The organization of the retinal nerve fiber layer of the cynomolgus monkey was studied by electron microscopy. Nerve fiber size spectra were obtained from measurements of every fiber in enlarged montages of selected bundles. Variation in spectra among nasal, arcuate, and papillomacular bundles was examined, and variation of spectra of a given bundle as it crossed the retina was determined. Among the three types of bundles, the papillomacular contained relatively more small fibers, nasal bundles relatively more large fibers. Systematic variation of fiber spectra was observed as the disc was approached by arcuate and papillomacular but not nasal bundles. Arcuate bundles sampled in the peripapillary area contained relatively more small fibers than when sampled at a greater distance from the disc. In contrast, papillomacular bundles sampled near the disc contained relatively more large fibers than near the fovea.

The nerve fiber layer of the primate retina contains the axons of ganglion cells and the processes of astrocytes and Muller cells, aggregated into bundles by radial fibers of Muller. The nerve fibers within a bundle are of different lengths since they originate at varying distances from the optic disc margin. Recent studies in the cynomolgus monkey have shown that long fibers, whose cells of origin are outlying, pass to the optic disc on the vitreal surface of the bundles, overlying shorter fibers that originate from cells located closer to the disc.

Although long-standing interest in nerve fiber layer retinotopy has led to a number of qualitative studies of this important retinal structure, no quantitative data concerning the variation of nerve fiber layer morphology across the retina have been published. In particular, the proportions of nerve fibers of various sizes (nerve fiber spectra) and their organization within the nerve bundles have not been reported. Previous studies include those of Cohen who examined three adjacent bundles of fibers in a macaque monkey and found, respectively, 310, 317, and 330 fibers ranging in size from 0.2 to 3.0 microns. Ogden, in a study of primate nerve fiber layer astrocytes, described one fiber bundle that contained 508 nerve fibers but was not concerned with fiber dimensions.

Variation in fiber spectra across the retina could result from variation in the proportions of large and small ganglion cells at different retinal locations. This could be of significance because ganglion cells of different size probably have different functions. Variation in ganglion cell size with retinal position has been reported and may contribute to the peculiar susceptibility of ganglion cells of the central retina to the effects of toxic chemicals and nutritional deficiency or to the selective loss of axons of Bjerrum area ganglion cells in glaucoma and peripheral ganglion cells in papilledema. Thus the availability of more detailed data concerning nerve fiber layer structure could facilitate understanding of a number of important retinal problems.

The purpose of this study is to provide quantitative data concerning the variation in size, number, and distribution of ganglion cell axons in the nerve fiber layer of the cynomolgus monkey at various positions across the retina. These data complement the results of recent studies that revealed variations in the thickness and glial content of the nerve fiber layer, traced the paths of individual nerve fibers and bundles, and showed their retinotopic organization in the macaque and owl monkeys.

Materials and Methods

The tissues studied were obtained from ten adult cynomolgus macaque monkeys of both sexes, anesthetized with ketamine (10 mg/kg) and pentobarbital (40 mg/kg). The posterior hemisphere of the enucleated eye was rapidly incised, pinned flat in the form of a Maltese cross and immersed in cold fixative (2% glutaraldehyde, 2% paraformaldehyde, 1% osmium in 0.1 M phosphate buffer, pH 7.2). After 1 hour, the lightly osmicated tissues were post-fixed in 2% osmium, supported by NIH grants EYO-2637 and EYO-3040. Submitted for publication March 4, 1983.
washed in cold buffer, dehydrated in alcohol, and sectioned into blocks whose position in the retina was carefully noted. Each block contained a complete nerve fiber bundle projection. Thus the superior and inferior temporal blocks were arcuate in form to accommodate the arcuate fibers. The temporal and nasal blocks were wedge shaped. The tissues were embedded in epon-alaraldite and thin sectioned on an LKB V ultratome. Sections were stained with uranyl acetate and lead citrate, and examined with a Zeiss 10B electron microscope. The data presented below are not corrected for shrinkage; however, previous studies of retinas embedded in this manner showed linear shrinkage of 12–15%.14

Nerve Fiber Layer Morphometry

Measures of nerve fibers were obtained from photographic montages of the nerve fiber layer. These were constructed from electron micrographs with a total enlargement of 16,000X. The montages varied from 25 cm square to 100 × 300 cm. Each montage was first divided into square sectors, 21 cm on a side. A microcomputer-based graphics tablet was used to perform the measurements, one sector at a time. The diameter of each fiber was traced on the tablet. The minimum diameter that could be registered reliably at this magnification was about 0.1 micron. These data, converted to microns, were stored by the computer to form the measurements, one sector at a time. The diameter of each fiber was traced on the tablet. The minimum diameter that could be registered reliably at this magnification was about 0.1 micron. These data, converted to microns, were stored by the computer together with the X-Y coordinates of each diameter center point. In the case of elliptical fibers, presumed to have been cut tangentially, the length of the shorter axis was taken as the pertinent diameter. Every fiber in a bundle was measured, then checked off on the montage to prevent overlap of measurements.

A major goal of this study was to determine to what degree the proportions of large and small fibers in bundles change as they cross the retina. Thus it was necessary to compare a number of fiber diameter histograms. Quantitative comparison of fiber distributions was facilitated by fitting a mathematically defined function, the log-Weibull probability density function (PDF), to the data. The log-Weibull PDF is well suited to modelling of skew distributions, such as those found in this study.

The procedure for fitting a log-Weibull PDF to a frequency distribution used in this study is described in detail by Oyster et al.15,16 The Weibull function is:

\[ P(x) = \beta x^{-1} \left[ (x - \gamma)/\alpha \right]^{\beta-1} \exp\left[-(x - \gamma)/\alpha\right] \]

where \(\alpha\), \(\beta\), and \(\gamma\) are parameters of the distribution. The parameters \(\alpha\) and \(\beta\) determine the position of the curve maximum and the broadness of its base. The parameter \(\gamma\) is a shift parameter held constant at the smallest histogram bin width, 0.1 \(\mu\)m. These parameters were estimated by a graphical technique,16 which also yielded a correlation coefficient (r) for each curve. Proper choice of the parameters consistently resulted in r-values greater than 0.9 (Table 1), indicating an acceptable fit of the curves to the data.

Terminology

Nerve fibers are called nasal if they enter the optic disc along its nasal half. Fibers that originate within 0.5 mm of the fovea are called papillomacular. Note that nasal papillomacular fibers, so-called because they originate in the nasal half of the macula, pass directly to the optic disc. The temporal papillomacular fibers arch above and below the macula then join the nasal fibers to enter the disc temporally. These curvilinear fibers are not considered arcuate.17 Fibers called arcuate are partly those that originate temporal to but outside the macula, within about 2 mm of the horizontal meridian. They enter the optic disc between 12 and 1 or 5 and 6 o’clock. The remaining arcuate fibers are those that originate in retina underlying the arcuate bundles. Fibers that originate within 1 mm of the disc are called peripapillary or short; those that originate more than 5 mm from the disc are called long. The terms central and peripheral are used with reference to the fovea. Obviously, temporal papillomacular fibers are much longer than nasal papillomacular fibers, although both may be equally central.

Results

The axons of the nerve fiber layer were partitioned into discreet bundles by sheets of Müller cell fibers.1 At progressively greater distances from the optic disc, the nerve fiber layer became thinner,13,17 and the amount of glial tissue between the bundles increased. About 5 mm from the disc, ganglion cells were observed in the fiber layer, and the identity of individual bundles became somewhat ambiguous.

A typical nerve fiber bundle is illustrated in Figure 1. This section was obtained 3.8 mm from the disc margin, along the inferior arcuate ridge,13 where the nerve fiber layer was 15–20 \(\mu\)m thick. Nerve fibers were easily distinguished from astrocyte and Müller cell processes within the nerve fiber layer. Most axons

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Weibull curve shape parameters for figure 2*</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(\alpha)</td>
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<tr>
<td>Total bundle</td>
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<td>Inner third</td>
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<td>Outer third</td>
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* Parameters used to calculate the smooth curves of Figure 2.
contained mitochondrial profiles, and all contained neurotubules. The glial profiles contained varying amounts of glial filaments and, unlike the axons, were seldom oval or round in form. Only an occasional profile smaller than 0.1 μm in diameter could not be identified. Such processes were rarely encountered and
AXON DIAMETER (μm)

Fig. 2. Fiber size histograms and calculated log-Weibull probability density functions (PDF) for the fiber bundle shown in Figure 1. A, total population, 0.73 μm sample mean; B, outer third subpopulation, 0.79 μm sample mean; C, inner third subpopulation, 0.68 μm sample mean; D, core subpopulation, 0.79 μm sample mean; E, histograms from A, B, and C are superimposed; F, PDFs from A, B, C, and D are superimposed. In each graph, the relative frequency of fibers of different diameter is indicated on the abscissa. Diagrams indicate sampled area and numbers of fibers in the sample.

Fig. 3. X/Y plotter write-out indicating the position of every fiber of the bundle shown in Figure 1. A, fibers smaller than 0.3 μm are circled; B, fibers larger than 1.4 μm are circled. The area enclosed by lines was included in the “core” sample of Figure 2D (Calibration: 2 μm).

Axon diameter (μm)

Fig. 2. Fiber size histograms and calculated log-Weibull probability density functions (PDF) for the fiber bundle shown in Figure 1. A, total population, 0.73 μm sample mean; B, outer third subpopulation, 0.79 μm sample mean; C, inner third subpopulation, 0.68 μm sample mean; D, core subpopulation, 0.79 μm sample mean; E, histograms from A, B, and C are superimposed; F, PDFs from A, B, C, and D are superimposed. In each graph, the relative frequency of fibers of different diameter is indicated on the abscissa. Diagrams indicate sampled area and numbers of fibers in the sample.

Nerve fiber bundle structure was examined with stepped serial sections at three retinal regions, including three papillomacular, three arcuate, and three nasal projections, each containing 3-5 bundles. These nine studies involved the retinas of eight monkeys. Less detailed studies involved an additional 32 papillomacular, 30 arcuate, and 21 nasal bundles. The arcuate bundle shown in Figure 1 will be used to demonstrate the details of the analysis applied to each of the bundles. At this location, the bundle contained 1,029 fibers. The histogram of the fiber population was unimodal, as it was in every bundle examined, and had a peak frequency at 0.6 μm (Fig. 2A). The bundle was unusual in that the fiber spectra of different regions showed a variation of dominant fiber size with position of the fibers. The outer third of the bundle (Fig. 2B) contained relatively more large fibers than the inner third (Fig. 2C). The central core of the bundle also contained relatively more large fibers than the inner third (Fig. 2D). The core region included in Figure 2D is shown in Figure 3B.

Histograms of raw data are inherently noisy due to inevitable biologic variation among the samples. An
attempt to compare histograms directly is confounded by this variability (Fig. 2E). The smooth curves superimposed on the histograms of Figures 2A–D represent the PDF for each population. Comparison of populations is greatly facilitated by superimposition of these curves, which effectively smooth the noise inherent in the histograms (compare Figs. 2E and 2F).

The differences in the PDFs shown in Figure 2 can also be characterized by the differences in the parameters \( \alpha \) and \( \beta \) used for their calculation (Table 1). The shift from small to large fibers and broadening of the base of the curves was associated with an increase in these parameters. Thus the parameter \( \alpha \) was 4.171 for the inner third of the bundle and 5.362 for the outer third. This increase was associated with a shift of the population peak to larger fibers and a broadening of the base of the probability density function. Table 1 also shows the correlation coefficient associated with each parameter determination. The high r-values reflect the closeness of fit of the curves to the data.

The computer-based measurement system used in this study stored the position and size of every fiber within a given bundle. These data could be displayed graphically with an X-Y plotter, and it was convenient to designate fibers of a given size in such a plot to reveal subtle aspects of bundle organization. Figure 3 shows plots of the bundle shown in Figures 1 and 2. The position of every fiber in the bundle is accurately shown, and the position of every fiber smaller than 0.3 \( \mu m \) also is indicated in Figure 3A. These 36 very small fibers were predominantly located at the bundle surface. Figure 3B shows the location of every fiber larger than 1.4 \( \mu m \). These 60 large fibers were clearly not localized in any particular part of the bundle.

The analysis shown in Figures 1–3 was applied to a total of 58 nerve fiber layer montages. Segregation of fibers by size was found in three samples and only one bundle. Thus the results shown in these figures are exceptional. In the remaining samples, no significant differences in fiber population was found within a given bundle at one retinal location, and fibers of all sizes were equally represented in all parts of the bundles.

Variation among Nasal, Arcuate, and Papillomacular Bundles

There was a significant difference among fiber spectra of nasal, arcuate and papillomacular bundles. This difference is illustrated in Figure 4A, which shows samples obtained 2–3 mm from the disc. The papillomacular bundle (p) contained proportionately many more small fibers than the arcuate bundle (a), which in turn contained proportionately more small fibers than the nasal bundle (n). These differences were consistently found among bundles sampled in each of the 11 retinas, at all distances from the optic disc. Mean fiber diameter in 44 papillomacular bundles sampled within 1 mm among bundles sampled in each of the 11 retinas, at all distances from the optic disc. Mean fiber diameter in 44 papillomacular bundles sampled within 1 mm

Table 2. Shape parameters for weibull curves of figure 4

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<th>( \beta )</th>
<th>( \gamma )</th>
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<td>a</td>
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<td>3.244</td>
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<tr>
<td>n</td>
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<td>0.995</td>
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</tr>
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<td>Fig. 4D—Comparison of adjacent nasal bundles</td>
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* Each smooth curve is derived from a single bundle. Three bundles are represented in Figures 4B and C. Figure 4D includes four bundles.
AXON DIAMETER (μm)

Fig. 5. Variation with retinal position of fiber spectra and Probability density functions (PDF) of one arcuate bundle. The location of each sample (A–E) with respect to the disc and fovea is indicated in the diagram in A. Sample means were, respectively, 0.44 μm, 0.51 μm, 0.53 μm, 0.68 μm, and 0.76 μm. F, the PDFs, each appropriately labeled, are superimposed to demonstrate a systematic shift of the population to smaller fibers as the disc was approached, and the number of fibers (N) increased from 542 to 18,351.

Variation among Adjacent Bundles is Small

The fiber spectra of adjacent bundles at one retinal location were determined for the three projections in each of the retinas. The spectra of adjacent bundles were very similar. The PDFs superimposed in Figure 4B, obtained from 3 adjacent arcuate bundles sampled 4 mm from the disc margin, are seen to be nearly identical. The superimposed PDFs of 3 papillomacular and 4 nasal bundles (Figs. 4C and D) also show relatively little variation among adjacent bundles sampled at the same retinal location. This is reflected in the similarity of the shape parameters for these curves, shown in Table 2.

Bundle Variation across the Retina

The fiber spectrum of individual papillomacular and arcuate, but not nasal, bundles varied as the bundles crossed the retina en route to the disc. The degree of variation was dependent on the position of the bundle with respect to the area centralis. Bundles that picked up papillomacular fibers as they crossed the retina varied more with position than bundles whose fibers all originated at about the same retinal eccentricity. Fiber histograms and probability density functions from a single arcuate bundle sampled at five locations are shown in Figures 5A–E. The location of each sample is indicated in the diagram of Figure 5A. As this bundle approached the disc, it increased in size from 542 to 18,351 fibers and showed a striking increase in the proportion of smaller fibers. Superimposition of the probability density functions demonstrates the trend clearly (Fig. 5F). Curve A, adjacent to the disc, has a narrow base and tall peak indicating a large proportion of fibers were 0.2 to 0.6 μm in diameter. Curve E, from well out in the periphery, shows proportionately many more fibers larger than 0.6 μm in diameter. These changes in curve shape were associated with changes in the α and β parameters of the log-Weibull functions.

The graphs of Figure 6A show the means of the shape parameters, calculated from adjacent arcuate bundles at each location. Both parameters decreased as the disc was approached.

Figure 7 shows a similar study of a temporal papillomacular bundle sampled at three locations between the disc and macula. In contrast to the arcuate bundle,
this bundle acquired relatively more large fibers as it approached the optic disc and increased from 1,317 to 13,088 fibers. This is reflected by the relatively broader probability density curve at position A as compared to the curve of position C (Fig. 7D). The trend of Weibull curve parameters from small to large with approach to the disc is shown in Figure 6B, and clearly differs from the graph of arcuate parameters shown in Figure 6A. Nasal bundles, followed up to 4 mm from the optic disc, showed no consistent variation of fiber spectra with distance from the disc.

Segregation of Fibers by Size within Bundles

There is recent evidence that fibers of more peripheral origin are segregated from peripapillary fibers within arcuate bundles. The above results show that the arcuate bundles near the disc contain proportion-
Fig. 8. X/Y plotter write-out of an arcuate bundle to show the position of every fiber (dots) and every fiber larger than 1.4 μm (large circles) at various distances from the disc: A, 5.5 and 5 mm; B, 4 mm; C, 3 mm; D, 2 mm; E, 1.5 mm; F, 1 mm. The large fibers were randomly distributed throughout the bundle (Calibration: 20 μm).

ately many more small fibers than they do in the periphery. It should be noted that the development of the retina is from center to periphery. It was of interest to determine where the large fibers, which are proportionately more common in the periphery, come to lie within the bundles as they approach the disc. It was predicted that the large fibers should be segregated either at the vitreal border of the bundles or at the scleral border of the bundles. In fact, the large fibers were randomly scattered throughout the bundles all along their course to the disc. This is illustrated in Figure 8, which shows plots of an arcuate bundle sampled at seven locations across the retina. The positions of all fibers greater than 1.4 μm in diameter are specifically indicated, and it is apparent that they are not segregated in one particular part of the bundle. An analysis of the position of fibers smaller than 0.3 μm gave similar results. Also, similar studies of 11 other arcuate bundles, eight papillomacular bundles, and nine nasal bundles did not show evidence of segregation of either large or small fibers within the bundles. The arcuate bundle shown in Figures 1–3 was the only bundle encountered in this study that showed such an effect.
Comparison of Cynomolgus and Rhesus Monkey Fiber Spectra

The range of fiber diameters observed in this study was 0.1 to 3.9 \( \mu m \). This includes the range (0.2–3.1 \( \mu m \)) found in the rhesus monkey by Cohen.\(^3\) The histogram of Figure 9A shows his data, obtained by summing the spectra of three adjacent bundles (see ref. 3, Table I). A probability density curve was calculated for these data and is shown superimposed on the histogram in Figure 9A. The same family of curves used in the above study also describes the data of Cohen. This is shown in Figure 9B, where the log-Weibull curves from Cohen’s data and an arcuate bundle of the cynomolgus monkey, sampled 4 mm from the optic disc, are superimposed. The similarity of the curves shows that the data obtained from these different species and laboratories is highly comparable.

Discussion

It has been shown that bundles of retinal nerve fibers at all retinal locations contain populations of fibers with a skewed unimodal distribution of sizes. The fiber spectra of adjacent bundles were relatively uniform within a local area. The proportion of small fibers in a bundle depended on the relative contribution to it of the area centralis. This is in agreement with the observations of Polyak\(^10\) and others that the proportion of small-sized ganglion cells is substantially higher in the area centralis than in the periphery.\(^5,11,18\) The great preponderance of small fibers in the papillomacular bundle is also consistent with past studies of the retrolaminar optic nerve, which showed similar fiber spectra in the temporal quadrant of the nerve.\(^19,20\)

Substantial and systematic variation in the spectra of single arcuate and papillomacular bundles sampled at serial positions across the retina was found. The extent of the variation along a bundle was dependent on its trajectory. Bundles that passed close to the area centralis varied more than those whose path remained equidistant from the fovea. Absence of positional variation of spectra in the nasal bundles was associated with their extreme peripheral position and suggests uniformity in proportions of different sizes of ganglion cells at eccentricities greater than 20°.

Nerve fiber spectra obtained from the inner thirds of most fiber bundles were very similar to those obtained from the middle and outer thirds. Thus fibers were not segregated within the bundles by size. This was an unexpected finding in view of recent evidence of retinotopical labeling of fibers within fiber bundles of the cynomolgus monkey.\(^2,21\) Ogden\(^2\) labeled long fibers of arcuate bundles with local, outlying retinal injections of horseradish peroxidase and was able to follow the fibers to the optic disc margin in serial sections of the retina. The labeled long fibers maintained a vitreal position in the bundles as they crossed the retina. Since the bundles have proportionately more large fibers in the periphery, it was predicted that the vitreal portion of arcuate bundles should have a spectrum shifted toward large fibers, but this was not found. A possible explanation for the similarity of the spectra of inner and outer portions of bundles is that the peripheral contribution of a few hundred relatively larger fibers is masked by the many thousands of relatively smaller fibers added to the bundles as they approach the disc. Thus the expectation of large fiber segregation in the nerve bundles may have been unwarranted, and the absence of segregation does not invalidate the earlier findings of nerve fiber layer retinotopy, which showed unequivocal evidence of gross segregation of short from long fibers.

Ganglion cells may be classified as of several types on the basis of functional or structural characteristics. Classifications that include both types of properties are clearly the most helpful in emphasizing meaningful differences among cells. Thus the association of X and Y receptive field properties with the morphologic \( \beta \) and \( \alpha \) ganglion cell types in the cat\(^2\) had led to a substantial advance in our understanding of ganglion cell function. Primate ganglion cells also have X-like and Y-like response properties.\(^6,9,23\) The midget ganglion cells of Polyak\(^10\) are clearly X-type\(^5\) and have been shown to project to the parvocellular layers of the lateral geniculate nucleus in the primate.\(^5\) These cells are analogous to the \( \beta \) cells of the cat and have been called B cells by Leventhal et al\(^5\) or \( P \beta \) cells by Perry and Cowey (1981). A high percentage of ganglion cells of the macula are of the midget type and have X-like properties. Thus, the papillomacular bundles sampled near the the fovea should contain primarily the axons of the B cells. Average fiber diameter in the papillomacular bundles sampled in this study was 0.4

![Fig. 9. Comparison of fiber spectra and PDFs of rhesus and cynomolgus monkeys. A, nerve fiber spectrum and PDF from rhesus retina obtained by summing the data of Cohen, Table I. B, superimposition of PDFs of Cohen’s data and an arcuate bundle from the cynomolgus monkey, sampled 4 mm from the disc.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933111/)
also be trimodal. Examination of whole cat optic nerve
tional to soma diameter,24 nerve fiber spectra should
than 5-10% of the total population. A second deflection
which constitute perhaps half the total. The late de-
the form of the compound action potential.19'23 There

to the spectrum of fiber diameters in a population of
about 0.4 \mu m to about 0.6 \mu m.

Functional Significance of Nerve Fiber Spectra

The fiber spectra of cat and monkey optic nerves
are unimodal19'20'25; however, their compound action
potentials are strikingly multi-modal, suggesting the
activity of clearly distinct groups of fibers of different
sizes. The relationship of a compound action potential
to the spectrum of fiber diameters in a population of
fibers is complex. Even a cursory examination of pub-
ished data provides convincing proof that the relation
is nonlinear. For instance, most recordings from the
optic disc or nerve show a short latency deflection of
large amplitude that represents the largest fibers of the
population. Yet these large fibers constitute no more
than 5-10% of the total population. A second deflection
of similar amplitude represents medium-sized fibers,
which constitute perhaps half the total. The late de-
fection, representing the smallest fibers, probably
almost half the total, is usually poorly defined.19 A num-
ber of factors contribute to this noncorrespondence of
action potentials and population statistics. The use of
bipolar stimulation in the brain results in unequal ex-
citation of different-sized fibers at different distances
from the cathode, so placement of the stimulating elec-

trode is of crucial importance, and slight changes in
electrode position may result in dramatic changes in
the form of the compound action potential.19,23 There
can never be assurance that all fibers at a given location
are activated by electrical stimulation in the optic
chiasm, optic tract, or lateral geniculate nucleus. The
problem is compounded by electrode bias at the re-
cordng site, where large fibers generate more extra-
cellular current flow per unit time than small fibers.
This results both from the geometry of the large fibers
and temporal dispersion of activity in the small fibers.

The lack of correspondence of electrophysiology and
morphology is more apparent in studies of cat than
primate. There is sound morphologic evidence that
cat ganglion cells are of at least three distinct size pop-
uations.22 Since axon diameter is generally propor-
tional to soma diameter,24 nerve fiber spectra should
also be trimodal. Examination of whole cat optic nerve
reveals a unimodal population of fibers. A peripheral
zone of the nerve, however, may have discreet pop-
pulations of fibers of different sizes,25 in keeping with
the measurements of ganglion cell somata. The mon-
key, in contrast, has an essentially unimodal population
of different-sized ganglion cells.11 Thus morphometric
studies of optic nerve fiber size and ganglion cell soma
size are in agreement in the case of the primate.

Landau et al24 successfully modelled the optic nerve
compound action potential of the cat on the pragmatic
assumption that the contribution of different-sized fi-
bers to the extracellularly recorded potential was pro-
tional to the third power of fiber diameter. They
based their modelling exercise on the spectra published
by Bishop et al.27 An attempt of Stone and Hollander28
to apply this approach to spectra of retinal nerve fibers
in the cat gave poor results. However, this should not
be surprising. In any particular recording situation,
the relationship of fiber size to extracellular current
flow through the surrounding tissue must be influenced
not only by the nature of the active fiber population,
but also by the particular geometry and impedance of
the tissues at the recording site, which differ greatly
between optic nerve and retina.

The conduction velocities of the X-type and Y-type
ganglion cell axons of the monkey show considerably
more overlap than is the case with the cat,9,29 so it is
not clear from the single cell electrophysiology that
the primate should have a polymodal distribution of
fiber sizes. The conclusion that the primate has a uni-
modal population of optic nerve fibers, as indicated
by this study, is also supported by the observations of
Ogden and Miller,19 who recorded antidromic com-
 pound action potentials from several locations around
the optic disc of the rhesus monkey. Several conduction
velocity groups were observed at each location. How-
ver, multiple recordings revealed a wide range of
overlapping groups consistent with a locally biased
sampling of a unimodal population. Thus, unlike the
cat, the morphologic and physiologic data for the pri-
mate are probably not in conflict in suggesting an es-
tentially unimodal population of different-sized fibers.

Validity of Nerve Fiber Spectra

Studies of nerve fiber diameter are based on the tacit
assumption that the data are meaningful despite fiber
shrinkage and distortion caused by histological pro-
cessing. This assumption has been seriously questioned
by Freeman30 who studied with the electron microscope
serial sections of seven cat optic nerve fibers. The di-
ameters of some of the fibers varied as much as 110%
over distances as small as 4 micra. He suggested that
random variation of fiber diameter along the length
of fixed fibers might mask the appearance of separate
fibre groups in nerve fibre spectra obtained from the optic nerve.

The irregular shrinkage observed in the cat optic nerve by Freeman\textsuperscript{20} may have been the result of his method of fixation. The optic nerve undergoes substantial distortion during fixation unless it is mechanically stabilized or fixed in situ. Freeman excised his optic nerves and fixed them by immersion without stabilization. Also, he did not measure the shrinkage of his tissue or evaluate its uniformity. Non-uniform shrinkage of tissue floating in fixative or dehydrating fluids could result in longitudinal fiber size variability. In the present study, the retina was mechanically stabilized prior to fixation and linear shrinkage was 15\% or less. These procedures should not have been associated with large fluctuations in fiber diameter. Retinal nerve fibres processed without these precautions and stained with silver or methylene blue frequently exhibit beading at regular intervals. This beading results from agonal collections of intracellular organelles and does not occur in properly prepared tissues, where long uniform diameter segments of fibers may be seen. Thus it is concluded that the unimodal fiber spectra observed in this study are not artefactual. As noted above, this conclusion is supported by studies of ganglion cell soma size.\textsuperscript{11}

It is probable, however, that some longitudinal variability in fiber size is always present in fixed tissues. It was not sufficient to mask the similarity of adjacent bundles or the variation of spectra along a bundle observed in this study. Longitudinal fiber variation may have contributed to the variability of shape parameters along single bundles, as illustrated in Figure 6. Point-to-point variation of shape parameters could also result from interchange of fibers among bundles, a common feature of nerve fiber bundle organization in the primate.\textsuperscript{2,12}

Key words: primate, retina, nerve fiber layer, morphometric analysis, electron microscopy

References