Architecture of the Optic Chiasm
and the Mechanisms That Sculpt Its Development

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I. Introduction

II. The Mature Structure of the Mammalian Chiasm
   A. Chiasmatic pathways and the naso-temporal division in the retina
   B. Patterns of fiber order in the optic nerve
   C. Wilbrand's knee in the proximal optic nerve
   D. Changes in fiber order between the optic nerve and optic tract
   E. The course of crossed axons through the chiasmatic region
   F. The course of uncrossed axons through the chiasmatic region

III. Development of the Mammalian Chiasm
   A. Potential mechanisms for chiasm generation
   B. Ganglion cell generation and optic chiasm formation
   C. Axon extension and morphological development of retinal axons
   D. Adhesion molecules and pathway markers
   E. Genetic regulation of chiasm development
   F. Role of chiasmatic neurons in chiasm pathway selection
   G. Role of chiasmatic glia in chiasm pathway selection
   H. Interactions between fibers from each eye at the developing chiasm

IV. Chiasmatic Abnormalities Found in Albinism
   A. Role of temporal ordering in patterns of retinal cell addition and outgrowth

V. Mature Structure and Development in Nonmammalian Vertebrates
   A. Amphibians
   B. Birds

VI. Conclusion

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I. INTRODUCTION

At the optic chiasm, axons of retinal ganglion cells either cross the midline and innervate the contralateral hemisphere or remain uncrossed and project ipsilaterally. There is renewed interest in the chiasm because the binary decision made by optic axons may cast light on fundamental mechanisms of axon guidance in the central nervous system (CNS). However, surprisingly little is known about the mature organization of the chiasm, how
this varies between mammals, or the developmental mechanisms that sculpt its architecture. This review focuses on the chiasm in animals with partial patterns of decussation, where fibers from each eye give rise to both crossed and uncrossed hemispheric pathways. In these animals chiasmatic organization is more complex, and from a developmental point of view probably more interesting, because each fiber is required to make a choice as to whether it crosses the midline or not.

It is thought that optic fibers project across the midline because early in evolution they innervated motor neurons on the contralateral side of the body in primitive bilaterally symmetrical animals. Any change in illumination, such as the movement of a shadow, was interpreted as indicating the potential presence of a predator. Hence, the crossed fibers provided a simple reflexive pathway for turning away from the potentially threatened side (136). Although this assumes that the crossed chiasmatic pathway predates the evolution of the uncrossed projection, ipsilaterally projecting fibers are not the preserve of mammals, but can be found, to a greater or lesser extent, in adult members of all vertebrate classes including the agnathans (jawless fish), amphibians, and birds (170). Hence, it can be assumed that partial patterns of decussation have a long ancestral history.

Although there are extensive data in a range of vertebrates on the existence of crossed and uncrossed projections, few consider the organization of fibers through the chiasm and how they develop. The literature on this subject is marked by a limited number of detailed studies undertaken in a few specific animal models, which with a few exceptions are almost exclusively confined to mammals. These studies do not always tell a consistent story, and there is growing evidence that even within mammals, the organization of the chiasm is variable, and as such, the factors that influence its development are probably not universal. Despite this, considerable advances are being made in our understanding of the mechanisms of chiasmatic pathway selection in the mouse. The advantage of using the mouse is the relative ease with which methods of molecular genetics can be applied to addressing the problems of CNS development.

This review considers the fiber organization of the chiasm and how this might arise. This cannot be undertaken without reviewing the organization of the optic nerve and tract as well as patterns of retinal ganglion cell production. The role of fiber-fiber interactions and fiber-glia relationships in the chiasm is also reviewed. Although there is an ever-expanding literature on chemical signals/markers in development, these are not considered extensively, but only where they are directly relevant to the chiasm. Likewise, the literature on differential neural adhesion chemicals are not extensively reviewed because these appear to play a smaller role in pathway selection than was originally thought. Consideration is given to abnormalities of the chiasm where these cast light on mechanisms of normal development.

II. THE MATURE STRUCTURE OF THE MAMMALIAN CHIASM

A. Chiasmatic Pathways and the Naso-temporal Division in the Retina

Optic decussation was first postulated by Albert the Great, who in the 13th century noted the loss of contralateral visual fields after unilateral damage to the back of the head (158). The partial pattern of decussation at the chiasm was first proposed by Newton (107), but was formulated more explicitly by Taylor almost 100 years later (117, 153). Such a pattern forms the basis of binocular vision, such that in humans, fibers originating from the temporal retina remain uncrossed at the chiasm, whereas those originating from the nasal retina cross. This pattern of partial decussation results in the continuity of the visual field representation in central visual structures in the brain. The line that divides these two populations of ganglion cells in the retina passes vertically through the fovea. The projections from each eye are in conjugate register in the lateral geniculate nucleus, which projects a map of the visual hemifield to the primary visual cortex (43, 85, 117).

The retinal line that segregates ipsilaterally from contralaterally projecting cells is termed the naso-temporal division and has varying degrees of precision depending on the species examined. In all cases the crossed projection is larger than the uncrossed. Generally, in mammals, the majority of fibers that give rise to the uncrossed chiasmatic pathway are located in the temporal retina, but complete spatial segregation of cells giving rise to crossed and uncrossed pathways is primarily the preserve of primates (43). In most mammals other than primates, the crossed projection arises from the entire retina, whereas the uncrossed projection is mainly confined to the region temporal to the area of maximum ganglion cell density, the area centralis (29, 37, 55, 81). In these animals the temporal retina contains a mixed population of cells in terms of their chiasmatic pathways. The relative proportion of cells within the temporal retina that project ipsilaterally varies with the degree of retinal specialization and the location of the eyes in the head, and hence the size of their binocular visual field. In the cat, which has frontally placed eyes, cells with an uncrossed pathway form a greater proportion of the total ganglion cell population in the temporal retina than they do in rodents, who have a less specialized retina than that commonly found in carnivores and laterally placed eyes. Rabbits have specialized retinæ, but very laterally placed eyes, and hence a small uncrossed pathway (29, 37, 70, 78, 118).
Cells giving rise to the uncrossed projection terminate in the main visual nuclei that receive retinal input. These include the lateral geniculate nucleus (LGN), the superior colliculus (SC), and pretectal nuclei (171). They also project to the superachiasmatic nucleus and the accessory optic system (183). In primates, retinal projections to both the LGN and SC respect the sharp naso-temporal division, in that only cells located nasal to the fovea project to the contralateral LGN and SC (31, 43). Although the projection to the LGN respects the naso-temporal division in carnivores, the projection to the SC does not. Hence, although cells projecting ipsilaterally to the SC are confined to the temporal retina, cells projecting to the contralateral SC are found distributed across the entire retina (173). The only exception to this is the projection pattern found in large fruit-eating bats, which have a retinal projection to the SC with a vertical hemidecussation of the kind commonly only found in primates (115). This pattern is not found in other bats and has given rise to considerable speculation about the evolutionary relationship between mega bats and primates (116).

In the cat, the line that segregates the uncrossed cells projecting to the LGN varies depending on the subtypes of ganglion cell considered. In this animal, as in most mammals, ganglion cells can be grouped roughly into three classes on the basis of morphology and physiological properties (15). β-Cells form almost half of the ganglion cell population and have brisk sustained responses and medium-sized cell bodies. They project primarily to the LGN (171, 172). These cells have a relatively sharp naso-temporal division as the line segregating those with different chiasmatic routes is <1° wide (89). However, α-cells that form a much smaller percentage of the total population and have large cell bodies and a more transient response (172) have a much wider naso-temporal division, with some cells still found projecting across the midline over 15° into the temporal retina (89). These cells project to both the LGN and the SC (171). The different way that the separate cell types straddle the naso-temporal division in the cat appear also to be present in primates (43, 93).

The separate chiasmatic configurations of the different cell types demonstrate that the extent to which retinal location determines chiasmatic pathways can vary with the cell type that is being considered. This point may be of significance when the development of these different cell types is considered in relation to their patterns of projection.

A small population of retinal ganglion cells has been identified in rodents and cats that have bifurcating axons that project to both sides of the brain. It is not clear where the axon bifurcation occurs. These cells are rare and are confined to the temporal retina (73, 78). Cells that project bilaterally do not appear to arise from any particular cell type.

B. Patterns of Fiber Order in the Optic Nerve

Patterns of fiber order through the optic chiasm are dependent on those in the optic nerve. Considerable debate has taken place as to whether the nerve is retinotopically organized or not. It had been proposed that in both cat and primate fibers were not organized in a meaningful way but were relatively random (62, 69). The limitations of these studies were that they examined only a small sample of optic fibers either anatomically or physiologically. When larger populations are considered following tracer injections into the brain, or retinal lesions, it is clear that for the greater length of the nerve the majority of fibers are organized in a roughly retinotopic manner (5, 55, 68, 76, 104, 105). Occasionally fibers can be identified that do not conform to this pattern, but they remain in the minority and do not appear to arise from any specific functional class (76). This pattern appears to be present in all of the mammals studied to date. However, depending on the animal type, it can change in the proximal optic nerve just before fibers enter the chiasm.

In rodents and carnivores, gradients in ganglion cell density across the retina are far less marked than they are in primates. In primates a much greater proportion of ganglion cells arise from the central retina due to the highly developed degree of retinal specialization in this animal (43, 70). Consequently, fibers from this region occupy a greater proportion of the nerve. A number of studies have traced the course of fibers from the papillomacular region. These have shown that behind the eye they occupy a wedge-shaped region in the temporal sector of the nerve, but more caudally they are located in a central core of the nerve (17, 33, 104, 117). There is some discrepancy regarding the precise distribution of these fibers prechiasmatically, as just before the nerves meet at the chiasm they may be distributed more widely (67).

Although optic fibers are grouped within fascicles from the optic nerve head into the intracranial segment, in some animals these patterns are no longer present in regions adjacent to the chiasm. This change from a fascicular to a nonfascicular configuration has been seen in some primates, carnivores, rodents, and marsupials (4, 55, 76) and is associated with a change in the glial organization of the nerve at this location (52). In some animals the change in glial organization is associated with a change in fiber order such that the roughly retinotopic representation found along the majority of the length of the nerve is lost in this region, and fibers destined to project to different hemispheres become completely mixed (4–6, 76). These significant changes in fiber order in proximal regions have been identified in carnivores (4, 76) and rodents (5) but may be the preserve of these animals alone. In some marsupials the fascicular organization of the nerve is lost in regions close to the chiasm, but there is no major change in nerve fiber order (55). Hence, the loss of
fascicular organization is not necessarily associated with a major change in fiber order. In the tree shrew, fascicular patterns found along the length of the nerve remain in proximal regions and are also found deep in the chiasm. In this animal there is no change in fiber order in the proximal nerve (81). These studies show that there are wide variations in the organization of the proximal nerve within mammals and that only within a limited range of mammals does there appear to be significant changes in fiber order in these proximal regions.

C. Wilbrand’s Knee in the Proximal Optic Nerve

The region of the proximal nerve where the fascicular configuration is lost in some animals has been thought to contain fibers from both eyes, as fibers from the other eye that have crossed the midline, course through here in a loop before turning back toward the optic tract (117). They were thought to form the anatomical basis of the anterior chiasmal syndrome resulting from a tumor or compression of the proximal nerve. In this condition, not only is there a loss of vision from the eye from which the nerve originates, but also a superior temporal hemianopia in the other eye (10).

Wilbrand first identified these fibers in silver-stained sections from a human chiasm where one eye had been lost a number of years before death (117, 176). Although Wilbrand’s knee fibers are mentioned in many text books and have been referred to frequently, recent research has demonstrated that they are an artifact resulting from a distortion of the chiasmatic region as a consequence of long-term degeneration following eye loss. These fibers almost certainly originate from more central regions of the chiasm but are shifted with time into regions previously occupied by the now absent proximal nerve. With modern tracing techniques, Wilbrand’s knee fibers cannot be identified in the normal primate chiasm or in those from primates after recent monocular enucleation. However, they are present after longer term survival after monocular enucleation (61). The author of this study also points out that as these fibers were thought to extend only ~2 mm down the contralateral nerve, it was most unlikely that any tumor would compress this region without involvement of the main body of the chiasm itself. Hence, even the clinical evidence in favor of the existence of these fibers was not compelling.

D. Changes in Fiber Order Between the Optic Nerve and Optic Tract

The roughly retinotopic pattern of organization of the nerve as revealed by degeneration or modern tracing methods is consistent with observations made of the distribution of axons of different sizes along the nerve. In general, mammalian optic fibers can be classified on the basis of size into three groups reflecting the three main types of retinal ganglion cell (171, 179). The majority of these are either medium or fine, but a smaller proportion is coarse. This trimodal distribution of fiber sizes reflects the size differences in the main types of ganglion cell (15).

Although there is a partial segregation of axons by size within the tract, it would be an oversimplification to say that this represents a change from a retinotopic to a nonretinotopic fiber distribution. Although the axon groups have become reordered, each group is roughly organized in an independent retinotopic pattern. This results in the dorsoventral retinal axis being represented across the mediolateral dimension in the optic tract and the center to periphery axis being represented across the depth of the tract. However, the precision of this representation is relatively poor (127, 159).

The observation that there are major differences in the organization of the three fiber classes between the nerve and the tract reveals that optic fibers not only segregate depending on whether they cross the midline or not. Within the chiasmatic region they also change their relative order before entering the optic tract. Hence, the chiasm is a region in which there are major changes in fiber order.

Although the analysis of the relative locations of optic nerve fibers of different size has been important in revealing the organization of retinal pathways, a qualification needs to be taken into account when considering studies that have relied on such methods. Baker and Stryker (7) demonstrated anatomically and physiologically in the ferret that optic axon size is not fixed, but that there is an increase in axon size between the nerve and tract. However, there are no grounds for assuming that different axon groups change their relative size. Rather, there are reasons for believing that all fibers increase in size along the length of the optic pathway because their fiber spectrum is trimodal in both the nerve and tract.

E. The Course of Crossed Axons Through the Chiasmatic Region

To date there has only been one major study that has traced the course of axons with different diameters...
through the mammalian chiasm to reveal the pathway taken by different ganglion cell types, and this has been undertaken in the ferret (123). It is important to note that although this animal is an established model, it differs in two important aspects from other animals studied. First, axons with different sizes begin to segregate in the most proximal region of the nerve, while in other mammals this appears to occur in the chiasm (4). Hence, proximally, coarse and fine fibers become increasingly confined to ventral regions of the nerve leaving the dorsal region occupied predominantly by intermediate-caliber fibers. It is not clear why the ferret initiates the segregation of axons here, but currently there is no reason to believe that this reflects a major difference in chiasm organization in this animal compared with others. Second, in the cat, rodent, and primate, ganglion cells from each of the three main cell types contribute components to the uncrossed chiasmatic pathway (43, 126, 171). Unusually, all of the cells with large axons that arise from α-cells in the ferret, including those in the far temporal periphery, have crossed chiasmatic pathways (162).

Despite these qualifications, the data provided by Reese and Baker (123) represent an important advance in our understanding of chiasmatic organization, as they have demonstrated that fibers of the three main ganglion cell types, as determined by axon diameters, cross the midline at different locations along the length of the chiasm (Fig. 1). The coarse fibers, all of which project across the midline, cross rostrally in the chiasm and move to take up a superficial location in the tract. Fine fibers that cross the midline do so at a relatively rostral location, from which they move to adopt a superficial position in the optic tract. Medium-sized fibers that project across the midline do so caudally and pass into deep regions of the tract.

Although it is possible to divide the midline axis according to where axons from specific classes decussate, it is also possible to divide the chiasm across the

![Figure 1. The course of fibers with different diameters through the ferret chiasm. Fibers with different diameters are mixed along most of the length of the nerve (1). Just in front of the chiasm the majority of medium-diameter fibers segregate into the dorsal part of the nerve, leaving the ventral part of the nerve occupied by coarse and fine fibers (2). As soon as the nerves fuse, the coarse fibers cross the midline in the rostral chiasm along with many of the fine fibers (3). At mid-chiasmal regions, most of the decussating fine and coarse fibers have crossed the midline, while the majority of medium fibers have yet to do so (4). Further caudally, medium fibers either decussate or enter the ipsilateral optic tract (5). At the caudal-most region of the chiasm, the fiber order found in the optic tract is established (6). ON, optic nerve; OX, optic chiasm; OT, optic tract. [From Reese and Baker (123). Copyright Blackwell Science Ltd.]](http://physrev.physiology.org/)

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dorsoventral axis in each hemichiasm in terms of whether fibers have crossed the midline or not. Generally, as optic axons cross the midline, they adopt a ventral location, such that progressively through the length of the chiasm there is a reduction in the dorsal component of fibers that are waiting to cross, and an increased proportion of fibers located ventrally that have decussated and are coursing toward the optic tract. Within this ventral component, fiber order increasingly adopts the configuration found in the tract (123). Although there is a paucity of data regarding the path that fibers with different diameters take through the chiasm in other animal types, there is evidence that the dorsoventral distinction between fibers that have crossed the midline and those that have not is common to a range of mammals (68).

There have been two detailed studies undertaken in old world primates that have traced projections through the chiasm with either degeneration techniques or using a retrograde tracer. The first used photocoagulation of defined retinal regions and silver degeneration methods (68). This revealed that the crossed fibers from peripheral dorsal and ventral retina cross the midline at different locations. Fibers from the dorsal retina cross in the caudal chiasm, whereas those from ventral locations tend to cross more rostrally. Once these fibers have crossed the midline they enter a lateral region where the chiasm is laminated, with the decussated fiber groups sandwiched between fiber groups from the other eye that have not crossed the midline. The macular fibers were found to occupy all regions of the chiasm except those in the inferior rostral and caudal regions but were more concentrated centrally and dorsally.

The second study used retrograde labeling to trace optic fibers projecting to the LGN (105). It also revealed that fibers from dorsal and ventral retina cross at different locations. Fibers from the peripheral ventral retina cross in more anterior chiasmatic regions than those from the dorsal retina periphery. The distribution of label from more central retinal regions and silver degeneration methods (68). This pattern with crossed and uncrossed projections being mixed in the proximal nerve and hemi-chiasm is not ubiquitous in mammals. In marsupials, uncrossed fibers are confined laterally in the nerve and chiasm with none approaching the midline. In some marsupials uncrossed fibers are segregated from fibers from the same eye that are going to cross the midline by thick astrocytic processes, which demarcate a morphologically distinct lateral region in the far proximal nerve and rostral chiasm. In this animal the chiasm has a tripartite organization, having a central core containing crossed fibers and two lateral segments that appear to have been added on to the main body of the chiasm, each exclusively containing uncrossed fibers (80). This finding gave rise to the notion that there may be a fundamental difference in chiasmatic organization between eutherian (placental) mammals and marsupials. Given that marsupials branched from the main line of mammalian evolution relatively early, this idea was not difficult to accept (18). Unfortunately, it was incorrect. Recently, it has become clear that uncrossed fibers in some eutherian mammals also remain confined laterally in the nerve and chiasm. Such patterns are found in tree shrews, which have highly specialized visual systems (81).

In rodents and carnivores there is a major change in fiber order in this region, such that uncrossed fibers spread throughout the nerve mixing with fibers that are destined to cross (4, 5, 76). The chiasm in these animals is relatively homogeneous, with no obvious internal features, and the uncrossed fibers remain widespread throughout each hemi-chiasm. This point is important in understanding the mechanisms that regulate chiasm development, as if fibers destined to project to different hemispheres are mixed in the proximal nerve, then spatial factors are unlikely to determine which hemisphere a fiber projects into. But if these fibers remain segregated, then such mechanisms may be significant.

This pattern with crossed and uncrossed projections being mixed in the proximal nerve and hemi-chiasm is not ubiquitous in mammals. In marsupials, uncrossed fibers are confined laterally in the nerve and chiasm with none approaching the midline. In some marsupials uncrossed fibers are segregated from fibers from the same eye that are going to cross the midline by thick astrocytic processes, which demarcate a morphologically distinct lateral region in the far proximal nerve and rostral chiasm. In this animal the chiasm has a tripartite organization, having a central core containing crossed fibers and two lateral segments that appear to have been added on to the main body of the chiasm, each exclusively containing uncrossed fibers (80). This finding gave rise to the notion that there may be a fundamental difference in chiasmatic organization between eutherian (placental) mammals and marsupials. Given that marsupials branched from the main line of mammalian evolution relatively early, this idea was not difficult to accept (18). Unfortunately, it was incorrect. Recently, it has become clear that uncrossed fibers in some eutherian mammals also remain confined laterally in the nerve and chiasm. Such patterns are found in tree shrews, which have highly specialized visual systems (81).

There is also strong evidence that uncrossed fibers remain confined laterally in the primate nerve and chiasm. Lesions confined to the temporal retina result in degeneration restricted to the lateral nerve and chiasm in Old World monkeys (67, 68). Naito (105) retrogradely labeled ganglion cells in Old World primates and traced their axons. In one example, a group of cells was labeled just temporal to the fovea. The axons of these cells were confined to the lateral chiasm with none approaching the midline. When anterograde tracers are injected into one eye in similar animals and both nerves and chiasm sectioned horizontally, labeled fibers can be identified segregating into two groups in the anterior chiasm. The medial group crosses the midline, whereas the lateral group enters the ipsilateral optic tract via a lateral course through the chiasm avoiding the midline region (61). These anatomical descriptions are consistent with clinical observations in humans. Pituitary tumors that compress the chi-
asmatic midline result in a specific loss of visual fields arising from the crossed projection and only affect the uncrossed projection when the tumor is very large and the crossed projection is almost completely lost (59). Compression of the lateral chiasm by aneurysm of the internal carotid artery results in a loss of visual fields arising from the temporal retina (32). Hence, there is growing evidence that the dispersed uncrossed projection found in the hemi-chiasm in some rodents and carnivores may be unique to these mammals and that this pathway remains confined laterally in most other mammalian types.

These findings support the notion that there is probably not a prototypical mammalian chiasm in terms of the course adopted by uncrossed fibers. This has important ramifications for mechanisms that regulate pathway selection. The potential mechanisms determining whether a fiber remains uncrossed or crosses the midline are likely to be very different depending on whether it is mingled with fibers that will behave in a similar fashion or not. Unfortunately, to date, research on chiasm development has almost exclusively been confined to animals in which the projections from each eye are mixed in the proximal nerve and hemi-chiasm (5, 6, 45). Hence, our picture of chiasm development may have to be revised.

III. DEVELOPMENT OF THE MAMMALIAN CHIASM

A. Potential Mechanisms for Chiasm Generation

Numerous factors probably regulate the development of the chiasm. The relative influence of each of these almost certainly varies between species. Furthermore, it is likely that some mechanisms are regulated temporally, such that they may be influential during one period but not necessarily in another. Despite this, it is possible to divide the potential developmental mechanisms into crude categories that are not exclusive and to some of which a temporal dimension may be added.

First, it is possible that chiasmatic pathways are partly specified by retinal location and that the fundamental mechanism has a significant genetic component. Second, it is possible that chiasmatic pathways are established as a consequence of interactions between retinal axon growth cones and elements in the chiasm that provide critical information in a time-dependent manner. These elements may or may not be located along the midline. Third, the pathways may be established by interactions between growth cones from the two eyes along the midline region, with some interactions being permissive allowing fiber crossing and some being inhibitory resulting in ipsilateral deflection. Fourth, it is possible that the temporal domain on its own is critical and that the time at which a developing fiber enters the environment of the chiasm determines which hemisphere to which it projects. Because these categories are unlikely to be totally exclusive or universal, they will not be dealt with in order, but they need to be considered in relation to the experimental evidence that is available on chiasm development.

B. Ganglion Cell Generation and Optic Chiasm Formation

Spatiotemporal patterns of ganglion cell production are important in chiasm development because cells that are generated early are most likely to reach the chiasm before those generated later. As such, early generated cells are likely to encounter a very different chiasmatic environment than those that are generated late once this region is established.

The retina develops with a center to periphery gradient (96, 121, 129, 134). The only significant exception to this is the accumulation of melanin in the retinal pigment epithelium (RPE) where this gradient is reversed (12, 13). There is strong evidence in primates, including humans, that the maturational focus is the presumptive foveal region (92, 96, 110, 120), whereas in mammals with less specialized retinas, such as rodents and marsupials, it is around the optic nerve head (36, 54, 71, 125). Hence, in primates, the first ganglion cells to enter the chiasm are likely to arise from the presumptive fovea, whereas in rodents they are likely to arise from around the nerve head.

The difference between the nodal points for retinal development between primates and rodents appears to be a real species difference. However, data generated from carnivores are problematic. A range of studies undertaken in the cat indicate an area in the upper temporal retina as being the focus of maturation. This includes not only the nodal point for ganglion cell production, but also a range of other developmental events such as the initiation of layer formation and the location of the first region where mitotic activity ceases (46, 121, 134, 166). However, experiments undertaken on the ferret clearly demonstrate that the optic nerve head is the nodal point for ganglion cell production along with many other features of development (129, 131).

It is possible that within carnivores there is a shift of the nodal point from the optic nerve head to the presumptive area centralis. This discrepancy may not be understood until a wider range of carnivores has been studied. It is also possible that real differences exist between closely related animals depending on other, as yet unidentified, factors. Hence, the nodal point for ganglion cell generation may vary depending on the gradient of cell density across the mature retina, its size, or the relative location of the area centralis. In the cat and primate, the
retina is large, gradients in cell density are steep, and the region of peak cell density is located closer to the nerve head than the temporal periphery (70). In the ferret, ganglion cell density at the area centralis is only half of that in the cat, and this region is located closer to the retinal periphery than the optic nerve head (57, 151).

Despite such differences, ganglion cell production in all animals occurs in a crude center to periphery pattern that harbors three separate but overlapping waves. It has been shown in a wide range of mammals that different ganglion cell classes are generated at different stages (2, 120, 125, 132, 139, 167). These studies commonly rely on the use of [3H]thymidine injected during development to identify the time at which cells leave the cell cycle. The cells are then classified into one of the three main ganglion cell classes at maturity on the basis of soma diameter, or morphologically following retrograde tracer labeling. Unfortunately, in many mammals, distinctions between ganglion cell types based on soma diameter are poor, and there are few studies in which there is a sharp picture of relative patterns of cell generation in the ganglion cell subtypes. Furthermore, it is only in the ferret that there is an understanding of the patterns of axon projection of the different ganglion cell types through the optic tract. In the cat and ferret retina is large, gradients in cell density are steep, and the region of peak cell density is located closer to the nerve head than the temporal periphery (70). In the ferret, ganglion cell density at the area centralis is only half of that in the cat, and this region is located closer to the retinal periphery than the optic nerve head (57, 151).

Patterns of ganglion cell production can be related to the distribution of axons of different size across the chiasm, providing a chronotopic history of chiasmatic development. In the ferret, β-cells are the first to be generated, and their medium-sized axons are located caudally in the chiasm. As ganglion cells are generated with a center to periphery gradient (121, 129), axons arising from β-cells from the central retina should be located toward the caudal pole of the chiasm and those from more peripheral regions deeper in the chiasm. The α- and γ-cells are generated later, and their respective large and fine axons are found at more rostral locations than the majority of axons from β-cells (123, 124). Axons from α- and γ-cells from the retinal periphery should be located toward the rostral pole of the chiasm. Hence, cells generated early in development are found caudally in the chiasm, whereas those generated later are located rostrally. This provides strong evidence that the chiasm develops along a caudal to rostral gradient.

These patterns of cell generation can also be related to the organization of fibers as they leave the chiasm and enter the optic tract. Fiber addition in the optic tract occurs preferentially along the pial surface, such that older fibers are displaced by younger ones into deeper regions away from the pia (165). Medium-sized fibers originating from β-cells are found deep in the tract, whereas large and small fibers are located more superficially (51). Analysis of the distribution of axons of different size across the chiasm has not been undertaken in another animal model. Despite this, a range of studies undertaken on the cat, rat, and primate have confirmed that different-sized fibers are differentially distributed in the optic tract but not in the nerve (35, 51, 122, 127, 128).

Patterns of ganglion cell generation in rodents and carnivores also become of interest when ganglion cells are segregated into those projecting to separate hemispheres rather than into different cell types. In these animals the temporal retina contains cells with crossed and uncrossed pathways. Although there is a center to periphery pattern of ganglion cell production, the cells destined to remain uncrossed through the chiasm are generated in front of the expanding wave of cell generation. Hence, in the temporal retina, they are produced before cells at similar eccentricities that will project across the midline. This has been demonstrated with different methods in mouse and ferret. In the mouse, patterns of ganglion cells production have been marked with [3H]thymidine and their chiasmatic pathways identified at maturity by tracer injections into one side of the brain (Fig. 2). Ganglion cell generation in the mouse occurs across embryonic days (E) 10–19. Birth occurs around 21 days after conception. The peak in the production of uncrossed cells occurs on E13, whereas for
Crossed and uncrossed ganglion cells are significant in understanding the development of the optic chiasm. Hence, if differences in the time of cell generation for crossed and uncrossed projections in the mouse between embryonic days (E) 11 and E19, the border of the temporal retina from which uncrossed cells arise is marked by the dashed line. Ganglion cells are generated in a crude center to periphery pattern. However, uncrossed cells in the temporal retina are generated abnormally early within this pattern. Most of the uncrossed cells are generated around E13, which is before the main wave of cell production that gives rise to the crossed projection from this location.

In developing ferrets, ganglion cells have been retrogradely labeled from one optic tract or their axonal pathways marked anterogradely by placing a tracer in the temporal retina. Early in development, ganglion cells in the temporal retina were only found to project to the ipsilateral optic tract, whereas later cells within this region projected bilaterally. Likewise, early in development only uncrossed cells could be labeled in the temporal retina from the ipsilateral optic tract, with no label found at similar temporal eccentricities in the contralateral retina. However, labeled cells were found at these locations in the contralateral retina later (6). Hence, not only do ganglion cells that are destined to remain uncrossed arise relatively early for their retinal eccentricity, but their axons also extend into the chiasm before those from similar locations that are destined to project across the midline.

Taken together, these studies demonstrate that within the temporal retina, cells that remain uncrossed through the chiasm come out of the cell cycle before those that will project across the midline. Hence, it is possible that uncrossed cells could be partly specified in terms of the number of cell cycles they go through or the point at which they exit the cell cycle in relation to their location. This point will be relevant when abnormalities in patterns of cell generation and chiasm formation are considered in albinism (see sect. iv).

The pattern of cell generation in the primate is markedly different from that found in rodents and carnivores. Here, ganglion cell generation is roughly symmetric across the naso-temporal division, with no indication that uncrossed cells that arise exclusively from the temporal retina are generated before contralaterally projecting cells that reside on the nasal side of the division. The only other comprehensive studies of ganglion cell generation in mammals have been undertaken in marsupials. Again, in these animals there is no indication of asymmetric patterns of cell production in the temporal retina (2, 54). Hence, if differences in the time of cell generation for crossed and uncrossed ganglion cells are significant in chiasmatic pathway selection, such mechanisms are restricted to only a limited range of mammals.

C. Axon Extension and Morphological Development of Retinal Axons

Optic axons extend during maturation via the progressive development of their growth cones along the optic pathway. These consist of a flat central area from which filopodia extend outward. Between the filopodia there are ruffled membrane regions, lamellipodia. The filopodia are active and are constantly extending and retracting as the cone moves and the axon extends.

Growth cones play an active role in enabling the developing axon to navigate through regions where pathway choices must be made. In the rodent retinofugal pathway, the morphology of the growth cones varies markedly depending on its location. Hence, along the course of the optic nerve the growth cones tend to have a relatively simple morphology, whereas in the region of the chiasm and close to their target structures where choices must be made, they are more extensive and morphologically complex (14). Similar results showing that growth cones have more filopodia when they encounter decision points have been obtained in both mammalian and non-mammalian species and in a wide range of systems (86).

Evidence that growth cone filopodia play a critical role in optic pathway guidance has come from studies undertaken on the retinofugal pathway of *Xenopus* embryos. Exposed brain preparations were maintained in vitro, and cytochalasin B was added to the medium. This drug disrupts microfilament cores of filopodia, resulting in retraction of the filopodia, but leaves the lamellipodia intact. The optic tract in these preparations became disorganized with the majority of fibers being unable to locate the tectum. Unfortunately, the optic chiasm was not examined specifically, although the authors report that some fibers growing through this region stalled with drug application (22).

Optic nerve growth cones appear to advance at a rate of ~60–100 μm/h (26), but there is evidence that this rate of growth may not be uniform along the length of the optic nerve.
pathway (45). In the fish optic nerve it has been proposed that the rate of fiber development changes as optic nerve fibers move between different glial environments (95). In the mouse the crossed and uncrossed pathways appear to travel at different rates between the eye and the optic tract. Uncrossed cells take ~5 days between leaving the cell cycle and their axons entering the optic tract, whereas cells generated at the same time but projecting contralaterally take only 3 days before entering this region (26, 36). It is possible that this difference can be partly accounted for by different types of interaction between the two fiber populations in the chiasm (see sect. mH).

Growth cones can be found in all regions of the developing optic nerve, although there is evidence that more are found in peripheral regions than toward the nerves core, reflecting the rough center to periphery gradient in ganglion cell production. Growth cones do not accumulate exclusively along preexisting bundles (26, 177, 178). In more proximal regions the new fibers accumulate in the superficial subpial regions between the endfeet of radial glia. It has been suggested that the growth cones use the stems of the radial glia at the junction between the nerve and chiasm as a substrate for directed growth (52). Despite this, growth cones in the superficial regions tend to be associated with one another rather than to the processes of the glia. Because there is no clear trend for growth cones to travel along radial glial processes over extensive distances, it is unlikely that they move toward the pial surface along the stems of these cells (24).

In the chiasm the glial endfeet line the surface of the chiasm and separate fibers from the underlying basement membrane. The membranes of adjacent neuronal and glial processes have regions of specialization. These have been termed omega profiles and occur between axons or growth cones and glial processes. They are identified as membrane thickenings on radial glia processes that are invaginated. Within the invaginations growth cone processes can be found. However, there appears to be no fusion of the growth cone membrane with that of the radial glial cell, although dense material can sometimes be found spanning the cleft between the two membranes. On the basis of an analysis of the spatial distribution of these profiles it has been suggested that they represent the basis of a signaling mechanism that results in growth cones losing their preference for travelling among radial glial endfeet (24). This represents a transition away from the chronotopic distribution of fibers present prechiasmatically.

It was originally proposed that growth cones advanced through the chiasm exclusively along the pial surface (14, 164). It now appears that they can be found through the full depth of the rostral chiasm, with a tendency to be more numerous toward the pial surface (24, 131). The finding that new growth cones pass preferentially through the rostral chiasm is consistent with studies that have compared the differential distribution of various axon sizes across the chiasmatic midline in relation to patterns of ganglion cell production. This is consistent with the evidence that the chiasm develops caudorostrally (123, 131).

Unfortunately, during development it is not possible to differentiate the different ganglion cell classes on the basis of axon size, because at these stages they are uniformly thin, with no morphological criteria for differentiating them. Because of this, it is not possible to determine whether the cells that will mature into the separate ganglion cell classes have different rates of axon growth or distinctive growth cone morphologies. Differential growth during the postnatal period results in the fiber spectrum seen in the adult (11, 26).

Many more retinal ganglion cells are produced than are found in the adult. There are approximately twice as many optic axons at early stages compared with the number found at maturity (109, 114, 177). Behind the eye these fibers appear to be arranged in a retinotopic pattern as far as the four retinal quadrants are concerned (21). Hence, the cells destined to die during the period of natural ganglion cell death are not organized in a nontopographic manner. Detailed examination of individual growth cones and fibers has been undertaken in the developing monkey nerve. This showed that within specific regions, growth cones and fibers lost half of their original neighbors over a distance of only 8–10 μm. A total of 500 serial, transverse, ultra-thin sections were collected, and between the first and last sections of the series 92% of all initial contacts were lost. Furthermore, in the majority of cases the fibers touched by growth cone filopodia had no common neighbors (181). Hence, although there appears to be quadrant specific order, optic nerve fibers do not maintain a particular group of immediate neighbors during outgrowth, but are highly promiscuous within subregions of the nerve.

The retinotopic pattern of fiber organization is eroded along the length of the developing mouse optic nerve, and closer to the chiasm there is a major reorganization of the axon population (21). The change in order seen proximally marks the location where new growth cones segregate from being mixed with older nerve fibers to adopt a more exclusive ventral location adjacent to the pial surface. In the ferret, this change in fiber order is associated with a change in glial organization (Fig. 3). In the distal nerve, glial cells have an interfascicular distribution, with axon bundles separately encircled by glial cytoplasm. In the proximal nerve, chiasm, and optic tract, glia adopt a periventricular location with radial processes projecting down to the subpial surface. These processes pass between axon groups. The boundary between these two regions of glial organization shifts during develop-
ment with the interfascicular region progressing toward
the chiasm with development (52). The age-related pat-
terns of fiber order seen in the chiasm and optic tract can
be understood in relation to this arrangement.

One aspect of the developing nerve and chiasm that
has not been widely appreciated is the changing configu-
ration of this region during optic ingrowth. When the first
optic fibers enter the chiasm, the eyes of the embryo are
more laterally placed than at later stages of development.
Hence, the first fibers at the chiasm, which arise from
central retinal regions of each eye, meet almost head on
as the nerves approach the midline at \( \pm 90^\circ \) to it. This
occurs within a relatively loose cellular environment.
Later in development, the angle between the two nerves
declines markedly. Around the time of birth, when in
rodents the last ganglion cells are being generated in the
peripheral retina (36, 71), new fibers grow through a much
tighter environment and approach the midline at \( \pm 30–40^\circ \)
(87, 137). It is not known whether this change in the
configuration of the chiasm affects the probability of a
cell crossing the midline or not. However, it may result in
differential patterns in fibers that do cross, as early de-
cussating fibers would be expected to cross the midline at
an angle close to perpendicular, while later decussating
fibers would be expected to cross at a more acute angle.

D. Adhesion Molecules and Pathway Markers

A substantial body of literature exists on the role of
agents that provide adhesive or repellant surfaces that
influence the direction of fiber growth both in vivo and in
vitro (84, 133). Such agents have been shown to be im-
portant in the regulation of midline commissural path-
ways (140, 149, 150, 152), and although there is little doubt
that these, or related agents, probably play a role in
chiasm development, there is only limited understanding
of what it might be. Consequently, no attempt will be
made here to review this literature comprehensively as
this has been done well by others (150).

Optic nerve fibers grow out from the eye in fascicular
groups, with contact between them mediated in part by
membrane glycoproteins. The first of these glycoproteins
to be identified was neural cell adhesion molecule
(NCAM) (84). In both bird and mammal, the course of the
optic pathway from the eye into the brain is marked by
the presence of NCAM. In chicks NCAM is present on
neuroepithelial cells and preferentially distributed on the
marginal endfeet of the radial glia, which are contacted
directly by optic growth cones (141). That this molecule is
important in the regulation of patterns of fiber order along
the pathway is demonstrated by the finding that intraoc-
ular injection of anti-NCAM in the chick results in distortion of growth cone-neuroepithelial cell relationships leading to a disruption of fiber order in the optic pathway (141). This supports the idea that NCAM plays a role in axonal fasciculation and substrate adhesion, although doubts have been raised regarding its role in directional guidance. Considerable additional evidence in favor of this comes from work on fasciclin II, which in Drosophila is the immunoglobulin most similar in structure to NCAM. Less evidence is available for the mammal, although its presence is consistent with this notion (161).

NCAM is expressed along the length of the ferret visual pathway during development but appears to be distributed differently in the nerve when compared with the optic tract (130). In the nerve, NCAM distribution is axonal and around fascicular groups, whereas in the tract it is diffuse. In the chick, NCAM is present on the surface of radial glial endfeet along the visual pathway (141). However, this is not the case in mammals (132). Hence, it is unlikely that this molecule is responsible for the change in fiber order found in association with a change in glial organization in the proximal nerve (6, 50). Despite this, evidence that it may play an important role in the development of this pathway is underlined by the finding that in the chick, blocking NCAM results in degradation of fascicular order in the nerve, and of nerve fiber order along the length of the optic pathway. However, there is no evidence that it disrupts pathway selection (157).

It has been possible to look at the effects of mutations in NCAM and its isoforms in mice. Surprisingly, the effects of such mutations have been very limited, and in general have not resulted in significant disruptions to developing fiber pathways in the brain. This implies that multiple adhesion systems may be responsible for the development of fiber pathways, and it is probable that when one agent is removed genetically, others may have the capacity to take its place. To complicate matters further, these agents may have different effects at various time points in development. Hence, a knock-out model for investigating these molecules may not be as suitable in revealing their functions as was originally thought (161).

Although we know little about adhesion molecules and markers in the chiasm, this is a rapidly expanding field, and any review of them will soon be dated. Information from other systems reveals that some markers appear to change in relation to the midline. In the spinal cord the cell surface glycoprotein TAG-1 is expressed by axons that will cross the midline, but not by those that will not cross. After crossing, TAG-1 expression ceases and these fibers start to express the immunoglobulin L1 (34). L1 is expressed in optic axons that are grouped into fascicular bundles and by their growth cones, but not by the growth cones that are in contact with glial cells (8). It has been proposed that L1 may be playing a part in the changing relationship present as optic fibers pass through the chiasm (27), although the limited experimental evidence available does not support this idea (130).

Chondroitin sulfate proteoglycans (CSPGs) are extracellular matrix molecules that appear to provide an unfavorable environment for axon growth and have been implicated in the changing patterns of fiber order in the developing retinofugal pathway (16). The differential distribution of CSPGs during development appears to complement the chronotopic segregation of axons in the optic pathway and the underlying change in glial architecture of this region. Other cell adhesion and extracellular matrix molecules have been examined (L1, NCAM, and TAG-1) and appear to show no differential distribution related to changes in fiber order. CSPGs are present in older groups of optic fibers, but not in newly developing ones (130). These agents may act by providing a relatively unfavorable environment for axon addition, perhaps by disrupting adhesion molecules that promote fiber growth. In support of this idea, it has been demonstrated that both the glial architecture and the pattern of CSPG’s labeling form as a consequence of the arrival of the first optic fibers, but fail to occur in the absence of optic fibers (130).

That these molecules are involved in pathway selection at the chiasm is confirmed by observation that their removal in tissue slices results in many axons deviating from their normal course (23).

Roundabout (Robo) is a transmembrane receptor identified in Drosophila that is expressed on axons. It is involved in preventing ipsilaterally projecting axons from crossing the midline and commissural axons that have crossed the midline from recrossing (88). It is a receptor for a midline repellent molecule termed Slit, which has been identified as an inhibitory extracellular matrix molecule that is secreted by midline glia. Robo and Slit both have variants. Slit2 collapses and repels retinal ganglion cell growth cones. In rodents it is expressed at a range of locations but is absent from the ventral midline in the regions where the chiasm forms (108). The spatial and temporal expression of Slit and Robo are consistent with their playing a role in chiasm formation (41). However, the nature of this role has yet to be defined.

E. Genetic Regulation of Chiasm Development

An increasing number of genes are being identified or implicated in the mouse as players in the development of the visual system, but only those directly relevant to the problems addressed here are considered. The Pax family of genes encodes a highly conserved group of transcription factors (Pax1 to Pax9, Ref. 168). These genes have dynamic patterns of expression in the developing nervous system, and it has been proposed that they play a role in defining regions and regulating differentiation of the neural primordium (47).
**Pax6** is also expressed early in the developing mouse anterior neural plate and optic vesicle. Mutations in this gene were found to be responsible for small eye mutants in animals and for some being born without eyes (119). Targeted expression of this gene has also been shown to result in the development of ectopic eyes (53).

Similarly, **Pax2** is expressed in the developing mouse eye. It is present in the ventral half of the optic vesicle but is lost when the retina invaginates. However, it remains along the borders of the choroid fissure and extends into the ventral half of the optic stalk at E9, which is just before the first optic fibers accumulate along the nerve. The region of the ventral forebrain where optic fibers will create the chiasm is formed at roughly this stage by neuroepithelial cells that express the Sonic hedgehog (**Shh**) gene, which marks the midline region. Optic fibers progress toward the chiasm within an optic stalk that continues to express **Pax2**. As the fibers from each eye enter the midline region, **Pax2** expression becomes continuous from one eye to the other eye across the midline, but at this stage **Shh** expression is lost (Fig. 4, Ref. 160).

The fact that these patterns of expression are important for development of the chiasm is reflected by the consequences of generating a **Pax2** null mutant. In this situation the optic fissure fails to close, the separation of **Shh** expression along the chiasmatic midline is less marked, and optic fibers project directly into the ipsilateral optic tract with none crossing the chiasmatic midline. In these animals, pigmentation at the back of the eye extends throughout the length of the optic sheath, and cells of the optic stalk do not proliferate. As a result, the mutant nerve is devoid of glia. This is of interest given that glia play such an important role in changes in fiber order and pathway selection (see above). However, the significance of their absence in this situation has not been determined (160).

Mutants of the **Pax2** gene in nonmurine species also show CNS defects. In humans, the mutation is associated with coloboma and a range of serious visual defects (135), although not all the optic chiasms of these individuals have not been examined. Disruption to the normal pattern of chiasm formation has been identified in zebrafish with mutations of No-isthmus, which is a transcription regulator that is highly homologous in sequence to **Pax2** (94).

An inability to form an optic chiasm has been reported in both dogs and in some human conditions. A strain of Belgian sheep dogs has been found to be achiasmatic. These animals have relatively normal retinas and optic nerves; however, each optic nerve projects only into the ipsilateral hemisphere without the formation of a chiasm (58, 180). Achiasmatic humans were first reported by Vesalius in 1543 (see Ref. 3 for discussion). More recently, Apkarian et al. (3) have documented two cases of “nondecussating retinal-fugal fiber syndrome.” These individuals are not albino and have a normal fovea and macular region, which are commonly underdeveloped in albinos (77). Unfortunately, nothing is known about chiasmal development in either these dogs or humans.

The spatial domains of a range of other regulatory genes have been mapped in relation to the development of the chiasmatic region. Lateral to the midline retinal axons course along part of the Nkx-2.2 domain in a region that overlaps with expression domains of BF-2 and Dlx-2. Closer to the midline the axons traverse a zone of overlap between Nkx-2.2 expression and BF-1 expression (99). However, it is not clear whether these domains influence retinal ganglion cell trajectory or not, as the authors themselves point out that changes in axon pathways are commonly associated with boundaries of, or interfaces between expression domains, which was not clearly the case here.

**F. Role of Chiasmatic Neurons in Chiasm Pathway Selection**

Before the arrival of optic axons at the mouse chiasm (E11–E12), cells expressing a range of neuron-specific markers can be identified in the ventral diencephalon in the region that will develop into the chiasm. They are organized in a V-shaped pattern with the tip of the V located at the midline and pointing anteriorly. These cells

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**FIG. 4.** Schematic diagram of the patterns of **Pax2** and Sonic hedgehog (**Shh**) expression in the mouse retinofugal pathway. At E9.5 **Pax2** is expressed along the optic pathway, but at this stage it does not approach the chiasm, where **Shh** is expressed. At E11.5 **Pax2** expression is continuous across the midline through a gap in **Shh** expression. If **Shh** expression is maintained across the midline at E11.5 in a mutant, optic axons are unable to cross and project ipsilaterally. **vh**, Ventral hypothalamus; **poa**, preoptic area. [From Torres et al. (160). Reprinted with permission of the Company of Biologists Ltd.]
express two factors affecting neuronal outgrowth: 1) an immunoglobulin, L1, which is known to promote retinal ganglion cell outgrowth; and 2) a glycosylated cell surface molecule CD44, which has an opposing inhibitory effect on retinal axon outgrowth. It has been proposed that these cells act as a chiasmatic template (145, 148).

Incoming optic axons do not enter or cross regions where neurons express these markers. Rather, they turn away from them to form the cross-shaped morphology of the chiasm. The fact that these cells play a role in chiasm formation is demonstrated by the consequence of their selective removal with an antibody to CD44 at E11. Following this manipulation it is reported that optic axons fail to advance just before the point of fusion of the two optic nerves (147).

The expression of GAP-43 in developing retinal axons is also critical for normal development of the chiasm. In GAP-43 knock-outs, retinal axons appear to be delayed in crossing the chiasmatic midline. However, more importantly, once across the midline they fail to progress normally through the region between the chiasmatic midline and the optic tract, resulting in disorganized patterns of trajectory into each hemisphere. There is evidence that this is retinal ganglion cell specific and may be restricted to specific developmental stages (91, 146).

G. Role of Chiasmatic Glia in Chiasm Pathway Selection

In rodents and carnivores fibers from the temporal retina that will remain uncrossed are already distinctive, as they are generated relatively early given their eccentricity, and probably enter the chiasm at a stage before those from similar eccentricities that will cross. Hence, any mechanism invoked to explain pathway selection has to be viewed within a temporal context. Furthermore, in these animals, fibers destined to project to different hemispheres are mixed in the proximal nerve (see above). Hence, spatial factors cannot be significant in pathway selection, and such cells may need additional information before they are able to select an appropriate pathway. Significant advances to understanding the factors regulating chiasmatic pathway selection in the mouse have been made by the ability to label axons and view the chiasmatic region in real time in isolated brain preparations. Under these conditions it is possible to monitor the behavior of individual growth cones in relation to the midline between E15 and E17 (45).

In such preparations, growth cones in the chiasm have been shown to have phases of rapid advance alternating with periods in which they pause. All optic growth cones, irrespective of whether they ultimately cross the midline or not, appear to approach this region. When adjacent to the midline, they pause for periods of several hours. During these periods, the growth cones remain highly mobile, extending and retracting their filopodia. Those that turn to project ipsilaterally have markedly extensive filopodia that are constantly being remodelled. On turning away from the midline they retract the filopodia that were extending proximally and extend a single process away toward the ipsilateral optic tract, which becomes its leading edge. These growth cones continue toward the ipsilateral tract with a much simpler morphology than they possessed at the midline region (45). These results are intuitively appealing, particularly in the light of the need for fibers to obtain information before committing themselves to a specific pathway. However, they are not in agreement with those of Sretavan and Reichardt (148), who using similar methods failed to find evidence of significant pausing of uncrossed axons before they turn at the midline.

The fibers that cross the midline do so at all dorsoventral locations and are not restricted to crossing along the subpial region (131). When they cross the midline, growth cones mingle with relatively mature fibers that have already crossed, but initially remain confined within monocellular fascicles lacking glia. This pattern of organization degrades further from the midline (24, 27). These results strongly suggest that in these animals there is a midline signal that ingrowing fibers must read to make the correct pathway choice. This notion has received some support from a study in which developing cells from different retinal regions in the mouse were cocultured with those extracted from the chiasmatic region (109). Cells from the temporal retina from which the uncrossed projection arise that were removed at E15 and maintained for 1–5 days were markedly reluctant to grow across chiasmatic cells compared with those derived from other regions. The reduction in neurite outgrowth and neurite length was ~50% in the cells taken from the temporal retina compared with those from other areas.

Although this finding is consistent with results obtained in isolated brain preparations (45), there are difficulties with its interpretation. Within the adult rodent temporal retina, 75% of ganglion cells still project across the midline (30, 78) and should have been present in these preparations as the majority of crossed and uncrossed temporal cells would have been generated by the time the extracts were taken (36). Hence, the magnitude of the effect is significantly larger than would be expected as the majority of fibers from this region project across the midline. Furthermore, at the stage of development studied, there is a significant transient uncrossed projection from outside the temporal retina from cells that do not survive to maturity (25, 74).

It is possible to divide the developing chiasm into a series of glial domains that differ architecturally and in terms of the composition of their intermediate filaments. There are differences in patterns of vimentin staining
marking two transition zones in the glial architecture along the ferret optic pathway. The first occurs between the developing optic nerve and the chiasm, and the second occurs between the chiasm and the tract, with the former being associated with the gradual transition from an interfascicular glial arrangement to a radial glial pattern. Later, labeled processes can be identified running doroventrally through the body of the chiasm. Subsequently, glial fibrillary acidic protein (GFAP)-positive processes appear that are confined to the chiasmatic midline. Although these processes mark the midline as a distinctive cellular region, their appearance is relatively late, occurring after the majority of optic axons have grown through this region (131).

In marsupials, crossed and uncrossed fibers are segregated in the proximal nerve and through most of the chiasm, with uncrossed fibers confined laterally from the main body of the chiasm by a thick vertical band of astrocyte processes. During development, as the fibers destined to remain uncrossed approach the chiasm, a population of distinctive glial cell bodies can be identified at the lateral chiasmatic margins. At this stage there are very few other chiasmatic cell bodies present. Uncrossed fibers pass lateral to these cells, while those destined to cross the midline pass medially. After the uncrossed projection has grown through the chiasm, the distinctive concentration of lateral glia can no longer be clearly identified, although their processes persist (56).

Developing fibers appear to respond to midline cues, and specializations have been identified in the developing mouse chiasm that may be the source of these signals. When the mouse chiasmatic region is stained with an antibody for mouse embryonic radial glial cells (RC2), a palisade of radial glial processes can be identified ~100–200 μm wide either side of the midline. Within this palisade, and confined to the midline, is a thin band of separate processes that express a different marker, a stage specific antigen (SSEA-1). The morphology of these two populations is different. When sections are stained for both RC2 and SSEA-1, and the course of individual axons is traced in relation to these patterns of staining, it is possible to trace fiber trajectories in relation to the glial palisade (95). Early axons enter the mouse chiasmatic region around E12–E13. The first uncrossed cells do not approach the midline but turn directly into the ipsilateral optic tract. They arrive in the ipsilateral tract before axons from the contralateral eye (98). A similar observation has been made in the developing primate chiasm (102). Hence, these cells navigate into the tract without information derived from the midline or from any interactions with fibers from the other eye. Many of these uncrossed cells do not originate from the temporal retina as in the adult, but from more central regions and do not persist beyond the late prenatal stage (25).

The first axons to cross the midline do so at the caudal end of the developing chiasmatic region, whereas those that cross later tend to pass further rostrally (98). In each case, growth cones move away from radial glial endfeet at the midline toward the ventricular zone (24). During the main period of axon ingrowth (E15–E17), cells that project contralaterally enter the palisade of RC2 positive cells and progress across the midline, while cells destined to remain uncrossed from the temporal retina turn within the palisade, but do not enter the SSEA-1 positive region. At this stage all growth cones appear to make contact with the processes of these cells (97). However, it has been shown that there are two components to the uncrossed pathway: the first from the central retina lost during development and the second from the temporal retina (25). The former group enters the ipsilateral tract without approaching the midline. It is unclear why it is possible to generate an early uncrossed pathway without midline interactions, which are necessary when the uncrossed projection arises from the temporal retina. This may be related to the changes in the geometry and cellular environment of the mouse chiasm between E12 and E15.

It is clear that growth cones and axons communicate and that the information derived is used to assist pathway selection. What is not clear is the route by which this communication might occur. This question remains largely unanswered. It is becoming increasingly apparent that in the adult, optic axons and glia are able to communicate without any form of anatomical specialization, possibly via the diffusion of chemicals that they release. There is evidence that glutamate may play a role in this (90). Furthermore, mGluR3 receptors have been localized to optic nerve astrocytes but not oligodendrocytes (82). Astrocytes are generated before oligodendrocytes during the period of chiasm formation (144), and their processes have been shown to form the boundaries between crossed and uncrossed axons in the marsupial chiasm (56).

H. Interactions Between Fibers From Each Eye at the Developing Chiasm

Interactions between the fibers from both eyes at the chiasmatic midline are important for the development of the normal partial pattern of decussation found in rodents and carnivores. If one eye is removed early in development while optic fibers are still growing through this region, the normal pattern of partial decussation at the chiasm is systematically disrupted. In rodents, only a very small number of axons project ipsilaterally (37, 74). However, following early monocular enucleation at E12, before significant chiasm formation, the number of uncrossed cells found at maturity is reduced by almost 50% (44). Similar results have been found in ferrets (19).

Monocular enucleation at E16 and later in the mouse
results in an increase in the number of uncrossed cells from the remaining eye by ~25% (44). This probably occurs because fibers projecting ipsilaterally at this stage enter visual structures that have had their major input removed due to the loss of the much larger crossed projection. The resulting increase in available trophic factors/space is probably responsible for the reduction in the magnitude of the wave of natural ganglion cell death (44, 74).

These results are in marked contrast to those of Sretavan and Reichart (148) who find evidence that midline fiber interactions can produce axon turning in the mouse, but not necessarily toward the optic tract. Moreover, they failed to find any significant change in the chiasmatic projection from the remaining eye if the other is removed during early chiasm formation. There is no obvious explanation for this result, which is in marked contrast to the findings of Godement et al. (44) and those undertaken in ferret (50, 156).

Studies of early eye removal in ferrets by Taylor and Guillery (156) examined animals before the main phase of natural ganglion cell death in the remaining eye. During early stages, uncrossed cells arise from both the temporal retina and more central regions, with the central uncrossed projection being generated before that from the temporal retina (20, 25). The projection from more central regions is lost as a consequence of natural cell death. Early enucleation results in almost total absence of an uncrossed chiasmatic projection irrespective of location. Enucleation undertaken slightly later results in an abolition of the uncrossed projection from the temporal retina of the remaining eye, leaving only that from more central regions. When the course of these axons is traced at the chiasm, many are seen to stop in regions lateral to the chiasmatic midline (156). Hence, the effect of monocular enucleation on the chiasm is progressively more severe the earlier the procedure is undertaken, with only those uncrossed cells that have already traversed the chiasm before enucleation surviving to form an ipsilateral projection.

If such midline interactions are critical for normal chiasm development, what happens in animals in which fibers from each eye destined for different hemispheres remain separate, with uncrossed cells restricted laterally? In marsupials where the uncrossed projection is confined laterally at all stages during development (56), enucleation has no effect on chiasmatic pathways irrespective of the time it is undertaken. The uncrossed projection from the remaining eye does not change in magnitude even if the enucleation is undertaken before the formation of the normal uncrossed projection from the temporal retina (155). This is in sharp contrast to the results of similar experiments in rodents and ferrets (20, 44). These results reveal that the different fiber patterns found in adult animals with crossed and uncrossed fibers either mixed or segregated are associated with fundamentally different mechanisms of chiasm formation. In the former, optic axons require information from the chiasmatic environment before selecting their pathway. In the latter group axons do not appear to need such information.

IV. CHIASMATIC ABNORMALITIES FOUND IN ALBINISM

Hypopigmented mutants have systematic abnormalities in their chiasmatic pathways. In all albino mammals that have been studied, there is a shift in the naso-temporal division of the retina toward the temporal periphery, with a population of ganglion cells that would normally project ipsilaterally, projecting inappropriately to the contralateral side of the brain. This results in abnormal binocular maps in central visual structures (49). This is not the only abnormality, because there is a rod deficit and the central retina is underdeveloped (77, 79). Deficits associated with albinism appear to be the preserve of mammals (83). However, not all mammals are affected as the central retina of the albino squirrel is normal. This may be related to the fact that this animal is highly unusual among mammals in having a cone dominated retina (42).

A. Role of Temporal Ordering in Patterns of Retinal Cell Addition and Outgrowth

Two factors provide evidence that the focus of the albino abnormality is located in the retina rather than in the chiasm. First, there are significant differences in the organization and development of the chiasm between rodents and carnivores on the one hand, and marsupials on the other. However, albinos in both groups of animals show an abnormally small uncrossed projection (48, 49). Hence, chiasmatic structure is not important for the development of the abnormality. Second, when developing retinal axons are grown in vitro and presented with cells from the immature chiasmatic region, no differences in patterns of growth can be found between growth over cells derived from pigmented compared with albino animals (100). Consequently, it is unlikely that albino chiasmatic cells produce a chemical signal that differs from that found in normally pigmented animals.

It has previously been claimed that there are differences in fiber organization in the optic stalk during development between pigmented and albino rodents and that ectopic fibers from the temporal retina are misrouted at the chiasm (142). However, this has been shown to be incorrect, and the patterns of outgrowth from the eye are the same in both types of animal (28). Also, no significant differences have been found in the organization of the uncrossed projection through the nerves of these animals.
(75). Hence, there are no significant spatial differences in the organization of the optic nerve of the albino compared with that found in normally pigmented animals.

Evidence strongly suggests that the albino abnormality is related to a temporal disruption in development. It is known that there is a disruption in the center to periphery pattern of maturation in the albino retina (174). There is a clear delay in the center to periphery gradient in cell production in the ganglion cell layer, which is not obvious very early in development but becomes increasingly more marked toward the end of the period of ganglion cell production (71). Also, there is a delay in the development of some of the terminal projections in central structures of hypopigmented animals (9). Recently, it has been shown that cells in the developing albino retina may not exit the cell cycle at appropriate times but are delayed by the absence of dopa, a melanin precursor normally present in the RPE that is known to be a cell cycle regulator (1, 72).

As in some rodents and carnivores, uncrossed cells are generated before those from similar eccentricities that project across the midline; the probability of a cell projecting ipsilaterally declines with time (6, 36). If there is a delay in patterns of ganglion cell production, it is conceivable that it might increase the probability of a cell projecting contralaterally, resulting in an aberrant crossed projection.

During development there are two uncrossed projections. The first arises from dorso-central retina but is lost later in development. The second arises from the temporal retina and is produced after that arising centrally. From the earliest stage the uncrossed projection arising from the temporal retina is abnormally small. However, the initial uncrossed projection from central retina is normal in albinos (19). This reinforces the idea that the problems associated with albinism are focused later in development rather than earlier. But it is not clear how the delay results in the systematic shift in the naso-temporal division found in these albinos.

The data from albinos point strongly to there being a temporal disruption in the form of a delay in ganglion cell production in these animals, with cells leaving the cell cycle at inappropriate points, resulting in abnormal patterns of projection through the chiasm. This reinforces the notion that any mechanism proposed to explain chiasm development must be viewed within a dynamic temporal framework.

V. MATURE STRUCTURE AND DEVELOPMENT IN NONMAMMALIAN VERTEBRATES

Many nonmammalian vertebrates have highly developed visual systems with substantial ganglion cell populations. Furthermore, because uncrossed pathways exist in members of all vertebrate classes (170), many of these animals are likely to have complex chiasms. As with mammals, there have been few substantial studies of the nonmammalian chiasm where cells giving rise to different hemispheric projections have been traced through this region. Those studies that have been undertaken have mainly been focused on three animal types: frog (mainly *Xenopus laevis*), bird (mainly the chick), and fish. Only the first two will be considered, since the fish chiasm is qualitatively distinct, in that in many fish the chiasm does not exist as recognized in other animals. Rather, the optic nerves lap over one another and become the optic tracts, without fusing (117, 163).

A. Amphibians

The frog retinofugal pathway has been studied in some detail, mainly because it is a classic animal model used in studies of the development of the retino-tectal system. Unfortunately, it does not possess a sophisticated visual system, but it does have a clear partial pattern of decussation. Cells have been described in the frog *Xenopus laevis* that give rise to the uncrossed chiasmatic pathway to the thalamus. The cells that give rise to this projection are large multipolar and confined to the temporal retina. At the optic nerve head they occupy a relatively central position. Behind the eye they regroup around the circumference of the nerve. At this location there are pigmented processes and an increase in nerve diameter. Through the majority of the nerves length, uncrossed fibers remain located around the circumference. They reorganize in the intracranial segment and adopt a ventral location. At the point where the nerves meet, uncrossed fibers turn directly into the optic tract without approaching the chiasmatic midline (138, 154).

In *Rana pipiens*, uncrossed cells have been described following unilateral tracer injections into the tectum rather than into the thalamus (143). Uncrossed cells were found in all retinal regions in this species and appeared to have a more diverse morphology than those identified in *Xenopus*. The different retinal location of these cells is probably due to differences in their projection, with those in *Xenopus* projecting to the thalamus and those in *Rana* projecting to the tectum. No attempt has been made to trace their pathway through the chiasm in *Rana*.

As in the retinofugal pathway of some mammals, there is evidence that changes in fiber order within both projections are associated with changes in glial architecture. This is particularly true of the change in fiber order just in front of the chiasm where fibers initiate a shift in organization toward the pattern found in the optic tract (154). When chiasmatic pathways are traced during development, newly growing axons can be found through...
the most ventral regions of the proximal nerve. Initially, the chiasm is formed from a loose meshwork of glial processes and optic and nonoptic axons. With development, axons that have grown through the chiasm are displaced dorsally and caudally to form the deeper layers of the chiasm. There is no clear glial segregation between new and old fibers (182).

Interestingly, the development of the uncrossed projection in *Xenopus* is regulated by thyroxine, the hormone that is responsible for metamorphic induction in this animal (63). Small injections of thyroxine into the eye can induce the precocious development of an uncrossed pathway through the chiasm in an animal before metamorphosis. Hence, an uncrossed projection can be induced in one eye while that from the other has still to develop. The development of this projection can also be retarded by blocking thyroxine systemically. Furthermore, it is then possible to induce development of an uncrossed pathway at will following withdrawal of systemic thyroxine blockade. These results reveal that interactions between fibers from both eyes at the chiasm are not necessary for normal chiasm development in *Xenopus*. These data also support the notion that in this animal there is no specific temporal window for the development of the uncrossed pathway (64–66). However, there are some qualifications to the above manipulations, in that it appears that it is not possible to induce an uncrossed projection very early in development, only in a time window just before metamorphosis (106).

Ephrins and Ephs are ligand and receptor molecules shown to be involved in key developmental processes including topographic ordering of retinal projections (60). Studies undertaken in *Xenopus* have shown that EphB expressing retinal ganglion cells appear at metamorphosis in a subpopulation of temporal retinal ganglion cells, which have an uncrossed pathway. When such cells are grafted to younger embryos, these cells project across the midline, implying that an appropriate chiasmatic signal is absent at this early stage. Ephrin-B is expressed at the chiasm of metamorphic animals, but not before. When it is expressed prematurely in the embryonic chiasm it induces a precocious uncrossed pathway from EphB expressing ganglion cells (106). Interestingly, the authors of this study also state that they could not detect Ephrin-B in the chiasm of fish or birds, but did detect weak label at E13.5 and a more marked label at E16.5 in the mouse, consistent with the time at which uncrossed fibers grow through the chiasmatic region (25). However, the role of Ephrin-B in the mammalian chiasm has still to be explored.

**B. Birds**

A common feature of the bird chiasm is that it is formed by a series of interdigitated fingers that represent grouped optic fascicles from each eye that alternate across the midline (117, 163). Although this feature has been seen in numerous bird species, it has only been systematically examined in the chick. Here, the fascicles are separated from each other by blood vessels and connective tissues. There appears to be little or no change in fiber order within or between fascicles along the nerve and through the chiasm (40). The total number of fascicles crossing the midline is ~34, although there is considerable variability in this number (38). A similar, although less marked, pattern of interdigitation can be seen in marsupials and tree shrews (80, 81). In tree shrews the fascicles are also segregated by connective tissue (81). That these common features exist in such diverse animal types says little about their relationship to one another, but it does highlight the large diversity in chiasmatic architecture.

During development, early optic axons enter the bird chiasm along the outer limiting membrane and pass between radially oriented processes of neuroepithelial cells (39). As more fibers accumulate in this region, the majority of growth cones, but not all, are identified in the ventral-most fascicles. This distribution gradient becomes more marked with progressive development so that the chiasm appears to develop predominantly from the pial surface.

It has commonly been assumed that birds do not have an uncrossed chiasmatic pathway. However, a transitory projection can be found in the developing chick, and careful analysis has revealed that this does not regress completely. A small uncrossed projection can be identified in restricted regions of the thalamus and the pretectum at maturity (111). Unfortunately, the retinal origin of this projection in the mature animal is not known, nor is its chiasmatic course. But during development optic axons can be identified entering the ipsilateral optic tract at the dorsal margin of individual fascicles. These fibers appear to arise from fascicles that are mainly composed of fibers destined to cross the midline (101). The chick is not alone in having an uncrossed projection to the thalamus and pretectum. A similar pattern of projection has been identified in the quail. In this study a number of different tracer methods were used to reveal the projection, but it could only be identified with the most sensitive of these, which is consistent with the projection being very small (175).

**VI. CONCLUSION**

Our understanding of the organization of chiasm and the factors that sculpt its development has been restricted by the very limited range of animal models that have been used to analyze this structure. It is increasingly clear that there is no such thing as the mammalian chiasm; rather,
there is a diverse series of structures that are put together in different ways in different animals. Although significant advances have been made in the fields of cellular and molecular biology in relation to chiasm development, these have only been undertaken primarily on mice, whose chiasm is probably not representative of the majority of other mammals including humans.

Different laboratories perceive chiasm formation in terms of the methods they employ. However, irrespective of the animal type considered, a myriad of different factors probably play a role in chiasm development, and the different methods employed to analyze its maturation whether histological, molecular, or cellular reveal only different facets of a complex interactive process. Even in the mouse there has been little effort to try to understand how these different features interact and too great a tendency to interpret chiasm formation in terms of a single mechanism. Little effort has been made to see the dynamic nature of this region. The axis of fiber ingrowth rotates by almost 45° between the first and last ganglion cell entering the chiasm. Early axons navigate through a loose cellular environment, while late fibers are packed in a much tighter framework with older cohorts. It is unlikely that the nature of navigational decisions in pathway selection remain the same over these periods within any individual animal model.

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STRUCTURE AND DEVELOPMENT OF THE OPTIC CHIASM


