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Functional Characteristics of Juvenile Lamprey Photoreceptors

A thesis submitted in satisfaction of the  
requirements for the degree Master of Science  
in Physiological Science

by

Gabriel Elijah Pollock

2017

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# ABSTRACT OF THE THESIS

## Functional Characteristics of Juvenile Lamprey Photoreceptors

by

Gabriel Elijah Pollock

Master of Science in Physiological Sciences

University of California, Los Angeles, 2017

Professor Gordon L Fain, Co-Chair

Professor Alapakkam P Sampath, Co-Chair

The visual system of freshwater vertebrates shifts photoreceptor sensitivity to longer wavelengths and extends visual sensitivity beyond the range of human vision. One mechanism of enhancing sensitivity to long-wavelength light is to replace the 11-cis retinal chromophore in photopigments with 11-cis 3,4-didehydroretinal. Migratory species of amphibians and salmon can dynamically tune their visual system by altering the balance of 11-cis retinal and 11-cis 3,4-didehydroretinal. Here it is shown that the same process is available in sea lampreys. The ratio of vitamin A<sub>2</sub>-to-A<sub>1</sub> increases as the animals migrate from the open ocean into inland freshwater environments. Suction-electrode recording was used to measure the spectral sensitivity in both

adult and juvenile *Petromyzon marinus*. Primarily, these data show that juvenile lamprey possess a duplex retina. Secondly, these data demonstrate a strong correlation between the red-shifted light environment of freshwater habitats and the use of the red-shifted vitamin A<sub>2</sub> in this primitive vertebrate.

The thesis of Gabriel Pollock is approved.

Mark Frye

Gordon L Fain, Committee Co-Chair

Alapakkam P Sampath, Committee Co-Chair

University of California, Los Angeles

2017

## **Dedication**

This thesis is dedicated to:

My father, a true eternal optimist.

Dr. Cari Meyer, the professor who enabled me to actualize my potential.

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## INTRODUCTION

Flux in the spectral composition and intensity of light in dynamic natural settings presents fundamental difficulties for vision. Consequently, many characteristics of the vertebrate eye are adaptations to cope with these challenges. In particular aquatic environments such as actuatories, streams and rivers, shorter wavelengths of light are scattered or absorbed by sediment and dissolved organic matter, resulting in a significant shift in the spectral composition of light toward longer wavelengths (Jerlov 1976; Jokela-Määttä et al. 2007). Over the course of evolution, aquatic organisms have optimized visual sensitivity and acuity by coordinating the sensitivities of their photoreceptors to the available light spectrum (Lythgoe 1979 & 1984; Cronin et al. 2014). As the light environment in the course of an organism's life is altered over space and time, many species have evolved mechanisms of visual system plasticity that allow them to shift photoreceptor sensitivity dynamically, maintaining an adequate fit to their surroundings.

Pertinent examples encompassing this spectral plasticity are robust in visual systems of anadromous species such as pacific salmon (*Oncorhynchus sp.*), which spend the majority of their adult lives in the ocean, where short-wavelength (blue-green) light predominates; but migrate to spawn in inland waterways ruled by long-wavelength (yellow-red) light (Beatty 1966). To cope with this shift in the available light spectrum, salmon red-shift the sensitivity of their photoreceptors by switching their visual pigment chromophore from 11-cis retinal (a derivative of vitamin A<sub>1</sub>) to 11-cis 3,4-didehydroretinal (a derivative of vitamin A<sub>2</sub>) (Figure 1a) (Beatty 1966; Bridges 1972). This shift in the visual system has classically been referred to as the 'rhodopsin-porphyrpsin' switch, because vitamin A<sub>1</sub>-based photopigments have traditionally

been called 'rhodopsins', whereas the red-shifted vitamin A<sub>2</sub>-based photopigments are collectively referred to as 'porphyropsins' (Bridges 1972). Chromophore switching allows for adaptation of the visual system to a greater number of spectral niches, and is utilized by a medley of fishes, amphibians, and reptiles (Bridges 1972; Reuter et al. 1971; Provencio et al. 1992).

The earliest vertebrates diverged from chordates during the Cambrian radiation (Smith et al. 2001) and were agnathans—fish-like organisms lacking a jaw. About 500 million years ago, these animals or their vertebrae progenitors gave rise to cyclostomes, the group that includes the only agnathan species to have survived to the present day—hagfish and lamprey (Kuraku & Kuratani 2006). At about the same time, other lines of agnathans gave rise to the first gnathostomes or jawed vertebrates, from which fishes, amphibians, reptiles, birds and mammals were all later derived.

The sea lamprey, *Petromyzon marinus*, is an anadromous species that is an ideal experimental subject for the study of chromophore switching. After several years in fresh-water habitats as larvae, lamprey undergo a metamorphosis that allows young postmetamorphic juvenile lamprey to migrate to the sea and begin hematophagous feeding (Collin et al. 2003). Larval lamprey possess no discernable eye, while juvenile lamprey possess a fully developed camera-like eye comparable to adult lamprey. The eyes of larval lamprey (also called ammocoetes) are buried beneath the skin, where most of the retina is relatively undifferentiated and possesses a thick neuroblastic layer (Dickson & Graves 1979). Reviews by Lamb (2007) and Lamb (2009) suggest that lamprey inherited their eyes from an ancestor in common with hagfish, and that this hagfish-like larval eye is present in the lamprey larva but transforms to a vertebrate-like eye in the adult.

The eyes of fully metamorphosed lamprey are certainly prominent and well differentiated, following closely the design of the gnathostomatous fishes, with a multi-focal lens, intra- and extraocular eye muscles and a complex retina (Collin et al. 1999). Additionally, the lamprey retina has three nuclear layers comprised of photoreceptors, bipolar cells, horizontal, amacrine, and ganglion cells. Adult lamprey also express opsin genes that are extremely similar to those of jawed vertebrates (Janvier 1996). Given these similarities, we reach the inescapable conclusion that the last common ancestor of jawless and jawed vertebrates (see Fig. 2) already possessed an eye that was comparable to that of extant lampreys and gnathostomes. Accordingly, a vertebrate camera-like eye must have been present by the time that lampreys and gnathostomes diverged, around 500 million years ago (Lamb 2007). Because the evolutionary lineage of lampreys diverged from the rest of the vertebrate clade so long ago, the lamprey is a valuable organism with which to make inferences about the properties of early vertebrate progenitors by comparing lamprey eyes to those of other vertebrate species.

Photoreceptors such as cones are extant in many invertebrate phyla as well as in chordata, and rods evolved from cones (Fain et al. 2010, Lamb 2013), but the progression of adaptations is not well understood. Due to the fact that duplex retinas are present in both agnatha and gnathostomata, which diverged in excess of 500 million years ago, functional characteristics of ancestral rods may be deduced via a comparison of cells in these two groups. Morshedian and Fain (2015) effectively ended the debate of whether lamprey possess a duplex retina. Suction recordings and sensitivity measurements demonstrated that lamprey possess rods with a primitive cone-like morphology. These rods were as sensitive as the rods of other vertebrates. This observation makes the question of how and when high-sensitivity transduction evolved all

the more interesting. What exactly was the sequence of events that gave rise to rod photoreceptors? Experiments performed in Morshedian and Fain (2015) showed that short photoreceptor cells present in lamprey retinas are more sensitive to light than the long photoreceptors. It was also demonstrated the short photoreceptors are able to amplify weak light signals. Also, the short photoreceptors send signals to the long photoreceptors in a similar way to how rod cells send information to cone cells (Asteriti et al. 2015). The similarities between lamprey photoreceptor cells and those of jawed animals support the idea that they have a common origin in evolutionary history. Taken together the ability to see in low light evolved before these groups of animals diverged about 500 million years ago. Hagfish and lampreys are the living representatives of the agnathan lineage that gave rise to gnathostomes, and therefore, studies of their eyes enable us to contemplate the type of light environment perceived by our vertebrate ancestors. The picture that emerges is one in which our remote ancestors inhabiting the Cambrian seas already possessed dim-light vision. This would have allowed them to colonize deep waters or to move at twilight, an adaptation suggestive of intense competition or predation from other life forms.

Was it a 'rhodopsin-porphyropsin' switch, analogous to what occurs in pacific salmon that allowed vertebrates to colonize fresh water habitats and perhaps by logical extension, land? Morshedian and Fain (2015) conclusively demonstrated that adult lamprey possess a duplex retina. Using this work as a foundation and the sea lamprey as an experimental model, the subject of this thesis was to determine if juvenile lampreys possess a duplex retina, and if there is a corresponding shift in spectral sensitivity as juvenile lamprey migrate to the sea.

## **MATERIALS AND METHODS**

### **Animals and Tissue Extraction**

Experiments were performed in accordance with rules and regulations of the NIH guidelines for research animals, as approved by the institutional animal care and use committee of the University of California, Los Angeles, USA. Juvenile lamprey were from 2-5 years of age of the genus and species *Petromyzon marinus*, which were captured by drift net in the St. Mary's River while in the process of migrating downstream to Lake Huron to begin the parasitic stage of the life cycle. Adult lampreys were captured in tributaries of Lake Huron (Ocqueoc River and Cheboygan River) in the process of their upstream spawning migration. Lamprey were kept in approved aquaria, supplied with substrate for burrowing, and maintained under cyclic 12-on-12 off lighting at 4°C. All the animals were dark adapted prior to every experiment for at least 3 hours. Lamprey were sacrificed by decerebration, and the eyes were enucleated under dim red light. The anterior portion of the eye was cut and the lens and cornea were removed under infrared illumination with infrared image converters. The retina was isolated from the eyecup; the retinal pigment epithelium was removed with fine tweezers, and the retina was chopped into small pieces with a razor blade. The pieces were then transferred to the recording chamber with a pipette in complete darkness by means of night-vision infrared goggles (American Technologies Network Corp).

### **Solutions**

During recording photoreceptors were continuously perfused with the same solution used for



dissection and which contained 93 mM NaCl, 2.1 mM KCl, 2.6 mM CaCl<sub>2</sub>, 1.8 mM MgCl<sub>2</sub>, 2.0 mM NaHCO<sub>3</sub>, 10.8 mM HEPES and 4.0 mM glucose. The solution was bubbled with 5% CO<sub>2</sub>/95% O<sub>2</sub> at pH 7.4. The recording electrodes were filled with Locke's solution, which contained 93 mM NaCl, 2.1 mM KCl, 2.6 mM CaCl<sub>2</sub>, 1.8 mM MgCl<sub>2</sub>, 2.0 mM NaHCO<sub>3</sub>, and 10.8 mM HEPES at pH 7.4.

### **Pipette electrode**

Fire polished borosilicate glass (Sutter Instrument) with an inner diameter of 0.86 mm was pulled with a micropipette puller (Sutter Instrument), to produce pipettes with rapidly tapering shanks. We viewed the tip under a compound microscope and moved the pipette close to a platinum heating wire until the tip had melted to an inner diameter of approximately 1.7 μm or 1.9 μm. This was close enough to provide a good seal onto juvenile lamprey rods and cones, which have a diameter of about 1.4-1.5 μm and 1.7-1.8 μm, respectively. A smaller pipettes damages the integrity of the membrane after the cell is pulled inside, and a larger diameter pipette produces noisy recordings. The resistance of a good pipette when filled with solution was about 3-4MΩ.

### **Suction-electrode recording**

Single rod photocurrents were recorded with the suction-electrode technique (Baylor et al. 1979a). The recording chamber was stabilized on the stage of an inverted microscope, and the entire apparatus was enclosed in a light-tight Faraday cage. Infrared illumination and a closed-

circuit television system were used to locate cells suitable for recording. The outer segment of the rod or cone was pulled into a suction electrode, and the change in membrane current was recorded with a current-to-voltage converter (Axopatch 200A; Axon Instruments, Inc.). Recordings were low-pass filtered at 30 Hz with an 8-pole Bessel filter (Kemo Limited Electronic Filters) and sampled at 100 Hz. Digitized data were recorded via Clampex 8.0 and were analyzed with Origin Pro 8.5 (OriginLab Inc, Northhampton, MA, USA). Curve fitting and plotting of data were also done with Origin.

Cells were stimulated with a dual-beam optical bench with FCR 12V 100W halogen lamp bulbs (Ushio), whose outputs were passed through different interference filters. The intensity of the light was attenuated and controlled with absorptive neutral density filters. The light was calibrated by means of a 3 mm pinhole placed in the position of the photoreceptor and a digital silicon photodiode (UDT Instruments, San Diego, CA, USA, formerly Graseby Optronics). An electronic shutter (UniBlitz Model D122) under the command of a pulse generator was used to control light duration.

### **Determination of Spectral Sensitivity**

Spectral sensitivity was determined relative to the sensitivity at a reference wave-length (500 nm) by the method of criterion response described in (Baylor et al. 1984). The sensitivity at the reference wave length was measured repeatedly throughout the experiment in order to avoid errors arising from slow changes in the quantum sensitivity of the cell.

The fraction of pigment molecules bleached during an entire spectral-sensitivity determination was estimated from the total applied photon density and the photosensitivity, which we assumed to be the same for lamprey photoreceptors as it is for the rods and cones of other vertebrates, ( $5.7 \times 10^{-9} \mu\text{m}^2$  — see Nymark et al. 2012). The fraction bleached,  $F$ , obtained from the relation

$$F = 1 - \exp(-IPt),$$

was about 0.03. Where  $I$  is the light intensity in photons  $\mu\text{m}^{-2}\text{sec}^{-1}$ , and  $t$  is the duration of the light exposure in seconds.

For an outer segment 2-4  $\mu\text{m}$  in diameter with a specific pigment density of  $0.016 \mu\text{m}^{-1}$ , there is negligible self-screening for the transverse illumination used in the experiments. Assuming that the quantum efficiency of excitation is not wave-length dependent over the range of wave-lengths used, the measured spectral sensitivity should be proportional to the molecular extinction coefficient, the probability that a photon will be absorbed as a function of its wave-length (Dartnall 1972). The average normalized spectra were plotted on double log coordinates (sensitivity vs. wave number) in order to obtain the coefficients of the polynomial expression. These resulting points were then used to calculate the coefficients of the best-fitting sixth-order polynomial by least-squares criterion, using singular value decomposition (Press et al. 1987). The wave length maximum,  $\lambda_{\text{max}}$ , was obtained directly from the peak of the polynomial.

## Measurements of sensitivity of the rods

When recording the photoresponse from a single rod, stimulation results in closure of cGMP-gated cation channels and hyperpolarization of the outer segment membrane as a result of reduction in the inward current of these cells. As the intensity of the stimulus light is increased, more channels will close. When a bright enough light is used to close all the channels, the cell becomes saturated and the recorded value of current (it's maximum value) is known to be equivalent to the circulating current or the dark current (Baylor et al. 1979a). Increasing the intensity of the light further has no effect on the value of the light response, but maintains saturation for longer times (Fig. 3A). When the peak amplitude of the current response is plotted against the logarithmic value of the light intensity used to elicit that response, the data can be fit with a general three-parameter logistic equation (more commonly known as the Michaelis Menten equation).

This equation has the following general form:

$$\frac{r}{r_{max}} = \frac{I}{I+I_{1/2}}$$

In the analysis of photoresponses at various intensities of light,  $r$  is response amplitude for current in pA,  $r_{max}$  is the maximum value of  $r$ ,  $I$  is the flash intensity, and  $I_{1/2}$  is the light intensity required to produce a half-maximal response.

In electrophysiological recordings from light-stimulated rods, this value for  $I_{1/2}$  is routinely used as a measure of the cell's gain in phototransduction. Calculating this number is however not easily achieved in all experimental protocols. To generate a well-fit curve requires data at wide ranges of intensities, which may not be always possible. As a result, an alternative approach can be adopted. At values of response approximately 20% of the peak current response, current and intensity display a linear relationship. The slope of this line measures the change in current in pA as a result of the change in the intensity of light. This ratio is a representation of the sensitivity of the photoreceptor. To measure sensitivity, we used test flashes at intensities that would generate currents that were below 20% of the cell's maximum dark current value. The generated values fell within the linear portion of the intensity-response curves, and allowed us to measure sensitivity by dividing the maximum amplitude of response by the test flash intensity.

### **Measurement of rod response kinetics**

A widely used parameter for measuring the kinetics of photoreceptors is the integration time. The integration time value reflects the duration of the signal from initiation to termination. It is calculated by dividing the area under the response curve by the maximum amplitude of the generated current.

The termination phase of photocurrent and the recovery of the dark current in mouse rods normally follow a time course well fit by a single exponential function.

The time constant of this decay function, known as  $\tau_{REC}$ , reflects the slowest or the rate limiting step in deactivation of the phototransduction cascade (Lyubarsky 1996; Krispel 2006)

and is widely used as one of the parameters reflecting the kinetics of the rods.

### **Statistical Analysis**

The results are expressed as means  $\pm$  SEM. Continuous variables were compared by means of a two-tailed student's T test for unpaired variables. Asterisks and crosses in the figure panels refer to statistical probabilities, measured in the various experimental conditions as detailed in the figure legends. Statistical probability values of less than 0.05 were considered significant.

## RESULTS

### Current Response Profiles

Morshedian and Fain (2015) showed adult lamprey possess short, rod photoreceptors, and long, cone photoreceptors. In Fig. 3, mean current responses from the two classes of juvenile lamprey photoreceptors to brief flashes of light are shown. The “short photoreceptors” (Fig. 3A) had responses resembling amphibian rods (for example, Crescitelli 1956). The “long photoreceptors” (Fig. 3B) had responses resembling mammalian cones.

### Sensitivity & Kinetics of Juvenile Lamprey Photoreceptors

In Figure 3C, I compare of the normalized waveforms of responses of rods and cones to flashes that, for each photoreceptor type, produced a response of about half-maximal amplitude. Data for both cell types were fitted with the Michaelis-Menton equation,  $\frac{r}{r_{max}} = \frac{I}{I+I_{1/2}}$  where  $r/r_{max}$  is the normalized current amplitude,  $I$  is the flash intensity, and  $I_{1/2}$  is the flash intensity producing a half-maximal response. When values of  $r_{max}$  and  $I_{1/2}$  were estimated by fitting the response-intensity curves cell by cell, the best-fitting values of  $r_{max}$  and  $I_{1/2}$  were 10.1 pA and  $1.52 \times 10^{-2}$  photons<sup>-1</sup> μm<sup>2</sup> for rods (n = 11), and 10.4 pA and  $2 \times 10^{-4}$  photons<sup>-1</sup> μm<sup>2</sup> for cones (n = 8). These measurements were made at wavelengths of stimulation that were somewhat different from our estimates of the wavelengths of maximal sensitivity of the two kinds of photoreceptors. For this reason, we adjusted the sensitivities by a factor of 1.1 for rods and 1.24 for cones based on template curves for the photopigments (Wald 1957), in order to estimate sensitivities at the  $\lambda_{max}$  of the pigments. This gave mean values of  $I_{1/2}$  of  $1.4 \times 10^{-2}$  photons<sup>-1</sup> μm<sup>2</sup> for rods and  $2.0 \times 10^{-4}$

$4 \text{ photons}^{-1} \mu\text{m}^2$  for cones, giving mean values of the intensity required to give a half-maximal response  $I_{1/2}$  of  $50 \text{ photons } \mu\text{m}^2$  for rods and  $3,500 \text{ photons } \mu\text{m}^2$  for cones. The ratio of the values of  $I_{1/2}$  at the  $\lambda_{\text{max}}$  of the pigments was therefore approximately 70. We conclude that rods at a wavelength of 500nm are of the order of 1.8 log units, or 70 times more sensitive than cones at 600nm. These measurements are within the range of values recorded for other vertebrate species (for example, Nikonov et al. 2006 & Fain 1973).

Because cones were less sensitive than the rods, the light intensities required to produce the cone responses in Fig. 3B were of the order of 90 times brighter than those used to stimulate the rods in Fig. 3A. The mean cone response had a much more rapid rate of activation and time to peak, probably in part the result of the brighter stimulus intensity. The decay of the cone response in Fig. 6 was fitted to a single exponential decay function with a best-fitting time constant of decay  $\tau_{\text{REC}}$  of 71 ms. Values of  $\tau_{\text{REC}}$  were also obtained by fitting the decay phases of small-amplitude responses cone by cone and gave a mean value of  $54 \pm 4 \text{ ms}$  (SEM,  $n = 8$ ). A similar fit to the rod response in Fig. 6 gave a value for  $\tau_{\text{REC}}$  of 793 ms, and cell-by-cell fits to small amplitude responses gave a mean value of  $\tau_{\text{REC}}$  of  $841 \pm 59 \text{ ms}$  ( $n = 12$ ).

### **Single-Photon Responses in Juvenile Lamprey Rods**

To determine whether juvenile lamprey rods respond to single photons of light, a series of dim flashes was given, and from these, the mean single-photon response was calculated from the squared mean and variance. Briefly, the form and amplitude of the single photon response was obtained by comparing the squared mean and the time-dependent ensemble variance of a series



of responses to dim flashes of fixed intensity (Baylor 1979a). The mean linear response to at least 50 dim flashes was squared and scaled so that its rising phase coincided with that of the time-dependent variance (Baylor et al. 1979a). The scaling factor for the squared mean response is  $1/n$ , where  $n$  is the mean number of photoisomerizations per trial. The mean response, divided by  $n$ , gave the estimated form and amplitude of the single photon response. The waveform of the response was divided cell by cell by the maximum current response to a bright flash, in order to give the fraction of channels closed as a function of time. These results are shown for lamprey rods in Fig. 7. In this cell type, 4%–5% of the channels of the outer segment are closed by a single photon. Although the peak response in lamprey is somewhat smaller than in mouse (Morshedian & Fain 2016), it is larger than in salamander (for example, Chichilnisky & Rieke 2005) and is well within the range of single photon response amplitudes of other vertebrate rods. Lamprey single-photon responses rise to a peak and decay more slowly than mouse responses, but the slower kinetics are probably mostly the result of the difference in the temperature of the recording (22°C versus 39°C).

### **Spectral Sensitivity of Photoreceptors Using Suction-Electrode Recording**

Spectral sensitivities of the short and long (rod and cone) outer segment classes of photoreceptor in both juvenile and adult lamprey gave complete spectral sensitivity curves. The relation between the peak amplitude of the response to a flash and the flash strength was determined for a test wave length and reference wave-length (500 nm). The difference in the positions of the two curves on the log abscissa gave the sensitivity at the test wave-length relative to that at 500 nm. Similar determinations were made with test wave-lengths in the range 460 to 640 nm. Final

spectra were shifted vertically so that the mean sensitivities (sum of the log sensitivities across wave-length) were the same for all curves of a given type. After normalization the average sensitivities and standard deviations were calculated at each wave-length. Averaged normalized spectra for short and long photoreceptors are plotted as the red squares in Figs. 4 and 5. The averaged, zero-line corrected spectrum from each individual was analyzed by fitting visual-pigment templates or nomograms of (Govardovskii et al. 2000), are designated a smooth blue curves. The wavelength of maximum absorbance is 504 and 551 for juvenile rods and cones, and 522 and 592 for adult rods and cones.

## **DISCUSSION**

### **The physiological responses of juvenile lamprey photoreceptors are similar to those of adults**

Juvenile lamprey are a small fraction of the size of adults, with eyes only about 20% as large in diameter. The physiology of juvenile lamprey photoreceptors had not been previously investigated. To examine the physiological response profiles of juvenile lamprey photoreceptors, suction-electrode recording was utilized to measure the responses of single photoreceptors. Responses of juvenile photoreceptors were nearly identical to those of adults (Crescitelli 1956) (Figs. 3A & 3B). When the peak amplitudes of the light responses for the two photoreceptor types as a function of stimulus intensity were plotted (Fig. 3C), juvenile rods were ~85-fold more sensitive than cones, comparable to the value obtained for adults (Morshedian & Fain 2015). Further analysis showed that juvenile rod responses decayed more slowly than cone responses (Fig. 6), and single photons produce a response of about 0.4 pA (Fig. 7), again similar to adult lamprey photoreceptors (Crescitelli 1956, Asteriti et al. 2015). Thus, the physiological properties of juvenile rods and cones showed no significant differences from those of their adult counterparts.

### **The spectral sensitivity of the rods and cones of adult migrant lamprey are red-shifted relative to those of the juvenile**

To determine whether the variance in retinoid content between juvenile and adult lampreys are

mirrored in the spectral sensitivity profiles of individual rods and cones, recordings of the light responses of photoreceptors across a range of wavelengths were performed. The results of these measurements are presented for juvenile rods and cones (red squares, Fig. 4) and for adult rods and cones (red squares, Fig. 5). In separate experiments of a recent collaboration (Morshedian et al. 2017), microspectrophotometry (MSP) was used to measure the mean normalized pigment absorbance of both juvenile and adult rods and cones. Data were superimposed on top of the spectral sensitivities (black points in Figs. 4 & 5). We found that juvenile rods have a peak sensitivity and absorbance ( $\lambda_{\max}$ ) at 504 nm, while adult rods have their  $\lambda_{\max}$  at 522 nm (compare Figs. 4A and 5A). In contrast, juvenile cones have  $\lambda_{\max} = 551$  nm, whereas adult cones have  $\lambda_{\max} = 592$  nm (compare Figs. 4B and 5B). Thus, the spectral sensitivity of adult rods and cones are red-shifted relative to those of the juvenile.

This study offers the first electrophysiologic account of the rod and cone photoreceptors of juvenile lamprey and indicates that this developmental stage possesses receptors that are physiologically comparable to those of adult lamprey (Crescitelli 1956, Asteriti et al. 2015). The spectral sensitivities of photoreceptors from upstream migrating adults were however notably red-shifted compared to the downstream juveniles. Consistent with foundational studies of lamprey visual pigments (Crescitelli 1956, Wald 1957), a recent collaboration (Morshedian et al. 2017) found that this red-shift is attributable to a switch in the visual pigment chromophore from 11-cis retinal (A<sub>1</sub>) to 11-cis 3,4-didehydroretinal (A<sub>2</sub>). Concomitant with the vitamin A<sub>1</sub>-to-A<sub>2</sub> shift, there was an observed increase in the expression of CYP27C1 in the retinal pigment epithelium of adult lamprey compared to juveniles. Taken together, electrophysiological and molecular biological data suggest that CYP27C1-mediated “chromophore switching” is an

evolutionarily primordial mechanism of sensory plasticity.

Vertebrates evolved in the ocean and subsequently colonized freshwater habitats (Halstead 1985). The spectral composition of light in fresh inland waterways, especially rivers, streams, and actuarios is red-shifted due to the existence of suspended sediments and dissolved organic matter in the water. This resulting turbidity could have posed a significant challenge to the visual systems of early vertebrates moving to colonize new niches. A strong possible conclusion of my work, supported by our lab's collaboration in Morshedian et al. (2017), is that “the CYP27C1-mediated vitamin A<sub>1</sub>-to-A<sub>2</sub> switch in the eyes of early vertebrates may have been among the suite of adaptations that facilitated the early invasion of brackish and freshwater habitats”.

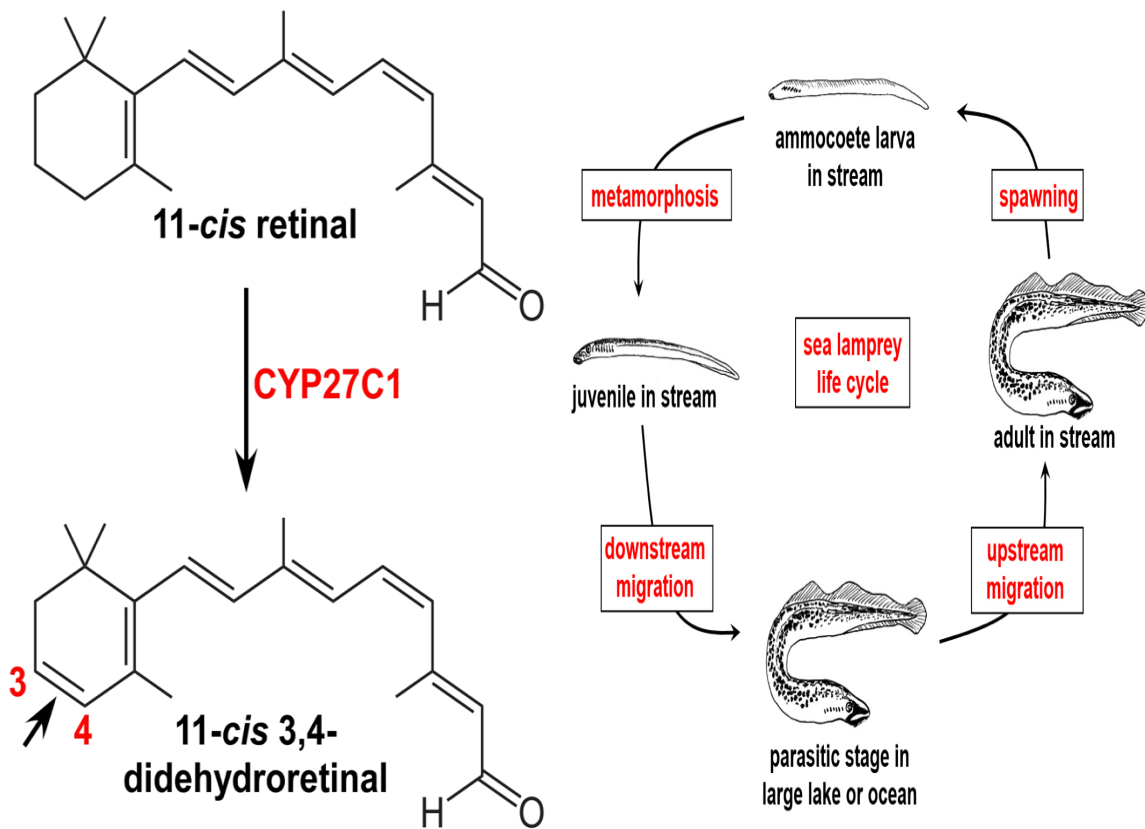


Figure 1. The eyes of adult upstream migrant lamprey are enriched with vitamin A<sub>2</sub> chromophore. (A), In teleost fish and amphibians, CYP27C1 mediates the conversion of 11-cis retinal (a derivative vitamin A<sub>1</sub>) to 11-cis 3,4-didehydroretinal (a derivative of vitamin A<sub>2</sub>) to red-shift photoreceptor spectral sensitivities. (B), The sea lamprey moves between streams and large lakes/ocean through the course of its life cycle.

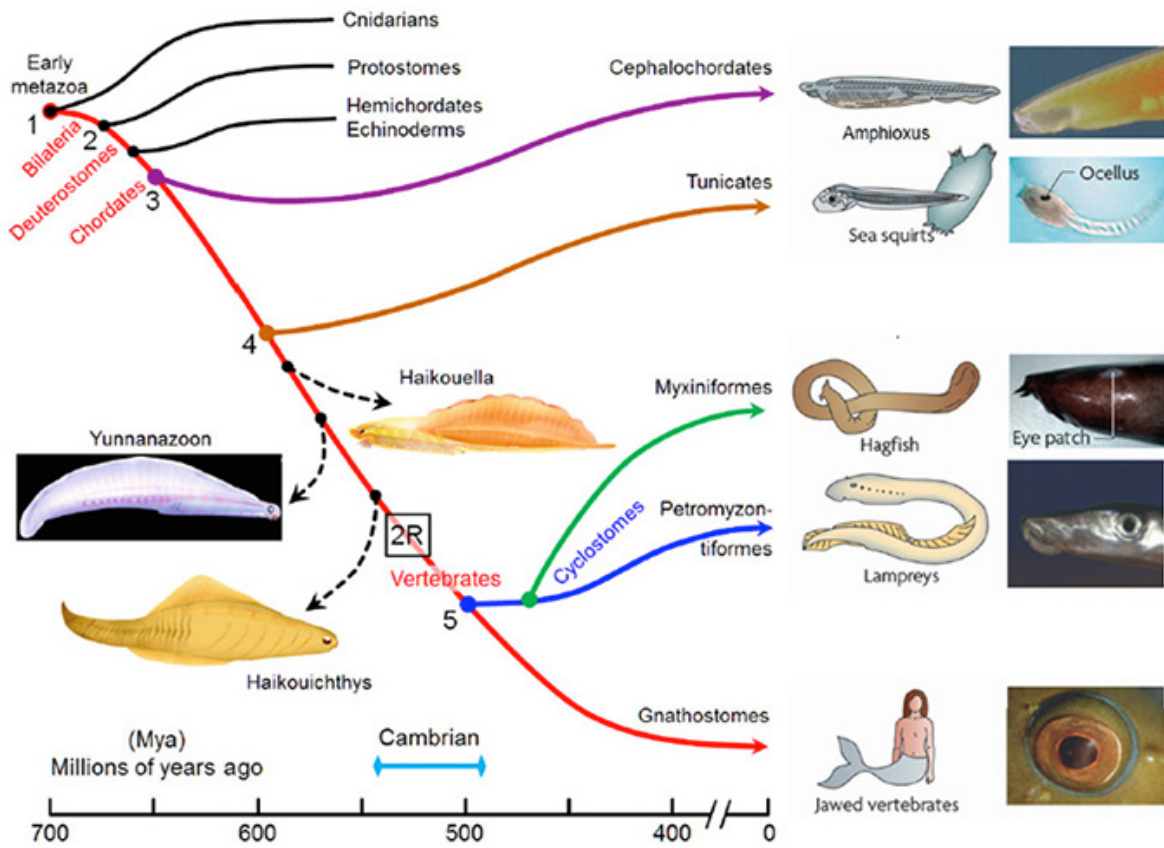


Figure 2. The origin of vertebrates, over a time-scale from roughly 700 to 400 million years ago (Mya), with timings of the branchings taken from a reconciliation of fossil and molecular data by Erwin et al (2011). The red curve indicates our direct ancestors, beginning with early metazoans, and the numbers along the curve denote major branchings that are significant to the evolution of the vertebrate eye. By the time that the ancestors of lampreys diverged around 500 Mya, they and our own ancestors were vertebrates. Subsequently (by around 420 Mya) our own ancestors evolved jaws and hence became jawed vertebrates (gnathostomes). Source of illustrations: Haikouella, Yunnanazoon and Haikouichthys from Chen (2012); remainder from Lamb et al (2007), where original sources are given.

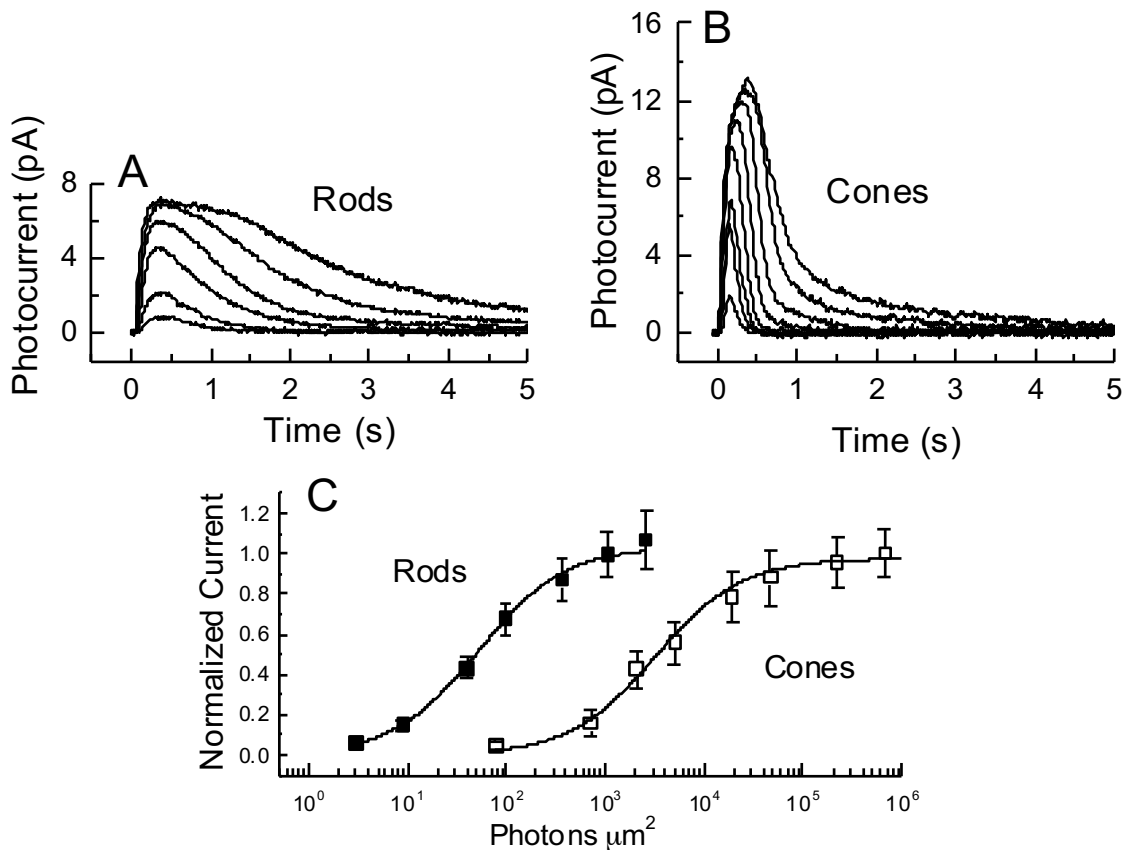


Figure 3. Current Responses and Sensitivity of Juvenile Lamprey Rod and Cone Photoreceptors to Brief Light Stimuli

For both photoreceptor types, response amplitude and duration increased with increasing stimulus intensity. (A) Mean responses of 13 rods to 20-ms 500-nm flashes given at  $t = 0$  at the following intensities (in photons  $\mu\text{m}^{-2}$ ): 9, 40, 98, 366, 1055, and 2591. (B) Mean responses of 6 cones to 20-ms 600-nm flashes given at  $t = 0$  at the following intensities (in photons  $\mu\text{m}^{-2}$ ): 78, 711, 2,051, 5036,  $1.91 \times 10^4$ ,  $2.20 \times 10^5$ ,  $6.71 \times 10^5$ ,  $6.96 \times 10^5$ . (C) Current response amplitudes were plotted against flash intensities for rods (closed squares) and cones (open squares). Error bars represent SEMs. Cells are the same as in A & B. Data were fitted with  $\frac{r}{r_{max}} = \frac{I}{I + I_{1/2}}$  with best-fitting values of  $I_{1/2}$  of 52 photons  $\mu\text{m}^{-2}$  for rods and 3210 photons  $\mu\text{m}^{-2}$  for cones.



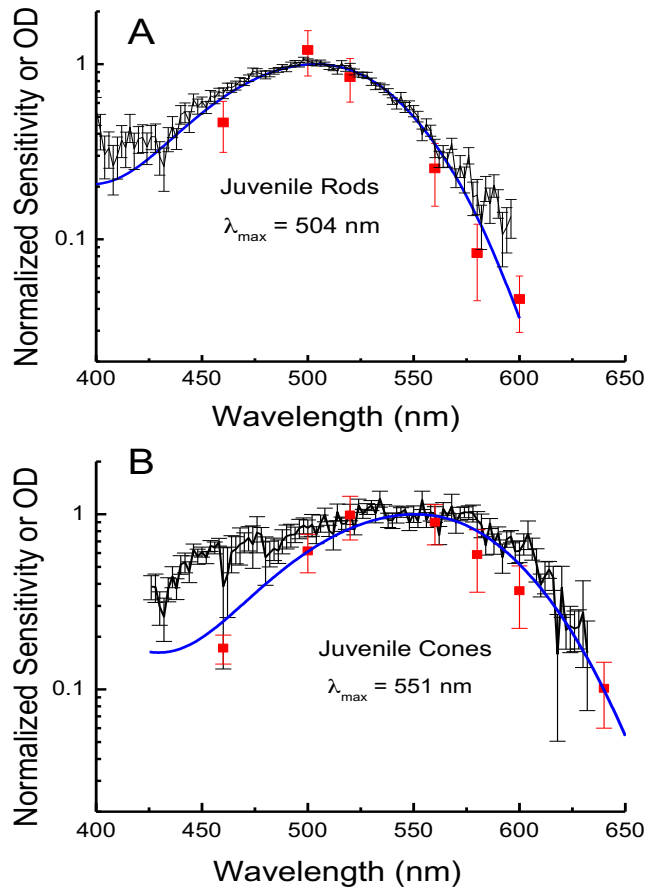


Figure 4. Superimposed Spectrum and Spectral Sensitivity of Juvenile Lamprey

Absorbance spectra of photoreceptors in the eye of juvenile lamprey were determined using microspectrophotometry (black data points). Optical densities were normalized and plotted as a function of wavelength for 58 rods (A) and 9 cones (B). Normalized Spectral sensitivity was also calculated using suction-electrode-recording by measuring flash sensitivities at different wavelengths for 9 rods (A) and 7 cones (B) (red boxes). The data was fitted with appropriate  $A_1$  visual pigment templates (Govardovskii et al., 2000) (blue curve).

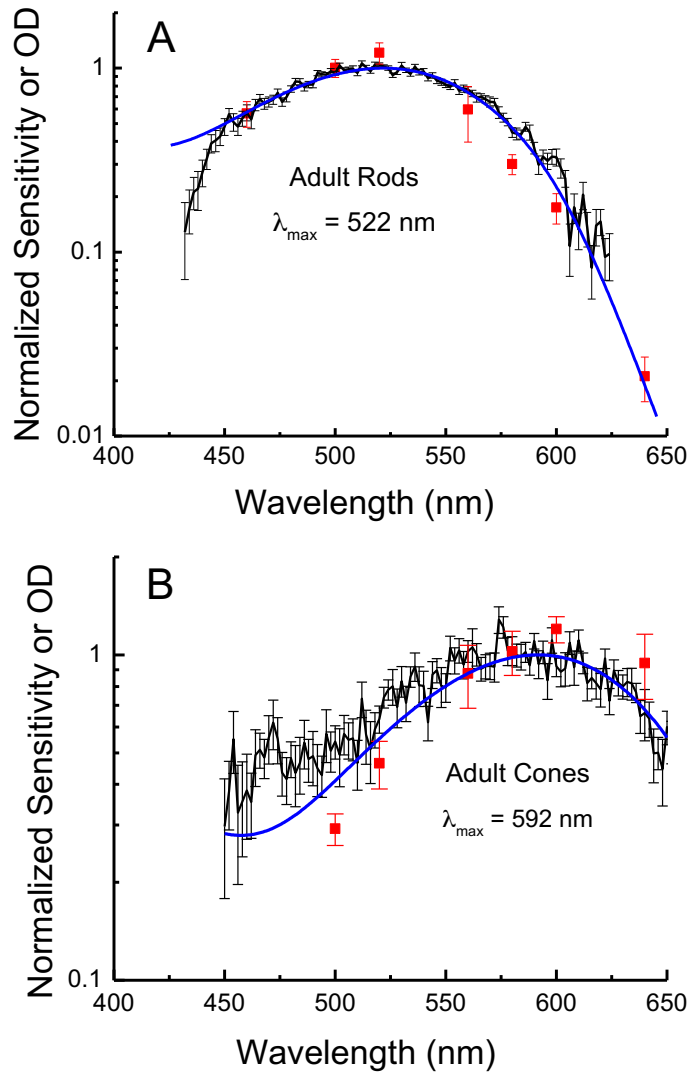


Figure 5. Superimposed Spectrum and Spectral Sensitivity of Adult Lamprey Photoreceptors

Normalized Spectral sensitivity was calculated with suction-electrode-recording by measuring flash sensitivities at different wavelengths for 13 rods (A) and 14 cones (B) (red squares). Absorbance spectra of photoreceptors in the eye of adult lamprey were determined with microspectrophotometry (courtesy of Dr. Frederiksen (black data points)). Optical densities were normalized and plotted as a function of wavelength for 70 rods (A) and 23 cones (B). The data was fitted with appropriate  $A_2$  visual pigment templates (Govardovskii et al., 2000) (blue curve).

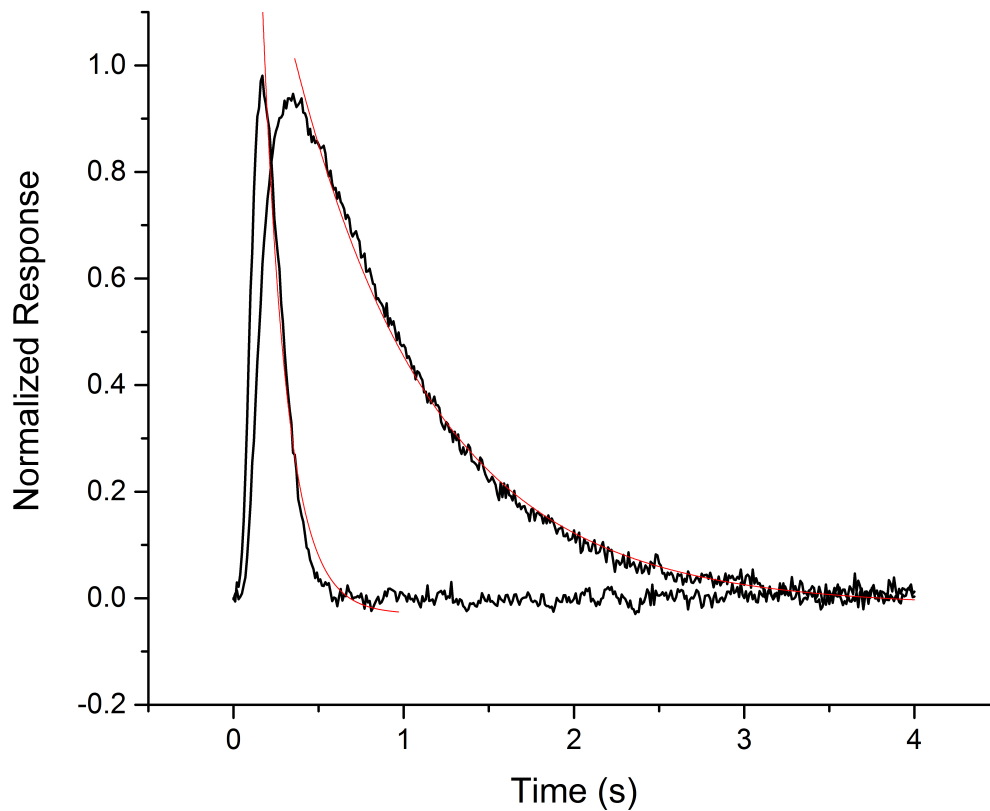


Figure 6. Response Waveforms of Rod and Cone Photoreceptors in Juvenile Lamprey

Normalized mean light response of 13 rods and 6 cones to 20-ms flashes that produced an approximately half-maximal response. Flash intensities were  $98 \text{ photons } \mu\text{m}^{-2}$  for rods and  $2051 \text{ photons } \mu\text{m}^{-2}$  for cones. The decay phases of both responses were fitted with a single exponential decay function with a time constant of recovery ( $\tau_{\text{REC}}$ ) of 564 ms for rods and 97 ms for cones.

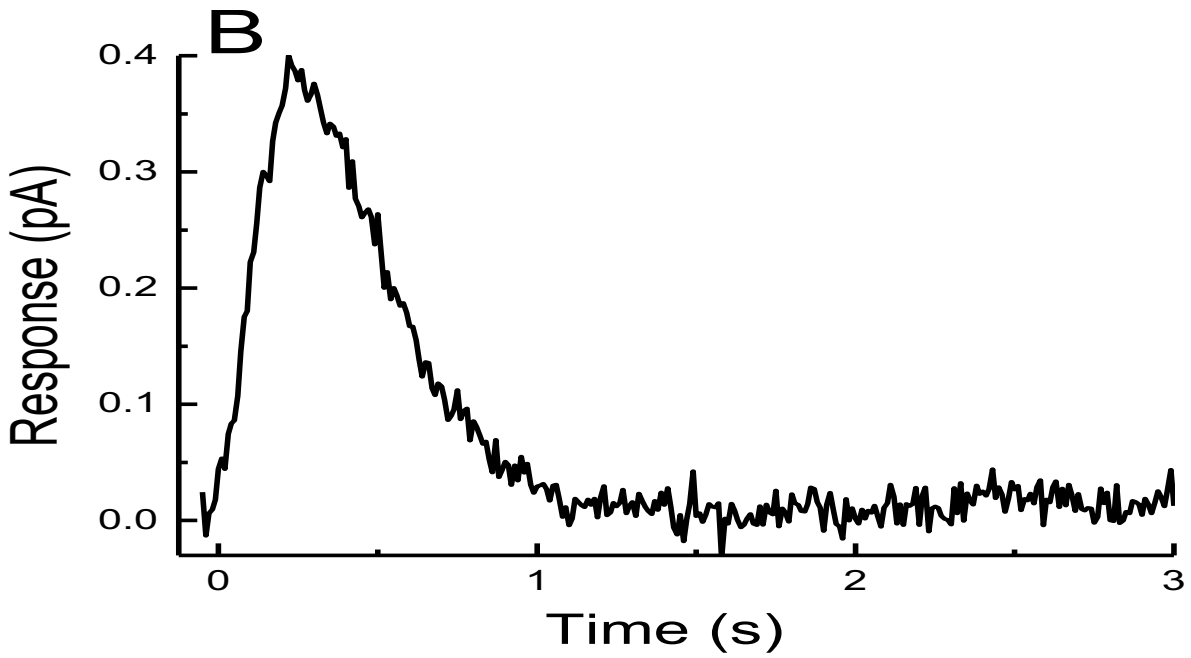


Figure 7. Single-Photon Recording of Rod in Juvenile Lamprey

Single-photon responses of juvenile rods were calculated from the squared mean and variance for 9 lamprey rods, normalized rod by rod to circulating current, and averaged to give the mean fractional closure of channels as a function of time (see text).

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