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Developmental Neurobiology: Preventing Midline Crossings

Dispatch

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A recent study has shown that EphB1, a receptor for ephrin-2, guides selected retinofugal axons into the pathway from the retina to the cerebral hemisphere on the same side of the body. Another shows that the transcription factor Zic2 is also important in this 'uncrossed' pathway. Does Zic2 regulate EphB1?

The long pathway from the eye to the brain has many thousands of axons, about 50,000 in mice [1] and a million in humans [2], each arising from one retinal cell. Each axon must find its correct terminal station(s), and in mammals there are six or more, depending on how one counts [3]. Further, there are three or more functionally distinct retinal cell types, each distributing to a characteristic set of terminal stations [4,5]. The mechanisms that guide axons into their correct course are only partially understood, but two recent studies [6,7] have clarified one important feature by showing molecular determinants of the 'uncrossed' course that some axons take at the midline.

In most mammals, as in many other vertebrates, each eye sees more than half the visual field (Figure 1), and each cerebral hemisphere receives a mapped projection of just half the visual field. The binocular part of the visual field is represented twice in each hemisphere, so that the messages from the two eyes can be matched centrally. Terminal stations receive from the visual hemifield of the opposite side, because each hemisphere, in general, deals with events on the opposite side of the body. For an orderly representation of the binocular hemifield, the pathway from each eye must split, most axons crossing at the optic chiasm and a minority not crossing.

Mice have laterally placed eyes and thus have small binocular fields and a small uncrossed component. Primates, with forward facing eyes have large binocular fields and large uncrossed components; carnivores like cats and ferrets are intermediate in this respect. Tadpoles have laterally placed eyes, with no binocular field and no uncrossed pathway, but during metamorphosis their eyes shift position and frogs develop a binocular visual field and an uncrossed pathway. For reasons not yet understood, the uncrossed component is significantly reduced in albino or hypopigmented mammals — such as mouse, mink, tiger, monkey and human — which lack the retinal pigment melanin [8]. These individuals commonly have visual problems and misaligned eyes.

Some of the constraints, or 'guidance cues' in the region of the optic chiasm that either attract or repel retinal axons are known [9–12], and it is also known that these must act during the limited time when the uncrossed component develops [13,14]. Nakagawa *et al.* [15] have shown in *Xenopus* and mouse that B-class ephrins are present at the chiasm and act to prevent the midline crossing of the uncrossed component. Further, ectopic expression of ephrin-B2 can induce an abnormally early uncrossed projection in *Xenopus*.

The recent study by Williams *et al.* [6] shows that the receptor for ephrin-B2, EphB1, is present in the ganglion cells of the temporal retina of mouse and

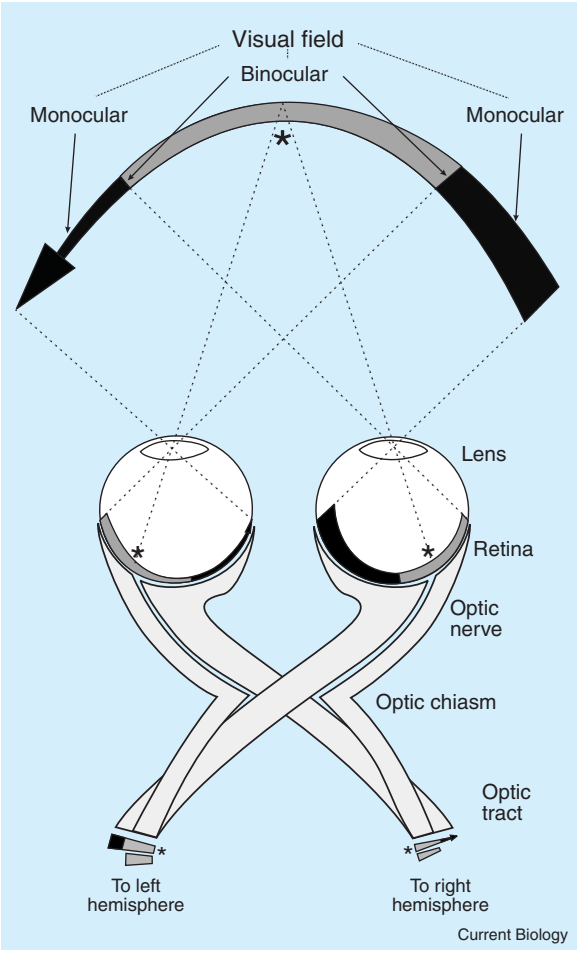

Figure 1. Schematic representation of the pathways from the eye to the brain.

The large arrow shows the visual field, with the parts seen by both eyes (grey, binocular) and parts seen by only one eye (black, monocular). The lens casts an inverted image of the visual field onto the retina, and retinal cells send axons into the optic nerve. Asterisks show the vertical midline of the visual field and its representation in the retina and optic tract. Retina medial to the asterisk is known as nasal retina (nearer to the nose), and that lateral to the asterisk is known as temporal retina (nearer the temple). The temporal retina gives origin to the uncrossed axons. In mice and frogs, a large part of the binocular field is dorsal, and, correspondingly, the uncrossed component arises from ventral and temporal retina.

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Xenopus during the relevant period. The match of spatial and temporal distributions makes a strong case for regarding the interaction of ephrin-B2 and its receptor as a key determinant of the uncrossed pathway, and the evidence from two such widely different vertebrates indicates an ancient and well-established mechanism. Further strong support for this mechanism comes from experiments in which the function of ephrin-B2 was blocked and from mutant mice that did not produce the receptor EphB1 protein.

In a closely related study, Herrera *et al.* [7] identified a zinc finger transcription factor, Zic2, which is also expressed specifically in ganglion cells of the temporal retina during the relevant developmental period in mice, ferrets and *Xenopus* — but not in the chick, which like other birds has no uncrossed pathway. The size of the retinal region expressing Zic2 is large in ferret relative to that in mouse, and smaller in albino mice than normal mice. These results correspond nicely to the size of the uncrossed projection. Zic2 is absent in the temporal retina of *Xenopus* tadpoles, but present during metamorphosis when the uncrossed component develops. Further, experiments using loss or gain of Zic2 function confirmed the role of this transcription factor in determining the course and the size of the uncrossed component. The next step, determining how the transcription factor Zic2 is related to the receptor EphB1, has yet to be taken.

The beauty of these two studies [6,7] is the rigorous way in which the proposed mechanisms for guiding the uncrossed axons were tested against the known facts of comparative anatomy, developmental timing and morphology of the visual pathways.

Many questions remain. One concerns the mechanism that defines the retinal line separating crossed from uncrossed pathways, and how this relates to the position of the eyes and, therefore, the visual fields. In frogs, eye position and chiasmatic pathways change concurrently in the seeing animal, but in mammals the pathways develop *in utero* when the eyes are shut. How then is eye position in the adult linked so accurately to this retinal line?

Another question concerns the controls that lead each functionally distinct component to its appropriate end stations. The albino abnormality presents further questions. It has been suggested that the abnormality may be due to a change in the rate at which retinal cells are generated [16,17], influenced by melanin production in nearby retinal pigment cells. This possible relationship can, perhaps, now be subjected to some rigorous tests. What happens to cell divisions in the region of Zic2 expression? What, precisely, are the temporal relationships of Zic2 expression, melanin production, cell division and the uncrossed pathway in different parts of the retina? Is cell production modified even in regions like the tapetal portion of the cat or ferret retina, which lacks melanin but gives rise to an abnormal uncrossed pathway?

A further, intriguing question concerns related actions of Zic2. The three Zic genes (Zic1, Zic2 and Zic3) are expressed in the brain and eyecup early in development before the uncrossed pathway forms, and mutations of Zic2 produce holoprosencephaly, a fused forebrain, occasionally associated with cyclopia in mutant mice [18,19]. Perhaps these are quite independent actions, but their relationship to earlier observations of Jacobson [20] merit thought and exploration. He found that in early development of *Xenopus*, a small group of cells crosses from one hemisphere to the other. These cells form the region of the optic chiasm as well as the retinal region that in the adult gives rise to the uncrossed pathway. How is this migration across the midline related to the separation of the hemispheres on the one hand, and to determining the course of the axons that stay in their own hemisphere, on the other?

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