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Pupillary light reflex of lamprey *Petromyzon marinus*

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The discoveries of the photopigment melanopsin and intrinsically photosensitive retinal ganglion cells (ipRGCs) have revealed novel mechanisms of light detection now known to control several kinds of non-image-forming vision, including regulation of mood, the circadian rhythm, and the pupillary light reflex (PLR). These remarkable discoveries have been made mostly on mammals, but many vertebrates express melanopsin and adjust the diameter of the pupil to the ambient light intensity to extend the operating range of vision and reduce spherical aberration[1]. We were curious to know whether a PLR controlled by melanopsin is also present in lamprey, which are members of the only remaining group of jawless vertebrates (agnathans) which diverged from all other vertebrates about 500 million years ago[2]. We now show that lamprey have a robust PLR mediated by melanopsin apparently without any contribution from signals of rods and cones, suggesting that non-image-forming perception emerged long before the radiation of present vertebrate lines and was already present in the late Cambrian.

To demonstrate a PLR in lamprey, we anaesthetized intact adult *Petromyzon marinus* with 100 mg per liter tricaine methane sulfonate. We placed the animals in darkness on the stage of a dissecting microscope equipped with infrared image converters, and we focused down on the eyes and captured images continuously with a CCTV camera. In experiments on isolated eyes (Figure 1E), lamprey and mice were euthanized by protocols approved by the UCLA Animal Research Committee. Illumination was delivered with optical-fiber cables directly over the surface of the eye, with LED light controlled by an OptoLED LITE system (Cairn Research, Faversham, UK). Pupil and eye size were measured in Adobe Photoshop® from screen-captured images (as in Figure 1A inset), and the ratio of the pupil area to the total area of the eye was plotted and normalized to this ratio in darkness.

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AUTHOR CONTRIBUTIONS

A.M. assisted in the design of the experiments and obtained and analyzed the data. T.H.H. assisted with experiments. R.F. assisted in the design of experiments and helped with analysis of data. G.L.F. and A.P.S. assisted in the design of experiments and wrote the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Lamprey display a robust PLR, which can reduce the area of the pupil to as much as 50–60% that in darkness (Figure 1A). Constriction was, however, slow: the mean time of half constriction even in bright light was 81 ± 22 s. Similar slow responses have been observed in elasmobranchs, but in birds and mammals, including man, the PLR is considerably faster; in the eel and frog, it is complete in a few seconds[1]. The PLR was most readily evoked by blue light. The lamprey genome is known to express *Opn4m* for one form of melanopsin[3], and lamprey appear to have retinal horizontal and ganglion cells containing melanopsin[4]. We therefore measured the action spectrum of the PLR by stimulating the eye with light at 8 wavelengths and evaluating the intensity required to reduce the normalized pupil area to 0.83, about half-maximum. These measurements were converted to relative sensitivities and are given in Figure 1B. They could be reasonably well described by an A1 rhodopsin nomogram[5] with peak at 480 nm (Figure 1B), the λ_{max} of expressed lamprey melanopsin[4].

The data in Figure 1B suggest that only a single pigment is responsible for the PLR. To show this more directly, we have plotted in Figure 1C response-intensity curves for each of the stimulating wavelengths. The abscissa gives the intensity for 480 nm and shows that the pupil began to constrict only when light intensities were elevated above 10^{13}–10^{14} photons cm^{-2} s^{-1}. For the other wavelengths, we have translated the data along the intensity axis by the ratio of sensitivity at each wavelength to that at 480 nm, as given in Figure 1B. These curves all have a similar shape, further supporting spectral invariance.

The intensity required to produce constriction is somewhat brighter than the intensity required in wild-type mice or humans[1] but is similar to that required to produce a PLR in *rd/rd* mice without functioning rods or cones[6]. It is considerably brighter than what is needed to saturate the single class of lamprey (522 nm) rods[7] for the short-duration light exposures we used[8], indicating that rods cannot drive the PLR in this species. Adult *Petromyzon marinus* have only a single spectral class of cone with peak sensitivity at 590–600 nm[7]. Figure 1B shows that the PLR is 100 times less sensitive at 600 nm than at 480 nm. The PLR cannot therefore be driven by cones. Lastly, the action spectrum does not resemble other pigments known to be expressed in lamprey, like UV-sensitive parapinopsin[9]. We therefore propose that lamprey use melanopsin and melanopsin alone to control pupil diameter, and that rods and cones make little or no contribution, unlike in mammals[1].

In humans and many vertebrates, the PLR is bilateral (or consensual), so that light in one eye constricts the pupil in the unilluminated eye. This is not the case for lamprey (Figure 1D). In this regard, the lamprey resembles other animals with eyes placed on either side of the head[1]. For many species including frogs, eels, and mice[1], an intrinsic PLR can be observed in the isolated eye. In mouse there is clear evidence that the iris can contract intrinsically, driven by melanopsin expressed in the iris sphincter muscle[10]. To establish whether the lamprey PLR is also intrinsic, we illuminated the isolated eye with bright light (Figure 1E). No change in pupil area was detected, even under conditions producing a clear constriction in an isolated mouse eye. Although it is possible that isolation of the lamprey eye damages the intrinsic response, the lamprey eye is considerably larger than that in mouse (Figure 1E) and easier to dissect[7,8]. We think it more likely that the lamprey PLR requires input from the central nervous system, possibly driven by melanopsin-containing retinal ganglion cells as in mammals.

We have shown that non-image-forming vision is present in lamprey, and that signals from the photopigment melanopsin are controlling the pupil area, possibly from ipRGCs as in mammals but without significant input from rods and cones. We think it unlikely that both melanopsin as a visual pigment and the PLR evolved by convergent evolution in cyclostomes and other vertebrates. Instead, we suggest that non-image-forming perception regulated by melanopsin emerged long before the radiation of present vertebrate lines and was already present in the late Cambrian.

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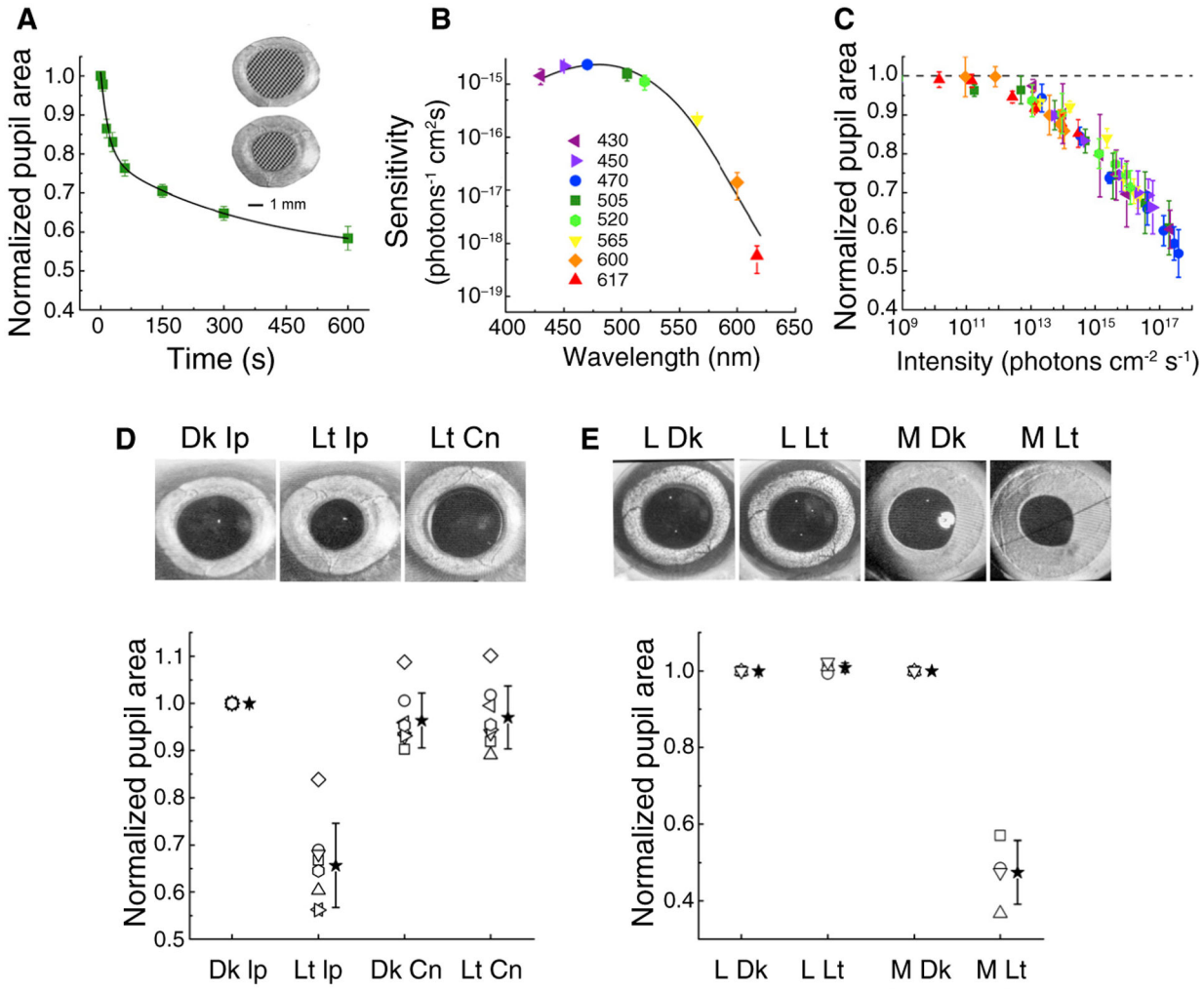

Figure 1. Pupillary light reflex (PLR) of the lamprey *Petromyzon marinus*.

(A) Pupil area as function of time. The eye in darkness (inset in A, above) was exposed to light (inset in A, below) and pupil area was measured from the checkered areas as shown. Eyes were exposed to 505-nm light of intensity 2.4×10^{17} φ cm^{-2} s^{-1}, and images were captured at the times shown on the abscissa. The measured ratio of pupil area to the area of the front of the eye was divided by the ratio in darkness to give the normalized pupil area (NPA). Curve is double-exponential fit with time constants of 29 and 180 seconds. (B) Action spectrum of PLR. Sensitivity of pupil constriction estimated from unadjusted data in (C) for light at 430 nm (n = 4), 450 nm (n = 4), 470 nm (n = 5), 505 nm (n = 7), 520 nm (n = 4), 565 nm (n = 5), 600 nm (n = 5) and 617 nm (n = 6). The 470, 505, 565, and 617 nm lights were produced by a monochromatic LED. The 430, 450, 520, and 600 nm lights were produced by a white LED with interference filters. The data are overlaid with an A1 rhodopsin nomogram with peak at 480 nm. (C) NPA plotted as a function of adjusted intensity. Measurements were made from dimmest intensity to brightest and were taken after 1 min of light exposure. NPA from individual experiments was plotted as a function of intensity on a logarithmic scale. The interpolated intensity required to reduce the normalized pupil area to 0.83 was calculated with a linear fit model. These values were then inverted,

averaged, and plotted as a function of wavelength in (B). Adjusted intensities were taken from ratio of sensitivity at each wavelength to sensitivity at 480 nm based on the nomogram in (B), and the value was used to shift the intensity axis for each of the wavelengths as plotted. (D) Ipsilateral (Ip) and the contralateral (Cn) eyes were imaged in darkness (Dk) prior to onset of a bright 505-nm light of intensity 2.4×10^{17} φ cm^{-2} s^{-1}. The ipsilateral eye was directly illuminated (Lt Ip), while the contralateral eye was covered and kept in darkness (Lt Cn). After 10 minutes, both eyes were imaged. Graph gives NPA; dark-adapted contralateral eye (Dk Cn) has also been included. Pupil areas were normalized to value of Dk Ip. (E). Eyes of lamprey (L) and wild-type (129S1/SvlmJ) mice (M) were enucleated and exposed as in (A) to 505-nm light of intensity of 2.4×10^{17} φ cm^{-2} s^{-1} for 10 minutes.