Eye (2007) 21, 1264–1270

www.nature.com/eye

CAMBRIDGE OPHTHALMOLOGY SYMPOSIUM

Chiasm formation in man is fundamentally different from that in the mouse

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Abstract

At the optic chiasm axons make a key binary decision either to cross the chiasmal midline to innervate the contralateral optic tract or to remain uncrossed and innervate the ipsilateral optic tract. In rodents, midline interactions between axons from the two eyes are critical for normal chiasm development. When one eye is removed early in development the hemispheric projections from the remaining eye are disrupted, increasing the crossed projection at the expense of the uncrossed. This is similar to the abnormal decussation pattern seen in albinos. The decussation pattern in marsupials, however, is markedly different. Early eye removal in the marsupial has no impact on projections from the remaining eye. These differences are related to the location of the uncrossed projection through the chiasm. In rodents, axons that will form the uncrossed projection approach the chiasmal midline, while in marsupials they remain segregated laterally through the chiasm. Histological analysis of the optic chiasm in man provides anatomical evidence to suggest that, unlike in rodents, uncrossed axons are confined laterally from the optic nerve through to the optic tract and do not mix in each hemi-chiasm. This is a pattern similar to that found in marsupials. Electrophysiological evidence in human anophthalmics shows that the failure of one eye to develop in man has no impact on the hemispheric projections from the remaining eye. This strongly suggests that the mechanisms regulating chiasmal development in man differ from those in rodents, but may be similar to marsupials. This implies that optic chiasm formation in rodents and ferrets is not common to placental mammals in general.

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Received: 7 March 2007
Accepted: 21 March 2007

Eye (2007) **21,** 1264–1270; doi:10.1038/sj.eye.6702839

Keywords: optic chiasm; visual evoked potential; anophthalmia; albino

Mammalian optic chiasm

The retinofugal fibres in the mammalian visual system make a binary decision to either cross the chiasmal midline and project to the contralateral hemisphere or remain uncrossed and project to the ipsilateral hemisphere. The percentage of fibres that remain uncrossed varies between species depending upon how lateral the eyes are placed in the head. This partial decussation of optic fibres at the chiasm forms the basis for normal binocular vision.

The developmental mechanisms that determine the choice of projection in rodents and ferrets are similar. In rodents and ferrets the retinotopic order present in the optic nerve is lost before the nerves fuse at the anterior chiasm[1–3] and the two projections from each eye mix through each hemi-chiasm (Figure 1).[4–6]

The marsupial chiasm is distinctly different from that in the rodent and ferret. The architecture of the chiasm is a central core and two lateral appendages. Axonal projections destined for different hemispheres remain strictly segregated along the complete length of the optic nerve and through each hemi-chiasm (Figure 2). Retinal ganglion cell axons destined for the ipsilalateral hemisphere never approach the chiasmal midline and remain confined laterally in the proximal optic nerve and chiasm. In the chiasm they course through the lateral appendages, which are segregated from the contralaterally projecting axons by astrocytic processes.[7,8]

Primate optic chiasm

There have been very few examinations of fibre order through the human optic chiasm. However, the results obtained remain controversial. One of the earliest studies by Wilbrand and Saenger[9] showed that some axons located laterally in the nerve cross the chiasmal midline and that many crossed axons coursed through the proximal contralateral nerve before approaching the optic tract. However, this analysis was undertaken in tissue from a subject who had lost one eye 24 years before death. Clinical studies cast further light on patterns of fibre organisation through the human optic chiasm. Patients with pituitary tumours have a suprasellar extension from the pituitary fossa, which gives rise to central chiasmal compression. This primarily disrupts the crossed projection, while aneurysms impinging on the lateral chiasm primarily disrupt uncrossed axons.[10,11] Such data are consistent with at least a partial separation of the two projections.

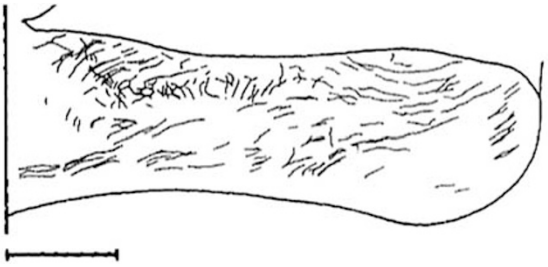

Figure 1 The distribution of uncrossed fibres in the rodent hemi-chiasm. The midline is to the left and the chiasm has been sectioned transversally. In rodents and ferrets these fibres are distributed widely rather than grouped laterally. Scale bar, 250 μm (from Baker and Jeffery[4]).

Fibres patterns in the non-human primate have been studied more extensively.[12–15] Horton[12] examined anterograde labelling patterns through the primate chiasm and showed that the findings described by Wilbrand and Saenger[9] in the human were an artefact resulting from neuronal degeneration following monocular enucleation. These data[12] supported a number of other studies undertaken in Old World Primates, suggesting that the two hemispheric projections from each eye are largely segregated through the chiasm, with the uncrossed projection confined laterally, similar to that seen in the marsupial. Early studies on the *Macaque* monkey induced retinal lesions at specific locations to trace the patterns of nerve fibre degeneration through the optic nerve and chiasm. The studies confirmed that temporal retinal lesions result in degeneration confined to the lateral optic nerve and lateral chiasmatic regions alone. Lesions of the nasal retina, that have no involvement from axons originating from the temporal region, result in degeneration only in the medial or central optic nerve and in the central chiasmatic regions.[13,14] Further studies by Naito[15] on the *Macaque*, showed that when retinal ganglion cells in the temporal retina are labelled following small tracer injections into the thalamus, the labelled axons course laterally through the chiasm, while the chiasmatic pathways of more centrally located ganglion cells do not enter this region. These data suggest that the fibre architecture of the primate optic chiasm is consistent with that seen in the marsupial.

Monocular enucleation studies

It appears that there may be at least two structural forms to the mammalian chiasm; one present in

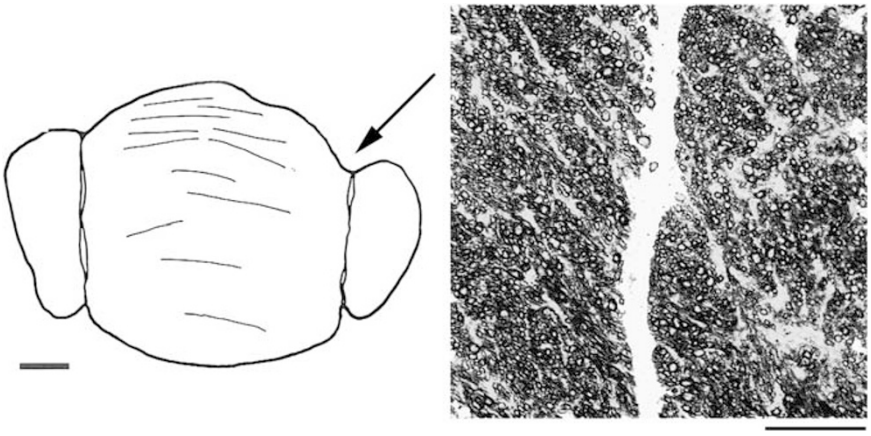

Figure 2 Coronal section through the marsupial optic chiasm, showing the main body of the chiasm and two lateral components (left, scale bar 0.25 mm). The fibres that will cross to the contralateral hemisphere are contained within the main body. The fibres that will project to the ipsilateral hemisphere are contained within the two lateral appendages. The arrow indicates the location of the fissure that divides the main body of the chiasm from the lateral body. This is detailed in the photomicrograph (right, scale bar 40 μm), where the fissure is continuous along the dorsoventral axis (from Jeffery and Harman[7]).

rodents and ferrets and the other in marsupials and perhaps man. Monocular enucleation studies have also shown that the developmental mechanisms of optic chiasm formation are fundamentally different in these animals. In rodents and ferrets, where crossed and uncrossed fibres are intermingled at the chiasmal midline, monocular enucleation early in development reduces the uncrossed projection in favour of the crossed.[16,17] This is similar to the fibre pattern found in albinism, where disruption of melanin synthesis, due to a mutation of the Tyrosinase gene, results in an abnormal projection of the retinofugal fibres at the optic chiasm (Figure 3).[18] However, in marsupials, where uncrossed axons are segregated laterally and do not approach the chiasmal midline, early eye removal has no impact on the projections from the remaining eye.[19] Hence, there are at least two separate mechanisms regulating chiasm formation. One in rodents and ferrets where midline interactions are significant, and another in marsupials where they are not.

Fibre patterns in the human optic chiasm

Rodent and ferret models of chiasm development have been regarded as typical of most mammals, including man.[20–23] However, there is evidence that the human chiasm is fundamentally different. A recent study by Neveu *et al*[24] showed that the human optic chiasm has two spatially distinct retinal axon trajectory patterns that probably represent the course of the crossed and uncrossed hemispheric projections.

Histological analysis of human nerve fibre patterns

Histological analysis of nerve fibres in the optic chiasm was carried out to investigate the decussation pattern of the visual pathways in the human.[24] Fibres were silver-stained and the fibre patterns at the proximal optic nerve, chiasm and distal optic tract were examined. Detailed analysis of these regions demonstrated that the human optic chiasm contains two spatially distinct retinal axon trajectory patterns that probably represent the course of the crossed and uncrossed hemispheric projections (Figure 4). These appear to be largely separate, with the uncrossed projection confined laterally, while the crossed projection occupies more central locations. Groups of axons in the central region are interdigitated in regular plaits across the full length of the midline, demonstrating that axons from each eye intermingle in this region as they course through to the contralateral optic tract. Axons located laterally could be traced in clear parallel lines through from the junction with the nerve towards the optic tract, and at no point did they appear to deviate from this lateral region and approach the midline (Figure 4). However, there was no obvious morphological feature separating the two projections as found in the marsupial.[7]

Further, there is no obvious change in axon order in the proximal optic nerve that might represent a shift away from the retinotopic pattern found along the length of the optic nerve. This is consistent with the notion that position alone may influence pathway choice rather than interactions at the midline. The fibre patterns seen in humans are consistent with axon patterns found in marsupials, but not those found in rodents and ferrets.[4,6,7,22,25]

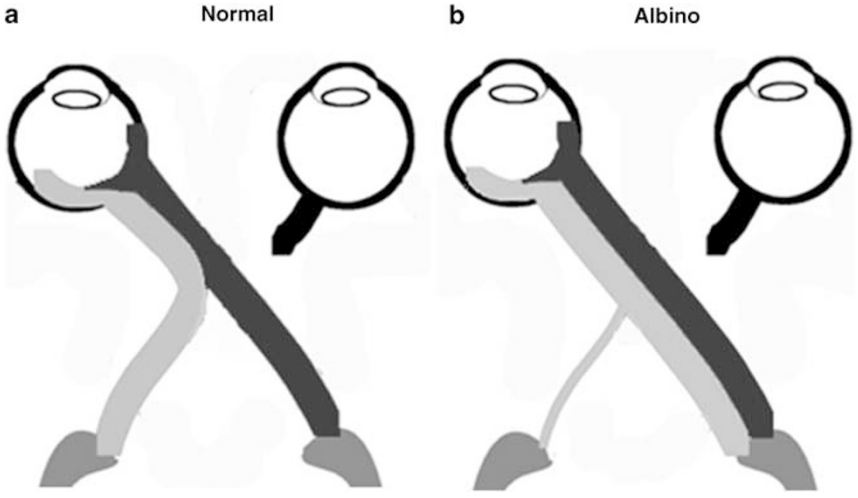

Figure 3 Schematic of normal and albino visual pathways. (a) Normal visual pathways. Nerve fibres originating from temporal retina project to the ipsilateral hemisphere (light grey line). Nerve fibres originating from nasal retina) cross at the chiasm and project to the contralateral hemisphere (dark grey line). (b) Albino misrouting. The majority of optic nerve fibres decussate to the contralateral hemisphere (light and dark grey lines).

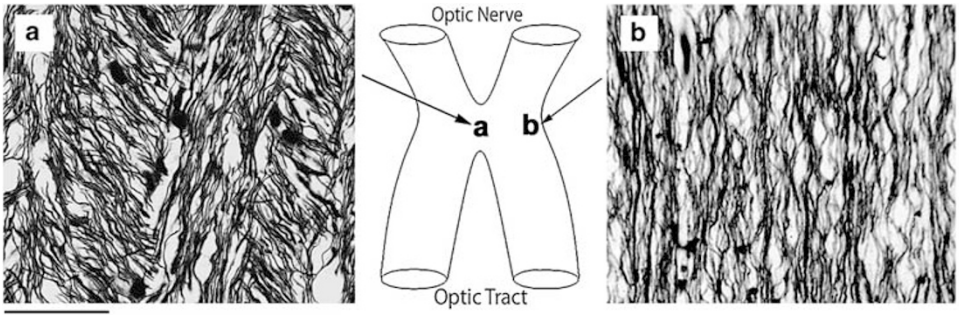

Figure 4 Silver-stained horizontal sections of the human optic chiasm. The schematic shows the relative location of each micrograph. (a) Optic axons from the midline region of the central chiasm. Here, groups of axons from each eye are interdigitated in regular plaits across the midline. The plaits are approximately 50 μm wide. (b) Stained axons from the same chiasmatic section but located laterally. Here, axons course in parallel groups from the junction with the nerve towards the optic tract, with no obvious change in axon order and can be traced along the length of the chiasm. At no point did they appear to deviate from this lateral region. Scale bar, 50 μm (from Neveu *et al*[24]).

Electrophysiological analysis of cortical hemispheric projections in anophthalmics

Unlike rodents and ferrets, which have a relatively small number of optic axons (approximately 110 000)[26] and a small uncrossed projection,[27,28] the retinogeniculate pathway in man is large, comprising of approximately 1.1 million optic axons,[29–31] and the chiasmatic pathways from each eye in primates divides almost equally between hemispheres.[32] This can be demonstrated using the visual evoked potential (VEP), an electrodiagnostic test that enables objective assessment of the visual pathways, including a representation of the hemispheric projections to the visual cortex. The relative size and timing of the responses recorded over each hemispheric projection gives an indication of the retinocortical fibre decussation pattern at the optic chiasm. Therefore, in the normal, the size and timing of the responses from each hemisphere are symmetrical, demonstrating an equal decussation of the retinocortical fibres to the ipsilateral and contralateral hemisphere. This is very different to the VEP pattern seen in albino patients. In the albino, a larger and faster conducting response is seen in the contralateral hemisphere to the stimulated eye. The responses in the ipsilateral hemisphere are smaller and delayed compared to those in the contralateral hemisphere, demonstrating an abnormal decussation pattern, where the majority of nerve fibres cross the chiasmal midline to innervate the contralateral hemisphere. VEPs therefore can be used to determine if the pattern of decussation in human monocular anophthalmics is similar to that seen in the rodent and ferret or if it is similar to the marsupial.

VEP techniques were used to examine a group of patients with anophthalmia or severe microphthalmia and compare them with age-matched albino and normal controls.[24] Anophthalmia is a genetically determined disorder where the patient is born without an eye or a chronically underdeveloped eye.[33] It is predominantly due to a mutation in the SOX2 gene,[34] although mutational analysis has shown that other genes such as OTX2 and Pax6 have also been implicated in malformation or the absence of an eye at birth. These genes play a significant role in the development of the eye.[35]

Anophthalmic patients were examined to study optic chiasm development when only one eye was present at birth, this situation being analogous to that of early unilateral eye removal in animals. However, it could be argued that in anophthalmic/severe microphthalmic subjects, two optic nerves were present at some early stage of development, during which the ground plan for chiasm formation was established, but that one was subsequently degenerated. Although possible, ultrasound scanning and orbital examination during surgery revealed no significant anatomical or histological remnant of viable neural tissue in the socket. Also, the failure of the orbit to develop without surgical intervention is an indication that an eye was not present. Even in those with severe microphthalmia rather than anophthalmia, a significant optic nerve is rarely formed. In addition, normal retinal function was demonstrated in the remaining eye of all individuals by recording the electroretinogram from this eye.

The VEP in anophthalmic and severely microphthalmic patients is symmetrical, reflecting a normal chiasmatic decussation of the nerve fibres from the eye to the brain, in the absence of the contralateral eye.[24] There was no evidence of an asymmetrical albino-like VEP in any of these patients, suggesting that normal development of the human optic chiasm, unlike the rodent and ferret, does not require the interaction of nerve fibres from both eyes to determine the correct choice of projection. Development of the human chiasm

is unaffected by the absence of the contralateral nerve fibres. Although the methods of analysis differ, data from the human and the marsupial show a consistency that distinguishes them from rodents and the ferret.

Discussion

The mammalian optic chiasm can be grouped into at least two basic forms, that seen in rodents and ferrets and that seen in marsupials (Figure 5). In rodents and ferrets retinotopic order is lost in the proximal nerve where there is a major change in axon order, and axons approach the midline before deciding whether to decussate or turn away and innervate the ipsilateral hemisphere.[4,6,36] Two mechanisms may influence this pattern of decussation. First, there are interactions between axons from the two eyes at the midline region, and second, there are interactions between midline glia.[5,37] The importance of the first mechanism is confirmed by monocular enucleation studies in these animals where the removal of one eye results in an albino pattern from the remaining eye.[16,17] Different mechanisms are described in marsupials. There is no change in the pre-chiasmatic axon order and axons that form the uncrossed projection remain confined laterally through the chiasm and are segregated by astrocytic processes.[7] Similar patterns are found in the tree shrew.[38] Therefore unlike the rodent, no axons destined for the ipsilateral hemisphere approach the chiasmal midline and it is the spatial orientation of these fibres, such as their position in the nerve determines their choice of projection. This is supported by the observations of early eye removal in marsupials over a range of developmental stages that has no impact on the projections from the remaining eye,[19] similar to the observations reported in man.

The segregated pattern of hemispheric projections through the mammalian chiasm were first identified in marsupials and tree shrews.[7,38] These animals represent the prototypic mammalian form, which was a shrew like marsupial.[39] Rodents and ferrets branch as separate groups at a later stage. Hence, it is probable that the segregated pattern of hemispheric projections found in the chiasm of primates including man, predominates among mammals and lower vertebrates, and studies in progress support this.

The optic chiasm is a popular model for studying axon guidance. Research on its development has been a key area of recent research activity, because at this location optic axons make a binary choice of whether to cross the midline or not. Chiasm formation in rodents is markedly different from that in man. Recent studies strongly suggest that the architecture of this region and the mechanisms that regulate its development differ

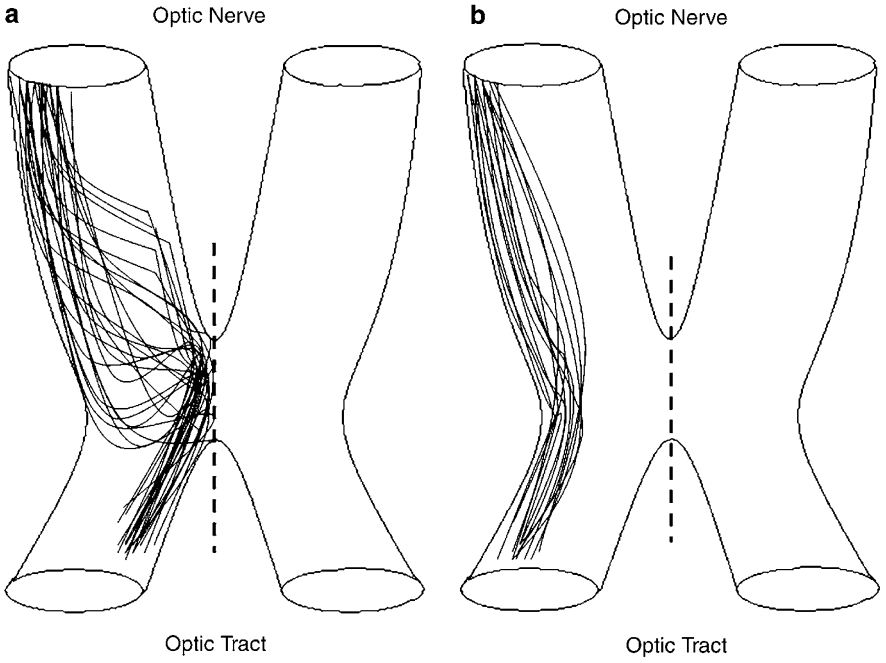

Figure 5 Schematic drawings showing two distinct forms of axon projection to the ipsilateral optic tract and chiasm formation. (a) Representative pattern of chiasm formation in the rodent, where axons that form the ipsilateral projection interact with axons from the other eye at the chiasmal midline (dashed line), before innervating the ipsilateral optic tract. (b) Representative pattern of chiasm formation in the marsupial and man, where axons that form the ipsilateral projection maintain a lateral course through the optic chiasm into the ipsilateral optic tract.

significantly between the two. This is of particular importance as chiasm development in the mouse is thought to reflect that in man. Studies in the marsupial and man suggest otherwise; therefore, caution should be exercised when attempting to extrapolate from rodents to higher mammals.

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