Efferent innervation of the retina
II. Morphologic study of the monkey retina*

Francisco M. Honrubia and James H. Elliott

Silver impregnation of the monkey's retina has been employed to study the presence and distribution of centrifugal fibers in flat mounts. This method has allowed identification of individual centrifugal fibers which appear to emerge from the optic papilla. Their course and distribution within the retina has been depicted. In the vicinity of the papilla, where the stained fibers in the optic nerve layer were very numerous, the centrifugal fibers could be differentiated from the ganglion cell axons by virtue of their thicker diameter, their irregular trajectory, and the presence of branches. By contrast in the mid and peripheral retina, the centrifugal fibers were readily identified.

Successive bifurcations and a descending course toward the inner nuclear layer were characteristic features of intraretinal centrifugal fibers. Additional studies of vertical retinal sections have confirmed their descending trajectory from the optic nerve fiber layer to the innermost aspect of the inner nuclear layer. The findings in the monkey's retinas have been compared with the previous findings in human and other mammalian retinas using the same silver impregnation technique.

Key words: retina, monkey, efferent innervation, retinal flat mounts, silver impregnation technique.

In a previous report, histologic evidence was presented for the presence of centrifugal fibers in the human retina. That report summarized the current morphologic evidence for existence of centrifugal innervation in primates and subprimates. In addition, a more recent report by Sacks and Lindenberg presented additional evidence for the possible origin and course of efferent fibers in the human retina. The present report pertains to results of studies of the monkey retina with the use of the technique of silver impregnation of whole retinal flat mounts. Retinal fibers with the same features attributed to reputed centrifugal fibers in the human retina have been identified. The similarity of these fibers in all species studied to date strongly supports the validity of efferent innervation of the primate retina.
Materials and method

The retinas of 28 rhesus monkeys (Macaca mulatta) were studied. After the animals were put to death, the eyes were enucleated and placed immediately in solutions of either 10 per cent neutral buffered formaldehyde or 20 per cent formaldehyde. The anterior half of the globe was removed and the retina was dissected free with the aid of a binocular dissecting microscope. The dissected retinal specimens were kept in one of the above fixatives for an additional month. Following appropriate fixation, all the vitreous body was removed from the retina under a dissecting microscope with a camel hair brush.

The silver impregnation technique used was Gallego's modification of the Cros-Bielchowsky method described in a previous study. In the present study where monkey retinas were used, a period of immersion in 20 per cent silver nitrate solution (40 to 50 minutes) at room temperature resulted in satisfactory silver impregnation.

After staining the retinas were dehydrated in a graded series of alcohol, cleared in a solution of phenol and xylene (25 mg. per 75 cc.), and mounted as whole flat mounts on microscopic slides with a 60 per cent solution of synthetic resin. After appropriate study, some of the whole retinal flat mounts were dismounted to facilitate a more definitive study of selected areas of the retina by vertical sections. The cover slips were removed in a carbol-xylol solution, appropriate dissection of the designated retina was made, and these pieces were embedded in celloidin. Cross sections of these selected retinal areas were cut 20 to 30 μ in thickness with a sliding microtome and remounted on microscopic slides with permount.

Results

The method used in this study has allowed the identification of the fibers of the optic nerve from their origin in the retinal ganglion cells. Their course converging toward the papilla was easily observed.

In the vicinity of the papilla, where the stained fibers in the nerve fiber layer were very numerous, some fibers could be differentiated from the ganglion cell axons by virtue of their thicker diameters (Figs. 1 and 2), the presence of branches, and their irregular trajectory (Fig. 3). The course of these presumptive centrifugal fibers from the region of the optic papilla toward the periphery of the retina was ascertained by the pattern adopted by the fibers at successive ramifications. In addition, there was a progressive diminution of their diameter as they coursed toward the retinal periphery.

In the mid and peripheral retina, where there was a decreased number of fibers in the nerve fiber layer, the presence of centrifugal fibers was readily identified. In some instances the centrifugal fibers were seen to bifurcate in the proximity of the ora serrata (Figs. 4 and 5).

Successive bifurcations and a descending
Fig. 2. Flat preparation, monkey retina. High power of branching indicated by arrow in Fig. 1. Note multiple successive bifurcations. (Silver impregnation. Original magnification x200.)

Fig. 3. Flat preparation, monkey retina. Area adjacent to papilla (P). Arrow indicates a centrifugal fiber running perpendicular to nerve fiber layer. (Silver impregnation. Original magnification x80.)

course toward the inner granular layer were characteristic features of intraretinal centrifugal fibers. The diameter of the secondary division was thinner than that of the primary branch. The direction of the secondary branch as it dipped deeper into the retinal layers might be vertical or oblique and crossed large sections of the retina before branching again at the level of the innermost boundary of the inner nuclear layer.

Additional studies of vertical retinal sections confirmed the descending trajectory of the centrifugal fibers from the optic nerve layer to the inner nuclear layer, after crossing the ganglion cell layer and the inner plexiform layers (Fig. 6). An interesting finding of qualitative nature deserves mention. When comparing the numbers of efferent fibers present in human and monkey retina as opposed to other mammalian species, it became apparent that the primate retina contained many more centrifugal intraretinal fibers.

In all instances, the suspected centrifugal fibers observed were carefully traced to ascertain if they originated in an intraretinal neuron, a fact easily confirmed in the case of the axons of the ganglion cells. This excluded the possibility of confusion.
of these fibers with atypical ganglion cell axons. No intraretinal neuronal origin could be traced for any of the presumptive efferent fibers observed. Therefore, it was assumed that the fibers surveyed were centrifugal in nature and originated from extraretinal neurons.

Comment

With classic degenerative techniques it has been difficult to obtain definitive anatomical evidence confirming the presence of efferent fibers to the retina. Monnier, summarizing the literature, felt that the existence of centrifugal nerve fibers in the retina and optic nerve in primates was suggestive but not conclusive evidence. However, Dowling and Boycott remain dubious that such innervation exists in any primate species.

The technique of silver impregnation of whole retinal flat mounts has permitted the identification in the monkey retinas of fibers with the same characteristics as the fibers described by us in the human retina in a previous publication. These fibers were identified as they emerged from the optic papilla and individual fibers could be fol-
Fig. 5. Flat preparation, monkey retina. (A) High power of bifurcation designated as No. 1 in Fig. 4. (Silver impregnation. Original magnification x200.) (B) High power of bifurcation designated as No. 2 in Fig. 4. (Silver impregnation. Original magnification x320.) (C) High power of bifurcation designated as No. 3 in Fig. 4. (Silver impregnation. Original magnification x320.) (D) High power of bifurcation designated as No. 4 in Fig. 4. (Silver impregnation. Original magnification x200.)

Fig. 6. Vertical section, monkey retina. Efferent fiber with long course in innermost aspect of bipolar cell layer (arrow); ganglion cell layer (G); inner nuclear layer (I). (Silver impregnation. Original magnification x320.)
allowed until their termination at the level of the inner nuclear layer. This descending trajectory from the optic nerve fiber layer could be confirmed by vertical sectioning of the retina. Curiously, some fibers could be seen reaching the ora serrata, but due to technical difficulties we are unable to say with certainty that any fibers cross the ora serrata.

These centrifugal fibers described in the monkey retina, as well as in the human retina, were similar in origin, course, and destination to the well-documented and established efferent retinal fibers in the subprimate species. These findings, although not conclusive, are corroborative evidence for the existence of centrifugal retinal fibers in the primate retina.

Whole monkey eyes were provided by Dr. C. W. Hill, Head of Research Service Division, Delta Regional Primate Research Center, Covington, La.

REFERENCES