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Translocation of progenitor retinal cells on bifurcation of the optic primordium: a contrarian view

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Abstract

Early in vertebrate embryogenesis a single medial primordial eye field divides to form bilateral optic vesicles. It is generally believed that this process involves the migration of progenitor retinal cells (PRCs) from the primordium to their prospective ipsilateral optic vesicles. A reappraisal of the published data, however, throws this belief into question and supports a new hypothesis suggesting that PRCs translocate contralaterally, in the process forming an incipient optic chiasm. This hypothesis can be tested with a high degree of confidence using a study design that employs a single cell fatemapping technique in zebrafish embryos that is both very sensitive and specific.

Keywords: Contralaterality, optic primordium, optic chiasm, Inversion Hypothesis.

Introduction

Modern expert consensus holds that a single embryonic primordial eye field in vertebrates bifurcates to become two lateral eyes. Such a consensus has firmed over the last few decades and follows a lengthy debate as to the initial nature of the primordium. It was argued by some since 1826 [1][2][3] that the two optic vesicles arose from two distinct primordia, and that the occasional developmental abnormality of cyclopia resulted from the downstream fusion of the two. The alternate view, now the accepted view, was that the formation of two lateral optic vesicles was preceded by a single medial field of precursor retinal cells, referred to as the optic primordium. [4][5][6][7][8][9][10][11] Cyclopia, from this point of view, is seen as the failure of the optic primordium to bifurcate to become two lateral vesicles, rather than a fusion of two primordia.

While it is now accepted that a single optic primordium bifurcates to become two lateral optic vesicles, there remains an underlying assumption in recent studies demonstrating this, that, by and large, the progenitor retinal cells from each half of the primordium migrate, on bifurcation, to the ipsilateral optic vesicle. [12][13][14] This view might be seen as intuitively satisfying, but it fails to provide a mechanism for the creation of the optic chiasm. A more logical explanation, albeit a counter-intuitive one, not only provides a mechanism for the creation of the optic chiasm, it is an essential prediction of the Inversion Hypothesis. The Inversion Hypothesis requires that on bifurcation of the optic primordium, the majority of precursor retinal cells (the quantity depending on the vertebrate class and its chiasmal architecture) must cross the midline to become mature retinal cells in the contralateral eye. [15][16] In so doing, an incipient optic chiasm is formed at the anterior border of the presumptive ventral diencephalon (Fig. 1). To help appreciate the need for such an extreme migration of cells, a brief overview of the Inversion Hypothesis follows.

The Inversion Hypothesis

The hypothesis proposes that in ancestral craniates, a single frontal eye, homologous to the single frontal eye of an amphioxus-like ancestor, developed increasing contralateral discernment of light photons due to the progressive increase in curvature of the expanding retina (Fig. 2) (For a simulation of retinal evolution see Nilsson and Pelger, 1994 [17]).

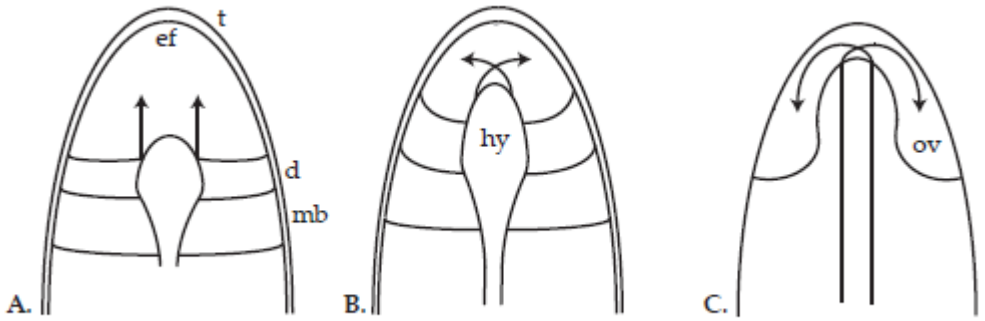

Fig. 1. Organisation of the Rostral Neural Plate and Forebrain in Embryonic Zebrafish.

Cartoons of the rostral neural plate of a zebrafish embryo with axial midline tissue shown moving rostrally to influence bisection of the primordial eye field. Arrows (based on the Inversion Hypothesis), represent the proposed movement of PRCs leading to the formation of the incipient optic chiasm. Figures (without arrows) are adapted from Wilson and Houart [13]

a;b;c: dorsal views. Rostral to the top. a; A uniform single primordial eye field prior to neurulation (75% epiboly). b; At 80% epiboly medial PRCs cross the midline as the presumptive hypothalamus moves rostrally. c; By closure of the neural tube and formation of the optic vesicles, the majority of PRCs have crossed the midline except at the anteromedial diencephalon where the incipient optic chiasm has formed (crossing of arrows).

*Abbreviation*s: d, diencephalon; ef, eye field; hy, hypothalamus; mb, midbrain; ov, optic vesicle; t, telencephalon.

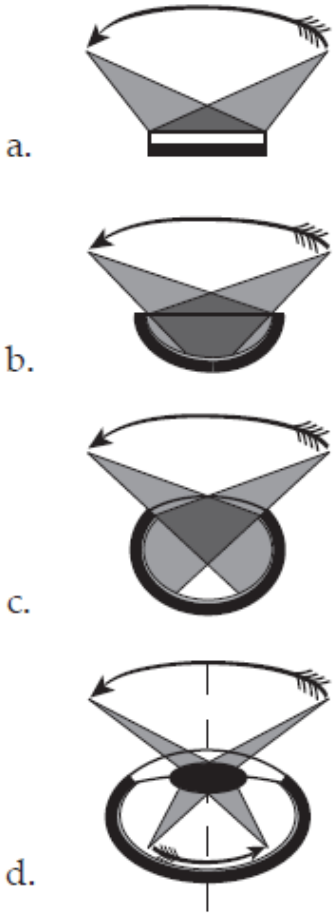

Fig. 2. The Evolution of Increasing Contralateral Retinal Discrimination of a Light Source in the Early Craniate Retina.

Dorsal cross-sectional view. Only the depiction of the retina is adapted from Nilsson and Pelger (1994)[17]. A rostral visual object is represented by an arrow. Shading represents all photon streams that reach the retina from the lateral extremes of the visual object.

a) A simple flat retina cannot discriminate the direction of a light source because such a retina receives photon streams from all point sources equally.
b) A deepening of the retinal pit favours increasing contralateral discrimination of light sources by ipsilateral deselection of photons.
c) Contralateral discrimination of light source extends, over generations, toward the midline as the retinal curvature increases.
d) With acquisition of a lens, all light is fully directed contralaterally and images are formed, and inverted.

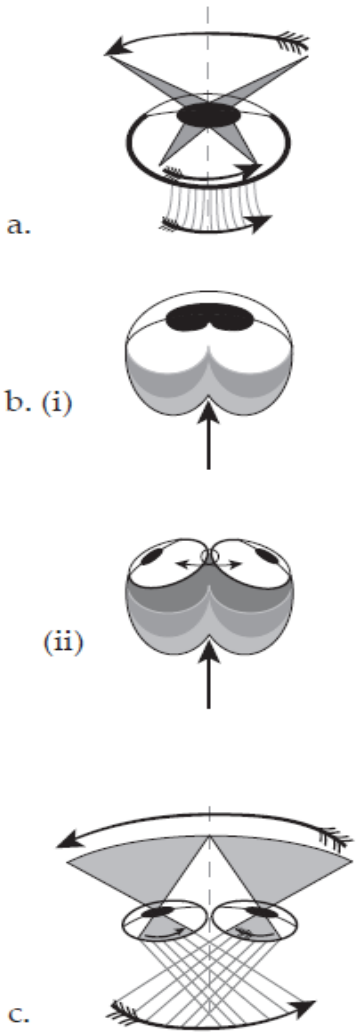

Fig. 3. From Inversation to Chiasmation. Dorsal cross-sectional view of the evolving primitive eyes from a single imaging eye. A rostral visual object (arrow) is projected inversely to the diencephalon and is not altered significantly by the very disruptive chiasmatic process. Rostral is to the top.

a) With the acquisition of a lens in the adult single-eyed craniate, the retina that previously received largely contralateral inputs now received images that were inverted and completely contralateral. Ipsilateral retinofugal ganglia would inform the diencephalon of the inverted images in an equivalent topological format (caudal arrow).
b) At chiasmation, anterior movement of the diencephalon to bifurcate the optic primordium in the embryo(i) resulted in bilateral eyes in the adult (ii), each formed from half the original retina but with positions switched across the midline (vertical arrows represent anterior movement of the embryonic diencephalon).
c) In the resultant two-eyed adult craniate, the crossing of the optic fires at the midline involves no significant change to the orientation of images received in the diencephalon (caudal arrow).

As proposed, this increasing retinal favouritism for contralateral light sources in a single frontal eye, led to increasing contralateral neural representation in the supporting forebrain and midbrain, culminating in complete contralateral imaging on acquisition of the inverting lens. Further improvement in the range of vision is likely to have resulted from a profound genetic mutation that, while threatening the viability of craniates by dividing the embryonic optic primordium and switching positions of the retinal halves, actually maintained and improved the visual status quo (Fig. 3). Since the forebrain had developed as the receptor of contralateral visual images, the switching of the initial retinal halves to the opposite side of the head to become separate eyes in their own right, allowed the now ipsilateral eyes to continue supplying unaltered visual images to their companion contralateral forebrain. In the process, the optic chiasm was formed. This, presumably, is the same process, recognised by Hatta et al (1994),[18] as the key step in the early evolution of vertebrates where anterior expansion of a CNS ventral midline signalling system involved cells specified by the gene *cyclops* .

Summary of primary mechanisms:

1 *Inversation*: Forebrain contralaterality developed in support of a single evolving retina in early craniates and preceded the development of bilateral vision.
2 *Chiasmation*: The optic chiasm resulted from the bifurcation of the craniate embryonic primordial eye field and the switching of retinal hemifield precursors across the midline.

Thereafter, bilateral retinas in adult craniates were situated ipsilateral to the animal's visual hemifields (previously contralateral in the single-eyed ancestor) while the forebrain remained contralateral. This change from contralateral retinal hemifields to ipsilateral retinas should be traceable by fatemapping the development of the eye in the modern vertebrate embryo. Here we should observe that the majority of PRCs cross the midline to become mature retinal cells in the contralateral eye. The proportion of such cells should be equivalent to the relative portion of crossed fibres in the chiasm of the adult class. In ancestral craniates, this crossing should have been complete. [16] Since the crossed fibres within the chiasm of teleost fish are near 100% of their total fibre content, [19] the zebrafish embryo is a suitable proxy in this regard for the ancestral craniate chiasm.

Eye morphogenesis

The debate as to whether the optic primordia/um begins as a single eye field or as bilateral entities was largely resolved by the end of the last century, especially after the publication of a paper by Varga et al (1999).[12] This study demonstrated by single cell fatemapping at 80% epiboly in zebrafish embryos, the presence of a single field of precursor retinal cells that divides to form two lateral optic vesicles under the influence of an anteriorly moving diencephalic anlage. A number of contemporary studies [20][21][22][23] made similar findings, albeit with different emphases. Varga et al,[12] however, was distinctive in its employment of a very specific fatemapping technique.

Further support for a single primordial eye field came with a review of early eye development in 2001 by Chow and Lang.[24] This review synthesised the classical work of the previous century by investigators such as Spemann and Adelmann with modern genetics, supporting the existence of a single eye field early in development that is bisected by the influence of the underlying prechordal mesoderm.

In 2004 a further review of the early development of the forebrain summarised the state of our ever growing mass of knowledge. [13] This overview by Wilson and Houart built on the observations of Varga et al and others, and broadened our view of the genetics surrounding eye development and their origins as a single primordial eye field.

In 2006 two studies [25][26] further advanced our understanding of early eye development in terms of 3D and 4D dynamic movement of cells by using timelapse microscopy to track the folding of neural sheets during development. These techniques, while exquisitely sensitive and visually captivating, lack the specificity of earlier fatemapping techniques employed by Varga et al (1999). [12]

Discussion

From the standpoint of the Inversion Hypothesis all these papers fail to observe the predicted major crossing of the midline by PRCs. A token crossing by medial PRCs is noted, but accepted only as support for the concept of a homogenous single primordial eye field by Varga et al, 1999.[12] The impressive 4D imaging studies by England et al, and Rembold et al, [25][26] show sheets of PRCs moving from lateral regions of the primordium toward the midline where they apparently suddenly dive ventrally and return toward the periphery without significant crossing of the midline.

How, then, can the primary prediction of the Inversion Hypothesis, that the majority of PRCs must cross the midline, possibly be correct?

Firstly, earlier pioneering work by Jacobson and Hirose [27] using a very specific fatemapping technique, demonstrated that there is actually more significant translocation of PRCs across the midline than more recent studies suggest. They showed, by labelling one of the first two blastomeres of the Xenopus frog embryo, that all downstream staining was confined to the ipsilateral brain, except for the ventral half of the contralateral eye and incipient optic stalk. More importantly, this demonstration understated the magnitude of the translocation across the midline because the technique (single cell injection of horseradish peroxidase) failed to capture the location of daughter cells due to dilution of the stain by mitosis. These cells presumably would also have matured within the contralateral eye. Even so, here was an unequivocal demonstration that the translocation of PRCs across the midline in vertebrates is actually quite substantial.

Secondly, studies such as Jacobson and Hirose [27] are very specific in that individual cells are injected with dye at their origin and later traced to identify their final destinations. There is no such specificity in studies such as England et al [25] or Rembold et al [26], where very sensitive but non specific techniques of mass fluorescent staining are used. Nor, according to Castro-Gonzalez et al, [28] is the technique of tracking gene expression an accurate means of detecting cell position. Under these circumstances it might not be possible to accurately discern whether cells at the midline have about-turned or actually crossed to the other side. In such instances of uncertainty, the most intuitively satisfying assumption is accepted.

Thirdly, while the most specific and elegantly constructed study of early eye development by Varga et al, [12] purportedly found that in zebrafish embryos an insignificant number of PRCs crossed the midline, a reassessment of the published data suggests otherwise.

This study sought primarily to demonstrate whether or not the primordial eye field was a single medial entity or bilateral entities. The incidental finding that some PRCs crossed the midline supported the conclusion that the primordial eye field was single but this was not investigated further for greater significance. While each PRC was labelled at 80% epiboly and its position plotted accurately on a grid overlying the optic primordium, and later checked at tailbud stage and at maturity, no calculations are supplied in the published paper analysing the exact quantity of their distributions. The results, however, are graphically presented in Fig. 4;a,b,c,d of the paper by Varga et al. [12]

Without mathematical analysis of the exact distribution of PRCs, the natural assumption appears to have been made by the authors that the majority of PRCs mature to the ipsilateral eye. However, it is clear from Fig. 4 of their paper (noting that 4a; and 4b; are inversions of each other) that about 89% of PRCs that mature in the left eye originated in the right eye field, and that about 53% of PRCs that matured in the right eye originated in the left eye field. More significantly, of the 17 lateral PRCs labelled in Fig. 4a; 88% crossed the midline. At tailbud stage (Fig. 4c,d;) all crossing of the midline by PRCs appears to have been completed, except at the anteromedial diencephalon where the incipient optic chiasm should be found.

Conclusion

Modern expert consensus holds that the vertebrate primordial eye field begins as a single medial entity and bifurcates to produce two bilateral eyes. The assumption however, that on bifurcation, the two halves of the primordium move primarily to the ipsilateral optic vesicles, has been shown by fate specific studies in zebrafish and Xenopus frog embryos to be erroneous. Such studies show that the majority of PRCs cross the midline between 80% epiboly and tailbud stages in zebrafish. Since at 80% epiboly in zebrafish there is substantial intermingling of PRCs near the midline before translocation of lateral PRCs becomes evident, we postulate that fatemapping from an earlier fraction of epiboly (say 75%) is likely to show that an even greater majority of retinal cells (than already observed) originate in the contralateral primordial eye field. Such is the required primary prediction of the Inversion Hypothesis.

This could most precisely be tested by repeating the study by Varga et al with a more specific focus on quantifying the exact distribution of PRCs migrating across the midline. The same technique of single cell fatemapping used in that study should be re-employed, as it is both exquisitely sensitive and specific. The difference would be that staining of PRCs should take place at an earlier fraction of epiboly (say 75%) in order to better capture the position of PRCs prior to crossing the midline, and that mathematical analysis of their fates should be calculated.

Conflicts of Interest

This paper is based on the research of a single author, without financial or research relationships to any other bodies.

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