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Changing Glial Organization Relates to Changing Fiber Order in the Developing Optic Nerve of 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

The structures of the developing eye-stalk and the relationships of early retinofugal fibers as they pass through the stalk, chiasm, and tract have been studied by light and electron microscopical methods in fetal ferrets aged 23–27 days. The early eye-stalk can be divided into two parts: a narrow extracranial part has a narrow lumen and is lined by few cells, whereas a thicker intracranial part has a wider lumen and is lined by several rows of cells. At the earliest stages no axon bundles are recognizable in the stalk, but fibers of the supraoptic commissure are already beginning to cross the midline in the diencephalon. Subsequently, as retinofugal axons invade the stalk, the glia of the extracranial part of the stalk have an interfascicular distribution and axon bundles are separately encircled by glial cytoplasm. In the intracranial part, as in the chiasm and tract, the glial cells occupy a periventricular position and send slender radial cytoplasmic processes to the subpial surface; these pass between groups of axons that here lie immediately deep to the subpial glia. Whereas axonal growth cones have no evident preferred distribution in the extracranial stalk, they tend to accumulate near the pial surface intracranially. The boundary between the two types of organization shifts as development proceeds so that the interfascicular glial structure of the early extracranial stalk first encroaches upon the intracranial parts and later appears in the chiasm. The characteristic adult arrangement of fibers in an age-related order in the optic chiasm and tract, but not in the optic nerve, can be understood if axonal growth cones are guided toward the pial surface by radial glia but not by interfascicular glia. From the distribution of the growth cones, this is what appears to happen.

Key words: retinofugal fibers, optic chiasm, optic tract

The retinofugal fibers undergo a significant rearrangement as they pass from the eye to the brain (Horton et al., '79; Naito, '86). In the optic tract of cats the fibers are arranged in an age-related order (Torrealba et al., '82; Walsh et al., '83; Walsh and Polley, '85), and this order is quite distinct from that seen in the nerve close to the eye where the fibers are in a roughly retinotopic order. In ferrets, which have central visual pathways generally resembling those of cats, the sequence of axonal growth into the pathways has been demonstrated, and it has been shown that in the optic tract the oldest fibers are those that lie deepest (Walsh and Guillery, '85). The age-related order is also seen in the optic nerve distal to the tract (Walsh, '86), and at early developmental stages the change from a retinotopic to an age-related order appears to occur roughly where the nerve passes through the optic foramen. The present study of ferrets was undertaken to see whether, at the time the retinofugal axons are growing past the optic foramen, there is a change in the structure of the eye-stalk that might be related to the change in fiber order, and to examine the structure and relationships of the growing axons themselves.

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

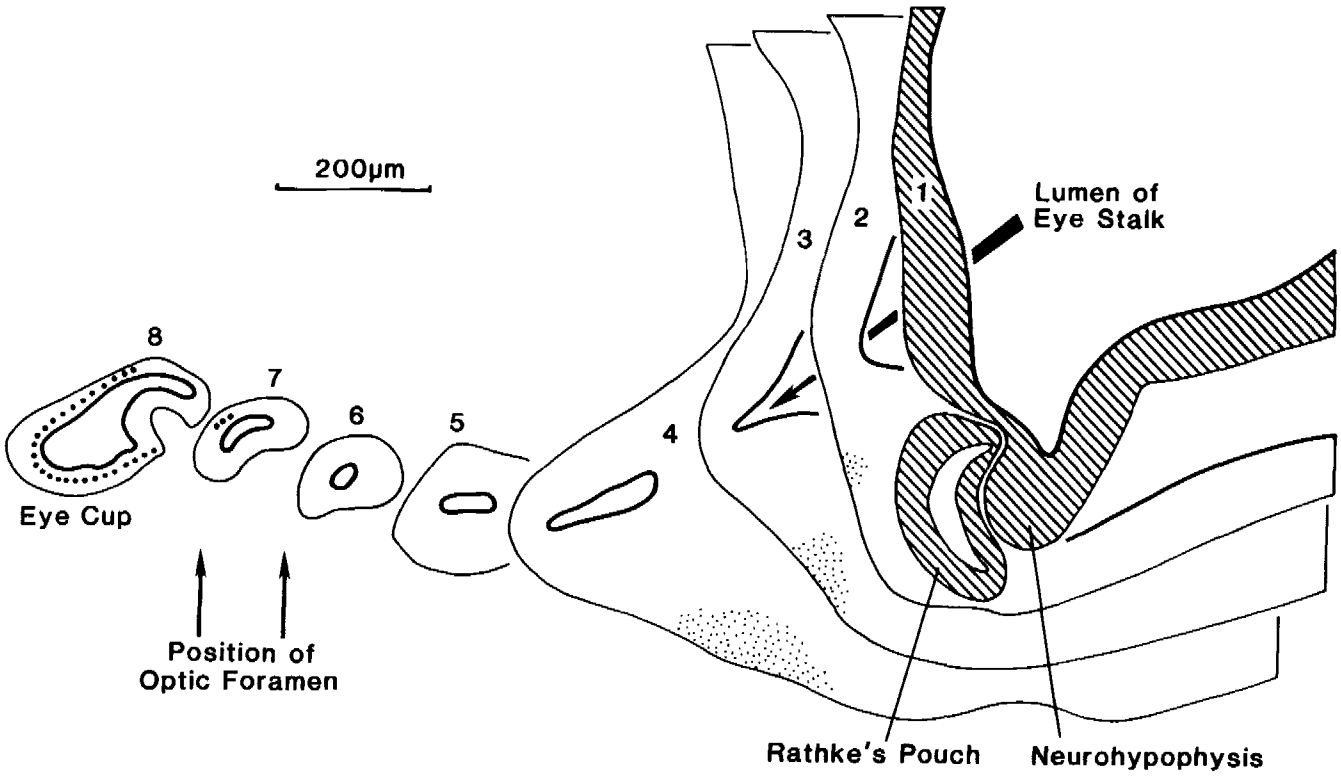


Fig. 1. Drawing made from 8-μm sagittal paraffin sections through the diencephalon, eye-stalk, and part of the eye-cup of a normally pigmented ferret embryo aged 22 days. The most medial of the sections is indicated by cross hatching. The sections are spaced 112 μm apart and are numbered in mediolateral sequence. Fine fiber bundles of the supraoptic commissures are shown as small dots. Melanin granules in the pigment epithelium of the eye-cup and distal eye-stalk are shown as coarse dots.

MATERIALS AND METHODS

Normally pigmented ferrets (*Mustela putorius furo*) ranging in age from 21–27 days in utero were obtained either from our own colony or from Marshall Research Animals (North Rose, NY). The day of mating has been counted as day 0. The pregnant females were anesthetized with sodium pentobarbital and the young were then rapidly removed from the uterus, separated from the placenta, and the head was fixed by immersion (or by perfusion through the heart for the older animals) in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde with 0.03% picric acid in a 0.1 M cacodylate buffer (pH 7.6) or with 1% glutaraldehyde and 4% paraformaldehyde in a 0.1 M phosphate buffer at the same pH. We thank Dr. G. Jeffery for fixing a number of the blocks used in this study. In some of the animals the head was divided midsagittally. After fixation at about 4°C for 24 hours or longer, the blocks were rinsed in the buffer, transferred to 1% osmium tetroxide in the same buffer, and then embedded in a mixture of "Epon" and "Durcupan." Sections were cut by hand until the relevant portion of the head (hypothalamus, eye) could be identified and then serial semithin sections were cut on an ultramicrotome. Thin sections were obtained at selected points, mounted on formvar grids, and stained with uranyl acetate (5% in 50% ethanol) and lead citrate (Reynolds, '63). Some of the younger animals were fixed in AFA (10% formalin, 20ml; absolute alcohol, 80ml; glacial acetic acid, 2ml), and the heads were then processed for paraffin sectioning and light microscopical study. Sections for light and electron microscopy were cut in the frontal and parasagittal planes and some of the blocks used for electron microscopy were also cut horizontally or obliquely. For each developmental age (counted in days), four or more animals were available. Since there is considerable variation in the developmental stage reached by the animals at any one age, the age groups given below serve as rough guides only and overlap one another.

RESULTS

General, light microscopic description of eye-stalk and chiasmatic region

22–23 days. At the earliest stages studied (22 days), the optic fissure of the eye-cup is almost closed and the neural retina is beginning to differentiate, but no fiber bundles can yet be seen beyond the region of the optic disc. The cavities of the eye-cup and eye-stalk are still continuous with the third ventricle (Fig. 1). The eye-stalk is funnel shaped, broad near the brain and narrow near the eye, not yet containing any identifiable fiber bundles. Whereas at its cerebral end, the eye-stalk has a thick periventricular zone containing many rows of closely packed nuclei, at its retinal end the eye-stalk has a thin wall, with only two or three rows of nuclei ventrally and a single row dorsally.

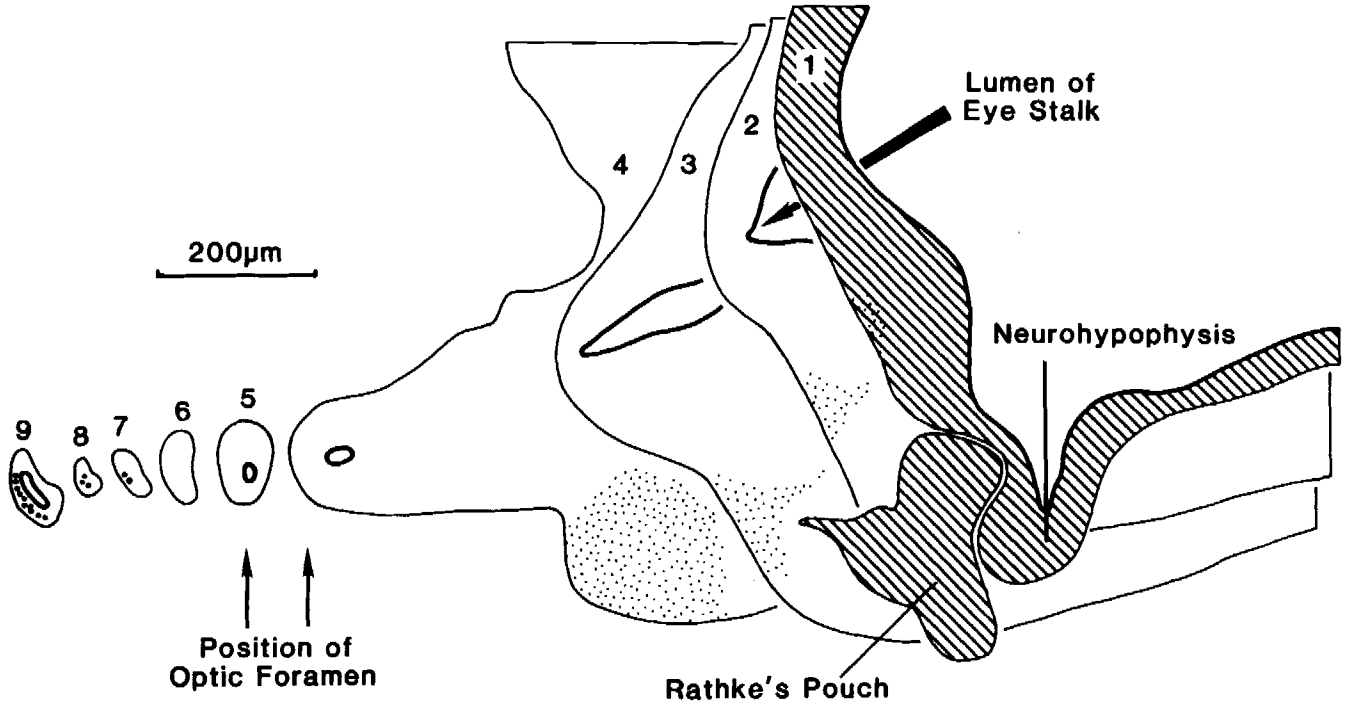


Fig. 2. Drawings made from 10-μm sagittal paraffin sections through the diencephalon, eye-stalk, and part of the eye-cup of a normally pigmented ferret embryo aged 23 days. The sections are spaced 120 μm apart. Other conventions as for Figure 1. The ventricular cavity can be traced as far as the level of the optic foramen, beyond which it becomes obliterated.

Melanosomes are present in the retinal pigment epithelium and also extend into the dorsal and distal part of the eye-stalk, gradually becoming sparser farther from the eye and reaching as far as the developing optic foramen. Many of the melanosomes close to the optic disc are grouped around darkly staining lysosomelike bodies, forming the "rosettes" previously described by Strongin and Guillery ('81), but these are limited to the most distal regions of the stalk. Apart from these rosettes there are no signs of cellular degeneration in the dorsal part of the eye-stalk. In contrast, in the ventral part of the eye-stalk there are some scattered pyknotic nuclei and many darkly staining lysosomelike bodies that are either contained in normal cells or lie within macrophages (Colello, '86). These are reasonably regarded as products of cellular degeneration (see Silver and Hughes, '74; Horsburgh and Sefton, '86). At this early stage they occur close to the eye and also in the peripheral, extracranial part of the eye-stalk, but not in the intracranial part.

In the brain, in the region of the optic chiasm itself, there are, as expected, no fibers. However, just posterior to the eye-stalks there are signs of a developing fiber system that extends from the lateral hypothalamic regions toward the midline (fine dots in Fig. 1). This fiber zone is poorly defined at this early stage, consisting of fine loosely organized bundles running through a narrow, subpial, cell-free zone. At later stages it forms the supraoptic commissures (see below).

Figure 2 shows a brain that is slightly more advanced than the one described above. The shape of the eye-stalk has changed. Its extracranial part is formed by a single layer of epithelial cells around a very narrow lumen that in places is obliterated. A few fiber bundles can be traced from the retina a short distance into the eye-stalk. Melanin is present as before in the dorsal cells of the stalk near the retina. It forms many rosettes near the eye and extends as a thin streak toward the brain, reaching almost as far as the optic foramen. Thus, the extracranial nerve differs from the intracranial nerve because for a brief period the former has some melanin-bearing cells, whereas the latter does not. Pyknotic nuclei and other signs of cellular degeneration are rare in the extracranial part of the eye-stalk, but are now more common near the optic foramen where the eye-stalk suddenly begins to broaden. Close to the brain in the broadest part of the eye-stalk, there are, again, few signs of degeneration. These two preparations suggest that there is a wave of cellular degeneration progressing from the eye-cup toward the brain. Material from the older embryos confirms this and suggests that in terms of the distribution of degeneration, the extracranial stalk only differs fleetingly from the intracranial part.

The fiber system that will form the supraoptic commissures is more extensive than before (small dots in Fig. 2). The system is heaviest in the lateral hypothalamus, becoming lighter near the midline. As yet there is no contact between these supraoptic fibers and the retinofugal fibers since the latter do not extend beyond level 8 of Figure 2.

23–24 days. Figure 3 (24 days) shows coronal sections obtained at a slightly later stage. The contrast between the

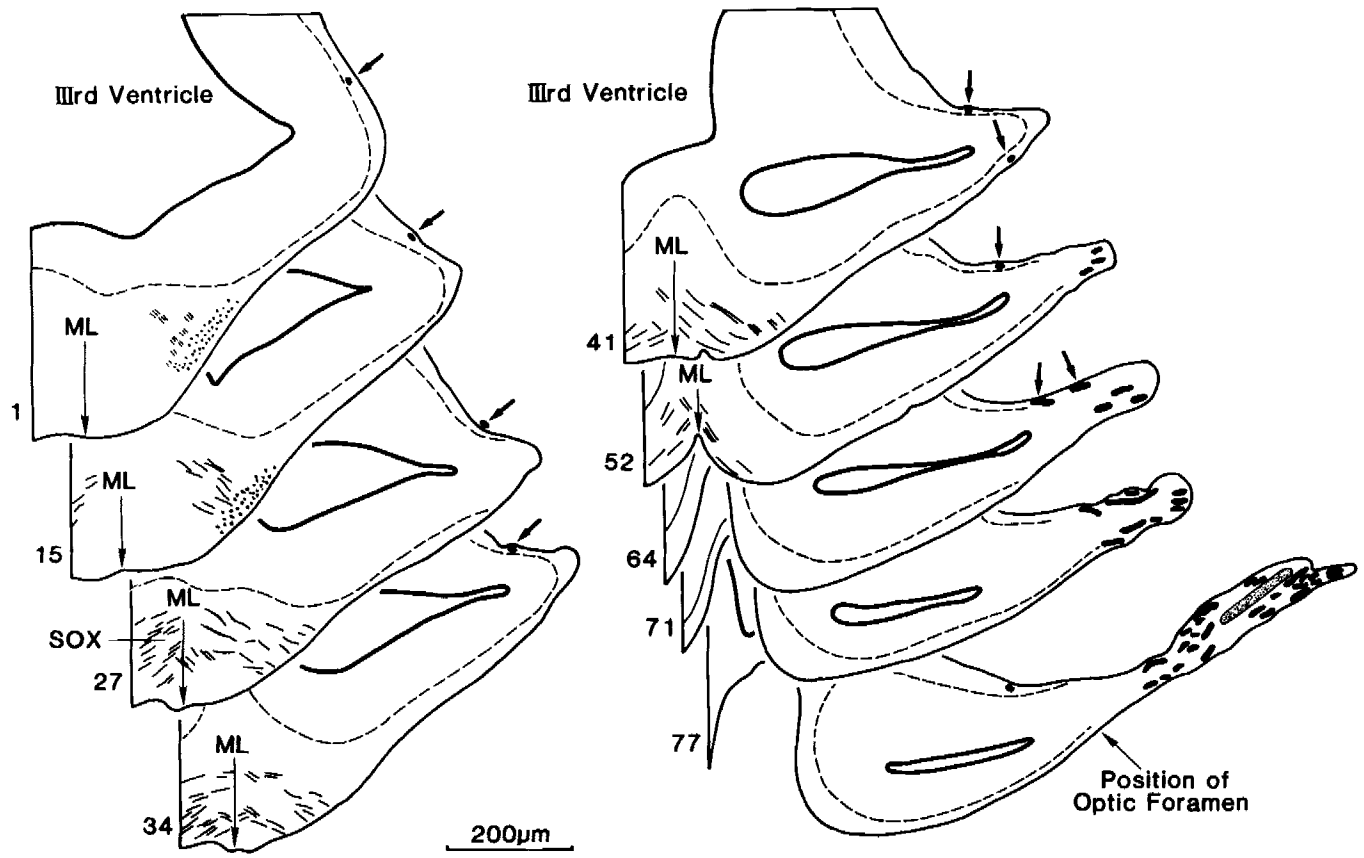


Fig. 3. Drawing of semithin (circa 1 μm) sections obtained from a normally pigmented 24-day-old ferret embryo. The sections are cut in a plane close to the coronal, but are somewhat oblique so that the lower parts of the sections represent ventral and caudal parts of the brain. The sections are numbered in serial order, section 1 being the most caudal (and dorsal). The cavity of the third ventricle can be traced into the intracranial part of the eyestalk, extending almost as far as the optic foramen. In the distal parts of the eye-stalk (level 77), where the retinofugal fiber bundles lie among the nuclei of the neuro-epithelial or glial cells (not shown), there is also a tongue of melanin-bearing cells (fine stipple on level 77) in the dorsal part of the eye-stalk (see text). Three individual fiber bundles that can be traced centrally toward the brain are indicated by unlabeled arrows. Two of the bundles run dorsally toward the lateral aspect of the hypothalamus (seen at levels 1–64) and one runs a short distance ventrally toward the midline at level 41. The region of the densest periventricular cell distribution is outlined by the interrupted lines. The approximate position of the midline is indicated (ML) at levels 1–52, and the decussating bundles of the supraoptic commissures (SOX) are shown at levels 27–52.

intra- and extracranial parts of the eye-stalk is now marked, the former being broad with a well-defined lumen that continues into the third ventricle. This part of the eye-stalk is beginning to look like a part of the hypothalamus and may later become incorporated in it. The extracranial part of the stalk is narrow and has lost its lumen. There are still some melanosomes in the distal part of the stalk (fine stipple in Fig. 3), a few forming rosettes near the eye. Signs of cellular degeneration are most evident on either side of the optic foramen.

The distal, extracranial part of the eye-stalk contains several bundles of fine axons (black in Fig. 3), and scattered interfascicular glial nuclei among the bundles (not shown in Fig. 3). The fine structural details are described below. In the proximal, intracranial part of the eye-stalk, the nuclei form a thick periventricular zone but leave a pale, narrow, subpial marginal zone free of nuclei (indicated by the interrupted lines in Fig. 3). (The contrast between these two parts is illustrated in Figures 7, 8, and 9 from more mature stages having more axonal bundles, see below.) The extracranial type of organization is here called "interfascicular glial," whereas the type seen intracranially at this stage is called "radial glial."

The few axon bundles that can be traced into the intracranial stalk at the early stages shown in Figure 3 enter the nucleus-free subpial zone. In Figure 3 there are only three of these bundles, one running ventrally and two running dorsally (arrows in Fig. 3). The ventral bundle is the smallest and is soon lost. The dorsal ones pass to the angle where hypothalamus and eye-stalk meet, and one can be traced dorsally and rostrally into the subpial parts of the hypothalamus. These dorsal bundles represent the "ipsilateral optic bundles" described in the accompanying paper (Guillery and Walsh, '87). They do not join the optic chiasm and do not contribute to the uncrossed component of the optic tract.

The supraoptic commissures form an extensive system of loosely arranged bundles of decussating fibers at this stage

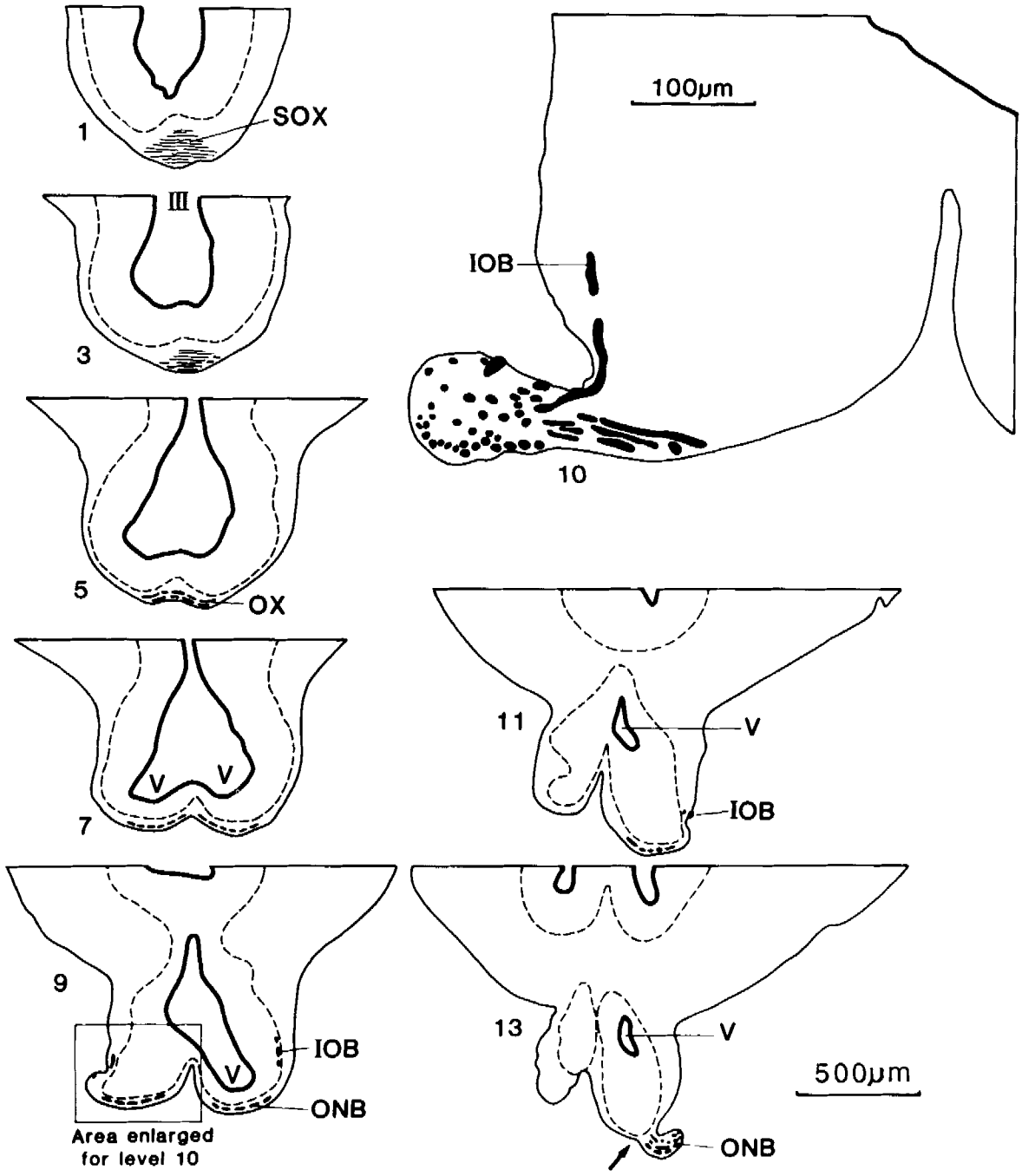


Fig. 4. Drawings of coronal semithin (circa 1 μm) sections through the ventral hypothalamus obtained from a normally pigmented ferret embryo aged 23 days. Note that in spite of the recorded age of this embryo, the pathways are in a slightly more advanced stage than those shown in Figure 3. The sections are numbered in caudo-rostral sequence to indicate the relative spacing. Notice the supraoptic commissures (SOX, level 1), the optic chiasm (OX, level 5), and the optic nerve bundles (ONB) in the intracranial (level 9) and extracranial (level 13) eye-stalk. Some of these bundles can be traced as ipsilateral optic bundles into the rostral and lateral parts of the ipsilateral hypothalamus (IOB). The proximal (cerebral) parts of the eye-stalk still have an open ventricular cavity (v) that can be traced into the third ventricle (III). The regions of the densest periventricular cell distributions are outlined by the thin, interrupted line. Level 10 is shown at five times the magnification used for the other levels in order to show the distribution of the fiber bundles. Note the marked change in thickness that occurs in the structure of the eye-stalk at level 13 (arrow), where it changes from the interfascicular to the radial glial structure. This is central to the optic foramen, which is not shown in this figure.

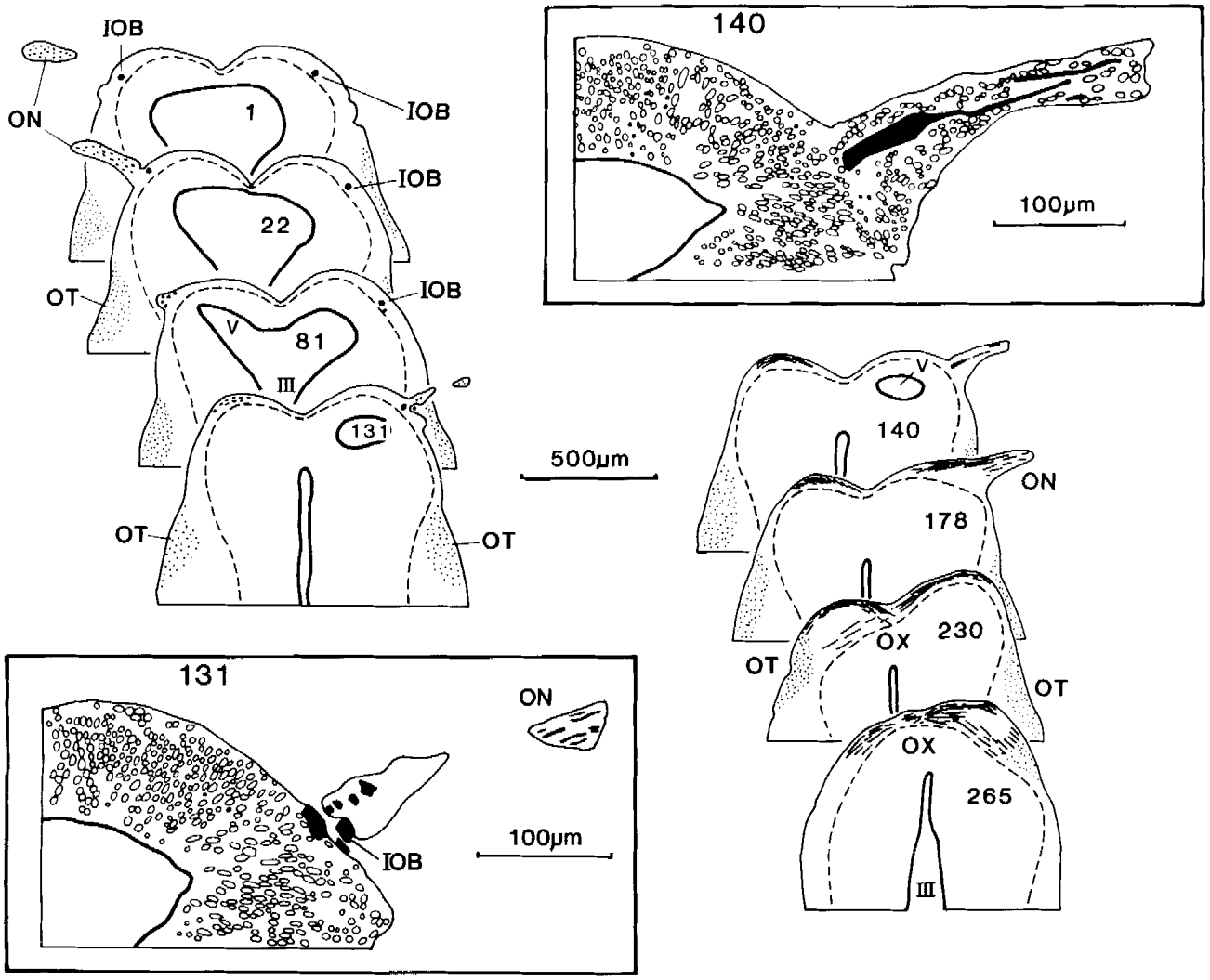


Fig. 5. Drawings of semithin (circa 1 μm) sections from a 25-day-old pigmented ferret embryo cut in an oblique plane between the horizontal and the coronal. The upper part of each section is rostral and ventral to the lowest part. The sections are numbered in sequence, the lowest numbers marking the most dorsal (and rostral) of the sections. ON, optic nerve; IOB, ipsilateral optic bundles (see Fig. 4 and text); OT, optic tract; V, ventricular cavity of the eye-stalk; III, third ventricle. Levels 131 and 140 are illustrated twice, once at low magnification and once at five times the magnification to show the arrangement of the fiber bundle (shown in black).

(Figs. 3, 4; see also Fig. 10). They are still quite distinct from the retinofugal system. At later stages the two systems become indistinguishable.

In Figure 4, which shows a more advanced stage than Figure 3, there is a clear distinction between the narrow extracranial part of the eye-stalk and the broad intracranial part that is beginning to become continuous with the hypothalamus (the border is shown by an arrow at level 13). In these sections the retinofugal bundles are again mingled with interfascicular glial nuclei throughout the cross-sectional area of the extracranial part (the glial nuclei are not shown in Figure 4 but the relationships are shown in Fig. 7), but become restricted to the outer nucleus-free zone of the stalk as they approach the chiasm (see Figs. 8 ,9).

Day 25 and later. As the number of retinofugal fiber bundles increases, the contrast between the interfascicular and the radial glial organization becomes more obvious. The border between the two types of organization shifts centrally, toward the brain, and the fiber bundles can be traced through the optic chiasm and into the optic tract. This is the earliest stage at which the retinofugal fibers can be traced through the optic chiasm and to the tract. In addition, some fibers can be traced from the nerve to the tract without any chiasmatic crossing.

Figures 5 and 6 show sections cut in a plane close to the horizontal (see figure legends). At this stage the extracranial optic nerve is densely packed with fiber bundles (shown in Fig. 6 but not in Fig. 5) and the intracranial part can be divided into two halves. The distal half, central to the arrow at level 15 of Figure 6 (see enlarged box) is becoming continuous in its structure with the extracranial part, showing an interfascicular glial organization. The fiber bundles tend to lie toward the outside of the nerve, but there is no longer

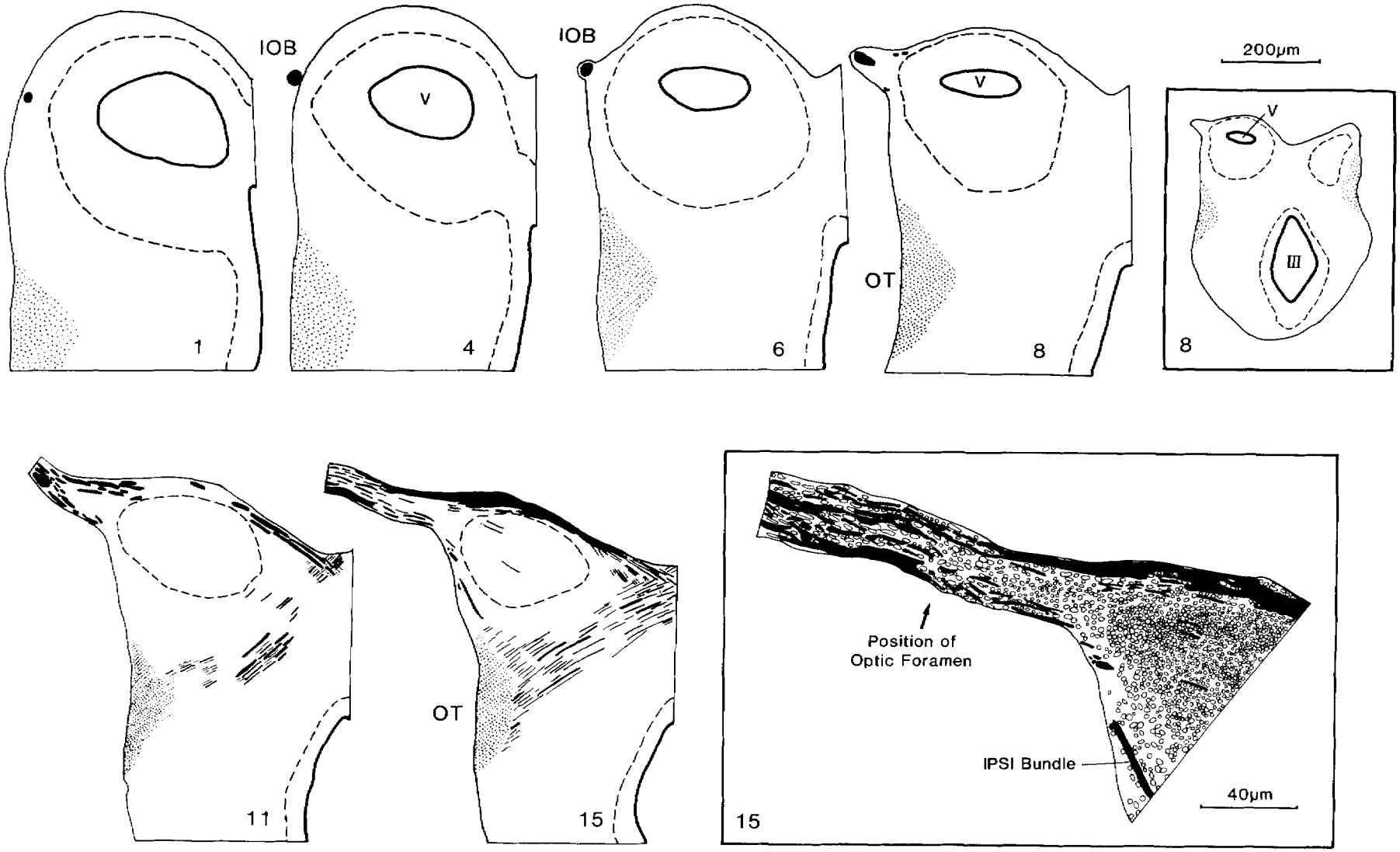
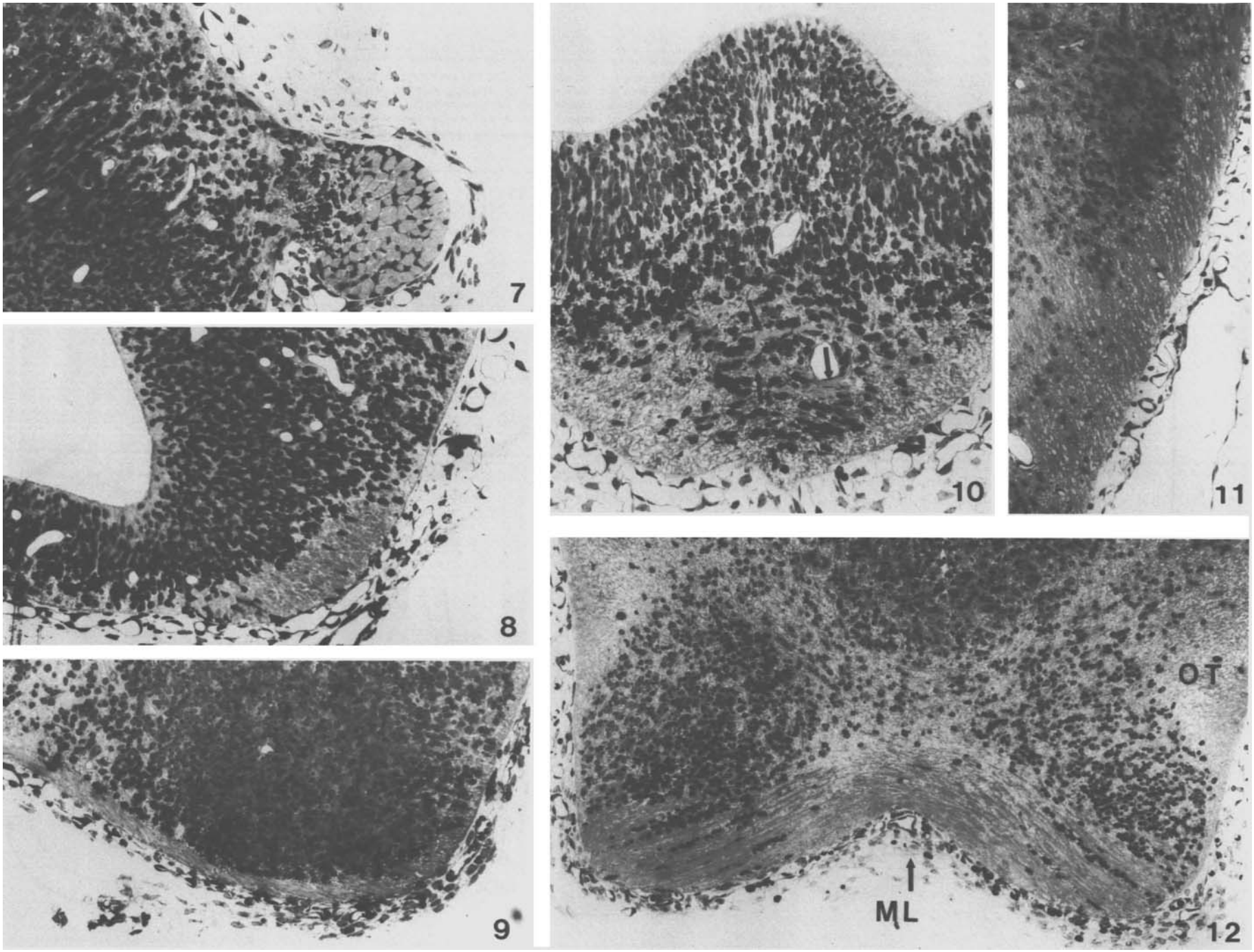


Fig. 6. Drawings of semithin sections (circa 1 μm) from a 25-day-old pigmented ferret embryo. The sections are cut close to the horizontal plane and are numbered in dorso-ventral sequence. Rostral is up. Level 8 is also shown at a low magnification to illustrate the relationship between the third ventricle (III) and the ventricular cavity of the eye-stalk (v). Note that this was a bilateral series but that only one-half is illustrated in the main part of the figure. Level 15 is also shown at a high magnification to show the detailed relationships between individual fiber bundles and the cell nuclei of the eye-stalk and hypothalamic junction. Fibers that pass from the eye-stalk to the ipsilateral optic tract are marked (IPSI bundle); other abbreviations are as in Figure 5. IOB, ipsilateral optic bundle; OT, optic tract; v, ventricle. The interrupted outlines indicate the regions of dense periventricular cells.



Figures 7–12

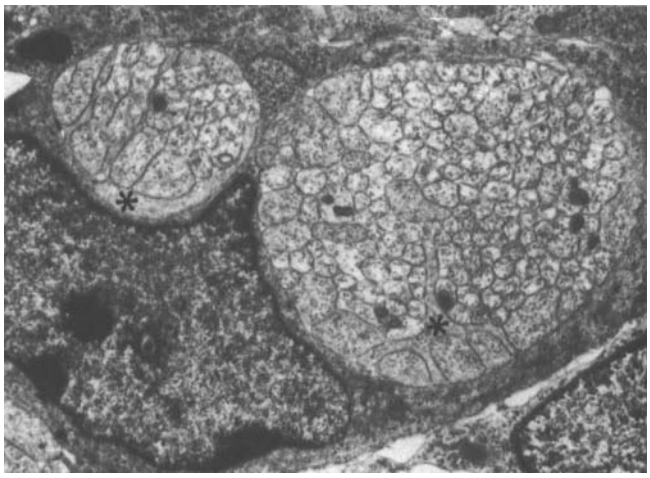


Fig. 13. Two fiber bundles from the optic nerve of a 25-day-old ferret just distal to its broad intracranial part. Compare with Figure 7. The asterisks indicate profiles interpreted as portions of growth cones. ×8,200.

a clear zone that is free of nuclei. The broad, proximal half of the intracranial nerve is now fusing with the hypothalamus and shows a radial glial organization. The ventricular extensions into the eye-stalk are still recognizable; they will presumably form the optic recesses of the adult.

Many of the retinofugal fibers can be traced through the optic chiasm and into the optic tract (OT in Fig. 6), and a few even at this early stage can be traced into the ipsilateral tract (ipsi bundle in Fig. 6). It is worth stressing that these uncrossed fibers appear very early, close to the time at which the tract itself is first formed. This accords with evidence obtained from birth dating (Dräger, '85), which shows the uncrossed component to develop at a very early stage.

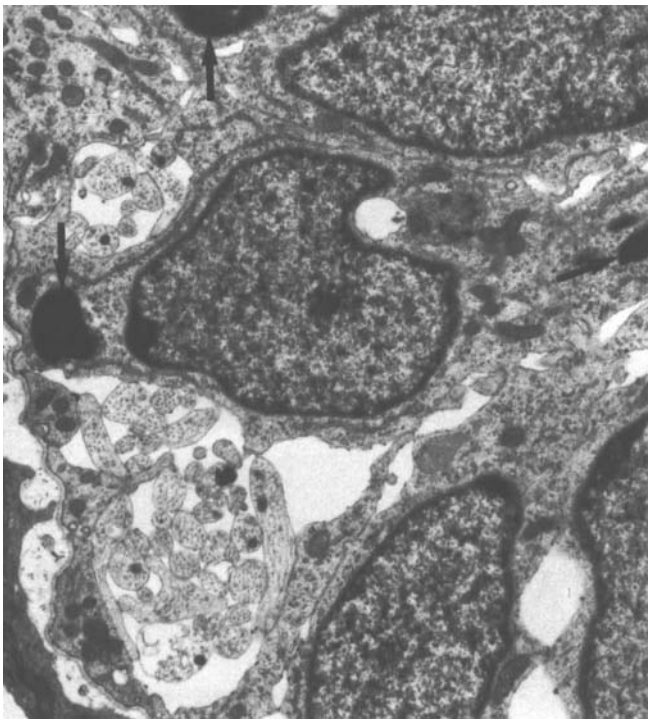


Fig. 14. Two small fiber bundles of a 24-day-old ferret from the distal parts of the eye-stalk in the region that contains melanosomes. The arrows show the melanosomes. The basal lamina is to the left. ×7,900.

Figure 7 shows the structure of the interfascicular part of the nerve as this approaches the broader radial glial portion, which is to the left but lies more caudally in the block (shown in Fig. 9). In Figure 7, the glial nuclei can be seen scattered among the axon bundles, whereas in Figure 9 there are no nuclei separating the fiber bundles. Instead the bundles form a broad sheet at the surface of the brain, with the glial nuclei all lying deeper in a broad periventricular zone. The nuclei that lie outside the fiber bundles in Figure 9 near the surface of the brain are not cerebral cells but cells of the developing meninges. The ventricular cavity is not shown in Figure 9 since the section passes tangential to the part of the ventricle in the cerebral end of the eye-stalk.

Figure 8 shows an intermediate region in a slightly older animal, where the interfascicular organization is changing into the radial glial organization. A few glial nuclei can still be seen among the fiber bundles, but the main fiber zone is separated into bundles by the extremely slender cytoplasmic process of the radial glia, which are more readily demonstrable with the electron microscope (see below).

The arrangement of fiber bundles separated by cytoplasmic processes continues across the chiasm at the early stages and into the optic tact (see Fig. 12). In the optic tract itself one sees an essentially continuous fibrous field, although in an appropriate plane of section a segmented appearance can be seen in the tract (Fig. 11) produced by the slender glial processes.

Fig. 7. Coronal 1-μm section through the developing optic nerve of a 25-day-old intrauterine ferret. The fiber bundles and interfascicular glial nuclei characteristic of the narrow, extracranial part of the optic nerve are shown on the right, approaching the broader intracranial portions, which are seen to the left but which contain no fiber bundles at this level. ×250.

Fig. 8. Coronal 1-μm section through the developing intracranial optic nerve of a 27-day-old intrauterine ferret. The fiber bundles are collecting in an outer, sub-pial zone that is almost free of nuclei. The outlines of the bundles are beginning to be lost. The ventricular cavity of the eye-stalk close to the third ventricle is seen to the left. ×250.

Fig. 9. Frontal 1-μm section through the junction of the eye-stalk and the hypothalamus of a 25-day-old intrauterine ferret to show the retinofugal fibers approaching the optic chiasm through a subpial nucleus-free zone. The midline is at the left border of the figure. ×250.

Fig. 10. Section cut at 1 μm close to the coronal plane to show the fiber bundles in the region of the supraoptic commissures at a stage before any of the retinofugal fibers have reached the chiasm. The bundles are indicated by arrows. The section is taken from the block illustrated in Figure 3 and comes from a level close to level 34 in that figure. ×335.

Fig. 11. Coronal section cut at 1 μm through the optic tract of a 26-day-old intrauterine ferret. Note that the field of the tract is essentially free of nuclei at this stage and that the bundles show a segmented arrangement. Glial nuclei invade the area later. ×245.

Fig. 12. Coronal 1 μm section through the optic chiasm of a 26-day-old intrauterine ferret. Notice that in the midline (ML) there are virtually no glial nuclei among the fiber bundles, but that laterally, where optic nerve fibers are approaching the chiasm, rows of glial nuclei have invaded among the fiber bundles (see text). Note that the field of the optic tract (OT) is essentially free of nuclei. The supraoptic commissures lie posterior to this figure. ×220.

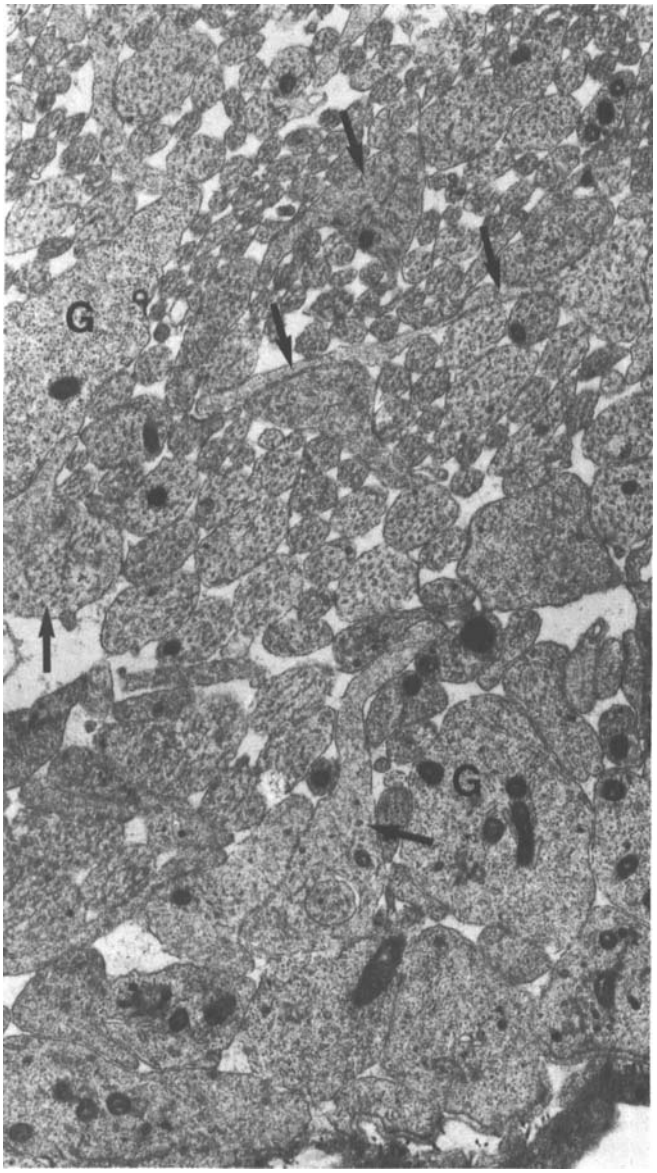


Fig. 15. Fibers from the ventral broad intracranial part of the optic nerve on the way to the optic chiasm. The figure comes from a section similar to that shown Figure 8, but from a younger animal (25 days in utero). The edge of a bundle is next to the pial surface in the lower part of the figure. Note basal lamina at the bottom right edge of the figure. Growth cones (small arrows) and thick axons tend to lie in the lower part of the figure, whereas fine axons are beginning to accumulate deeper near the top of the figure. G; glial profiles. × 12,900.

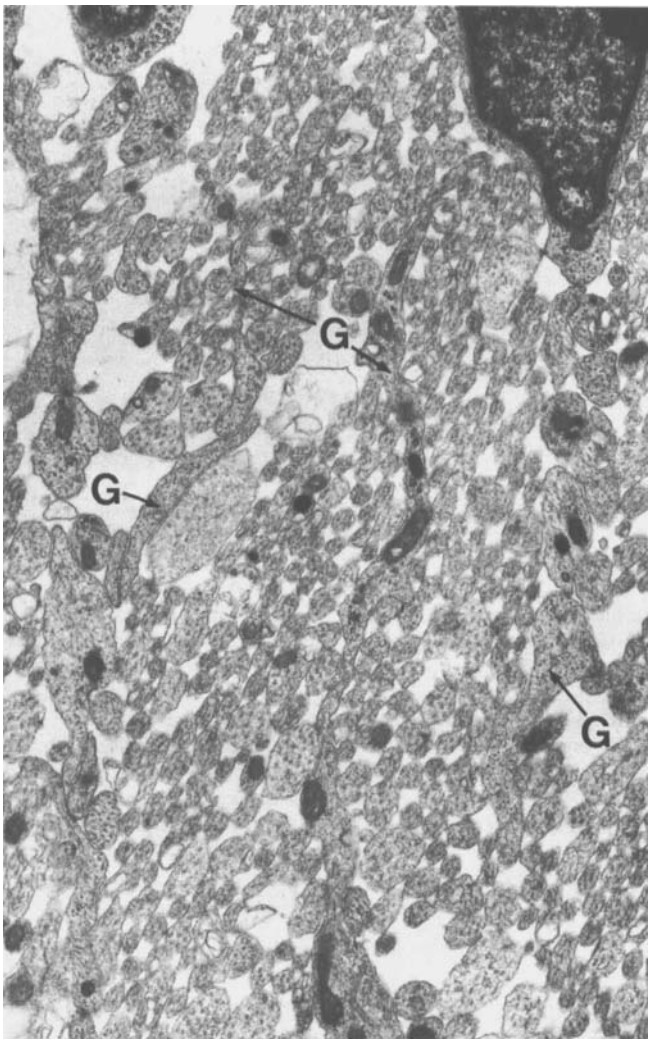
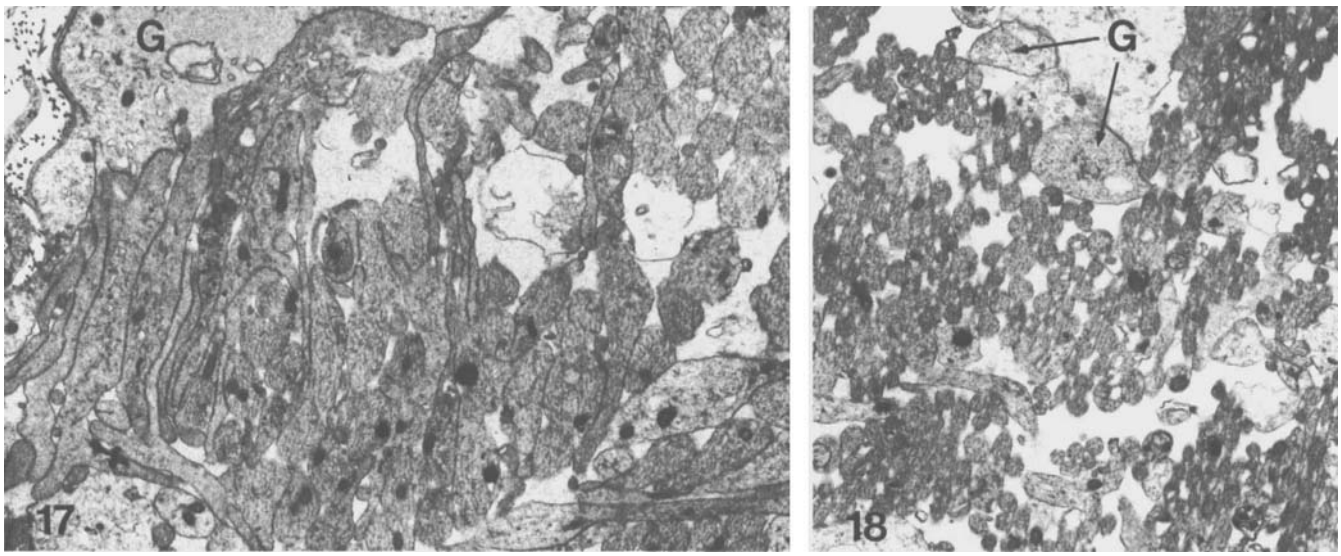


Fig. 16. A field of fibers from a region deep to Figure 15. The majority of the fibers are extremely fine. Slender radial glial processes are marked G. The cell nucleus at the top of the figure marks the ventral extent of the periventricular zone. ×12,900.

The relative movements that produce the changes between the earlier and later stages are undefined. That is, we do not know whether the optic foramen has moved relative to the nerve, or whether the structure of the extracranial portion invades the intracranial portion. There are reasons for favoring the latter view (see below). Nor do we know whether "hypothalamic" cells are invading regions earlier identified as "stalk," or whether stalk cells are forming hypothalamus. However, the relative shift of the border between the two types of glial organization can also be noted in the relationship of the ipsilateral optic bundle. This bundle leaves the brain within the radial glial portion at early stages (Fig. 3), but at later stages leaves at the junction between the interfascicular and the radial glial portions (Fig. 5).

At more advanced developmental stages, the change in structure of the radial portions of the pathway continues, so that at first glial nuclei are seen invading the zone between the bundles that approach the optic chiasm (Fig. 12) and later on one sees glial nuclei even among the fiber bundles of the optic tract, which at the stages under consideration (up to 27 days) is essentially free of nuclei as in Figure 11. That is, the interfascicular type of organization gradually proceeds from the extracranial nerve centrally through the intracranial nerve, chiasm, and tract.

The appearance of glial nuclei among fiber bundles could be produced by newly developing fibers running deep to the most superficial nuclei and so including them among the bundles, or it could be produced by a migration of glial



Figs. 17, 18. Fibers of the optic tract. Figure 17 comes from a region close to the pia (note the basal lamina at upper left) and Figure 18 comes from the deeper parts of the optic tract. Note the finer, deeper fibers and the slender (lamellopodial) extensions near the pia. G, glial processes. ×9,200.

nuclei into the fiber region. We favor the second interpretation because, as shown below, there is evidence that the newly entering fibers join the subpial parts of the intracranial fiber systems, not their deep parts.

Fine structural relationships of retinofugal fibers in eye-stalk and optic nerve

The relationships between the nerve bundles and the glial cells of the distal, extracranial eye-stalk resemble those described for other species (see Silver and Robb, '79; Silver and Sapiro, '81; Horsburgh and Sefton, '86; Williams and Rakic, '85; Williams et al., '86). With the light microscope one can see small fiber bundles scattered amongst the nuclei, and the electron microscope shows the fiber bundles lying next to glial cells, generally wrapped up by slender glial processes. The bundles vary in size, containing from a few fibers per bundle, up to 100 or more (Figs. 13, 14). Within the bundles the axons show regularly spaced microtubules, occasional small mitochondria, small profiles of smooth endoplasmic reticulum, and a few vesicles. Growth cones are recognizable as regions where the tubule containing axons give off slender extensions characterized by a dark, finely filamentous, tubule-free cytoplasm. These lamellipodia are generally distinguishable from tongues of glial cytoplasm, particularly where the latter contain ribosomes, but where the glial cytoplasm is free of ribosomes the distinction can be made only on the basis of serial sections (Williams et al., '86; Colello, '86). Axons and glia are intimately enwrapped with each other in the regions of the growth cones. There is no clear indication in any of the individual sections of the distal eye-stalk or optic nerve that the growth cones show any marked preferred distribution (central versus peripheral or dorsal versus ventral) in a bundle or in the optic nerve as a whole. They seem to be relatively evenly scattered throughout all of the bundles at all relevant stages. This is in sharp contrast to the relationships in the intracranial eye-stalk (see below).

We have seen that in the intracranial part of the eye-stalk having the radial glial organization, the axons no longer form distinctive separate bundles; instead they form a more extensive and continuous field among the slender glial processes that stretch from the nuclei in the periventricular zone to the subpial endfeet. There are fewer glial tongues wrapping individual bundles as the chiasm is approached. The electron microscopical appearances confirm the light microscopy. The change is gradual. At first where the eye-stalk becomes broader at the level illustrated in Figures 8 and 9, the bundles tend to collect near the surface, then the bundles become larger, and finally at the levels of Figures 11 and 12 they fuse, forming an almost continuous field in the chiasm and tract. As this change occurs there is an associated, gradual change in the relative distribution of different types of axonal profile. The finest axons tend to be deepest, most of the growth cones tend to lie superficially, and relatively large axonal profiles come to lie scattered among the growth cones or just deep to them. This separation of profiles continues into the optic tract where it is most marked.

Figures 13 and 14 come from the distal, interfascicular part of the optic nerve, Figure 14 being closer to the eye than Figure 13, and these show the nerve bundles lying between glial cells with growth cones individually distributed across the bundles, as demonstrated by others for the cat (Williams et al., '86) and the rat (Horsburgh and Sefton, '86). At these levels there is no clear segregation of fiber diameter classes. Figures 15 and 16 come from the distal portion of the nerve having a radial glial organization, comparable to the level illustrated in Figure 8. Figure 15 shows the region next to the surface, whereas Figure 16 shows the deepest fiber region. The contrast in the size of the axonal profiles is evident as is the difference in the

distribution of the neuronal growth cones and their lamellipodia. Growth cones are commonly encountered near the surface, but are not seen in the deeper regions.

Figures 17 and 18 are taken from the superficial (Fig. 17) and deep (Fig. 18) parts of the optic tract and show that the segregation present in Figures 15 and 16 is even more marked in this region, growth cones and thick axons being present superficially, fine axons being concentrated in the deep regions.

The change in the distribution of glial profiles can also be seen in these electron micrographs, with relatively slender radial glial processes characterizing the more central parts of the optic nerve and tract.

The relationship between the growing axons, the melanin, and the various signs of cellular or cytoplasmic degeneration is complex. Nearest the eye the axons never lie close to the cells that bear the melanin rosettes. However, occasionally a small bundle can be seen passing between melanin-bearing cells in which the melanosomes are not associated with lysosomes (Fig. 14). In more proximal regions, nearer the optic formamen, melanin-bearing cytoplasmic processes can also be seen among fiber bundles, but again these processes show no signs of degeneration or of lysosomes. We have seen that signs of cellular degeneration generally precede the ingrowth of axons. However, it is also common to see cellular debris, often in macrophage-like cells, among the advancing fiber bundles. Although this debris is rather widely distributed, it is most common in the regions where the eye-stalk broadens out and where the ipsilateral optic bundle leaves the main bundles that are heading for the optic chiasm.

The extent to which the cellular degeneration produces extracellular spaces is uncertain. It is clear that as axons are advancing there are extensive extracellular spaces (see Silver and Sidman, '80; Horsburgh and Sefton, '86) especially in the outer, subpial regions. These spaces are irregular, and even where sections are cut parallel to the ingrowing fibers, the spaces appear to be interrupted by the radial processes of glial or epithelial cells. There is no indication in our material that these spaces form oriented channels that might serve to direct advancing axons (see, e.g., Silver and Sidman, '80), or that the pattern of the degeneration changes between the peripheral and the central parts of the optic nerve.

Finally, the relationship between the axons and the basal lamina of the eye-stalk must be considered. As in other mammalian species that have been studied (Williams et al., '86; Horsburgh and Sefton, '86) and in contrast to some nonmammalian forms (Easter et al., '84), the axons are everywhere separated from the basal lamina by a continuous coat of subpial glial endfeet. Apart from the relationships of the ipsilateral optic bundles (see Guillery and Walsh, '87), we have seen only a single instance of a small axonal profile insinuated between two glial endfeet and lying next to the basal lamina of the eye-stalk.

DISCUSSION

Fiber arrangements in the prechiasmatic nerve

We have shown that the ferret's eye-stalk develops as a broad funnel-shaped outgrowth that narrows toward the eye and that can, at an early stage (24 to 25 days), be divided into two parts. The slender peripheral, extracranial part looses its ventricular cavity early, and in it the bundles of retinofugal axons pass among the perikarya of the interfascicular neuroepithelial or glial cells. The broader intracranial part retains its ventricular cavity for longer. It has an inner, well-defined periventricular zone within which are crowded a great many nuclei but initially no nerve fibers, and an outer, subpial zone within which the nerve bundles pass among the radial cytoplasmic processes of the neuroepithelial cells.

The border between the two types of glial organization shifts as development proceeds. The "radial glial" zone of the intracranial portion appears gradually to be invaded by glial nuclei and to take on the "interfascicular" appearance of the extracranial portion. During the period of development studied here, interfascicular glial nuclei invade all parts of the optic nerve and begin an invasion of the optic chiasm.

Whereas at 24 to 25 days growth cones and axons of varying diameters are more or less evenly distributed among the bundles and within the bundles of the extracranial part, the growth cones begin to accumulate toward the ventral pial aspect of the individual bundles and in the most ventral of the bundles as these enter the radial glial portion of the broad intracranial part of the eye-stalk. Here the growth cones are also accompanied by a population of particularly thick fibers. The growth cones and thick fibers continue in their preferred subpial position throughout the optic chiasm and into the tract, where their segregation from the deeper, very fine axons is more complete. The segregation of the growth cones and thick axons in the central parts of the pathway represents a major change, readily visible in the sections (Figs. 13–16) and distinct from the subtle gradients demonstrable in the intraorbital parts of the cat's optic nerve at relatively later developmental stages (Williams et al., '86).

The change in the position of the growth cones has also been noted in the mouse (Bovolenta and Mason, '86) and relates to earlier observations of the ferret (Walsh and Guillery, '85; Walsh, '86), which showed that as the retinofugal fibers approach the optic chiasm and tract, the newest fibers tend to take up a superficial, subpial position. The change in the position of the thickest fibers is less readily interpreted on the basis of the data presented here. These thickest fibers show none of the characteristics of the "cores" of growth cones (see e.g., Williams et al., '86), but rather resemble the thinner profiles in containing transversely cut microtubules. Our first interpretation was to consider that since these axons are thicker they must be older, but evidence obtained from serial reconstructions suggests that these thick fibers are the broad axonal portions that lie just proximal to growth cones. These have been dubbed axonal "wrists" by Maggs and Scholes ('86) in their study of developing retinotectal axons of cichlid fish, and it appears that these thicker regions can attain significant lengths and confuse a classification of axons made on the basis of diameter.

It is evident that a change in the distribution of the growth cones, and therefore a change in fiber order, occurs where there is a change in the character of the glial cells. This is closely comparable to the observation made by Maggs and Scholes ('86) of a change in fiber order occurring at a site where the nature of the glia is also changing. Their study dealt with the immunohistochemical nature of the glia, whereas ours deals with glial structure only. Unfortunately, the markers used by Maggs and Scholes develop later in mammalian development than the stages studied here, and the differences seen between the perinatal intra- and extracranial segments of the rat's nerve (Small, '86)

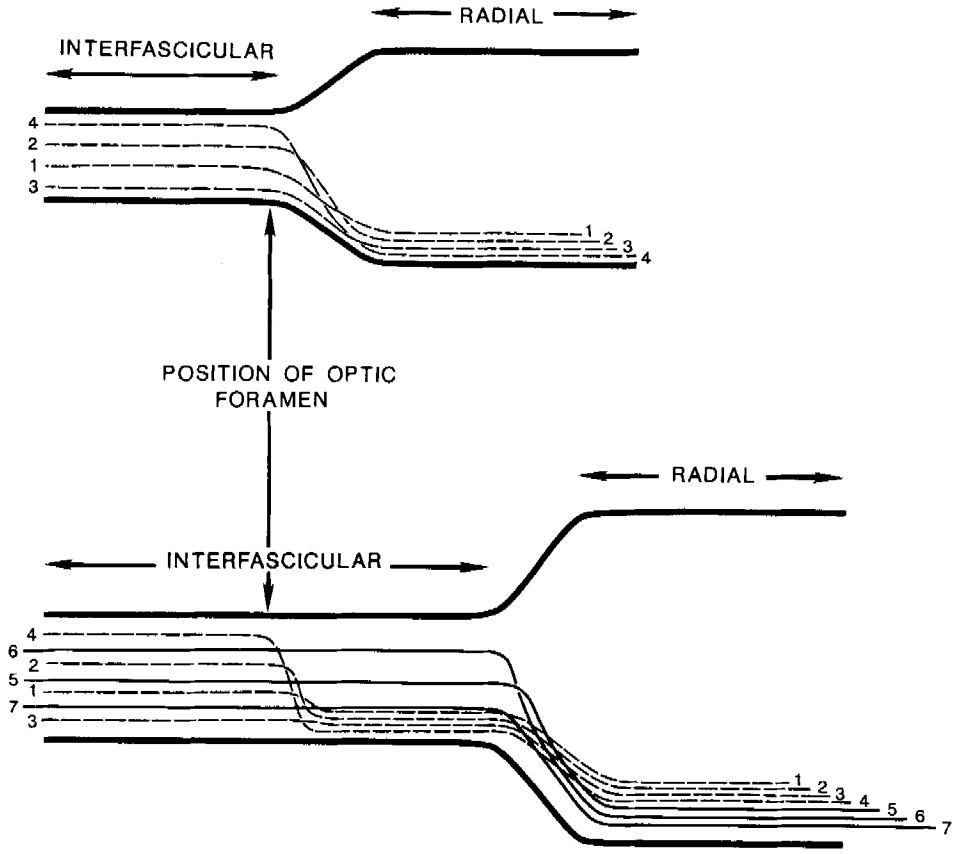


Fig. 19. Schema to illustrate the shift in fiber order that occurs within the optic nerve. The axons are indicated in order of age, 1 being the oldest. The first four are shown as interrupted lines and these reached the border between the two types of glial organization (interfascicular and radial) early, when the border was close to the optic foramen. The next three are shown as continuous lines and these reached the border later, when it was closer to the brain (to the right of the figure). It is postulated that the developing axon tips move toward the pial surface when they reach the radial glia, and that the partial reversal of order is produced by this and by the shift in the border of glial types.

cannot be readily related to the present studies. It is possible that the change in the appearance and structure of growth cones reported by Bovolenta and Mason ('86) also relates to the structure of the glial environment, but this remains to be defined.

The nature of the interaction between the glial cells and the growing axons is not known. One can postulate that the radial glia of the intracranial eye-stalk have subpial endfeet that are more attractive to growing axons than are the other glial surfaces, whereas the glia of the extracranial eye-stalk lack such a differential property. Silver and Rutishauser ('84) have described a distribution of NCAM on the endfeet of radial glia along the path of developing retinofugal axons in chicks, but they have not shown a change in the distribution or character of the NCAM between the eye and the chiasm that might relate to a change in axonal position comparable to that described here. This may indicate that NCAM is not relevant to the change in fiber order; it may indicate a difference between species, or it may indicate that the differential distribution is too subtle to be detected, or only occurs during a limited developmental period. The problem remains to be addressed.

The observations reported earlier (Walsh, '86) agree with the present observations in showing that newly growing nerve fibers move toward the ventral, pial surface before they reach the optic chiasm. The two sets of observations appear to differ because the earlier experiments showed that the youngest fibers first become segregated near the deep (inner) parts of the optic nerve as this passes through the optic foramen and then accumulate in a ventral, subpial position as they approach the chiasm. In the present material we have seen nothing in terms of the distribution of growth cones that corresponds to the first, deep segregation of the youngest fibers. The difference could be that we have not studied any stages after intrauterine day 27, whereas the earlier studies were concerned with changes occurring between days 27 and 34, but there is an alternative explanation. It is probable that the pattern of change in fiber position observed previously (Walsh, '86) is produced because the part of the eye-stalk containing radial glia becomes relatively shorter during development and the part having an interfascicular organization extends intracranially. Such an explanation was suggested in the earlier study and our observations on the pattern of invasion of interfascicular glial nuclei into the zone of radial glial fibers in the intracranial nerve confirm that the border of the two types of organization shifts during development (compare Figs. 3 and 4).

Figure 19 shows the effect that a moving border between interfascicular and radial portions of the nerve will have on fiber order if fibers are arranged according to age in the radial portions but not the interfascicular portions. The upper part of the figure shows an early stage of development with four representative axons (1 being the oldest, 4 the youngest) taking up an age-related order when they arrive at the border of the radial portion. The lower part of the figure shows a later stage when this border has shifted toward the brain (toward the right side of the figure). Axons numbered 5, 6, and 7 in the figure arrive later and do not take up their ordered positions superficially until they reach this now centrally displaced border. As a result, in the most peripheral part of the nerve (to the far left), the fibers are not arranged in a temporal order at all. There then follows a segment where older fibers (1 to 4) lie nearer the surface than younger fibers (5 to 7). There may be some order among these older fibers that reverses this sequence (that is 4 lies nearer the surface than 1), but the methods that have been used for studying the system would not discriminate this level of order. Finally, in the most central parts of the nerve one sees the age-related order that is characteristic of the optic tract, and continues into the tract.

We have not considered the distribution of the melanin or that of the degenerative changes in the eye-stalk as relevant to the production of the patterns of axonal growth in the nerve and tract. Although there are good reasons for thinking that melanin bears some relationship to the development of chiasmatic pathway patterns (see e.g., Strongin and Guillery, '81), this relationship is concerned specifically with the formation of the relatively small uncrossed component, not, so far as is known, with the "chronotopic" order that has been the focus of this study. Our material has shown that there is a brief period of development when a small population of melanosomes extends as a narrow tongue through the extracranial eye-stalk, that these melanosomes serve to distinguish extracranial from intracranial segments of the nerve, that some of these melanosomes appear to undergo lysis, and that some growing nerve fibers lie adjacent to the melanosome-bearing cells. However, the nature of the interaction between the nerve fibers and the melanosomes remains to be defined (see Silver and Sapiro, '81; Strongin and Guillery, '81).

The role played by the wave of degeneration that passes from the eye-cup to the brain is also undefined. It may serve to provide appropriately loose extracellular spaces for the advance of growing axons; it may contribute to the formation of channels that guide axons (see Silver and Sidman, '80); it may serve to bring in macrophages that, in turn, play a role in guiding axons (Colello, '86); or it may have a significance that is currently undefined. Given the distribution of the degenerative changes, there is at present no reason for relating it to the development of a chronotopic order in the retinofugal pathway.

Development of the optic chiasm and supraoptic commissures

The development of components of the supraoptic commissures before any of the retinofugal fibers reach the midline was at first sight surprising but should perhaps have been expected. We have argued here and elsewhere (Torrealba et al., '82; Walsh et al., '83; Walsh and Guillery, '85) that fiber order in the optic tract represents time of arrival, the deepest fibers being the earliest. Because the fibers of the supraoptic commissures lie deep to the tract, it is not surprising that they are the oldest. It would appear that throughout the development of the system of fibers crossing the midline in the chiasmatic region, new fibers are always added to the pial surface. Further, because in the adult there is a significant mingling of the chiasmatic and supraoptic fibers, it is reasonable to assume that the earliest retinofugal fibers grow along the surface of the supraoptic commissures, mingling with the more ventral of the commissural fibers and possibly being guided across the midline by them. The relationships of the earliest retinofugal growth cones as they cross the midline are likely to be of interest from this point of view.

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