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Age-Related Fiber Order in the Ferret's Optic Nerve and Optic Chiasm

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Although the mammalian optic tract shows a grouping of fibers by age, with newer fibers nearer the pial surface, the possible rules for fiber ordering in the mammalian optic nerve have not been well defined. In this study, preferential labeling of the older retinal fibers in the ferret, a close relative of the cat, shows that the age-related fiber order in the ferret's optic tract reflects a systematic sorting of fibers by age that occurs in the optic nerve, and that is maintained through the optic chiasm. The older retinofugal fibers, dispersed throughout the nerve near the retina, come to be limited to the perimeter of the nerve as it passes through the optic foramen, while newer fibers come to lie nearest the center of the nerve. These newest fibers approach the ventral surface of the brain nearer the optic chiasm. In the chiasm, as in the tract, the oldest fibers lie furthest from the pial surface of the brain, while newer fibers lie nearer the surface.

The age-related fiber ordering in the ferret's optic nerve, with the newest fibers initially being furthest from the surface at the optic foramen, differs from age-related orderings seen in non-mammalian vertebrates, where the newest fibers are always nearest the surface. The changing patterns of fiber ordering along the ferret's optic nerve may relate to changes in the underlying glial structure of the developing nerve.

A series of studies has established that the cat's optic tract represents a grouping of fibers by age, with newer fibers nearer the pial surface. Fibers in the tract are partially segregated in terms of size (Bishop and Clare, 1955; Bishop et al., 1953; Guillery et al., 1982), and this reflects the formation of retinal ganglion cells of different size classes as several, temporally shifted waves (Kliot and Shatz, 1982; Polley et al., 1981; Walsh et al., 1983). Moreover, the rough retinal mapping in the tract among fibers of each size class (Aebersold et al., 1981; Mastronarde, 1984; Torrealba et al., 1981, 1982) matches the topographic pattern of each wave of ganglion cell production (Walsh and Polley, 1985).

The cat's optic nerve does not show a segregation of fibers by size like that seen in the tract (Donovan, 1967; Hughes and Wässle, 1976; VanCrevel and Verhaart, 1963; Williams and Chalupa, 1983). Therefore, the sorting of fibers in the optic tract by size (and hence by *age*) must represent a systematic rearrangement of fibers between the nerve and the tract. The goal of this study was to determine where this age-related sorting occurs.

In the ferret, a close relative of the cat, the older retinofugal fibers in the tract have been labeled directly. These older fibers lie deep in the tract, with newer fibers nearer the pial surface, and this age-related grouping represents the sequence of axon arrival to the tract (Walsh and Guillery, 1985). In this previous study, some sections of the optic nerve also showed a tendency for newer and older fibers to be segregated. However, since the optimal plane of section for analysis of the nerve is perpendicular to that needed for analysis of the tract, this material was too limited to allow a full description. Further experiments using the same technique with frontal and parasagittal sections now allow a more complete description of the age-related fiber order in the ferret's optic nerve, and a comparison of the nerve and the tract.

Materials and Methods

The strategy of these experiments was to make intravitreal injections of wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) into the retinae of fetal ferrets, between the 27th and 29th days of life (E27–E29), while the retina is still adding new ganglion cells (Walsh and Guillery, unpublished observations of the ganglion cell layer after *in utero* administration of ^{3}H-thymidine). At this stage the retina is still very immature histologically (Greiner and Weidman, 1981), and many retinal axons have not yet reached the optic tract (Cucchiaro and Guillery, 1984). By allowing a long survival time during which the fetus developed normally, it was possible to label the older retinal cells while newer cells, formed after the WGA-HRP had been cleared from the retina, were exposed to less WGA-HRP and were unlabeled. Since most or all of the retinofugal axons have reached the ferret's optic tract by E34 or soon after (Walsh and Guillery, 1985), the animals were sacrificed at E34 or later.

Intravitreal injections of 0.3–0.4 μl of a 2% solution of WGA-HRP (Sigma) in 0.9% saline were made into fetal ferrets of known age, according to a surgical procedure described in detail elsewhere (Walsh and Guillery, 1985). Pregnant jills were purchased from Marshall Research Animals (North Rose, NY). Under Nembutal anesthesia, a midline abdominal incision was made to expose the uterus. Individual fetuses were located and the uterine wall incised to expose the head of the fetus. Injections were made into one or both eyes using glass micropipettes, after which the fetus was returned to the uterus and the uterine incision closed. After injections had been given to several animals, the abdomen was closed and the jill was allowed to recover.

After 5, 7, or 12 d (see Table 1), the jill was overdosed with Nembutal and the young removed and perfused with 0.9% saline followed by 4% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4). Each head was trimmed, embedded in a gelatin–albumen mixture, and allowed to sink in 30% sucrose. Sections were cut at 30 μm in the frontal, parasagittal, or horizontal planes, and were then reacted for HRP using the tetramethylbenzidine protocol of Mesulam (1984), with a neutral red counterstain. Since the optic nerve on the uninjected side showed scant or no label following unilateral injections (see Results), the 2 optic nerves of animals with bilateral injections were considered separately. In each

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Table 1. Animals used in this study

Animal	Age at injection	Age at sacrifice
82-123	E27	E28
82-125	E27	E28
81-349(2)	E27	E39
84-401	E28	E35
84-410R	E28	E35
84-410L	E28	E35
84-411R	E28	E35
84-411L	E28	E35
84-413	E28	E35
84-420	E28	E35
82-138	E29	E34
82-140	E29	E34
82-141	E29	E36
82-148	E29	E36
82-149	E29	E36
82-27	E34	E39
82-28	E34	E39
82-20	E35	E39
82-21	E35	E39

Each animal received unilateral or bilateral intravitreal injections of a 2% solution of WGA-HRP in 0.9% saline. Several animals were used in a previous study of the optic tract (Walsh and Guillery, 1985), and the nerves of these animals were either sectioned horizontally with the brain or were embedded separately and sectioned frontally. The brain, retina, and optic nerves of the other animals were sectioned as one block either frontally or parasagittally.

case, sections of the optic tract were also taken for comparison with the nerve and with previous results.

As controls, 4 animals received injections of WGA-HRP on E34 or E35, and were killed after a 4–5 d survival time, and 2 animals received injections on E27 and were killed 20 hr later, on E28. Each of these animals was then prepared as described above.

Results

Pattern of HRP label in the retina

All operated retinae were sectioned; in most cases, they looked histologically normal (*cf.* Greiner and Weidman, 1981). In 4 cases the retina was severely reduced in size and showed poor histological differentiation, while the ipsilateral optic nerve was smaller than that on the untreated side. These retinae appeared to have suffered severe damage, and these cases were not analyzed further and are not included in Table 1. The remaining description includes 19 cases, all showing normal retinal histology.

In 2 animals killed 20 hr after receiving WGA-HRP on E27, HRP label in the retina was uniform across all retinal layers and all the way out to the retinal periphery, indicating that initial distribution of WGA-HRP was uniform across the retina. Label was also uniform across the ganglion cell layer, and extended into other retinal layers, in animals given injections at later ages (E34, E35), even when processed after 4–5 d survival times. The dense packing of cells, and the cytoplasmic localization of the granular reaction product, precluded easy distinction of labeled and unlabeled cells.

The pattern of label in the retina differed markedly when intravitreal injections into immature animals (E28–E31) were followed by long (5–7 d) survival times. In these animals, the HRP reaction product was largely confined to the ganglion cell layer, being heaviest in central retina and gradually becoming more sparse in peripheral regions. Again, label could not be clearly localized to individual cells, and it is possible that, in some areas, unlabeled cells and labeled cells were intermingled. The entire periphery of the ganglion cell layer, near the ora serrata, was lightly labeled or unlabeled. This is illustrated in Figure 1, which shows the pattern of label in the retina of a ferret given WGA-HRP on E28 and killed 7 d later, on E35 (84-411R). Six other cases treated similarly showed the same pattern of label. In each case, the unlabeled region was wider ventrally than dorsally and tended to be larger in sections taken nearer the temporal edge of the retina (not shown). Three animals given injections on E29 and killed on E36 showed the same central–peripheral, nasal–temporal, and dorsal–ventral differences in label. A photomicrograph showing the peripheral retina of one of these animals (82-148) is illustrated elsewhere (Walsh and Guillery, 1985, Fig. 9). Another of these retinae (82-141) was sectioned horizontally and showed an unlabeled zone that was larger temporally than nasally. Two animals that received injections on E29 and were killed on E34 showed somewhat heavier label in the ganglion cell layer, with the unlabeled peripheral zone being narrower and of approximately the same size in all retinal quadrants. Finally, 1 retina prepared with a 12 d survival time showed very little HRP label in the retina, despite distinct label in the nerve and tract, and so this very long survival time was not used routinely.

Whereas the distribution of HRP label followed the same rules (in terms of central–peripheral and dorsal–ventral differences) in all animals treated similarly, the overall intensity of label and

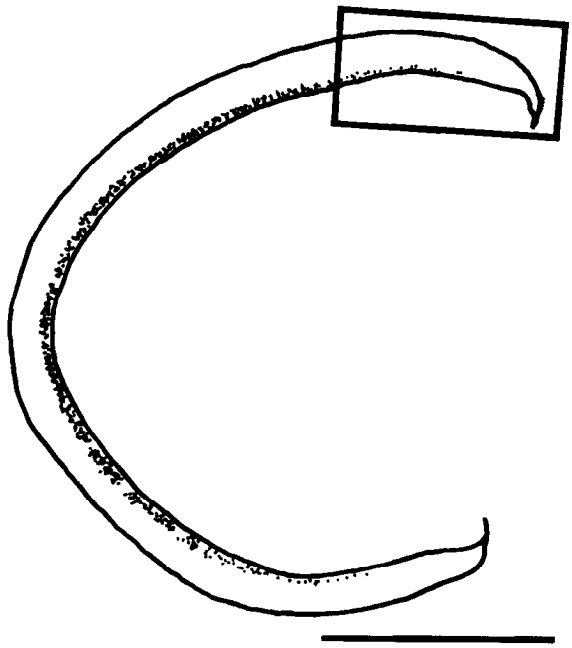

Figure 1. Illustration of the nonuniform pattern of retinal label produced when intravitreal injections of WGA-HRP into immature fetal ferrets are followed by long survival times. This drawing shows a frontal section of the retina of a ferret given WGA-HRP on E28 and killed at E35 (84-411R). The *dots* represent HRP reaction product. Because of the dense packing of cells in the ganglion cell layer, and because of the cytoplasmic location of the granular reaction product, separate labeled and unlabeled cells could not be distinguished with certainty. It is possible that, in some areas, unlabeled cells lie among labeled cells. A photomicrograph, taken from a different animal (82-148), of a region corresponding to that enclosed in the box is illustrated elsewhere (Walsh and Guillery, 1985, Fig. 9). Label is heavier in central retina than in peripheral retina, and an unlabeled zone of the ganglion cell layer lies next to the ora serrata, the peripheral edge of the retina. This unlabeled zone is larger in ventral retina (*down* in the figure) than in dorsal retina. Medial is to the *left*. Scale bar, 500 μm.

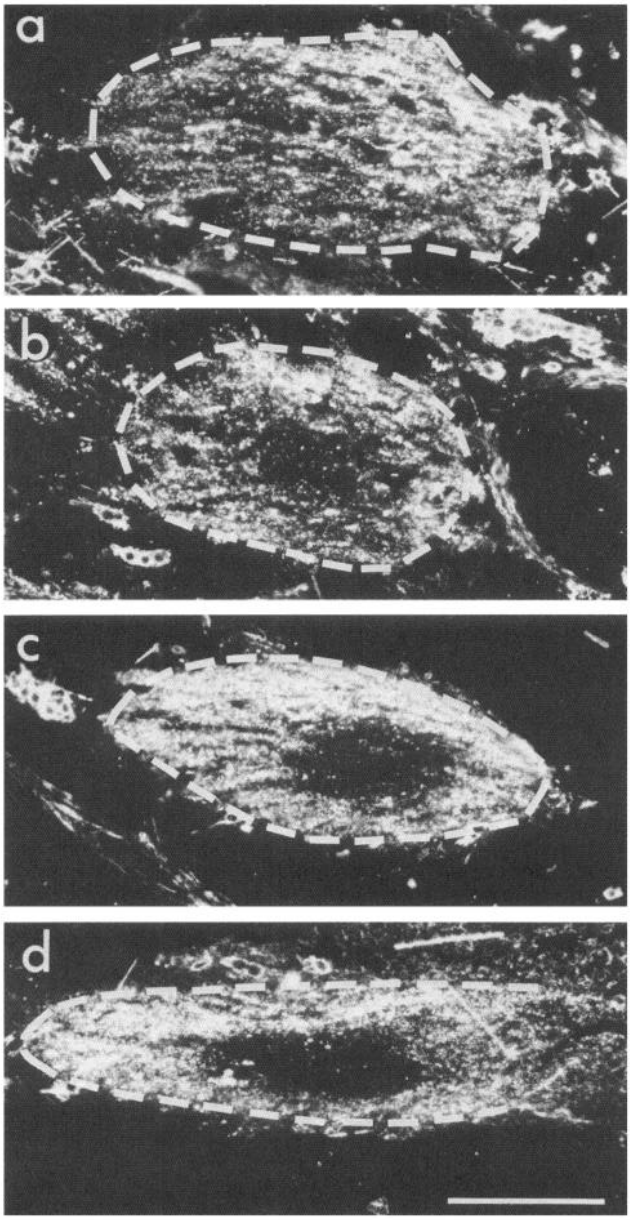

Figure 2. These dark-field photomicrographs illustrate the typical pattern of label in the optic nerve following the preferential labeling of the older retinofugal fibers. The figure shows 4 frontal sections taken from a ferret (84-411L) that received an intravitreal injection of WGA-HRP on E28 and that was processed on E35. *a*, Section 810 μm behind the optic nerve head, about two-fifths of the way from the eye to the chiasm. Whereas nearer the retina label tended to be darker in the center of the nerve than at the perimeter (not shown, but see Fig. 3*a*), at this level label was distributed over the entire cross section of the nerve, although somewhat darker dorsally. *b*, Section 1080 μm behind the eye, roughly halfway from the eye to the optic chiasm. Here, as the nerve entered the optic foramen, the older, labeled fibers were grouped around the perimeter of the nerve, especially dorsally, with unlabeled fibers in the center of the nerve. As the nerve left the optic foramen, three-fourths of the way to the chiasm (*c*), this segregation was sharper. Within 90 μm of the point where the 2 nerves joined to form the chiasm (*d*), the segregation of labeled and unlabeled fibers was even sharper, and label was quite light at the ventral surface of the nerve. In each part of the figure, dorsal is *up*; lateral, *left*. The outlines of the nerve are shown with *interrupted white lines*, except the medial border of the nerve in *d*, which could not be clearly defined. Scale bar, 100 μm.

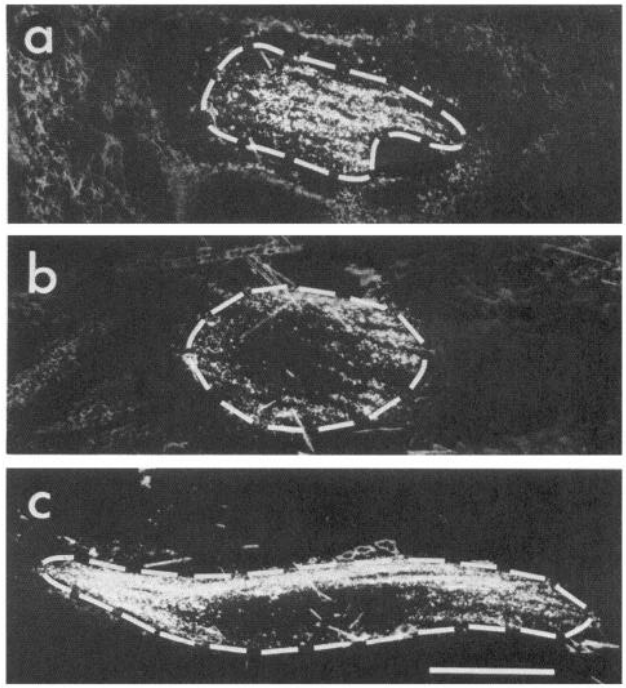

Figure 3. These dark-field photomicrographs illustrate the distribution of the older optic nerve fibers as seen in parasagittal sections taken from an animal that received an injection on E28 and that was processed on E35 (84-410R). *a*, Taken 180 μm behind the optic nerve head (about one-tenth of the way to the optic chiasm) shows label concentrated in the center of the nerve, with lighter label near the perimeter of the nerve. Sections taken further from the eye showed label distributed more evenly over the section (not illustrated, but see Fig. 2*a*). *b*, Taken 1080 μm from the eye, just over halfway to the chiasm, as the nerve passed through the optic foramen. At this level, labeled axons were limited to the perimeter of the nerve, with lighter label in the center of the nerve. *c*, Taken 1530 μm from the eye, just before the nerve fused with the wall of the diencephalon but still 360 μm from the optic chiasm. This section, which is rather oblique with respect to the axis of the retinal fibers, shows labeled axons now limited to the rostral, caudal, and dorsal perimeter of the nerve, with no label ventrally or in the center of the nerve. In each photomicrograph, dorsal is *up*; rostral, *right*. The outlines of the nerve are shown with *interrupted white lines*. Scale bar, 100 μm.

the size of the unlabeled zones varied somewhat, even in animals that were nominally of the same age at the time of the injection and of sacrifice. The intensity and topographic extent of label were closely correlated: Retinae with heavier label always showed a smaller unlabeled region in peripheral retina, while more lightly labeled retinae showed a larger unlabeled zone. This variability in label is taken up in the Discussion.

Pattern of HRP label in the optic tract

The pattern of label in the optic tract was entirely consistent with an earlier description (Walsh and Guillery, 1985), which included several of the animals used in the present study. The nonuniform pattern of retinal label was always accompanied by a nonuniform distribution of label in the contralateral optic tract. Labeling in the tract contralateral to the injection, when uneven, was always heaviest in its deepest zones, furthest from the pia, with lighter or no label nearer the pia. The proportion of the tract covered by label correlated with the intensity and topographic extent of the label in the retina: Animals with the lightest retinal label and with a large unlabeled zone in peripheral retina showed HRP reaction product only in the deepest parts of the tract, while animals with heavier and more extensive retinal label showed heavy label over a larger portion of the

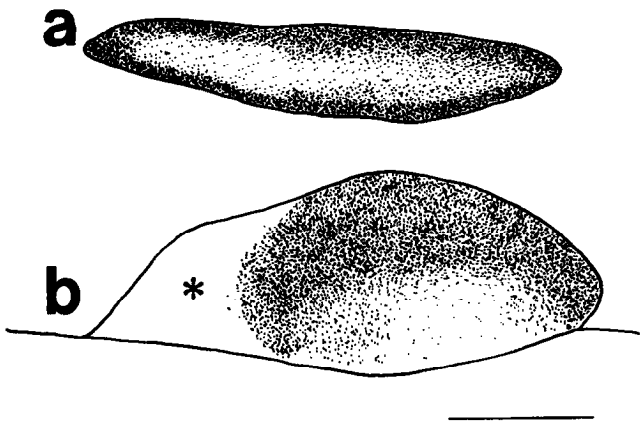

Figure 4. Drawings of parasagittal sections through the optic nerve (*a*) and optic tract (*b*) from an animal given WGA-HRP on E28 and killed on E35, but which showed relatively heavy label throughout the visual pathway. *a*, Section of the nerve 300–400 μm before the optic chiasm (same level as in Fig. 3*c*) shows a circumferential pattern of label that is fairly symmetrical and was only well seen in sections of the intracranial segment of the nerve. The unlabeled fibers in the center of the nerve shifted ventrally as the nerve fused with the diencephalic wall and remained ventrally adjacent to the pia through the chiasm (not shown) and into the optic tract (see *b*). In both *a* and *b* dorsal is *up*; rostral, *right*. Scale bar, 100 μm. *b*, Outlines show the pial surface of the brain ventrally, and the border of the tract, with the supraoptic commissures (the nonretinal component of the tract) indicated by the *asterisk*.

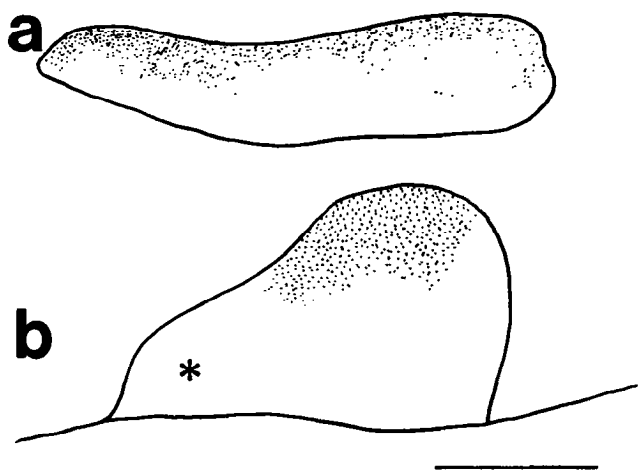

Figure 5. Drawings show the nerve (*a*) and tract (*b*) of another animal, which also received an injection at E28 before being processed at E35 and which showed the lightest label. *a*, Section of the nerve 300–400 μm before the chiasm (same level as in Figs. 3*c* and 4*a*) shows the labeled fibers scattered over the dorsal border of the nerve. These labeled fibers remained dorsally through the chiasm and in the optic tract (*b*), representing the deepest (and hence the oldest) fibers in the tract. Orientation is as in Figures 3 and 4, and the *asterisk* in the lower drawing indicates the supraoptic commissures. Scale bar, 100 μm.

tract. All animals in which retinal label was uniform also showed uniform label in the contralateral tract. Since details of the age-related fiber ordering in the tract have been established for several species by many different methods (Easter et al., 1981; Gaze and Grant, 1978; Herrick, 1941; Rusoff, 1984; Torrealba et al., 1982; Walsh and Polley, 1985; Walsh et al., 1983), the pattern of label in the tract was exploited as an indication in each experiment of the relative age of the retinal fibers labeled.

Pattern of HRP label in the optic nerve

All animals showing a nonuniform distribution of label in the retina and optic tract also showed a consistent pattern of nonuniform label in the optic nerve. For the purposes of describing this pattern, it is convenient to distinguish the intraorbital segment of the nerve, which extends from the retina to the optic foramen, and its intracranial segment, which extends from the optic foramen to the point where the two nerves join to form the optic chiasm.

Whereas near the retina label was widely scattered across the nerve, nearer the brain the label was limited to the perimeter of the nerve. This changing pattern is illustrated in frontal sections in Figure 2, which shows the optic nerve of an animal given WGA-HRP on E28 and killed on E35. Sections taken immediately behind the eye showed a modest tendency for label to be heavier in the center of the nerve than at its perimeter (not shown, but see Fig. 3*a*). However, such a central–peripheral gradient was not seen in sections taken further posteriorly along the intraorbital segment of the nerve. At this level, label was widely scattered all across the nerve, although somewhat heavier dorsally than ventrally (Fig. 2*a*). As the nerve approached the optic foramen, the pattern of label changed. Label in the center of the nerve became lighter, as labeled axons became limited to the perimeter of the nerve (Fig. 2*b*). This segregation continued as the nerve passed through the optic foramen, so that at the beginning of the intracranial segment of the nerve, the label was restricted to the circumference of the nerve, with little or no label in the center of the nerve (Fig. 2*c*).

As the nerve approached the brain, the segregation of labeled and unlabeled fibers continued to sharpen, while the pattern of label also changed. Whereas label ventrally in the nerve tended to be lighter than label dorsally near the retina, this tendency became more marked as the nerve approached and then fused with the wall of the diencephalon (Fig. 2*d*). At this point of fusion and nearer the optic chiasm, the ventral border of the nerve always showed very light label.

A similar pattern of label is illustrated in parasagittal sections in Figure 3, which shows sections of the optic nerve of another ferret that received WGA-HRP on E28 and was killed on E35. Near the retina, label was heavier in the center of the nerve than at the circumference (Fig. 3*a*), but this central–peripheral difference was not seen further posteriorly in the intraorbital segment of the nerve (not shown). As the nerve passed through the optic foramen, the labeled axons became limited to the perimeter of the nerve (Fig. 3*b*). Nearer the brain, the intracranial segment of the nerve showed a progressive sharpening of the segregation of labeled and unlabeled fibers, along with a loss of label at the ventral extreme of the nerve (Fig. 3*c*).

This same, nonuniform pattern of label in the nerve was seen in 11 cases, including animals with bilateral or unilateral injections, animals with 5, 7, or 12 d survival times, and in sections taken frontally, parasagittally, or horizontally. The unlabeled area in the nerve always developed gradually, apparently reflecting a gradual sorting of labeled and unlabeled fibers, and did not correspond to any specific anatomical structure (e.g., cells, blood vessels) in the nerve. This unlabeled area was never seen when the retinal axons were labeled uniformly (see below).

Although these 11 cases all showed the same pattern of label, the intensity and extent of label in the retina and contralateral optic tract varied among animals treated similarly, and this was reflected in the intensity and extent of label in the nerve. Label was generally heavier after 5 d survivals than after 7 d, but even among the latter group there was some variability. Cases with heavier and more extensive label in the retina and contralateral optic tract (Fig. 4*b*) only showed the circumferential pattern of label after the nerve had exited the optic foramen (Fig. 4*a*), with the intensity of label at the dorsal and ventral borders of the nerve being nearly equal and with lighter label at the ventral

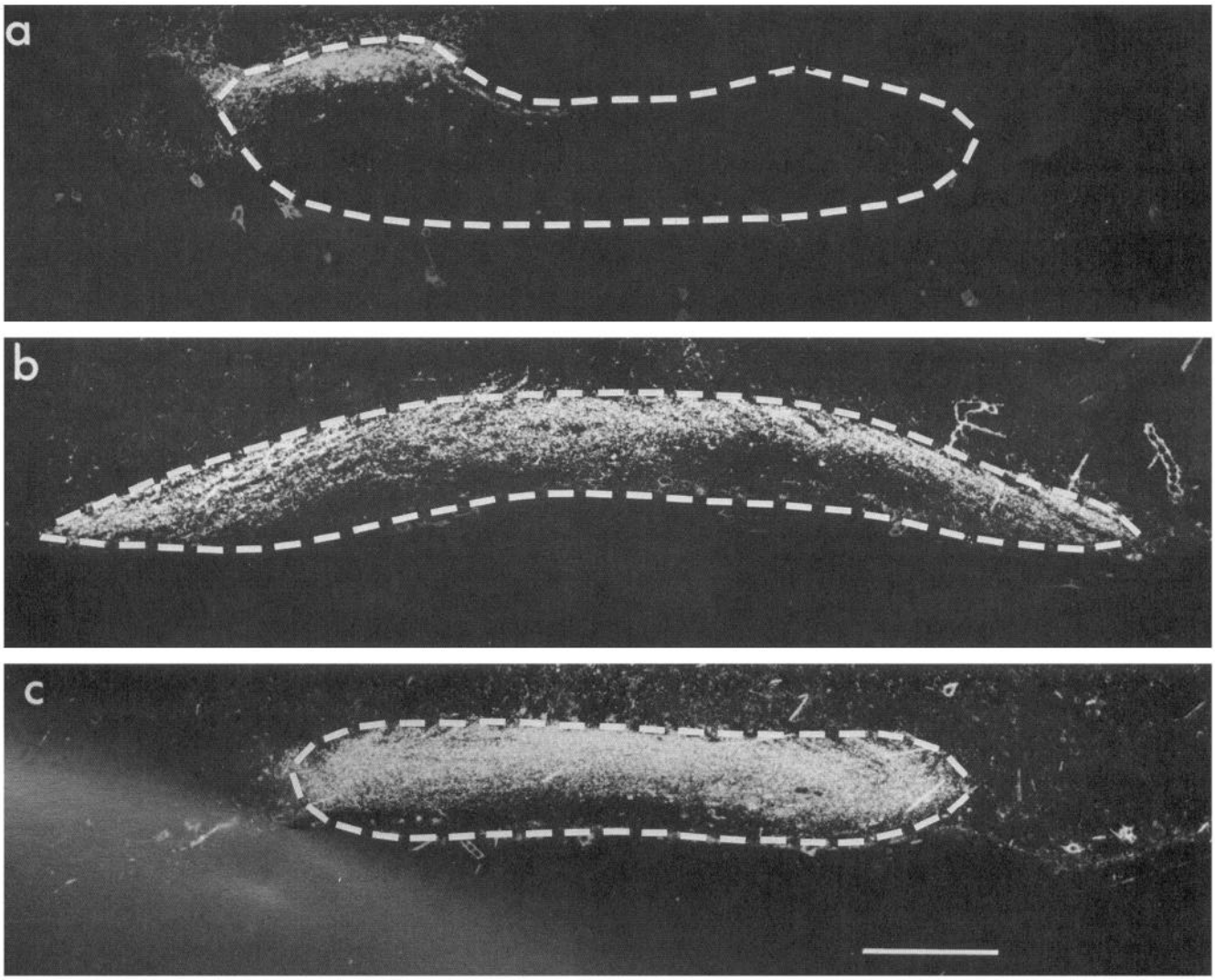

Figure 6. Dark-field photomicrographs illustrating the pattern of label in the optic chiasm following preferential labeling of the older retinofugal fibers. *a*, Frontal section through the chiasm of an animal given WGA-HRP on E28 and processed on E35. The ipsilateral optic nerve showed a circumferential pattern of label near the optic foramen, with label being heavier dorsally than ventrally. Here, in the chiasm, labeled fibers were restricted to the dorsal extreme of the chiasm, furthest from the pia, and maintained that position into the optic tract. *b* and *c*, Two frontal sections from an animal that received bilateral injections on E28 before being killed on E35. Both nerves showed similar, circumferential patterns of label near the optic foramen, with label becoming limited to the medial, dorsal, and lateral borders of the nerve near the chiasm (see Fig. 2*d*). As the nerves fused to form the chiasm (*b*), the same pattern of label was maintained, producing an "m" of label. Further caudally in the chiasm (*c*), all labeled fiber shifted dorsally and maintained that position into the tract. Dorsal is *up* in all figures, and the *interrupted white lines* indicate the outlines of the chiasm. Scale bar, 100 μm.

border of the nerve only being seen immediately before the chiasm. On the other hand, cases with lighter label tended to show the circumferential pattern of label as the nerve first approached the optic foramen, and showed a greater dorsal–ventral asymmetry in the intensity of label around the circumference in the intracranial segment of the nerve.

The 2 cases that showed the lightest and least extensive label in the contralateral tract did not show a complete circumferential pattern of label in the nerve. In these animals, label was again widely scattered in the nerve near the retina, but became limited to the dorsal perimeter of the nerve near the optic foramen. In the animal with the lightest label, the HRP reaction product was limited to the dorsal border of the nerve (Fig. 5*a*) and to the deepest, dorsal-most region of the contralateral optic tract (Fig. 5*b*). Another case showed label around the entire circumference of the nerve except the ventral extreme. While these 2 cases might represent incomplete injections, the limitation of label to the deepest extreme of the contralateral tract is interpreted as indicating that these cases showed preferential labeling of the very oldest retinofugal fibers (Walsh and Guillery, 1985).

To eliminate the possibility that the unlabeled area in 1 optic nerve might represent unlabeled retinoretinal fibers from the other eye (Bunt and Lund, 1981; Bunt et al., 1983) or else decussating optic fibers from 1 eye that enter the base of the other optic nerve before turning posteriorly (Aebersold et al., 1981; Polyak, 1957), the pattern of label was studied following uniform labeling of 1 retina. In animals killed 1 d after receiving WGA-HRP on E27, or in animals killed on E39 following injections on E34 or E35, label in the nerve ipsilateral to the injection was dark and uniformly distributed. No unlabeled zones were seen anywhere along the nerve. Label in the contralateral nerve was very light and sparsely scattered. Near the chiasm, some labeled fibers did enter the base of the unlabeled

nerve before turning posteriorly, but these fibers never reached further than 80 μm rostral to the chiasm.

Pattern of HRP label in the optic chiasm

The pattern of label in the optic chiasm can be regarded as a logical transition between the patterns of label in the optic nerve and optic tract. In cases showing light label, this label was limited to the deepest, dorsal region of the optic chiasm (Fig. 6*a*), and these fibers entered the deepest, dorsal portion of the optic tract. Those cases that showed circumferential label in the nerve near the optic foramen showed the heavily labeled fibers along the anteromedial, dorsal, and posterolateral borders of the nerve as the nerves fused to form the optic chiasm (Figs. 6*b*, 2*d*, 3*c*). Further caudally, labeled fibers were shifted dorsally in the chiasm (Fig. 6*c*). Fibers adjacent to the ventral border of the chiasm were labeled only following uniform labeling of the retina. The supraoptic commissures, the nonretinal components of the optic chiasm, were not labeled in any of these experiments, and could be demonstrated (following uniform retinal labeling) posterior to the retinal fibers.

Discussion

This study shows that the age-related fiber order in the ferret's optic tract reflects a systematic fiber rearrangement that occurs in the optic nerve and that is maintained through the optic chiasm. Whereas older and newer fibers intermingle in the nerve near the eye, near the optic foramen the older fibers occupy the perimeter of the nerve, with the very oldest fibers at the dorsal border, and newer axons at the ventral border. The very newest axons lie in the center of the nerve near the optic foramen, but shift ventrally nearer the optic chiasm, remaining nearest the pial surface through the chiasm and tract (see Fig. 7). This interpretation arises from the changing pattern of label in the optic nerve following the preferential labeling of the older retinofugal axons with WGA-HRP. Although a detailed discussion of this method is available elsewhere (Walsh and Guillery, 1985), a few technical points will be discussed briefly.

Technical considerations

The method used here has been applied previously with ^{3}H-proline in the frog (Cima and Grant, 1982; Gaze et al., 1979; Jacobson, 1977; Scott and Lazar, 1976). In the ferret's optic tract, WGA-HRP and several different ^{3}H-amino acids all produced a preferential labeling of older fibers, and all of these tracers produced qualitatively similar patterns of label in similar experiments (Walsh and Guillery, 1985). In the present study, WGA-HRP was used exclusively because the absence of background label allowed the clearest possible delineation of the gradual sorting of labeled and unlabeled fibers along the length of the optic nerve. However, 2 animals were prepared similarly using the autoradiographic technique (Walsh, unpublished observations) and showed the same nonuniformities of label both nearest the retina (with label deep in the nerve) and furthest from the retina (with label near the surface) seen in the WGA-HRP material presented here. These consistent nonuniformities of label suggest that the changing pattern of label is not due to some technical artifact, such as extra-axonal transport of the marker, but instead reflect the changing distribution of a group of preferentially labeled fibers.

Sections of the retina and optic tracts permitted a direct evaluation of the preferential labeling of the older retinal ganglion cells and their axons. The production of ganglion cells in the cat, a close relative of the ferret, shows dorsal–ventral, central–peripheral, and nasal–temporal gradients (Walsh and Polley, 1985; Walsh et al., 1983). In the present study, the pattern of retinal labeling, in experiments where this labeling was nonuniform, showed similar gradients, consistent with the interpretation that the older ganglion cells were preferentially labeled.

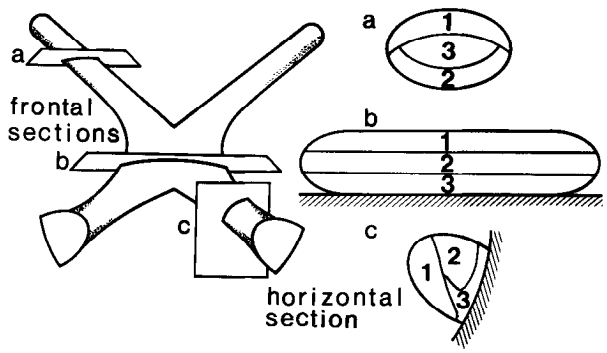

Figure 7. Schematic illustration summarizing the age-related fiber order in the intracranial portion of the optic nerve, the optic chiasm, and the optic tract (Walsh and Guillery, 1985). *Left,* Schematic view of the optic chiasm as viewed from above, with the optic tracts curving upward out of the plane of the page. *Right,* Drawings show frontal sections through the nerve (*a*) and chiasm (*b*), and a horizontal section through the tract (*c*). After the nerve leaves the optic foramen, the oldest fibers (*1*) occupy the nerve's dorsal perimeter, with newer fibers ventrally (*2*) and the newest fibers (*3*) nearest the center of the nerve. Nearer the chiasm, the newest fibers shift ventrally, so that in the optic chiasm the oldest fibers are dorsally furthest from the pia (*1, 2*), with newer fibers ventrally (*3*). In the tract, as shown previously (Walsh and Guillery, 1985), the oldest fibers (*1*) occupy the deepest, posteromedial corner of the optic tract in horizontal sections (these fibers lie dorsally and medially in parasagittal or frontal sections), with newer fibers anteriorly (*2*), and the newest fibers (*3*) next to the pia, and also forming a "notch" in the posterolateral corner of the tract. In *a* and *b*, dorsal is *up*; in *c*, rostral is *up* and lateral is *right. Diagonal hatching* in *b* and *c* indicates the pial surface of the brain.

The age-related fiber order in the optic tract has been documented extensively in many vertebrates with several other methods (Easter et al., 1981; Gaze and Grant, 1978; Herrick, 1941; Rusoff, 1984; Torrealba et al., 1982; Walsh and Polley, 1985; Walsh et al., 1983), and thus the labeling of the deeper axons in the tract served as an internal control of the preferential labeling of the older retinofugal fibers in each experiment.

The preferential staining of the oldest retinal cells and fibers probably relates to the kinetics of uptake and breakdown of WGA-HRP, with newer ganglion cells, formed after the WGA-HRP has been cleared from the vitreous, encountering little or no available marker. Whereas clearance of extracellular HRP (and presumably WGA-HRP) occurs within 1 d following injections into the brain (LaVail and LaVail, 1974; Mesulam, 1984; Vanegas et al., 1978), the rate of clearance from the vitreous probably depends on many factors, such as the size and developmental stage of the retina, the dimensions of the hole made by the pipette, the amount of retinal or vascular damage, and the degree to which WGA-HRP may be sequestered (e.g., in the anterior chamber) and slowly released. Therefore, it is not surprising that the overall proportion of fibers labeled can vary among animals treated similarly (see also Walsh and Guillery, 1985). However, the pattern of label in the retina (in terms of central–peripheral and dorsal–ventral differences) and in the tract (in terms of deep–superficial differences) was always similar, and always consistent with the interpretation offered. Furthermore, the consistent change in the pattern of label in the optic nerve, in association with this nonuniform label in the retina and tract, is what signaled a systematic rearrangement of the axons in the nerve, and suggested some important rules guiding this rearrangement.

It is important to indicate some limitations of the results presented here. The technique described preferentially labels a large population of fibers and cannot show patterns of distribution or growth of individual axons. The low resolution of the

method would allow small numbers of fibers that behave differently from the rest to be missed. Since fibers are labeled only in terms of age, no retinotopic information is provided. While previous reports have not shown any simple retinal mapping in the cat's optic nerve (Horton et al., 1979), the relationship of the age-related sorting in the nerve to some rougher mapping, or to the sorting of crossed and uncrossed fibers, is unknown. Finally, whereas many axons are lost from the nerve during development (Cucchiaro, 1984; Henderson et al., 1985; Ng and Stone, 1982; Rakic and Riley, 1983), the method used here neither detects that loss nor allows an assessment of the significance of that loss in determining the ordering of the remaining fibers.

Fiber order and glial structure

The systematic, age-related sorting of retinal fibers in the optic nerve may reflect changes in glial structure along the length of the fetal nerve. In the intraorbital segment of the fetal nerve, bundles of axons are embedded among early glial cells, and growth cones are scattered throughout the cross section of the nerve (Walsh et al., 1985; Williams and Rakic, 1985a, b). In the optic tract, where axons are grouped by age (Walsh and Guillery, 1985), axons do not form bundles, and growth cones tend to grow near the pial surface, in relation to the outer processes of radial neuroepithelial cells (Walsh et al., 1985; Williams and Rakic, 1985b). We have recently found that a radial neuroepithelial structure is present in part of the optic nerve of the immature fetal ferret, extending out to the optic foramen at early stages (E23–E25), and occupying a smaller proportion of the nerve's intracranial segment at later stages (Walsh et al., 1985). This transition in glial structure (from axon bundles to radial neuroepithelial cells), which occurs near the optic foramen at those ages when many new retinal fibers are added to the nerve, may bear an important relationship to the fiber reordering that occurs in the nerve near the optic foramen.

The pattern of the age-related fiber sorting in the ferret's nerve differs from that seen in all nonmammalian vertebrates studied (Bunt, 1982; Easter et al., 1981; Herrick, 1941; Rager, 1980; Reh et al., 1983), where newer fibers always abut the nerve's ventral surface. These differences might reflect species differences in the rules for axonal growth. Alternatively, growing axons in the ferret may follow familiar rules by growing near the nerve's surface where they meet radial neuroepithelial cells, with the peculiar fiber pattern in the ferret's nerve reflecting instead the changes in glial structure with age described above. For example, the oldest fibers, which meet the radial neuroepithelial cells near the optic foramen, seem to add to the ventral surface there (Walsh et al., 1985), and newer fibers may add ventrally to the older ones. At later stages, however, the very newest fibers do not encounter the radial neuroepithelial cells near the optic foramen, but only very near the optic chiasm. This may relate to the tendency for newer fibers not to be located near the surface in sections taken near the optic foramen (Figs. 2*c*, 3*b*), though these new fibers do shift ventrally near the chiasm. The details of the position of the growth cones in relation to the changing glial environment in the fetal nerve remain to be clarified.

The finding that retinal fibers are grouped by age in the ferret's optic nerve is intriguing, since axons of different age show distinct patterns of central projection. In the cat, the oldest, medium-sized ganglion cells (Walsh and Polley, 1985; Walsh et al., 1983) show the sharpest line of decussation in the retina (Illing and Wässle, 1981), with progressively younger ganglion cell types showing a decussation line that is less precise and is shifted into the temporal retina (Torrealba et al., 1981; Walsh et al., 1983). Similar rules apply for the mouse (Dräger, 1985). The newest retinofugal axons show, by their position in the prechiasmatic nerve (near the ventral edge), by their pattern of retinal decussation (mainly crossed), and by their central connections (mainly to the tectum) a formal analogy to the axons of ganglion cells in nonmammalian vertebrates. Whereas this superficial similarity might reflect similar developmental mechanisms at work, the older axons of the mammal, differing from those of nonmammalian vertebrates both in their development and adult connections, may be subject to separate developmental mechanisms unique to the mammal. A fuller understanding of the basis for the age-related fiber ordering in the optic nerve may shed important light on the developmental mechanisms affecting the chiasmatic decussation and central connections of the retinofugal fibers.

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