How the retina sorts colours into cones and rods
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Abstract: Light incident on the vertebrate retina crosses reflecting and scattering layers before reaching the photoreceptors. Previous experiments and simulations showed that glial Müller cells function as optical fibers to guide the light. Our new study shows that Müller cells concentrate the green and red light into cones, and at the same time scatter the bluer part of the spectrum to the surrounding rods. We performed experiments in isolated guinea pig retinas, where this effect is demonstrated very clearly. Colour separation was also supported by an optical computational model, in the guinea pig and the human parafoveal retina. Thus light conduction by Müller cells through the retina can be considered as an integral part of the first step in the visual process, optimising cone- and rod-mediated vision. It also means that both day vision and night vision gain from this spectral sorting.

Introduction: Vision starts with the interaction of light with the retina. After diffracting through the cornea, crystalline lens and humours, light crosses the retina and impinges on the cones and rods. Vertebrates’ retinas (except for the small foveal areas) are strangely organized in a reverse order: the photoreceptors turn out to be the last cells in the path of light, rather than the first. The incoming light must propagate through reflecting and scattering layers of cell bodies and neural processes before reaching the photoreceptors, reducing the vision acuity. Careful microscopic examination of the retina fails to see the effect of these nearly transparent layers: being phase objects close to the focal plane renders them invisible. That little light that is scattered back comes from nuclei and somata, as is well seen in OCT and SLO scanning. In comparison, weak phase perturbations have negligible effect on returned intensity. As a result of this failure, the very important optical role of glial Müller cells was missed until recently.

Müller cells were known for their metabolic and mechanical support of the retina, but they also carry an important optical purpose. Being of higher refractive index, they act as light guides, transferring light across the retina, from the vitreo-retinal border towards the photoreceptors1-4. A single Müller cell collects light in an extended area of the vitreo-retinal surface, and guides it onto one coupled cone of diameter ≥ 2-3 µm, located at its outer end. The wider receiving end of the Müller cell (12-20µm) covers also ~15 rods surrounding the central cone (Fig. 1a), so light concentration into the cone seems to reduce the share of the rods, responsible for dark conditions. Lossy guiding into cones could reduce the sensitivity of the cones, responsible for day-time colour vision. There must be a deeper reason for this arrangement, and we probed the optical aspect to it and found it to be essential.

Fig. 1. (a) Müller cells and photoreceptors in the human parafoveal retina. (b-d) Simulations results for three wave lengths before photoreceptors have different transmissions. (e) Combination of actual colours on cone and surrounding rods.
Simulation: We simulated monochromatic light propagation through the peripheral human retina. Using a measured refractive index profile, we constructed a 1000×256×256 data cube containing a Müller cell and its vicinity in the parafoveal retina. In different realization, we added 5% random perturbations of the cell’s width and for its center line, to simulate the uneven boundaries and undulations of the cells. We also added random perturbations of the cell’s refractive index on a scale of 1 µm and of its extracellular vicinity by ~5% of the local refractive index. We developed and applied the Fast Fourier Transform Beam Propagation. An initial light distribution was a diffraction pattern from the pupil, broadened by cornea’s aberrations, to create an average Gaussian distribution of ~40 µm width. Next, the field was propagated down the medium, plane by plane at 0.13 µm steps.

The results of the simulation show that Müller cells are concentrating light in a varying efficiency according to wavelength (Fig. 1b-d), the maximum being in the yellow. We repeated the analysis for 25 visible wavelengths (400–700nm). We defined a concentration factor for light in the center of the Müller cell, \( M(\lambda) \), and in its surrounding neural layers, \( S(\lambda) \), as the mean light intensity normalized by the mean intensity of a uniform illumination.

The light concentration \( M(\lambda) \) has a maximum of \( \approx 11 \) at 570nm (Fig. 2a). The spectrum of light transmitted by Müller cells is remarkably consistent with the measured spectral absorbance of human red cones and closely overlaps with that of green cones (Fig. 2b). Interestingly, this result is consistent with the natural abundance of red and green cones, which accounts together for ~90% of all cones. As a result of the funnel shape of Müller cells (Fig. 1a), red-green light impinging upon the retina is being gathered from a large retinal region of ~20 photoreceptors, and concentrated onto a single central cone. Less light, mostly blue, propagates in the surrounding area and hitting the rods. The intensity in the green-red band (520-600nm) reaching the rods is lowered by 30-40% (Fig. 2c), but only by 5-15% for the blue region of the spectrum (\( \lambda < 500\text{nm} \)), a region to which rods are most sensitive (Fig. 2d). Thus, Müller cells are sorting the visible spectrum so as to maximise the cone-mediated vision, and at the same time preserve the rod-mediated vision. Similar results were obtained for mice and guinea pigs, and should be relevant for most vertebrates.

Experiments: We measured the spatial and spectral distribution of white light, propagating in the guinea pig retina. Since the retinal refractive indices and Müller cells dimensions are known, we could also simulate them for cross-validation. We mounted a piece of a guinea pig retina that had been isolated from the pigment epithelium in an inverted confocal microscope with photoreceptors facing the objective. First, we identified the outer segments of the photoreceptors (Fig. 3b, in blue) by their intense auto-fluorescence. Then, the microscope configuration was switched to record the spectral transmission of light in the retina (Fig. 3a), at optical sections of 5µm thickness spanning a total of 150µm, the average length of Müller cells. Guided, straight light beams show clearly across the retinal depth (Fig. 3b, in purple). The visible range (400-700nm) was measured in 27 spectral bands. Three representative images are shown in Fig. 3c.

In each band we located the Müller cells by their higher intensity, and took the ten brightest ones. This was repeated for five retinas. The average spectrum of light transmitted inside Müller cells shows remarkable agreement with the corresponding calculated spectrum, obtained by the optical simulation.
performed for the guinea pig retina (Fig. 4). Similar correspondence is found for the light leakage, measured outside the hot spot areas of Müller cells. The measured and simulated curves stray apart in the blue, probably due to Rayleigh scattering, which was not simulated.

**Analysis:** We assessed the effects of Müller cells spectral separation on photon absorption by cone- and rod-photoreceptors. We calculated light absorption in cones' outer segments as a product of the Müller cell’s concentration factor (Fig. 2a) by the normal cone absorbance profile (Fig. 5a-c). We similarly calculated the photon absorption in the rods' outer segments by multiplying the spectrum of light scattered outside the Müller cell into the surrounding area (Fig. 2c), by the absorption spectrum of the rod visual pigment (Fig. 5d). The spectral gain in photon absorption shows an increase by a factor of ~7.5 for the red- and green-cones, and by a factor of ~4 for the blue cones (Fig. 5e). In contrast, Müller cells’ properties cause loss in photon absorption by rods, but only by ~20% (Fig. 5e).

**Discussion:** We showed by simulations that white light incident on the human parafoveal retina, and on the guinea pig retina, splits according to its spectral components by retinal Müller cells (Fig. 2). Imaging experiments in guinea pig retinas (Fig. 4) further support this notion. The Müller cell light guide concentrates the red-green part of the spectrum inside to reach the cones, while diffracting and scattering blue-violet light towards the surrounding rods. The spectrum of light transmitted through the Müller cells matches almost perfectly the pigments of the medium- and long-wave length cone photoreceptors. The scattered spectrum matches the absorption spectrum of rhodopsin, the rods' visual pigment. Hence light concentration into cones is not impeding significantly light absorption in rods' outer segments (Fig. 5).

It turns out that the retina is inverted on purpose, so that it will be thick enough that the glial Müller cells will be long enough to perform colour sorting. This way both day and night vision are supported, even if not at the same time. Different vertebrates with different pigments suited for their own needs will probably have slightly different geometries and refractive indices to fit these pigments.
Fig. 5. Gain in photoreceptors absorption by colours sorted in Müller cell. (a-c) Müller cell concentration into cones \(M(\lambda)\), gray bars, multiplied by the cones absorption \(C(\lambda)\), curves results in the gain of cone absorption \(A_{\text{cone}}(\lambda) = M(\lambda)C(\lambda)\) (red, green and blue respectively). (d) Spectral transmission outside Müller cells \(S(\lambda)\), gray bars multiplied by the rod absorption \(R(\lambda)\) results in the gain of rod absorption (black) \(A_{\text{rod}}(\lambda) = S(\lambda)R(\lambda)\). (e) Absorption gain is highest for the red cones, then green cones, and lowest for the blue cones. Rod absorbance (black) is reduced by ~20% because of the wave-guiding.

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References