

SEGREGATED HEMISPHERIC PATHWAYS THROUGH THE OPTIC CHIASM DISTINGUISH PRIMATES FROM RODENTS

G. JEFFERY,^{a*} J. B. LEVITT^b AND H. M. COOPER^{c,d}

^a*Institute of Ophthalmology, University College London, Bath Street, London EC1V 9EL, UK*

^b*Department of Biology J526, City College of New York, 138th Street and Convent Avenue, New York, NY 10031, USA*

^c*INSERM, U846, Stem Cell and Brain Research Institute, Department of Chronobiology, F-69500, Bron, France*

^d*University of Lyon, Lyon I, UMR-S 846, 69003, Lyon, France*

Abstract—At the optic chiasm retinal fibers either cross the midline, or remain uncrossed. Here we trace hemispheric pathways through the marmoset chiasm and show that fibers from the lateral optic nerve pass directly toward the ipsilateral optic tract without any significant change in fiber order and without approaching the midline, while those from medial regions of the nerve decussate directly. Anterograde labeling from one eye shows that the two hemispheric pathways remain segregated through the proximal nerve and chiasm with the uncrossed confined laterally. Retrograde labeling from the optic tract confirms this. This clearly demonstrates that hemispheric pathways are segregated through the primate chiasm.

Previous chiasmatic studies have been undertaken mainly on rodents and ferrets. In these species there is a major change in fiber order pre-chiasmatically, where crossed and uncrossed fibers mix, reflecting their embryological history when all fibers approach the midline prior to their commitment to innervate either hemisphere. This pattern was thought to be common to placental mammals. In marsupials there is no change in fiber order and uncrossed fibers remain confined laterally through nerve and chiasm, again, reflecting their developmental history when all uncrossed fibers avoid the midline. Recently it has been shown that this distinction is not a true dichotomy between placental mammals and marsupials, as fiber order in tree shrews and humans mirrors the marsupial pattern.

Architectural differences in the mature chiasm probably reflect different developmental mechanisms regulating pathway choice. Our results therefore suggest that both the organization and development of the primate optic chiasm differ markedly from that revealed in rodents and carnivores. © 2008 IBRO. Published by Elsevier Ltd. All rights reserved.

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At the mammalian optic chiasm, fibers from each eye meet and separate into those that cross the midline and those that turn away to innervate the ipsilateral hemisphere. There have been many studies of chiasmatic architecture and development (reviewed in Jeffery, 2001; Jeffery and

Erskine, 2005). However, they have been undertaken in a limited range of mammalian species, primarily rodents and ferrets. Here, the optic nerve's retinotopic order is lost proximal to the chiasm and crossed and uncrossed fibers mix completely (Baker and Jeffery, 1989; Baker, 1990; Jeffery, 1990). In each hemi-chiasm, fibers with different hemispheric pathways remain mixed and there are no obvious internal landmarks defining their separate course (Baker and Jeffery, 1989; Reese and Baker, 1992; Baker and Reese, 1993). This pattern of fiber order reflects the developmental history of this region. In mouse, each developing fiber, irrespective of its destination, approaches the midline and interacts with fibers from the other eye, before deciding to cross or turn back (Godement et al., 1994). The importance of these interactions is highlighted by the finding that early unilateral eye removal results in a systematic disruption in chiasmatic pathways from the remaining eye (Godement et al., 1987; Guillery, 1989).

These fiber patterns are not ubiquitous in mammals. In marsupials and tree shrews no change in fiber order occurs in the proximal nerve, and uncrossed chiasmatic fibers remain segregated laterally, a pattern that is present from the earliest developmental stages. In marsupials early eye removal has no impact on projections from the remaining eye, underscoring the fact that here midline fiber interactions between the eyes play no role in chiasm development (Harman and Jeffery, 1992; Jeffery and Harman, 1992; Taylor and Guillery, 1995; Jeffery et al., 1998; Knabe et al., 2008). These results demonstrate that the marsupial chiasm and the mechanisms that sculpt it are fundamentally different from those in mice. While it is possible to argue that tree shrews and marsupials represent minor subsets of the mammalian class, their importance in mammalian phylogeny is highlighted by the fact that the first mammals were shrew-like marsupials (Novacek, 1992).

If there are two fundamental plans of the mammalian chiasm, this raises the question of which mammals can be classified into each group. Surprisingly, it has recently been shown that fiber order through the human proximal optic nerve and chiasm is similar to that in marsupials, and unlike that in mice (Neveu et al., 2006), although the two pathways have not been selectively labeled. This is nevertheless supported by clinical evidence, as midline pituitary tumors disrupt crossed fibers (Holder, 1991), while aneurysms that put pressure on the lateral chiasm disrupt uncrossed fibers (Day, 1990). That regulation of chiasm development is different in human compared with mouse is reinforced by consideration of cases where one eye fails to develop, a situation analogous to that where one eye is

*Corresponding author. Tel: +44-207-6086837; fax: +44-207-6086850. E-mail address: g.jeffery@ucl.ac.uk (G. Jeffery).

Abbreviations: LGN, lateral geniculate nucleus; PBS, phosphate-buffered saline; VEP, visual evoked potential.

removed early in mice and marsupials. Unlike in mice where this results in a systematic disruption to hemispheric pathways, it has no impact on humans, similar to that found in marsupials (Neveu et al., 2006).

It is not known whether humans are representative of primates in general terms of chiasmatic organization. Further, no primate studies have traced hemispheric projections and compared these with fiber pattern orientations or structural features. Here we examine these elements in a New World primate, the marmoset, to reveal whether this animal has a segregated pattern of hemispheric pathways through the chiasm.

EXPERIMENTAL PROCEDURES

All of the marmoset tissue used in this study was obtained from animals used primarily for other purposes. It came either at post-mortem from animals used in unrelated physiology or anatomy studies, or from tissue already processed for a comparative study of the ocular innervation of the suprachiasmatic nucleus (Magnin et al., 1989; Mick et al., 1993).

Fiber and collagen staining

Optic fibers through the nerve and chiasm were stained in eight common marmosets (*Callithrix jacchus*). These animals had been used for electrophysiological experiments; tissue was retrieved at their termination following trans-cardiac perfusion with phosphate-buffered saline (PBS, pH 7.2) followed by 10% formalin in phosphate buffer (pH 7.2). In each case the optic chiasm was dissected free from the brain with the optic nerves and eyes attached. The proximal optic nerves (~5–10 mm) and chiasms were then processed for wax embedding and sectioned horizontally at 10 μm . These sections were then processed with Marsland, Glees and Erikson's method for silver staining axons (Bancroft and Stevens, 1990) or fiber stained with p-phenylenediamine (Hollander and Vaarland, 1968). The extra-fascicular matrix of the optic nerve is collagen rich (Jeffery et al., 1995) and the limits of individual fascicles are easily identified in collagen-stained material. A sample of paraffin sections collected above was processed with the phosphotungstic acid method (Bancroft and Stevens, 1990) to reveal the extra-fascicular matrix.

Anterograde tracing

The full methods for the anterograde tracing of optic fibers have been published previously in a study in which retinal projections to the telencephalon were traced following intraocular injections of [^3H] proline (Mick et al., 1993). Briefly, two animals were anesthetized with ketamine (30 mg/kg), and approximately 0.5 mCi of an equal mixture of [^3H] proline and [^3H] leucine (CEN Saclay) was injected into the vitreal chamber of one eye. Following kill after 2–3 days survival, animals were perfused with 4% paraformaldehyde. One brain including the chiasm and proximal nerves was sectioned horizontally and the other coronally. The sections were developed using standard autoradiographic procedures.

Retrograde tracing

Two marmosets used primarily in cortical imaging studies unrelated to the aims of this study, were also used to trace retinal pathways from one hemisphere through the chiasm. They were premedicated with atropine (0.05 mg/kg) and anesthesia induced with alphaxolone/alpadolone (saffan: 12–18 mg/kg i.m.). The trachea, femoral artery and veins were cannulated and the animals maintained on propofol (3.2–4.7 mg/kg/h) plus sufentanil (0.17–0.27 $\mu\text{g/kg/h}$) in lactated Ringer's solution. Levels of anesthesia

were monitored via alterations in blood pressure and cardiac rhythm. Rectal temperature was monitored and the animals kept on heated blankets. The animal's head was secured in a stereotaxic frame and a small craniotomy and durotomy made unilaterally above the lateral geniculate nucleus (LGN). A tungsten-in-glass electrode was directed vertically into the brain and action potentials conventionally amplified and displayed. Once a number of recording penetrations had been made to map the LGN, the electrode was replaced with a microsyringe filled with 30% horseradish peroxidase and 5% wheat germ horseradish peroxidase (HRP) in PBS containing 2% dimethyl sulfoxide (DMSO). The total volume injected was approximately 15 μl in each animal. The skull defect was then packed with Gelfoam (Pharmacia, UK) and the scalp sutured. The animals were maintained under anesthesia for approximately 40 h, at the end of which time they were killed by a lethal dose of sodium pentobarbital and perfused transcardially with 4% paraformaldehyde in PBS (pH 7.2). The retinae were removed and post-fixed flat in 2% paraformaldehyde and 1% glutaraldehyde in PBS (pH 7.2). The brains were removed with the optic nerves and chiasm attached and placed in 25% sucrose in PBS. The chiasms were cut free and frozen sectioned horizontally at 40 μm and the tissue reacted with the TMB method (Olucha et al., 1985). The retinae were reacted whole as with the same method. The brain sections were sectioned coronally at the same thickness and reacted to reveal the injection site using the Hanker-Yates method (Hanker et al., 1977). The sections were mounted onto slides, air dried and coverslipped.

RESULTS

In horizontal section, the marmoset optic chiasm was approximately 4 mm wide and approximately 3 mm long from the point of fusion of the two optic nerves to the point where the chiasm ends and the optic tracts become established. In central coronal section its depth was approximately 2.5 mm.

Nerve fiber patterns through the caudal nerve and chiasm

Optic fibers were well stained with both of the methods used along the length of the nerve and into the chiasm. In the proximal nerve there was no indication of any change in fiber order similar to that seen in rodents and carnivores. Rather fibers were largely aligned in a parallel manner and coursed directly into the chiasm (Fig. 1). However, the manner in which fibers entered the chiasm was unusual, as the fascicular organization of the nerve was retained deep into the rostral chiasm (Fig. 2), which would seriously restrict changes in fiber order. This result was confirmed in the sections stained for collagen fibers. In the marmoset, extra-fascicular collagen was present deep into the chiasm and was continuous with that found around fascicles in the proximal nerve. These patterns were reminiscent of patterns seen in the tree shrew (Jeffery et al., 1998). At the interface between the nerve and the chiasm, there was no indication of any fibers coursing across the width of the nerve that might correspond to the anterior knee of Wilbrand, which was supposed to represent fibers coursing from the contralateral nerve that had already crossed the midline and partially obscured fibers from the other eye as they enter the chiasm.

Throughout the main body of the chiasm, two distinct and spatially separate fiber trajectories could be identified.

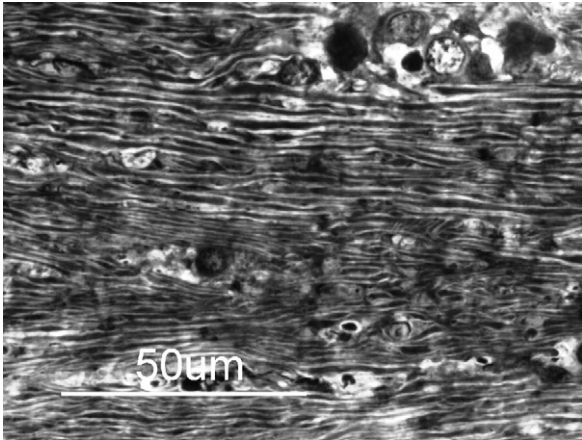


Fig. 1. Fiber alignment at the junction of the optic nerve with the optic chiasm in horizontal section. Optic fibers in this region were aligned in a parallel manner with no indication of any significant change in fiber order as seen in rodents or ferret. The pattern shown here is representative of the full medio-lateral and dorso-ventral extent of this region. Posterior is to the right and anterior to the left. Medial is down and lateral is up. The tissue is stained with p-phenylenediamine (Hollander and Vaarland, 1968).

First, fibers originating from the medial half of the nerve entered the chiasm to form a regular herringbone pattern with fibers from the other eye across the midline region (Fig. 3). Second, fibers originating from the lateral side of the nerve remained aligned parallel along the rostro-caudal axis of the chiasm and coursed directly through the lateral chiasm toward the ipsilateral optic tract (Fig. 4). The orientation of these lateral fibers was less obvious caudally where they mixed with fibers from the other eye that had crossed the midline and were converging toward the optic tract. Hence, optic fibers enter the chiasm without any

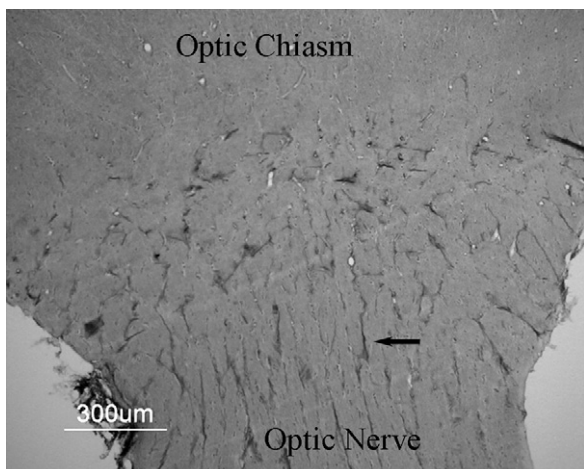


Fig. 2. Low power micrograph of the junction of the optic nerve with the optic chiasm in horizontal section. The nerve ends approximately where the scale bar is located. The tissue has been stained to reveal collagen fibers which mark the limits of fascicles (example arrowed). The fascicular organization of the retinofugal pathway is still present deep into the rostral chiasm. The presence of a fascicular configuration in the tissue would markedly restrict any significant changes in fiber order. Lateral is left and posterior is up.

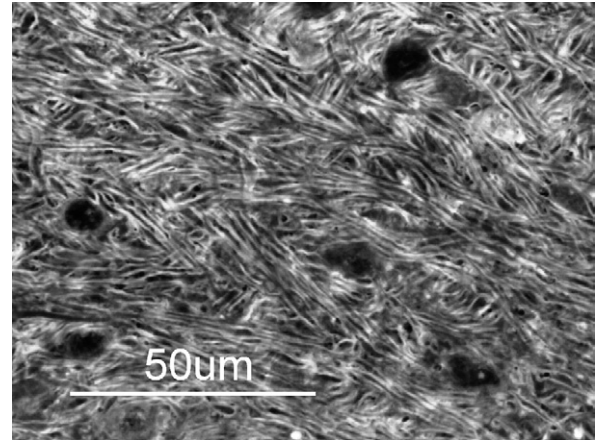


Fig. 3. Patterns of fiber order across the chiasmatic midline, approximately halfway along the length of the chiasm in horizontal section. Here crossed fibers from the two eyes inter-digitate to produce a herringbone pattern extending along the length of the midline. Orientation, scale and staining are as in Fig. 1.

significant change in fiber order, but within the chiasm can be divided into two distinct populations, one medial and one lateral.

Anterograde labeling

The patterns of anterograde labeling through the chiasm were similar in each of two animals that were injected. These patterns showed a distinct and marked separation of the two hemispheric pathways from each eye through the chiasm reflecting the two distinct fiber patterns seen using the staining methods above.

On the side of the injected eye, fibers were labeled across the whole optic nerve and into the rostral chiasm. From the tissue sectioned horizontally it is clear that in the rostral chiasm the labeled fibers clearly divided into the two hemispheric pathways. Labeled crossed fibers straddled the midline and interdigitated between unlabeled fibers from the uninjected eye. Labeled fibers that coursed toward the ipsilateral optic tract remained confined laterally and made no

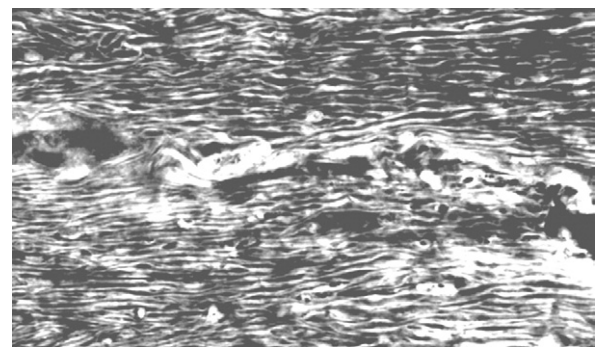


Fig. 4. In lateral chiasmatic regions, patterns of fiber order were markedly different from those across the midline. Here fibers remained aligned along a similar axis as that in the optic nerve and appeared to pass directly from the nerve toward the optic tract without approaching the midline. Orientation, scale and staining are as in Fig. 1.

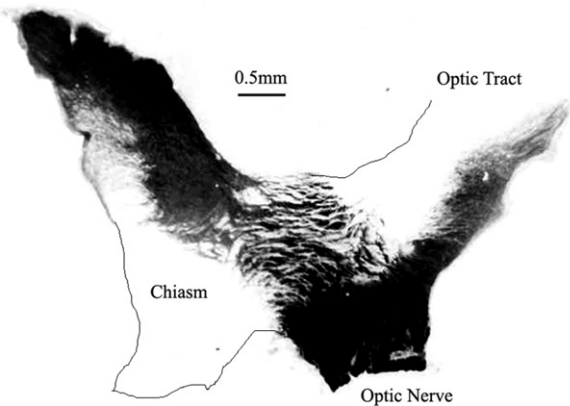


Fig. 5. Anterograde labeling of the retinofugal pathway from one eye in horizontal section. The right optic nerve is heavily labeled with proline. However, toward the optic tract the label separates into two pathways. The larger crosses the midline, while a smaller component remains restricted to the right lateral chiasm and approaches the right optic tract without approaching the midline. The outline of the chiasm has been drawn onto regions of the figure which would otherwise not be revealed on such a low power photomicrograph.

attempt to approach the midline (Fig. 5 and Fig. 6). The regions occupied by the two hemispheric pathways corresponded to the regions where the two different fiber patterns were identified using the two histochemical staining methods.

The patterns found on one side of the chiasm were reflected in a mirror fashion on the other side. Hence, on the uninjected side label accumulated caudally, however, it did not occupy the lateral chiasm, which presumably, was occupied by uncrossed fibers from the uninjected eye. Taken together, this spatial configuration confirms that not only does the uncrossed projection remain confined laterally, but that this projection occupies an exclusive region through this part of the chiasm. The patterns of label found in the chiasm sectioned coronally were identical to those found in the tissue sectioned horizontally.

Retrograde labeling

Retrogradely labeled fibers could be identified in the same regions as the anterograde label from the retina, with similar orientations and trajectories. However, as the retrograde label was less obvious and could only be followed through the chiasm by changing the plane of focus, this is shown in camera lucida representation (Fig. 7). Labeled fibers destined for the ipsilateral optic nerve were present only on the lateral side of the chiasm. The region that they covered was similar to that identified in the anterograde proline-labeled tissue above, and was similar to the lateral region containing axons with direct trajectories between the optic nerve and tract. Similarly, the label that straddled the midline was similar in its geography to that defined with anterograde tracing. The label did not extend to the retinae, presumably because of the limited survival times, which restricted the transport distance.

DISCUSSION

This study is the first to systematically trace hemispheric pathways and fiber patterns through a primate optic chiasm.

It reveals that there are major differences in the architecture of the marmoset optic chiasm when compared with rodents and carnivores, which have been the main animal models used to study this region and its development (Baker and Jeffery, 1989; Baker, 1990; Jeffery, 1990; Baker and Reese, 1993; Godement et al., 1994). However, the fiber patterns found in this New World primate are very similar to those described in humans (Neveu et al., 2006). Unlike in the rodents and carnivores studied, we reveal that there is no obvious change in fiber order in the marmoset proximal optic nerve, and that optic axons pass directly into the chiasm. Those destined to be uncrossed remain on the lateral aspect of the chiasm. These data are consistent with the notion that the optic chiasm of Old and New World primates shares the same architectural configuration with respect to the pathways taken from the eye to each cerebral hemisphere, and that this differs markedly from the patterns found in rodent and ferret.

In this study uncrossed fibers have been traced both anterogradely and retrogradely in marmoset. However, there are three studies that have used Old World macaque monkeys that cast light upon the question addressed here, and each presents data consistent with the spatial segregation of hemispheric pathways through the optic chiasm.

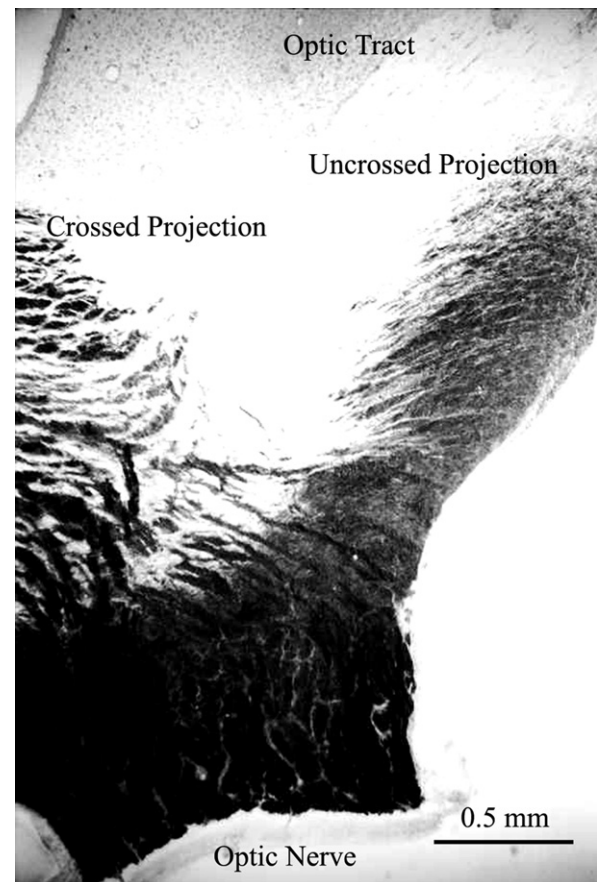


Fig. 6. A higher power picture of the region in which the crossed and the uncrossed projections divide in a section adjacent to that shown in Fig. 6. This confirms that the uncrossed fibers originate laterally in the nerve (right side) and do not approach the midline, which is located on the left of the figure.

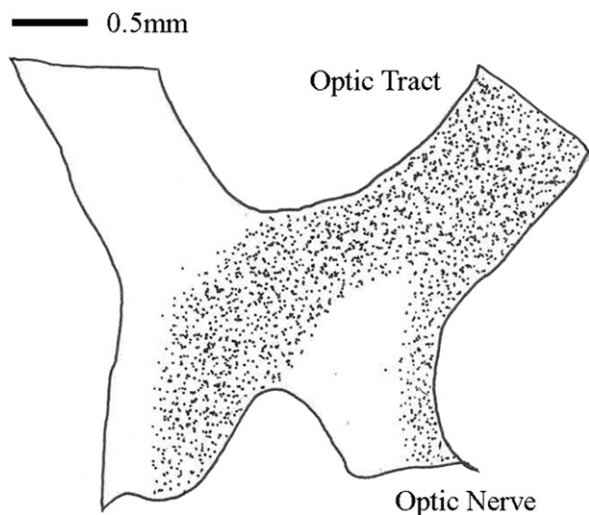


Fig. 7. A camera lucida drawing of retrogradely labeled fibers through the optic chiasm following a tracer injection into the right hemispheric pathway as seen in horizontal section. The tract on the right side adjacent to the injection is heavily labeled, but once the label enters the chiasm it divides into two pathways, a slightly larger crossed projection and a smaller uncrossed pathway. From the point at which the uncrossed pathway can be distinguished from the crossed, it remains confined laterally through the chiasm and into the optic nerve. The figure is presented from a camera lucida drawing because label could not be discerned along its pathway at low magnification.

Naito (1994) labeled small populations of retinal ganglion cells from the thalamus and traced their pathway through the chiasm to the retina. The patterns described for cells located in either the nasal or temporal retina are consistent with those described here, with temporal retinal cells having axons passing through the lateral chiasm and those located in the nasal retina possessing axons passing through more medial locations. They are also consistent with an older study in which relatively small retinal lesions were made in defined areas and the patterns of axonal degeneration traced along the retinofugal pathway (Hoyt and Luis, 1963). Here, temporal retinal lesions consistently resulted in degeneration along the lateral nerve and through the lateral chiasm. Further, Horton (1997) made intra-ocular injections of proline in the macaque and squirrel monkey and sectioned the nerves and chiasm horizontally. Again, these results are consistent with data presented here, with an apparent segregation of the two hemispheric pathways. This was particularly marked in Fig. 8A of the Horton study, which shows a laterally segregated uncrossed projection passing through the length of the chiasm. Additionally, Neveu et al. (2006) have undertaken an anatomical analysis of fiber patterns in the human optic chiasm and shown results very similar to those seen here in marmoset.

The evolution of the mammalian optic chiasm: two basic types

Early mammals were shrew-like marsupials. The eutherian (placental) line of mammalian evolution did not develop until after the marsupials were an established group (Novacek, 1992). Interestingly, tree shrews are like marsupials in lacking any change in fiber order in the pre-chiasmatic

region of the optic nerve and have segregated hemispheric pathways through the chiasm (Jeffery et al., 1998). Hence, it is possible that rodents and carnivores may form a separate and distinct group in terms of this feature. However, the reasons for this are unclear and the subject of future investigation in the form of a comparative analysis of chiasmatic fiber order across a range of mammals. Consequently, it is probable that there are at least two basic configurations for the mammalian chiasm. In man, marsupials, tree shrews and marmosets there is no loss of fiber order pre-chiasmatically, and hemispheric pathways remain segregated; in contrast, in rodents and ferrets they mix. The configuration of these two forms is represented schematically in Fig. 8.

Are the marked differences in chiasmatic organization between primates and rodents/carnivores, the product of different developmental mechanisms?

The mixed hemispheric projections found in each hemichiasm (Baker and Jeffery, 1989) reflect the developmental history of this region. In rodents and ferrets, all new fibers growing into this region make contact with the midline before deciding which hemisphere to project into (Baker and Reese, 1993; Godement et al., 1994). This process also involves key midline interactions between fibers from each eye that are important in determining pathway choice.

The behavior of individual growing axons in the mouse has been monitored along the midline and they have been shown to stall here before being committed to cross or turn back (Godement et al., 1994). Such interactions probably operate within specific temporal windows, as the signaling factors are unlikely to remain constant. Evidence for a form of temporal specificity comes from the finding in both

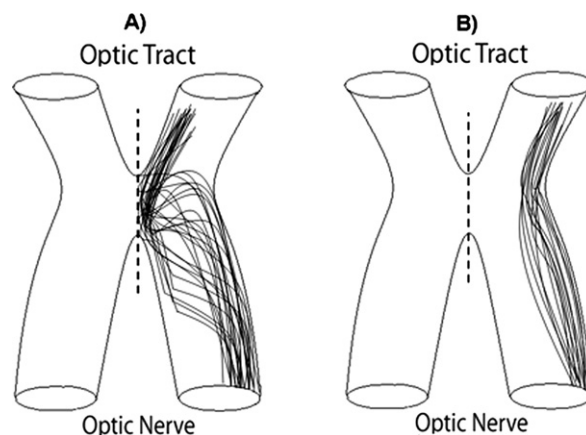


Fig. 8. A schematic representation of the uncrossed optic pathway through the optic chiasm in rodents/ferrets (A) and that described here in primate (B). In A there is a change in fiber order in the caudal optic nerve leading to the uncrossed fibers dispersing across this region and mixing with the crossed projection. These fibers then remain widespread across this hemi-chiasm before turning into the ipsilateral optic tract. This does not happen in primate. Here the uncrossed fibers enter the chiasm without significant changes in fiber order and remain confined laterally through the chiasm without approaching the midline.

mouse and ferret that ganglion cells destined to remain uncrossed are generated earlier than cells at similar eccentricities that are destined to cross (Drager, 1985; Baker and Reese, 1993). In both animals uncrossed cells in the temporal retina overlap with those that project contralaterally. In the mouse, the difference in the time between which the two populations are generated at similar eccentricities in this region can be as much as 2 days, which represents 20% of the period of ganglion cell generation (Drager, 1985). However, the first ganglion cells to enter the chiasm are still those from central retina that are destined to cross the midline.

This is not the case in primates. Here ganglion cells destined for different hemispheres are spatially segregated (Fukuda et al., 1989). Ganglion cells giving rise to the crossed and uncrossed pathways are generated at the same time either side of the naso-temporal division straddling the presumptive fovea (Rapaport et al., 1992). Hence, in primates the first ganglion cells to enter the chiasm are likely to contain components of both the crossed and uncrossed pathways. As there is no change in fiber order pre-chiasmatically, cells from the temporal retina are likely to be located lateral to those from the nasal retina. This is supported by experimental observations, as early generated uncrossed cells have been labeled in the developing primate chiasm and shown to be located laterally and not approach the midline (Meissirel and Chalupa, 1993). As ganglion cell addition progresses from the presumptive foveal region toward the peripheral retina (Mann, 1964; Rapaport et al., 1992), uncrossed fibers are most likely to accumulate laterally in the chiasm. These studies, along with the data presented here, are inconsistent with the notion that all primate ganglion cells approach the midline during development. Given the mature configuration of their chiasm and the data on ganglion cell generation (Rapaport et al., 1992), the most parsimonious explanation is that the majority of ipsilaterally projecting cells do not make contact with the midline during development as their location is restricted laterally by the presence of the contralateral projection. A complex argument involving the physical displacement of the entire uncrossed projection late in development would have to be invoked if it were to be argued that all cells irrespective of their trajectory made contact with the midline.

Could a significant difference between the two chiasmatic configurations reside in the retinal location of the ganglion cells forming the two hemispheric pathways? Ipsilaterally projecting ganglion cells are spatially mixed with contralaterally projecting cells in the temporal retina in rodents and ferrets (Jeffery et al., 1981; Thompson and Morgan, 1993), while in primates the two populations are segregated (Fukuda et al., 1989). While this notion is initially appealing, it is undermined by the fact that in both marsupials and tree shrews, that have segregated hemispheric pathways through the chiasm, ipsilaterally and contralaterally projecting ganglion cell populations are mixed in the temporal retina (Harman and Jeffery, 1992; Jeffery et al., 1998). Hence, the organization of the naso-

temporal division is independent of the configuration of the hemispherical pathways at the chiasm.

There is further compelling evidence suggesting that the primate chiasm is subject to different developmental mechanisms from those found in rodents and carnivores. In the latter group of animals the importance of midline interactions between fibers from each eye is highlighted by the finding that early eye removal results in a disruption in the chiasmatic projection from the remaining eye, systematically reducing the uncrossed projection in favor of the crossed. This is very similar to the disruption found in albinism (Godement et al., 1987; Guillery, 1989). In marsupials this does not happen, as early eye removal fails to alter the projections from the remaining eye (Taylor and Guillery, 1995), presumably because here fibers from each eye do not interact along the midline during development (Harman and Jeffery, 1995). Interestingly, a similar situation can be found in man. Here, visual evoked potentials (VEPs) have been used to examine the symmetry of chiasmatic projections. With such methods the reduced uncrossed chiasmatic projection found in albinos can be clearly demonstrated (Neveu et al., 2006). Examination of VEPs in humans where one eye has never developed, demonstrates perfectly symmetrical responses, suggesting that the projections to each hemisphere are normal and not disrupted into an albino pattern (Neveu et al., 2006). Taken together with the results of this study, such data argue strongly that both the organization and development of the primate optic chiasm are markedly different from that revealed in the rodents and carnivores studied.

In rodents and carnivores the midline plays a key role in pathway selection. This is based not only on interactions between fibers from the two eyes, but also on a range of molecular markers that are expressed by glia at this location (Jeffery and Erskine, 2005, for review). While it is clear that in primates, tree shrews, and marsupials that interactions between the two eyes do not occur at the developing midline, there is evidence for glia specializations that separate the crossed from the uncrossed pathway in the developing proximal optic nerve and rostral chiasm. Such glia are not located at the midline but laterally along the nerve/chiasm junction. They have been identified in both marsupials (Harman and Jeffery, 1995) and tree shrews (Knabe et al., 2008). In trying to establish key similarities and differences between the two different chiasmatic configurations it would be of great value to know if such glia express the same molecular markers as found in the mouse, but shifted laterally away from the midline.

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REFERENCES

- Baker GE (1990) Prechiasmatic reordering of fibre diameter classes in the retinofugal pathway of the ferret. *Eur J Neurosci* 2:24–33.

- Baker GE, Jeffery G (1989) Distribution of uncrossed axons along the course of the optic nerve and chiasm of rodents. *J Comp Neurol* 289:455–461.
- Baker GE, Reese BE (1993) Chiasmatic course of temporal retinal axons in the developing ferret. *J Comp Neurol* 330:95–104.
- Bancroft JD, Stevens A, eds (1990) Theory and practice of histological techniques. Edinburgh: Churchill Livingstone.
- Day AL (1990) Aneurysms of the ophthalmic segment: a clinical and anatomical analysis. *J Neurosurg* 72:677–691.
- Drager UC (1985) Birth dates of retinal ganglion cells giving rise to the crossed and uncrossed optic projections in the mouse. *Proc Roy Soc Lond B Biol Sci* 224:57–77.
- Fukuda Y, Sawai H, Watanabe M, Wakakuwa K, Morigiwa K (1989) Nasotemporal overlap of crossed and uncrossed retinal ganglion cell projections in the Japanese monkey (*Macaca fuscata*). *J Neurosci* 9:2353–2373.
- Godement P, Salaun J, Metin C (1987) Fate of uncrossed retinal projections following early or late prenatal monocular enucleation in the mouse. *J Comp Neurol* 255:97–109.
- Godement P, Wang LC, Mason CA (1994) Retinal axon divergence in the optic chiasm: dynamics of growth cone behaviour at the midline. *J Neurosci* 14:7024–7039.
- Guillery RW (1989) Early monocular enucleation in fetal ferrets produces a decrease of uncrossed and an increase of crossed retinofugal components: a possible model for the albino abnormality. *J Anat* 164:73–84.
- Hanker JS, Yates PE, Metz CB, Rustioni A (1977) A new specific sensitive and non-carcinogenic reagent for the demonstration of horseradish peroxidase. *Histochem J* 9:789–792.
- Harman A, Jeffery G (1992) The distinctive pattern of organisation of the retinofugal pathway of a marsupial: I. retina and optic nerve. *J Comp Neurol* 325:47–56.
- Harman A, Jeffery G (1995) Development of the chiasm of a marsupial, the Quokka wallaby. *J Comp Neurol* 359:507–521.
- Holder GE (1991) Chiasmatic and retrochiasmatic lesions. In: Principals and practice of clinical electrophysiology (Heckenlively JR, Arden GB, eds), pp 557–564. St Louis, MO: Mosby Year Book.
- Hollander H, Vaerland JL (1968) A reliable staining method for semi-thin sections in experimental neuroanatomy. *Brain Res* 10:120–126.
- Horton JC (1997) Wilbrand's knee of the primate optic chiasm is an artefact of monocular enucleation. *Trans Am Ophthalmol Soc* 95:597–609.
- Hoyt WF, Luis O (1963) The primate optic chiasm. Details of visual fibre organisation studied by silver impregnation techniques. *Arch Ophthalmol* 70:69–85.
- Jeffery G (1990) The distribution of crossed and uncrossed retinofugal axons in the cat optic nerve and their relationship to patterns of fasciculation. *Visual Neurosci* 5:99–104.
- Jeffery G (2001) The architecture of the optic chiasm and the mechanisms that sculpt its development. *Physiol Rev* 81:1393–1414.
- Jeffery G, Cowey A, Kuypers HGJM (1981) Bifurcating retinal ganglion cell axons in the rat, demonstration by retrograde double labelling. *Exp Brain Res* 44:34–40.
- Jeffery G, Evans A, Albon J, Duance V, Neal J, Dawidk G (1995) The human optic nerve: fascicular organisation and connective tissue types along the extra-fascicular matrix. *Anat Embryol* 191:491–502.
- Jeffery G, Harman A (1992) The distinctive pattern of organisation of the retinofugal pathway of a marsupial: II optic chiasm. *J Comp Neurol* 325:57–67.
- Jeffery G, Harman A, Flugge G (1998) First evidence of diversity in eutherian chiasmatic architecture: tree shrews like marsupials have spatially segregated crossed and uncrossed chiasmatic pathways. *J Comp Neurol* 392:183–193.
- Jeffery G, Erskine L (2005) Variations in the architecture and development of the vertebrate optic chiasm. *Prog Retin Eye Res* 24:721–753.
- Knabe W, Washausen S, Happel N, Kuhn HJ (2008) Diversity in mammalian chiasmatic architecture: ipsilateral axons are deflected at glial arches in the prechiasmatic optic nerve of the eutherian *Tupia belangeri*. *J Comp Neurol* 508:437–457.
- Magnin M, Cooper HM, Mick G (1989) Retinohypothalamic pathway: a breach in the law of Newton-Müller-Gudden? *Brain Res* 488:390–397.
- Mann I (1964) The development of the eye, 3rd edition. London: British Medical Association.
- Meissirel C, Chalupa LM (1993) Organisation of pioneering retinal axons within the optic tract of the rhesus monkey. *Proc Natl Acad Sci U S A* 91:3906–3910.
- Mick G, Cooper HM, Magnin M (1993) Retinal projections to the olfactory tubercle and basal telencephalon in primates. *J Comp Neurol* 327:205–219.
- Naito J (1994) Retinogeniculate fibres in the monkey optic chiasm: a demonstration of fibre arrangement by means of wheat germ agglutinin conjugated to horseradish peroxidase. *J Comp Neurol* 346:559–571.
- Neveu MM, Holder GE, Ragge NK, Sloper JJ, Colin RO, Jeffery G (2006) Early midline interactions are important in mouse optic chiasm formation but are not critical in man: a significant distinction between man and mouse. *Eur J Neurosci* 23:3034–3042.
- Novacek MJ (1992) Mammalian phylogeny: shaking the tree. *Nature* 356:121–125.
- Olucha FF, Martinez-Garcia C, Lopez-Garcia C (1985) A new stabilizing agent for the tetramethyl benzidine (TMB) reaction product in the histochemical detection of horseradish peroxidase (HRP). *J Neurosci Methods* 13:131–138.
- Rapaport DH, Fletcher JT, LaVail MM, Rakic P (1992) Genesis of neurons in the retinal ganglion cell layer of the monkey. *J Comp Neurol* 322:577–588.
- Reese BE, Baker G (1992) Changes in fibre organisation within the chiasmatic region of mammals. *Vis Neurosci* 9:527–533.
- Taylor JS, Guillery RW (1995) Does early monocular enucleation in a marsupial affect the surviving uncrossed retinofugal pathway? *J Anat* 186:335–342.
- Thompson ID, Morgan JE (1993) The development of retinal ganglion cell decussation patterns in postnatal pigmented and albino ferrets. *Eur J Neurosci* 5:341–356.