
Figures and figure supplements

A Cambrian origin for vertebrate rods

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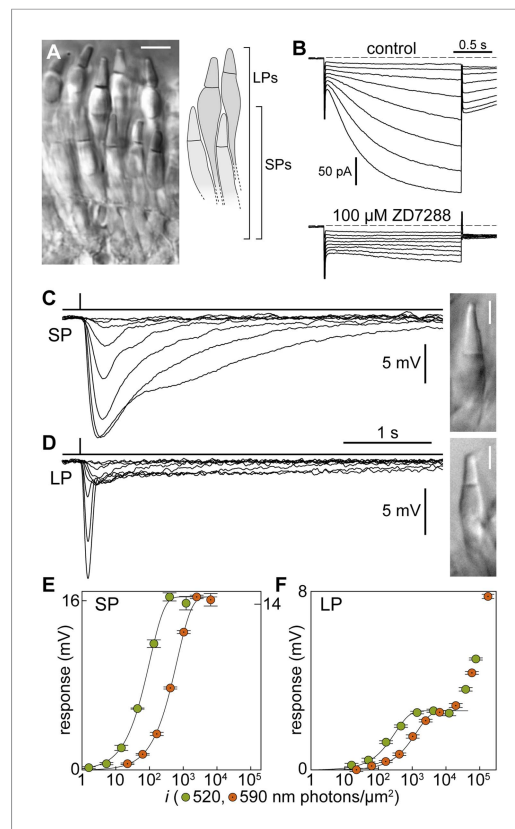


Figure 1. Signal processing in the inner segment of lamprey photoreceptors resembles that found in jawed vertebrates. **(A)** Image of a live retinal slice showing the layered organization of lamprey photoreceptors: short photoreceptors (SPs) in an inner tier and long photoreceptors (LPs) in an outer tier. Scale bar 10 μm . **(B)** Photoreceptors express the I_h current: membrane current of a SP in response to hyperpolarizing voltage clamp steps (from a holding potential of -53 mV to $-60/-67/-74/-81/-88/-95/-102/-109$ mV and repolarization to -65 mV) in control and during superfusion of the I_h blocker ZD7288 at 100 μM . Records are not averages. **(C–F)** Photovoltage responses reveal that SPs feed their signals into LPs. **(C and D)** Average responses to 520-nm flashes of a SP (0.5, 1.6, 5.4, 15, 45, 136, 398, 1128 photons $\cdot \mu\text{m}^{-2}$) and a LP (16, 51, 170, 469, 1413, 4314, 12,597, 38,847, 77,695 ph $\cdot \mu\text{m}^{-2}$). Insets show their outer segments (scale bars 5 μm). **(E and F)** Response amplitudes to 520-nm (green circles) and 590-nm flashes (orange circles with a dot) of a SP and a LP. Fits are exponential saturation functions (for the LP restricted to the first component: see text). In panel **E**, left and right ordinate values refer to left and right data sets, respectively, which were adjusted to match saturating amplitudes. In panel **F** such an adjustment could not be performed. Error bars are SEM. Action spectra templates for SPs and LPs are shown in **Figure 1—figure supplement 1**.

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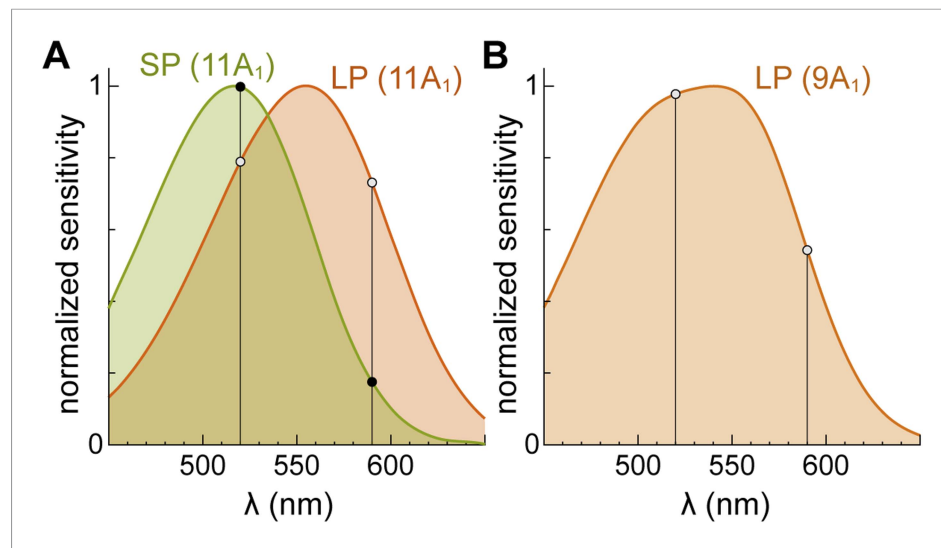


Figure 1—figure supplement 1. Predicted action spectra of SPs and LPs and their relative sensitivities at 520 and 590 nm. (A) 11-cis-Retinal (11A₁) based visual pigment templates (Equation 1 in [Govardovskii et al., 2000](#)) centered at the absorption peaks of SP (517 nm, green curve) and LP outer segments (555 nm, orange curve) determined previously by microspectrophotometry ([Govardovskii and Lychakov, 1984](#)). Small circles indicate, for each of the two photoreceptor types, the normalized sensitivities at our two stimulus wavelengths (520 nm, left vertical line; 590 nm, right vertical line). The predicted sensitivities ratios (520/590 nm) match well with those found in our experiments (see 'Results'). (B) 9-cis-Retinal (9A₁) based visual pigment template for regenerated LPs (peak at 541 nm), obtained from that of red salamander cones (Equation 3 and Table 2 in [Makino et al., 1999](#)) by: (i) assuming full replacement of the native chromophore and (ii) introducing a slight hypsochromic shift (1 nm) so as to match our experimentally determined sensitivities at 520 and 590 nm.

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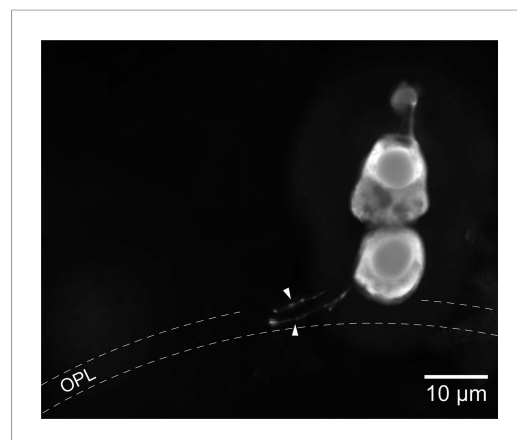


Figure 2. Lamprey photoreceptors extend telodendrial processes. An example of a lucifer yellow stain of a live SP showing two thin processes (arrowheads) extending laterally from the synaptic pole into the outer plexiform layer.

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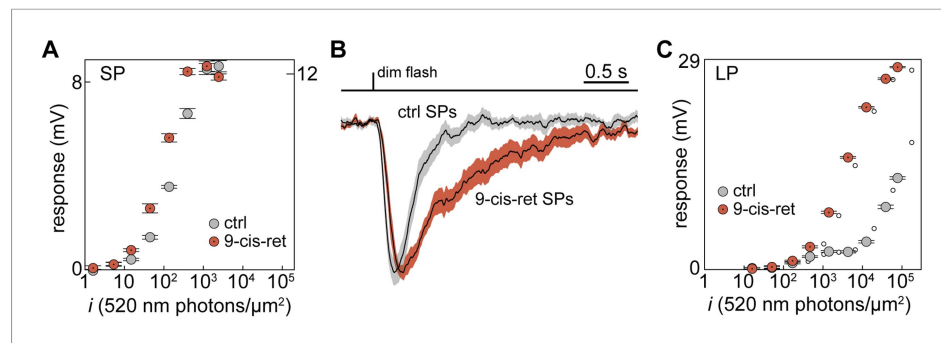


Figure 3. Visual pigment regeneration reveals the full sensitivity of photoreceptors in the upstream migrating river lamprey. **(A)** Photovoltage response amplitudes to 520-nm flashes before (gray circles) and after visual pigment regeneration with 9-cis-Retinal (red circles with a dot) of a SP. Left and right ordinate values refer to left and right data sets, respectively, which were adjusted to match saturating amplitudes. **(B)** Normalized-averaged-normalized dim-flash photovoltage responses in control ($n = 13$) and regenerated SPs ($n = 9$), highlighting the difference in integration time. These records were obtained as follows: (i) the average dim-flash response of each SP was normalized to its peak amplitude (always below 2 mV), (ii) normalized responses were averaged across cells, (iii) the final average was normalized to its peak. Shaded areas show ± 1 SEM. **(C)** Photovoltage response amplitudes to 520-nm flashes before (gray circles) and after visual pigment regeneration with 9-cis-Retinal (red circles with a dot) of a LP. Responses to 590-nm flashes are also shown (small empty circles; error bars are smaller than circle diameter). Error bars are SEM.

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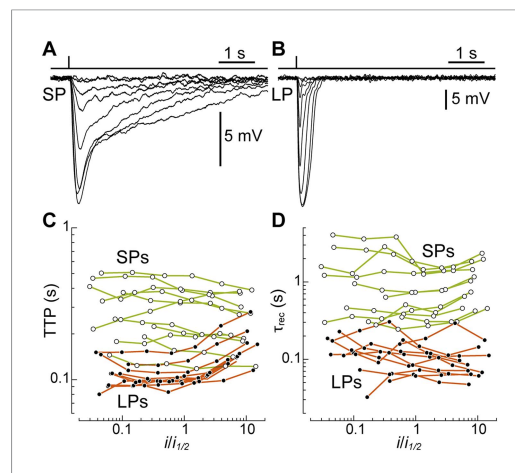


Figure 4. SPs are markedly slower than LPs. **(A and B)** Average responses to 520-nm flashes of a SP (0.5, 1.6, 5.4, 15, 45, 136, 398, 1128 photons/ μm^2) and a LP (16, 51, 170, 469, 1413, 4314, 12,597, 38,847, 77,695 photons/ μm^2), both recorded with patch clamp after visual pigment regeneration with 9-cis-Retinal. **(C and D)** Plots of time-to-peak (TTP) and decay time constant (τ_{rec}) vs flash strength, normalized to its half-maximal value $i_{1/2}$, in regenerated SPs ($n = 11$; empty circles) and LPs ($n = 10$; full circles). The data from each cell are connected by lines.

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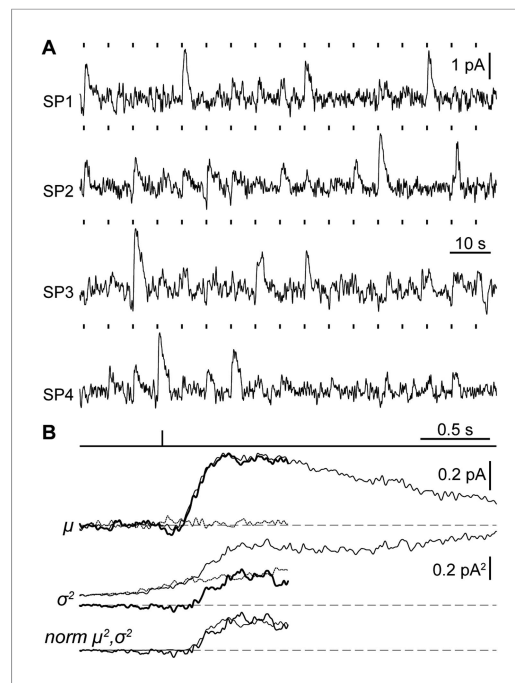


Figure 5. Suction electrode recordings of SP photocurrents in the single photon regime. **(A)** Samples of dim-flash response trains recorded from the outer segments of 4 SPs (SP1: flash strength = $1.64 \text{ photons} \cdot \mu\text{m}^{-2}$, $a = 0.25 \text{ pA}$; SP2: $3.26 \text{ ph} \cdot \mu\text{m}^{-2}$, 0.43 pA ; SP3: $1.64 \text{ ph} \cdot \mu\text{m}^{-2}$, 0.56 pA ; SP4: $3.26 \text{ ph} \cdot \mu\text{m}^{-2}$, 0.56 pA). **(B)** Single photon response analysis from one SP (SP3 in panel A), showing the mean response μ (thin trace: gross mean response, dotted trace: mean dark current, thick trace: net mean response), time-dependent variance σ^2 (thin trace: gross variance, dotted trace: dark current variance, thick trace: net variance), normalized squared mean response $\text{norm } \mu^2$ (thin trace) and variance $\text{norm } \sigma^2$ (thick trace). Dashed lines indicate the current baseline or zero level. Dark current records were taken from the last 2 s preceding each flash and where baselined in the first 1.1 s, therefore, dark and net current records cover up to 0.9 s after the flash (see 'Materials and methods'). DOI: [10.7554/eLife.07166.008](https://doi.org/10.7554/eLife.07166.008)

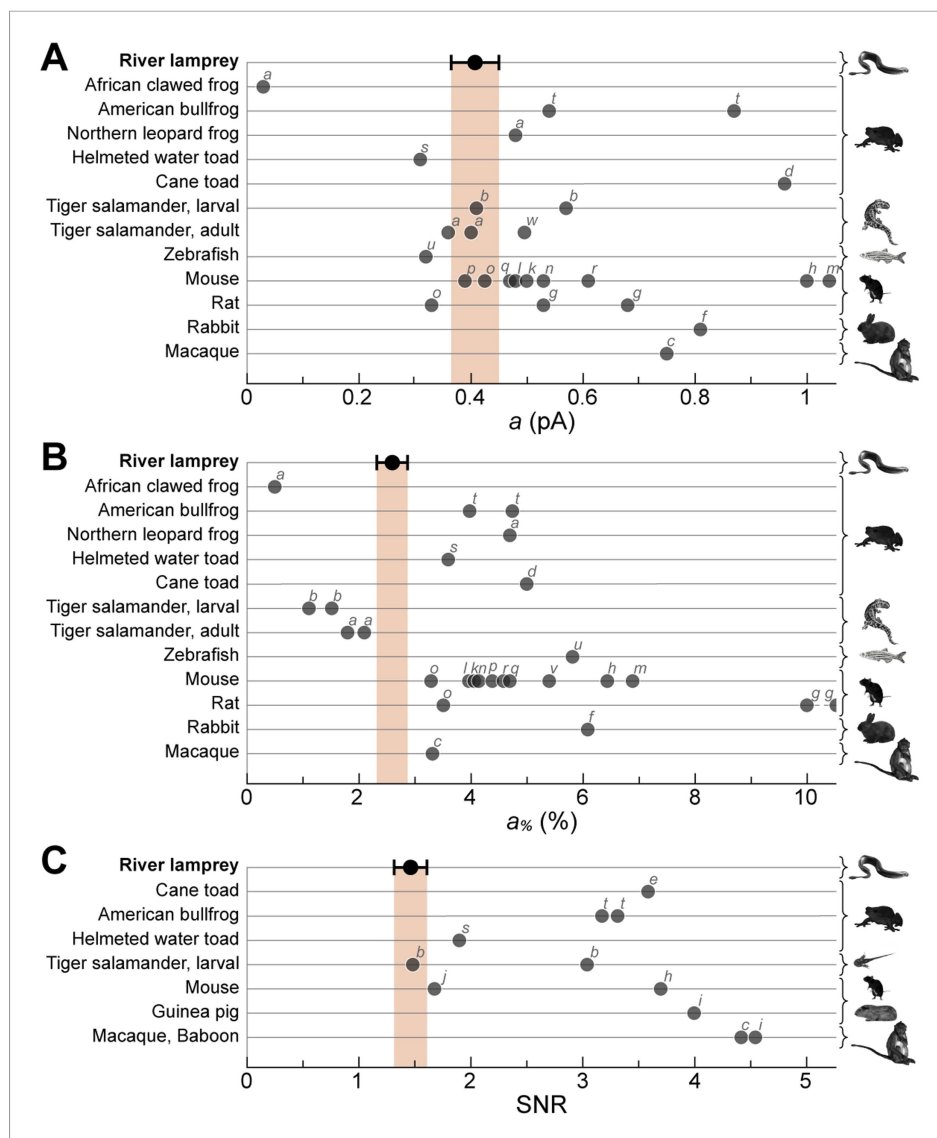


Figure 6. Lamprey SP single photon response parameters in the context of those of jawed vertebrate rods. River lamprey: this study; Jawed vertebrates: previous studies. **(A)** Absolute amplitude of the single photon response (a). **(B)** Fractional amplitude of the single photon response ($a\%$). **(C)** Signal-to-noise ratio (SNR). Data points shown in the figure were obtained with suction pipette recordings of photoreceptor outer segments. Error bars for lamprey are SEM. Letters next to the data points correspond to the following references: a (Palacios et al., 1998), b (Ala-Laurila et al., 2007), c (Baylor et al., 1984), d (Baylor et al., 1979a, 1979b), e (Baylor et al., 1980), f (Nakatani et al., 1991), g (Robinson et al., 1993), h (Field and Rieke, 2002b), i (Field and Rieke, 2002a), j (Okawa et al., 2010), k (Mendez et al., 2001), l (Burns et al., 2002), m (Azevedo and Rieke, 2011), n (Krispel et al., 2006), o (Luo and Yau, 2005), p (Makino et al., 2004), q (Wen et al., 2009), r (Gross and Burns, 2010), s (Palma et al., 2001), t (Donner et al., 1990), u (Vogalis et al., 2011), v (Nikonov et al., 2006), w (Rieke and Baylor, 2000).

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