) were made. Each injection resulted in heavy labeling of about five adjacent bundles at the injection site. The small opposed arrows indicate the extent of lateral dispersion of the temporal bundles (3 and 4) as their fibers approach the disc; in contrast, the nasal bundles stained by injections 1 and 2 retained a consistent width, ie, did not show lateral dispersion, as they approached the disc (see also Fig. 1C).

Although most temporal bundles passed directly to the disc, a few meandered considerably. Figure 1B shows an enlarged view of the papillomacular bundles stained by injection 4 (Fig. 1A). The large arrow indicates a meandering bundle that is seen to cross over adjacent bundles. This figure also shows more clearly the extent of lateral dispersion of the fibers in the bundles stained by injection 4 near the area centralis. These fibers, tightly grouped together just proximal to the injection site, spread to cover over 0.25 mm of retina at a distance of 1 mm from the optic disc, yet all of the ganglion cells associated with them were clustered in a small retinal area just temporal to the area centralis. As a result of lateral dispersion, these fibers entered the inferior pole of the optic disc between the 4 o’clock and 6 o’clock meridians and, thus, could be found in many pores of the lamina cribrosa.

Figure 1C shows an enlargement of the nasal bundles stained by injections 1 and 2. These bundles, although meandering somewhat as they crossed the retina, did not diverge. Also, all of the stained fibers remained within the same group of bundles. Thus, these nasal fibers showed little tendency to lateral dispersion.

Cross sections of the retina revealed the vertical position of labeled fibers in the nerve fiber bundles at various distances from the optic disc. Sections of the nasal bundles stained by injection 1 were obtained at the positions labeled 2A and 2B in Figure 1C; these are shown in Figures 2A and 2B. At location 2A, near the injection site, stained fibers filled the nerve fiber layer. These fibers, which originated at the injection site or at various distances into the far periphery, are referred to here as “long” and are contrasted below with parapapillary fibers that were unstained. At position 2B, near the optic disc, the nerve fiber layer doubled in thickness, due to the addition of unlabeled short fibers of peripapillary origin, yet stained fibers still were present throughout the full thickness of the fiber layer. Although there were rather more stained fibers in the scleral than in the vitreal portion of the fiber layer, stained and unstained fibers were intermixed at all levels. Sections at positions intermediate between the injection site and the disc all showed a random distribution of labeled fibers throughout the fiber layer. Thus, these nasal bundles did not have a vertical retinotopic organization, nor were any of the bundles stained by the eight nasal retinal injections so organized.

Cross sections of the temporal bundles stained by injections 3 and 4, in contrast, showed clear segregation of long fibers to the scleral portion of the bundles near the optic disc. This is illustrated in Figures 2C and 2D, which show sections obtained from positions 2C and 2D of Figure 1B. At position 2C, approximately 0.5 mm proximal to injection site 4, heavy labeling was present throughout the full thickness of the fiber layer. At position 2D, about 1 mm from the optic disc, all stained fibers were confined to the scleral half of the fiber bundles, adjacent to the ganglion cell layer (G). The stained fibers were separated from the internal limiting membrane by a clear zone of unstained short fibers that doubled the thickness of the layer at this position. Lamination of the fiber bundles, with long fibers in a scleral position deep to the short fibers, was found in every arcuate and papillomacular bundle studied in the owl monkey.

Discussion

This study of the owl monkey retina has shown an extensive lateral dispersion of fibers among temporal
Fig. 1. A, Low power micrograph of a plastic embedded retinal whole mount showing 4 HRP injections (labeled 1-4). The nasal bundles (1, 2) showed no lateral dispersion. The temporal bundles (3, 4) separated widely (small opposed arrows) as they approached the disc. B, High power micrograph of the papillomacular bundles stained by injection 4. The injection site (open circle) was adjacent to the area centralis (AC) and labeled a tightly clustered group of bundles (opposed arrows) and their ganglion cells (G). The extent of dispersion is indicated by the opposed arrowheads. Cross-over of bundles is indicated by the large arrow. C, High power micrograph of nasal bundles labelled by injections 1 and 2 (arrows). Note the bundles do not diverge as they approach the disc. Calibration: A, 4 mm; B, C, 1 mm.

but not nasal fiber bundles, an absence of vertical retinotopic organization in nasal bundles, and a well-defined vertical retinotopy in temporal bundles with long fibers scleral to shorter fibers that originate closer to the optic disc.

Comparison of the results of this and a previous study\(^5\) shows a significant difference in the vertical retinotopic organization of nerve fiber bundles of owl and macaque monkeys. In the macaque, long arcuate fibers are segregated in a vitreal rather than scleral position in the bundles. Also, the nasal bundles of the macaque have a well-defined vertical retinotopy that is lacking in the owl monkey.

These results are in partial agreement with those of Radius and Anderson\(^3\) who destroyed peripheral retinal nerve fibers by photocoagulation, then examined the retina for degenerating fibers. Both studies show temporal fibers of the owl monkey segregated
in the scleral portion of the nerve fiber layer near the disc. In contrast to the present study, they found a similar arrangement in the nasal bundles. It is possible that the apparent segregation of long nasal fibers observed by them was an artefact caused by early degeneration of the deeper fibers. In the present study, the position of the nasal long fibers within the bundles was observed throughout their length from far periphery to disc. Segregation would have been readily apparent near the disc where the fiber layer thickened to nearly 30 microns and unstained fibers predominated, yet the HRP stained fibers occurred at all levels...
within the layer. Radius and Anderson concluded in the same study that there is "little if any lateral dispersion of individual fiber bundles." This conclusion appears to be valid only with respect to the nasal fibers of the owl monkey. Temporal fibers are dispersed widely in Aotes, and a small percentage of arcuate fibers are dispersed horizontally in the macaque.

The growth processes that are the basis of retinotopic ordering of fibers in brain tracts and in the nerve fiber layer of the retina are poorly understood. It is known that the posterior pole of the retina develops first and that it increases in size by concentric growth at the retinal margin. Thus, successively longer axons are added to existing bundles, either randomly (owl monkey nasal bundles) or systematically on the scleral surface of the existing bundles (owl monkey temporal bundles) or systematically on the vitreal surface of existing bundles (all bundles of the macaque). Clearly, the rules of ordering and the axonal guidance mechanisms on which they depend may be different in these three cases. It is assured, however, that normal fibers make appropriate central connections and, thus, could share the same mechanism for long distance guidance. The results of this study in the owl monkey, which show differences in horizontal and vertical retinotopy in fibers of nasal as opposed to temporal retina, suggest that these features of fiber organization may vary independently and thus could be controlled by different mechanisms.

The owl monkey, by virtue of its large pupil and reflective fundus, has been a popular primate animal model for ocular research. Its retina, however, is highly specialized to support its crepuscular nature. The neural organization of its retina is much less similar to that of man than is the organization of the macaque retina. Therefore, caution must be used in extrapolation of the results of studies of retinal organization in the owl monkey to man. To date, there are no published studies of retinal nerve fiber layer retinotopy in man. It seems likely, however, that human fiber layer topography will prove to be similar to that of the macaque monkey, with long fibers in a vitreal position in the bundles overlying short fibers of peripapillary origin.

Key words: owl monkey, retina, nerve fiber layer, retinotopy, horseradish peroxidase

References