THE JOURNAL OF COMPARATIVE NEUROLOGY 265:218–223 (1987)

# Early Uncrossed Component of the Developing Optic Nerve With a Short Extracerebral Course: A Light and Electron Microscopic Study of Fetal Ferrets

R. W. GUILLERY AND C. WALSH
Department of Human Anatomy, Oxford University, Oxford, OX1 3QX, United Kingdom (R.W.G.); Department of Neurology, Massachusetts General Hospital, Boston, Massachusetts 02114 (C.W.)

ABSTRACT

During the study of the developing optic nerve described in the preceding paper (Guillery and Walsh, '87), small bundles of nerve fibers were seen passing between the optic nerve and the ipsilateral hypothalamus of 24-to 27-day-old prenatal ferrets. The bundles appear before any other fiber groups of the retinofugal pathway and are identifiable while the main portions of the retinofugal system are growing into the optic tracts. The bundles, made up of 50 or more axons, leave the optic nerve, emerge through the otherwise continuous layer of subpial glia and through the basal lamina of the nerve, run a short, naked, extracerebral course among collagen fibers and presumed fibroblasts, and then re-enter the central nervous system, passing rostrally and dorsally to the superficial parts of the ipsilateral hypothalamus away from the region of the chiasm. These fibers represent the earliest link between the optic nerve and the brain, but their course is not followed by the majority of retinofugal fibers developing later, which pass toward one or the other optic tract.

**Key words: retinofugal axons, hypothalamus, basal lamina**

In the accompanying paper (Guillery and Walsh, '87), the early development of the optic nerve in the prenatal ferret has been described. Small bundles of fibers (the ipsilateral optic bundles) that were consistently seen passing between the nerve and the brain and that formed the earliest neural pathway between the eye and the brain were noted during that study. These bundles are of interest for two reasons: (1) although they form the earliest link between the nerve and the brain, they appear not to act as "pioneer" fibers for later developing retinofugal axons, and (2) these bundles take a short, unusual extracerebral course, breaking through the subpial glia and the basal lamina before re-entering the substance of the central nervous system. In this paper we describe the detailed relationships of this curious bundle on the basis of light and electron microscopical studies.

## MATERIALS AND METHODS

The materials available for this study are described in the preceding paper (Guillery and Walsh, '87). The bundles under consideration were originally seen in electron microscopic sections in which a part of their extracerebral course was identifiable, but in which their three-dimensional pathway could not be traced. Subsequently, these bundles were traced through several series of semithin sections cut at 1 $\mu$m and stained by the Richardson method (Richardson et al., '60). Finally, some of the bundles were traced to the region close to the extracerebral part of their course, and then adjacent thin and semithin sections were cut so that the fine structural relationships of the bundles could be studied as they passed into the extracerebral part of their course.

## RESULTS

Four series of sections in which the ipsilateral optic bundles could be traced from the optic nerve, through the

Accepted April 1, 1987.

Address reprint requests to R.W. Guillery, Department of Human Anatomy, Oxford University, South Parks Road, Oxford OX1 3QX, U.K.

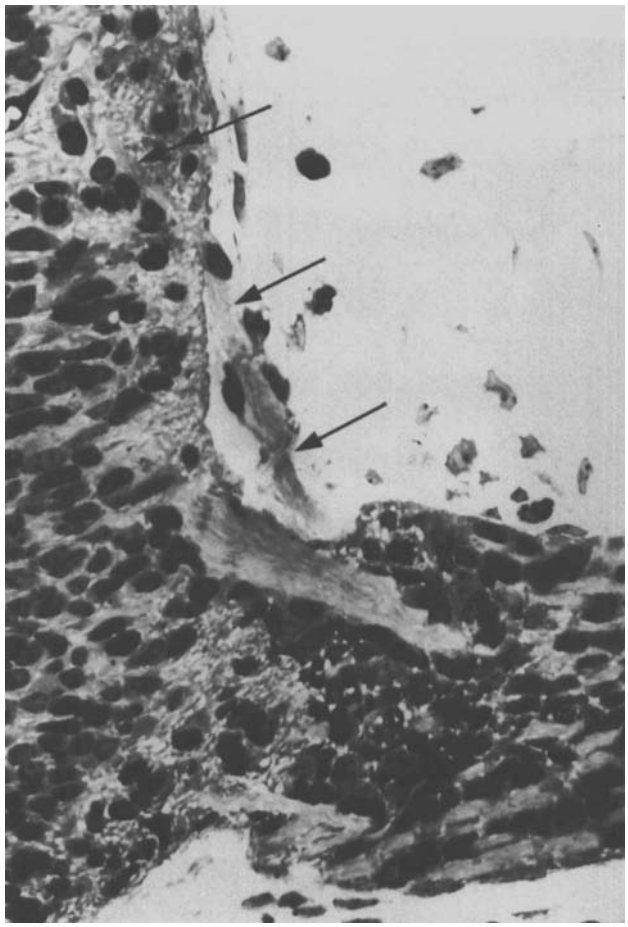

Fig. 1. Oblique 1-μm section through the eye-stalk of a 25-day-old intrauterine ferret. The narrow part of the eye-stalk to the right is joining the broad intracranial part, and some of the fibers of the ipsilateral optic bundles can be seen passing dorsally and rostrally toward the anterior hypothalamus. The single arrows show the extracerebral course of one of these ipsilateral optic bundles. The double arrow shows some of the fibers that have re-entered the brain. (×560)

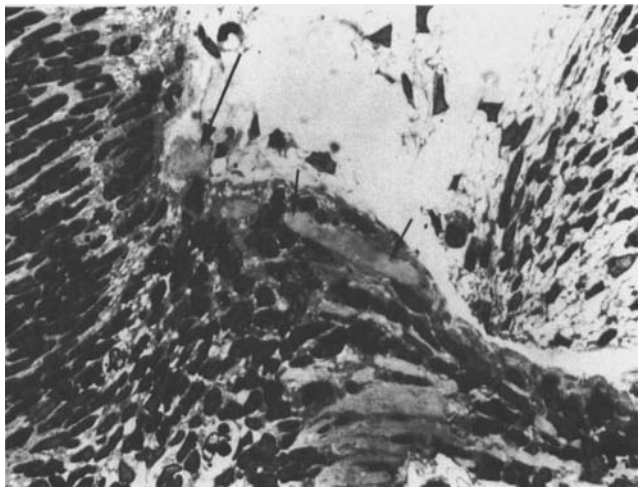

Fig. 2. In this coronal 1-μm section from a 25-day-old fetal ferret, the narrow part of the eye-stalk is to the right and the broad part is to the left. The ipsilateral optic bundles, one of which lies in an extracerebral position, are indicated by arrows, the large arrow marking the extracerebral bundle, the small arrows marking the others. ×230. (Electron micrographs from nearby thin sections of the same bundle are shown in Figs. 8 and 9.)

extracerebral part of their course, and then rostrally and dorsally into the hypothalamus are illustrated in the previous paper (Guillery and Walsh, '87, Figs. 3–6). Figure 3 from that paper shows one of these bundles (arrows at levels 1–34) that can be traced from the optic nerve into the brain before any other retinofugal fibers have reached beyond the optic foramen. The other figures show that at later stages one or more of these bundles run rostrally and dorsally, away from the developing optic chiasm and tract, and that the bundles can be seen to emerge from the surface of the brain or pass close to the surface in all of the animals. This short extracerebral course is seen in the more advanced animals at the junction of the interfascicular and radial parts of the nerve.

The appearance of these bundles in semithin sections is shown here in Figures 1 and 2. In both of these figures one bundle (indicated by two single arrows in Fig. 1 and by the larger arrow in Fig. 2) can be seen apparently lying outside the main part of the optic nerve, whereas other bundles (unlabeled in Fig. 1; small arrows in Fig. 2) clearly lie within the nerve but are approaching the region where bundles appear to emerge.

When these bundles are studied electron microscopically, they are seen to contain 50 to 100 or more closely packed fine axons, with only a few glial profiles among the axons. As the bundles approach the rostrodorsal aspect of the eye-stalk, they are surrounded by glial processes (Fig. 3), many of which represent subpial endfeet. In the regions where the bundles emerge from the CNS, the basal lamina is thrown into complex folds, some of which are filled by glial cytoplasm, whereas others are empty (Figs. 3, 4, BL). In these regions one can see individual fibers or fiber bundles lying adjacent to the basal lamina or emerging through it (A in Figs. 3–5). In other regions individual fibers or fiber bundles lie immediately next to the collagen at the surface of the brain, with no intervening glial layer or basal lamina (Fig. 5–7). Figures 8 and 9 (and see Fig. 2) show a bundle that is outside the brain, in the angle between the eye-stalk and the hypothalamus in which a few glial processes can be seen, but which show no evidence of any basal lamina.

Several observations support the view that the axons in these bundles must be regarded as fibers of the CNS that actually leave the brain for a short distance. They are not peripheral axons that pass close to the brain, nor are they central axons that appear to separate from the brain because of some artifactual distortion of the tissues. One point is that the bundles can be traced through serial semithin sections in brains that have not been removed from the skull and thus have suffered minimal distortion. Further, electron micrographs taken in the appropriate regions invariably confirm that the small, pale structures identified as bundles light microscopically are, in fact, axon bundles (Figs. 2, 8, 9). A second point is that one can see every stage of the apparent axonal escape, some axons leaving singly, others in bundles. Thus, there is a distinct change in the arrangement of the subpial glia as the axons approach the surface (Fig. 3), and in places there are signs of glial degen-

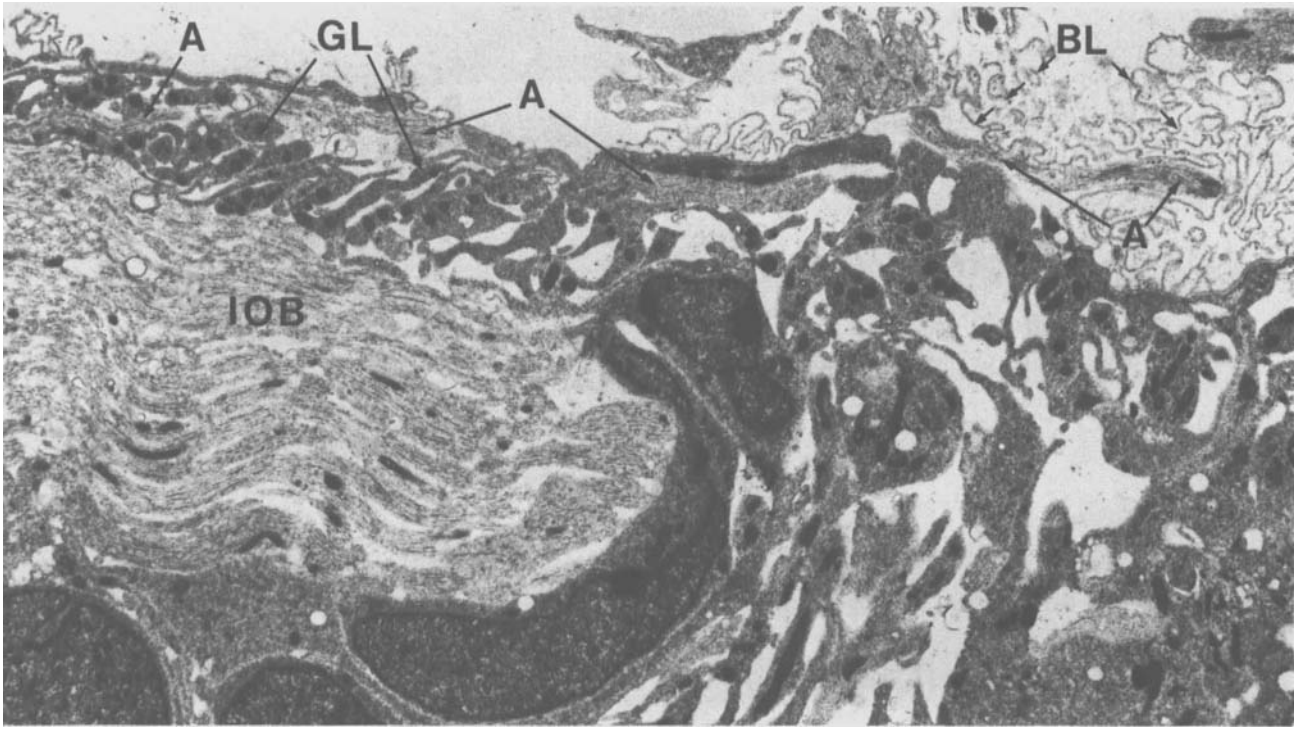


Fig. 3. Electron micrograph to show an ipsilateral optic bundle (IOB) approaching the pial surface of the eye-stalk. Note the highly folded basal lamina (BL) and the individual axons (A) breaking through the subpial glia (GL) and lying adjacent to the basal lamina. (×6,500)

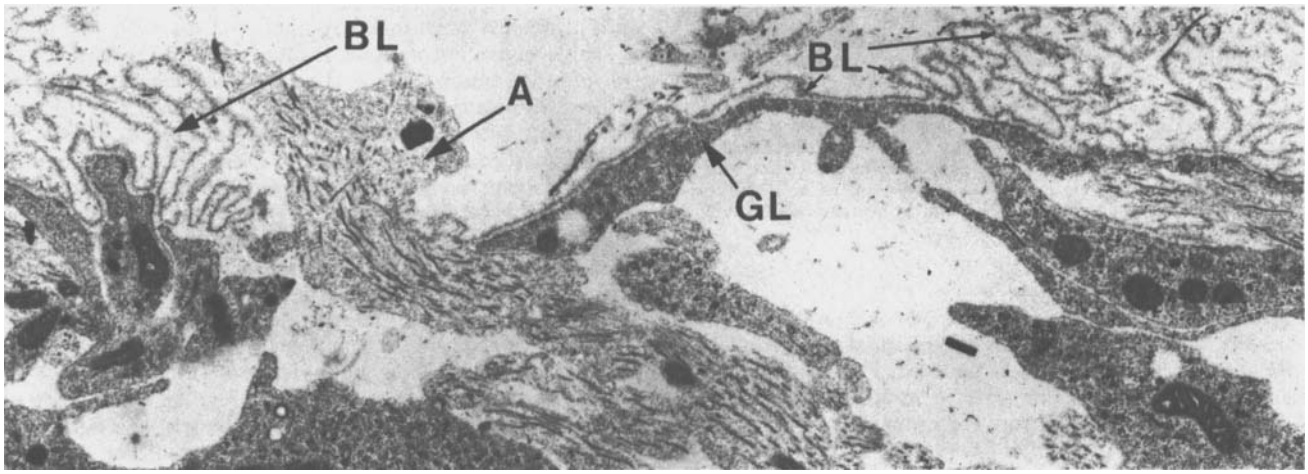


Fig. 4. An axon (A), possibly close to a growth cone, breaking through the highly folded basal lamina (BL) in the region where the ipsilateral optic bundles take a short extracerebral course. GL = subpial glia. (×15,200)

eration (Fig. 5) suggesting that the subpial glia may actually be lost, not merely penetrated by axons. Images like those in Figures 6 and 7 where the subpial glia and basal lamina are missing or interrupted, but where the outer covering of collagen fibers or cells is still present, are strong evidence against a change produced by tissue distortion. Finally, the naked axon bundles cannot be interpreted as peripheral nerve bundles because peripheral nerve bundles in the orbit are accompanied by Schwann cytoplasm, which tends to surround the fibers and which generally shows a basal lamina. Further, of course, we have traced the bundle out of the CNS and back into it.

## DISCUSSION

When we first saw the ipsilateral optic bundles in electron micrographs, we regarded them as the earliest components of fibers going from the retina to the ipsilateral optic tract. However, finding that the bundles are the first to pass between the eye-stalk and the brain, and seeing that they could be traced rostrally into the hypothalamus, not

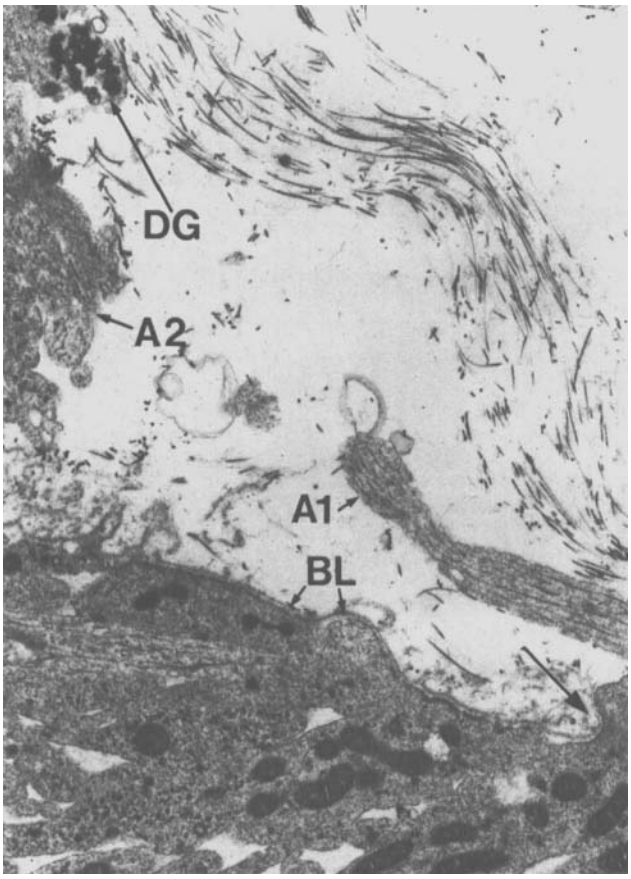


Fig. 5. An axon (A1) and a small axon bundle (A2) lying among the collagen fibers outside the cerebral borders defined by the basal lamina (BL). The cerebral tissue is continuous to the left of the figure with the small profile labeled DG. This may represent degeneration within one of the processes of the adjacent subpial glia. The unlabeled arrow shows a discontinuity in the basal lamina. (×11,200)

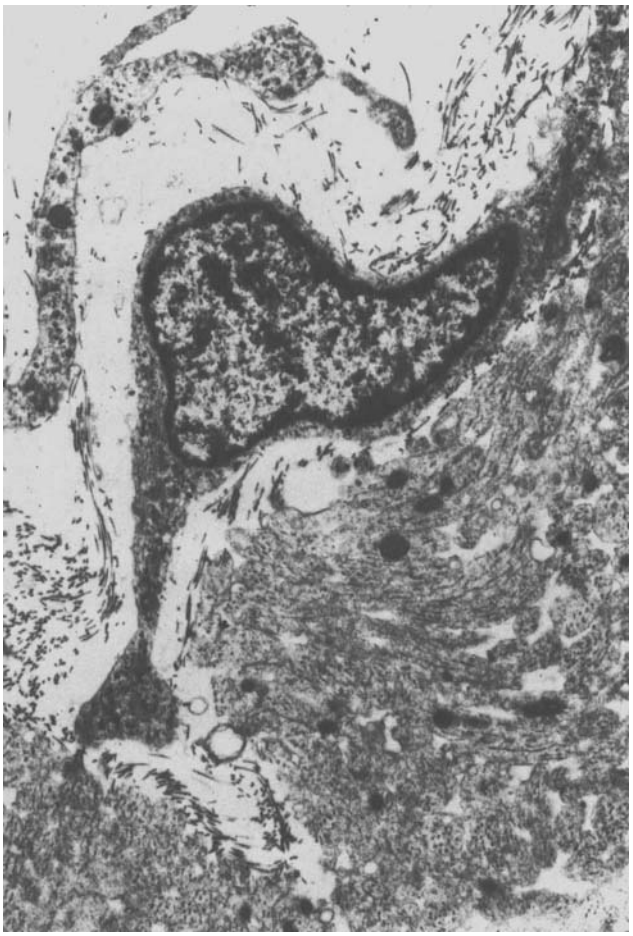

Fig. 6. An axon bundle in the lower right part of the figure lies adjacent to collagen bundles and to a connective tissue cell, with no intervening layer of glia or of basal lamina. Note that the cell covers the naked area entirely, indicating that the loss of glia and basal lamina is not the result of artifactual mechanical distortions. (×12,450)

caudally into the tract, convinced us that this was a misinterpretation. The evidence on the early development of the crossed and uncrossed components of the tract suggests that, if anything, the crossed develops earlier than the uncrossed component (see Cucchiaro and Guillery, '84 for evidence on the ferret). It would be surprising to find the uncrossed bundle to the tract in the lead. One might call these ipsilateral optic bundles the "aberrant" bundles because they run such an unexpected course, but since we have found such bundles in every one of the more than 12 blocks that have been closely studied at the relevant developmental stage, they can hardly be regarded as aberrant unless they prove unique to the ferret.

The fibers may prove to be an early component of the retinal input to the suprachiasmatic nucleus. Although we traced the fibers to only the lateral parts of the hypothalamus, they can occasionally be traced a short distance among the periventricular cells. Further, the hypothalamus and chiasm are still destined to grow to a very significant extent, so that the relationships of the fibers are appropriate for a suprachiasmatic component. However, the evidence that is currently available from axonal labeling suggests that the suprachiasmatic component develops rather late (Campbell and Ramaley, '74; Lund and Bunt, '76; Stanfield and Cowan, '76), and if these fibers are the retinohypothalamic input to the suprachiasmatic nucleus, then one would need to assume that they represent very small early bundles that develop demonstrable terminals later. Possibly the bundles represent a temporary pathway that fails to survive in the adult, or they could be fibers that arise in the brain and pass to the retina. These are problems that remain to be resolved.

The short extracerebral course of these bundles is entirely puzzling. We know of no fiber system in which a comparable loss of glial and pial covering during normal development has been demonstrated. One expects fibers of the CNS generally to stay within the glial borders of the CNS and not to break through the basal lamina. Since the fibers reenter the CNS after a very short extracerebral course, the significance of the extracerebral course is likely to be particularly difficult to understand. Not only will one want to know whether the developing system derives some benefits

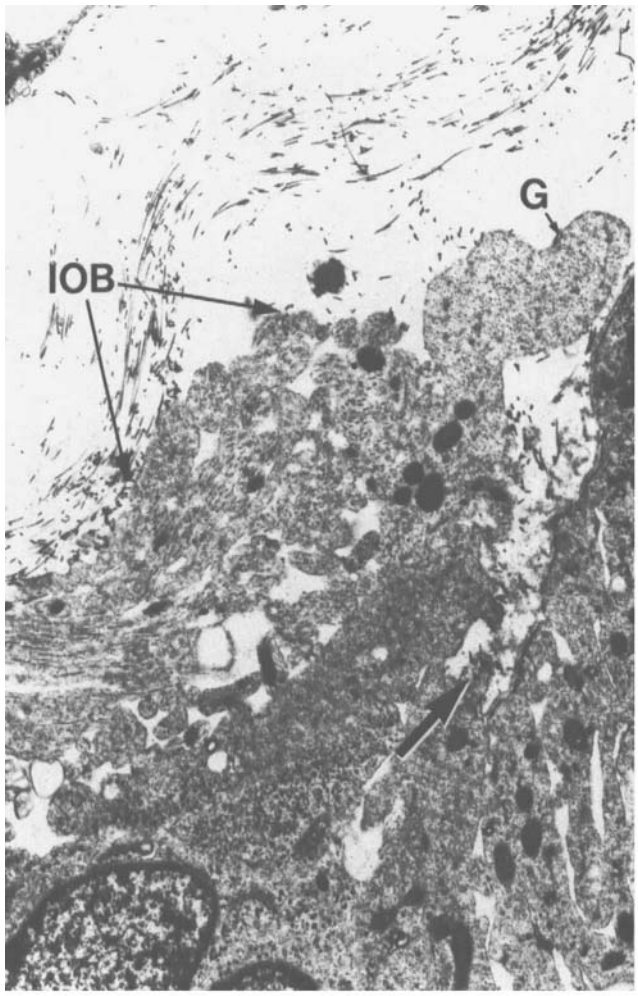


Fig. 7. An axon bundle (IOB) accompanied by a tongue of glial cytoplasm (G) is exposed on the surface of the brain with no glial covering and no basal lamina. Note that the basal lamina is present at the right of the figure and that it becomes discontinuous in the depth of the fold indicated by the unlabeled arrow. (×9,500)

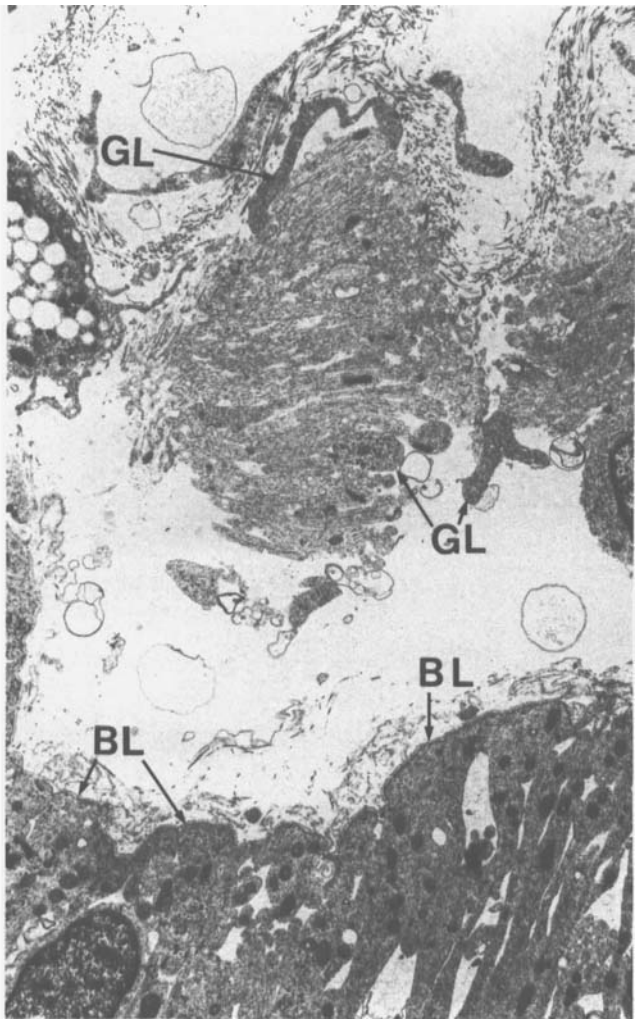


Fig. 8. An ipsilateral optic bundle accompanied by a few cytoplasmic processes that may be glial (GL) is seen in the center of the figure. The eye-stalk and its basal lamina (BL) is seen below and to the left. Some evidence of the complex folding of the basal lamina can still be seen. The subpial glia of the eye-stalk is continuous in this region. Section from a region close to that illustrated in Figure 20. (×5,000)

from the presence of these bundles, but also one can reasonably inquire about the developmental processes that lead first to the escape and shortly thereafter to the re-entry of the fibers. The relationship between the growth cones, the glial cells, and the basal lamina at the time that the axons leave and re-enter the CNS may prove of interest. Figure 4 looks as though it may be close to a growth cone that is just breaking through the basal lamina. However, it is possible that an intermediate part of a bundle pushes out of the CNS after the advancing tips are well past the region of the breakout. These are problems that remain to be studied. One point seems clear about these bundles: Although they are the "pioneers" that cross between the eye-stalk and the brain before any of the other fibers, the pathway that they establish is avoided by the majority of the later developing retinofugal fibers.

## ACKNOWLEDGMENTS

We thank M. Holley and M. Sanders for skilled histological assistance, T. Richards for the line drawings, C. Beesley for the photography, and R. Taylor for the typing. We thank B. Reese, V. Casagrande, and this journal's reviewers for helpful comments on a first draft of the manuscript. The research was supported by the MRC (Grant No. 8324037) and Medical Scientist Training Program Grant 5 T32 GM 07281.

## LITERATURE CITED

Campbell, C. B. G., and J. A. Ramalay (1974) Retinohypothalamic projections: correlations with onset of the adrenal rhythms in infant rats. Endocrinol. *94:* 1201–1204.

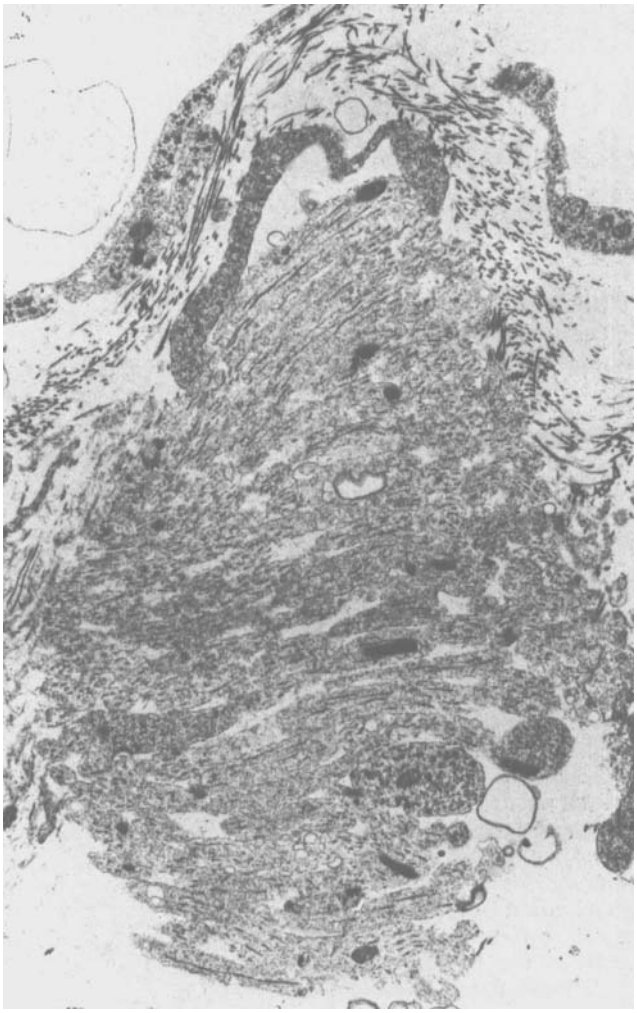

Fig. 9. Higher magnification of the bundle illustrated in Figure 8.

Cucchiaro, J., and R. W. Guillery (1984) The development of the retinogeniculate pathways in normal and abnormal ferrets. Proc. Roy. Soc. B. *223:* 141–164.

Guillery, R. W., and C. Walsh (1987) Changing glial organization relates to changing fiber order in the developing optic nerve of ferrets. J. Comp. Neurol. *265:*203–217.

Lund, R. D., and A. H. Bunt (1976) Prenatal development of central optic pathways in albino rats. J. Comp. Neurol. *165:* 247–264.

Richardson, K. C., L. Jarret, and E. H. Finke (1960) Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Technol. *35:* 313–323.

Stanfield, B., and W. M. Cowan (1976) Evidence for a change in the retinohypothalamic projection in the rat following early removal of one eye. Brain Res. *104:* 129–136.