Space-saving advantage of an inverted retina

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ABSTRACT

Vertebrate eyes are of the simple or camera type with a single optical system that creates an image on the retina in the back of the eye. There, the visual information is encoded as nervous signals by photoreceptors, processed by retinal neurons, and then sent to the brain via the optic nerve. Surprisingly at first sight, the retinal neurons are located between the lens and the light-sensitive parts of the photoreceptors. The tissue scatters some light, which leads to loss of light and image blur. The inverted retina has, therefore, long been regarded as inferior. Here, we provide evidence that the inverted retina actually is a superior space-saving solution, especially in small eyes. The inverted retina has most likely facilitated the evolution of image-forming eyes in vertebrates, and it still benefits especially small and highly visual species.

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1. Introduction

The image created by the optical system of a vertebrate eye is translated to nervous signals by the light-sensitive photoreceptors. The signals are conveyed to retinal neurons for processing, and eventually transmission to the brain. The intuitive option would be to place these neurons behind the photoreceptors, such that they are out of the way for incoming light. This is the case, for example, in cephalopod eyes (Land & Nilsson, 2002; Walls, 1942). Vertebrates, however, have inverted retinas with the retinal neurons being situated between the lens and the photoreceptors. This solution is usually considered to be disadvantageous because of the scattering of some light on its path toward the photosensitive layer. One may, therefore, wonder why just the vertebrate lineage has diverged into many highly visual species. By studying small fish eyes, we have identified a considerable functional advantage of the inverted retina.

The first vertebrate eyes were aquatic, which means that the cornea was of little optical importance. The refractive power of a thin cornea is negligible if it borders to aqueous solutions of high refractive index (sea water and aqueous humour) on both sides (Matthiessen, 1886). In such eyes, light is focused exclusively by a thick, often spherical (ball-shaped), and optically powerful crystalline lens (Kröger, Campbell, Munger, & Fernald, 1994; Matthiessen, 1882; Sroczyński, 1977). In many species, the eyes become functional early in life and are at that stage huge compared to body size. To make the eyes as large as possible in small animals, evolutionary optimisation had to meet stringent space-saving demands.

For well-focused vision, there has to be some distance between the lens and the photosensitive layer (Fry, 1960). In eyes with everted retinas, this space is filled with a clear, watery substance. In vertebrate eyes, there is also such a substance, the vitreous body. Some of the space, however, is used to accommodate the retinal cells. The amount of space saved by this arrangement was studied with histological sections of young zebrafish eyes that had just become functional and by geometrical modelling.

2. Materials and methods

2.1. Histology

Zebrafish (*Danio rerio*) fry (5 days past fertilisation; 5 dpf) were anesthetized on ice at 4 °C and immediately fixed in 4% paraformaldehyde in 0.2 M phosphate buffer, pH 7.4, for 1 h (4 °C). Fixed fry were dehydrated in a graded series of ethanol–water mixtures and embedded in resin (Technovit 7100; Kulzer, Wehrheim, Germany). Sections were prepared and mounted on poly-L-lysine-coated slides (Sigma). Sections were air dried at 60 °C, stained with Richardson's solution (Richardson, Jarett, & Finke, 1960), and coverslipped with DPX mounting medium (R-1340; Agar Scientific, Stansted, UK). At the age of 5 dpf, zebrafish start to show visually guided behaviour (Neuhauss, 2003). The procedures adhered to Swiss animal protection legislation and were approved by the local ethics committee.

2.2. Modelling

A model representing a simplified fish eye with a spherical lens and no cornea (Fig. 1) was used to study the space-saving effects of

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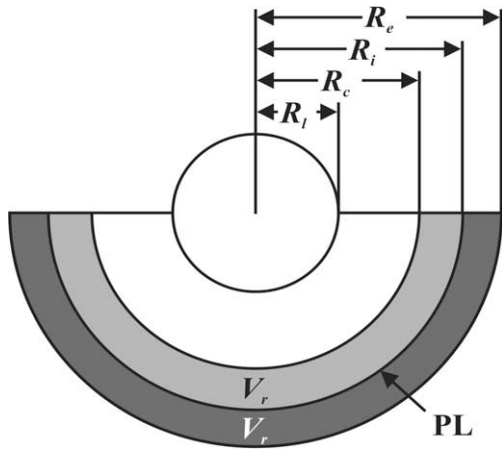
Fig. 1. Eye model used to study the space-saving effects of the inverted retina. R_l: radius of the lens; R_c: outer radius of the clear zone between the lens and the retina (with $R_c = R_l$ for the smallest possible eye with an inverted retina); R_i: outer radius of the inverted retina; R_e: outer radius of the everted retina; V_r: volume of the retina, which is the same for the inverted and everted retinas. PL is the photosensitive layer of cone outer segments.

the inverted retina as a function of eye size. Normalized focal length of the lens was set to 2.5 R_l (with R_l being the radius of the lens), which is a realistic value for both fish and cephalopod lenses (Matthiessen, 1882; Sivak, 1982). In vertebrate eyes, most of the retinal neurons process information with graded membrane potentials and have to be located close to the photoreceptors and each other for fast and reliable processing (Wirth, Cavallacci, & Genovesi-Ebert, 1984). It was, therefore, assumed that the retinal neurons are located in close proximity to the photoreceptors either on the outside (everted retina) or inside (inverted retina) of the photosensitive layer (PL in Figs. 1 and 2). The volumes of the everted (V_e) and inverted (V_i) retinas were kept equal to give room to equal numbers of cells. The thickness of the inverted retina was initially assumed to be 100 µm, which is a conservative estimate (compare to retinal thickness in Fig. 2). Species with complex visual behaviours have thicker retinas. Calculations were, therefore, performed also with a retinal thickness of 500 µm, which is about the thickness of the human retina (Rodieck, 1998). We concentrated on the effects of the orientation of the retina, thus ignoring the contributions of further ocular elements, such as the retinal pigment epithelium, choroid, sclera, and anterior segment.
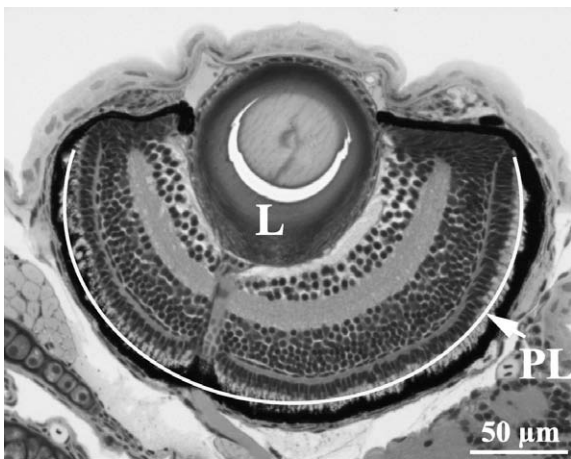
Fig. 2. Micrograph of the eye of a zebrafish (*Danio rerio*) fry (5 days past fertilisation). Note that the space between the lens (L) and the photosensitive layer of outer segments (PL) is completely filled with retinal cells. At this developmental stage, the swim bladder is inflated and the animals begin to feed on small food particles.

The following equations were used in the calculations:

$$R_{l\min} = D_r/(F - 1) \tag{1}$$

where $R_{l\min}$ is the radius of the smallest possible lens, D_r the thickness of the retina, and F the relative focal length of the lens expressed in units of lens radius (2.5 R_l).

$$R_i = F \cdot R_l \tag{2}$$

where R_l is the lens radius ($R_l \geqslant R_{l\min}$) and R_i the outer radius of the inverted retina.

$$R_c = R_i - D_r \tag{3}$$

where R_c is the outer radius of the clear zone between the lens and the retina.

$$V_r = \frac{2\pi \cdot (R_i^3 - R_c^3)}{3} \tag{4}$$

where V_r is the volume of the retina.

$$R_e = \sqrt[3]{\frac{3 \cdot V_r}{2\pi} + R_i^3} \tag{5}$$

where R_e is the outer radius of the everted retina.

$$V_i = \frac{2\pi \cdot (R_i^3 + R_l^3)}{3} \tag{6}$$

where V_i is the volume of the eye with an inverted retina.

$$V_e = \frac{2\pi \cdot (R_e^3 + R_l^3)}{3} \tag{7}$$

where V_e is the volume of the eye with an everted retina.

3. Results

In young zebrafish eyes, the space between the lens and the photosensitive layer of cone outer segments is completely filled with retinal cells, such that no noteworthy amount of vitreous body is present (Fig. 2).

The smallest eye that can accommodate an inverted retina of 100 µm in thickness is 330 µm in outer diameter and has a lens of 130 µm in diameter. With the same size and focal length of the lens, an eye with an everted retina is 420 µm in outer diameter and has almost twice the volume (with the lens included) of an eye with an inverted retina. As eye size increases, the relative amount of space saved decreases (Fig. 3).

A human eye is about 24 mm in diameter (Smith & Atchison, 1997) and has a retinal thickness of about 0.5 mm (Rodieck, 1998). In an eye of that size and retinal thickness, eye volume is 11.3% (=434 mm³) smaller with an inverted retina (see also Fig. 3). These values apply to the simplified eye shown in Fig. 1.

4. Discussion

In the smallest vertebrate eyes the space between the lens and the photoreceptors' light-sensitive outer segments is completely filled with retinal cells (Fig. 2). This is a highly space-efficient solution, because otherwise the retinal neurons would have to be placed distally to the outer segments, which would make the entire eye substantially larger. Alternatively, some other space has to be found in the body, such as in cephalopods where neurons with similar functions are organised in optic lobes separate from the eyes (Land & Nilsson, 2002). The cephalopod solution requires long-distance neural wiring between photoreceptors and higher order neurons, which is space-demanding as well as it leads to slower processing and noisier signals (Wirth et al., 1984).
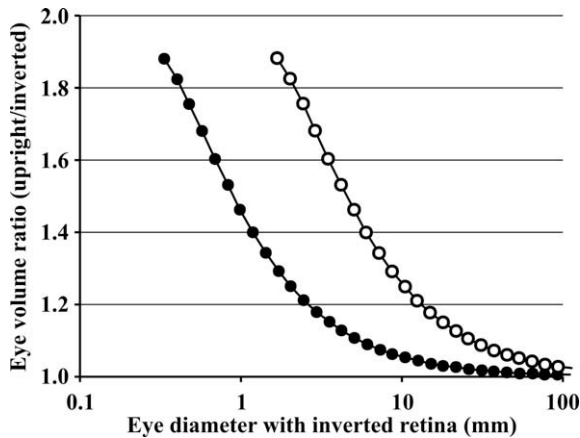
Fig. 3. Ratio of eye volumes (everted/inverted retina) as a function of eye radius with an inverted retina (R_i). For simplicity, the contributions to the total eye volume of additional ocular elements such as the retinal pigment epithelium, choroid, sclera, and anterior segment have been neglected. Closed symbols: retinal thickness = 100 μm; open symbols: retinal thickness = 500 μm.

The calculated amounts of space saved (Fig. 3) apply to the simplified eye shown in Fig. 1. They may be somewhat different in real eyes. The results from modelling nevertheless indicate clearly that the inverted retina offers a space-saving advantage that is large in small eyes and substantial even in relatively large eyes. The advantage also increases with increasingly complex retinal processing and thus increasing retinal thickness. Since the evolution of vision started with small eyes (Lamb, Collin, & Pugh, 2007), early vertebrates benefitted considerably from the inverted retina that they had inherited from their eye-less predecessors and that comes about by the invaginating type of eye formation in vertebrates (Lamb et al., 2007). This mode of eye formation has been considered to be the accidental reason for the apparently disadvantageous inverted retina (e.g. Land & Nilsson, 2002). We think that this evolutionary event has been a favourable incident because it allowed early vertebrates to accommodate relatively large eyes in small heads, thus favouring the evolution of vertebrates into a large group of animals of which many are highly visual.

Among the eyes of competing groups of animals, the complex eyes of arthropods offered a similar space-saving advantage as long as eye size was small. The interiors of small complex eyes are also entirely filled with visual cells, with the lower limit for eye size set by the minimum possible photoreceptor size (Kirschfeld, 1976). As eye size and spatial resolution increased, however, the camera-type eye turned out to be clearly superior (Kirschfeld, 1976). Eyes with inverted retinas perform well irrespective of their sizes, except for very small eyes with low spatial resolution (Kirschfeld, 1976). This type of retina has thus to be considered superior instead of inferior, with the latter having been the previously held general opinion. Only in large-eyed species, the scattering effect of the inverted retina may indeed pose a disadvantage and the everted retina of cephalopods may be superior, although it also has its problems (see above).

Franze and co-workers have put forward the theory that the Müller cells may act as light-guides in vertebrate retinas. The cells are hypothesised to pick up visual information at the inner limiting membrane and guide it undistorted and with little loss through the retinal neurons to the photoreceptors (Franze et al., 2007). In young zebrafish there is not enough space between the lens and the inner limiting membrane to create a well-focused image (Fig. 2). By contrast, the distance between the centre of the lens and the proximal end of the inner segments of the cones is about 2.5 lens radii (Fig. 2), a value that is well within the range of focal lengths of fish lenses determined by Matthiessen (1882). At least in young zebrafish a well-focused image seems to be created on the inner segments of the cones and the light is then guided within the cells to the light-sensitive outer segments. A similar mechanism has been described for the short-wavelength sensitive cones of humans (He & MacLeod, 1998).

In addition to the space-saving advantage, the invaginating mode of eye formation brings the photoreceptor outer segments in close proximity to the pigment epithelium that regenerates isomerised visual pigment and in many species regulates light flux to the photoreceptors (Douglas, 1982; Lamb et al., 2007; Lythgoe, 1979). It also allows for the nourishment of the metabolically highly active photoreceptors via the choroid, while keeping light-absorbing haemoglobin out of path of incoming light (Walls, 1942). The disadvantage of light scattering by retinal neurons can be reduced locally with a fovea from which most of the nervous circuitry and cell bodies are absent (Davson, 1990; Rodieck, 1998). A deep fovea can constitute a third refractive element – in addition to the cornea and lens – and bestows telescopic vision on some fishes, reptiles, and birds (Locket, 1992; Pettigrew, Collin, & Ott, 1999). These additional advantages, however, probably became of importance first when vision had evolved into a sophisticated sensory system and eye size had increased. They are, therefore, of little value for the understanding of the early evolution of vertebrate vision.

Instead of asking why vertebrates possess apparently problematic inverted retinas, one may ask why such space-saving retinas are limited to vertebrates and a handful of invertebrates (Duke-Elder, 1958). The answer is the same for both questions: animals have their group-specific eye and retina types because of common decent within each phylogenetic group. Vertebrates have evolved into the group of animals which most heavily rely on vision with high spatial resolution. The inverted retina has most likely been an important factor since it allows for massive retinal processing of visual information without investment of precious space and weight.

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