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Photoreception in pineal organs of larval and adult lampreys, *Lampetra japonica*

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Summary. A comparative study of the larval and adult pineal organs, which are sensitive to incident light, was carried out in the river lamprey *Lampetra japonica*, using intracellular recording from the pineal photoreceptors.

1. The tissue overlying the larval pineal organ is transparent, whereas that over the adult pineal is translucent. The optical density of this oval pineal window in the adult lamprey was 1.2.

2. In order to elucidate the early development of the larval pineal, the ratio r of the diameter (μm) of the pineal to the body-length (cm) was measured. The value of r was 62.5 in a small larva of 2.8 cm, 29.7 in a larger one of 14.3 cm, and 9.3 in an adult of 54 cm body-length.

3. The intracellular response to light of the larval pineal was a hyperpolarization, showing fundamentally the same pattern as that of the adult pineal. It was possible to record a typical response even from the pineal of the smallest larva, 2.8 cm in body length, used in this study.

4. The intensity-amplitude relationship was analysed after Naka-Rushton's hyperbolic equation. The value of σ of isolated larval pineals was 0.88 log unit higher than that of adults. The value of n was larger in larvae, suggesting a sensitive reaction to changing photic stimulus.

5. The spectral sensitivity was compared. The peak was at 505 nm in the larva, but 525 nm in the adult. A change of visual pigment in the pineal during metamorphosis is suggested.

Introduction

Morphological studies have indicated the presence of photosensory structures in the lamprey pineal (Collin 1969, 1971; Meiniel 1980; Pu and Dowling 1981; Cole and Youson 1982) and electrophysiological investigations have demonstrated the direct photosensitivity of the pineal in several species of lamprey (Morita and Dodt 1971, 1973; Morita 1975; Pu and Dowling 1981; Morita et al. 1985). The electrical response of the pineal to light consists of a hyperpolarization of photoreceptors and suppression of spontaneous spike discharges from ganglion cells. The chromatic type of response from ganglion cells suggests wavelength discrimination of photic components in incident light.

One of the characteristic features of larval lampreys is the absence of lateral eyes. Although vestigial eye bulbs are buried under the skin in the last larval stage, these undeveloped eyes are apparently not used as an effective photoreceptive organ. The pineal, on the other hand, is well-developed under the transparent covering tissue. The pineal organ in ammocoetes is important for the diurnal rhythm of body color change (Joss 1973) and for metamorphosis (Eddy 1969; Cole and Youson 1981).

In this report pineal photoreception in larval and adult lamprey is compared, using morphological and electrophysiological analysis. It became obvious that the pineal vesicle develops in size remarkably early compared to the body-length, and that the direct photosensitivity of the larval pineal is fundamentally similar to that of adults, but differs in the threshold and in the spectral sensitivity.

Materials and methods

Anadromous river lampreys, *Lampetra japonica*, captured in the Shiribetsu and Ishikari rivers in Hokkaido, were used in these experiments. Larvae were kept in an aquarium with a 5-cm sand layer, at a water temperature of 15 °–20 °C. Adult lampreys were kept in tanks filled with aerated and filtered water at ca. 10 °C. Both groups of animals were maintained

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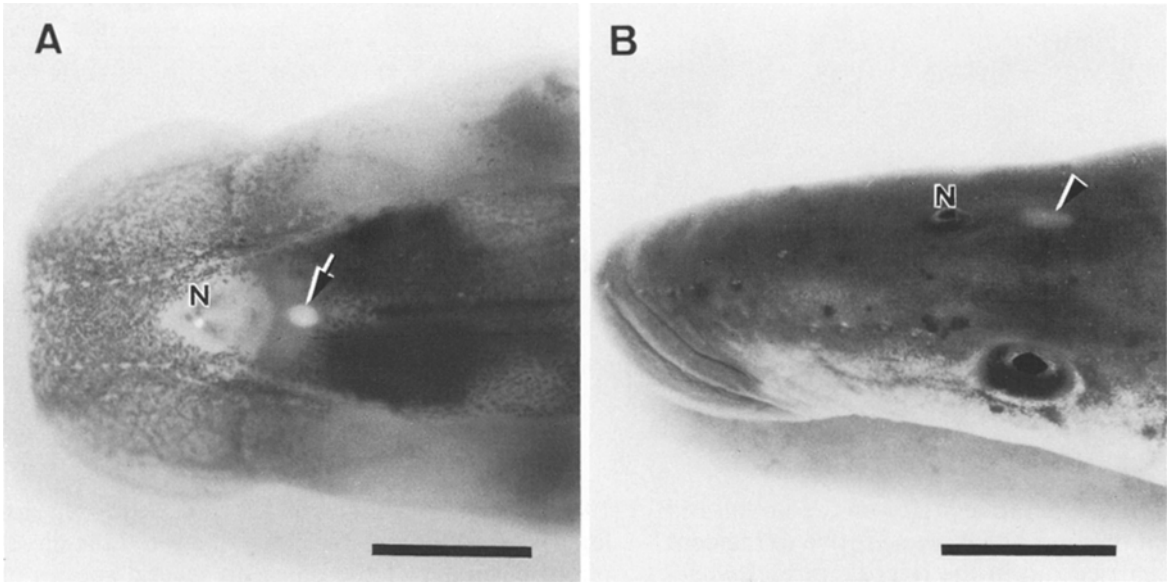

Fig. 1 A, B. Heads of larval and adult lamprey *Lampetra japonica*. **A** Larva. The pineal complex (arrow) is situated behind the nostril, *N*. The tissue covering the pineal is transparent. Eyes have not yet appeared. Body-length, 6 cm; calibration, 2 mm. **B** Adult. The pineal window (arrow-head) is translucent and free of pigmentation. The optical density is ca. 1.2. Body-length, 50 cm; calibration, 20 mm

under natural photoperiod. Ammocoetes were fed with compound powder food for fish culture. Adult lampreys during spawning migration do not take any food.

The experiments were carried out in July and September for larvae and between December and March for adults. After 30 min dark-adaptation the animals were decapitated under dim red light and pineals were isolated and placed dorsal side upward onto filter papers moistened with physiological saline. After 10–15 min in the dark, a glass capillary microelectrode filled with 4 mol/l potassium acetate was inserted into the pineal photoreceptor with a hydraulic micromanipulator (Narishige MO-102R). The electrodes had a resistance of 80–120 MΩ measured in physiological saline. An Ag-AgCl reference electrode was kept in contact with the moist filter paper. Intracellular responses were displayed on an oscilloscope (Tektronix D13) through a conventional capacity-compensating preamplifier. Data were recorded with a pen-recorder and stored on magnetic tape (Sony-Magnescale DFR-3515) simultaneously.

A stimulating light was delivered from a 300-W Xenon arc lamp. Its intensity was regulated with neutral density filters. The maximum intensity (ND = 0) available at the recording chamber was 3.5 mW/cm^2. Monochromatic stimuli were provided by interference filters (Nihon Shinku Kogaku, Type W). All of the monochromatic test light was equal-quantum calibrated to evaluate the relative spectral sensitivity. For the photometry, a vacuum thermopile (Pyro-Werk, Hannover) was applied for white light and RM-101 radiometer (Tokyo-Kohdenshi-Kogyo) for monochromatic light. Duration of light stimuli was 100 ms in all experiments.

The spectral sensitivity curve was calculated from the intensity necessary to elicit the same amplitude of response to all wavelengths. The criterion response was chosen in the linear range of the intensity-amplitude curve, being either 6 or 8 mV.

The optical density of the pineal window, the tissue covering the pineal organ, was determined with the SEI spot-photometer (Salford Electrical Inst.). Tracing paper was used as a diffuser in the control measurement.

Results

The pineal complex of the lamprey, consisting of the pineal and the parapineal organs, is situated on the dorsal surface of the diencephalon and beneath a nonpigmented region of integument and connective tissue. The part of this tissue covering the pineal is transparent in larvae and translucent in adults (Fig. 1). The optical density of this region was about 1.2 in adults. Under a stereo-microscope the isolated pineal organ is seen as a creamy-white flattened oval vesicle.

Figure 2 shows the correlation between the diameter of the pineal in major axis and the body-length of larvae. The mean values from 10 adults are plotted on the right side. The body-length of the smallest ammocoete was 2.8 cm and the diameter of its pineal organ was 175 μm. These values in adults were 54 cm and 500 μm, respectively. The pineal of the larva of 14 cm in body-length was already as well-developed in size as that of adults. The ratio, *r*, between the pineal diameter (μm) and the body-length (cm) (plotted with open circles in the figure) is 62.5 in the smallest larva of 2.8 cm, 29.7 in the larger larvae of 14.3 cm and 9.3 in adults of 54 cm.

Typical responses of a dark-adapted pineal of a larval lamprey are shown in Fig. 3. Photoreceptors responded to light by a hyperpolarization. With increasing light intensity the latency becomes shorter, the amplitude increases and the duration

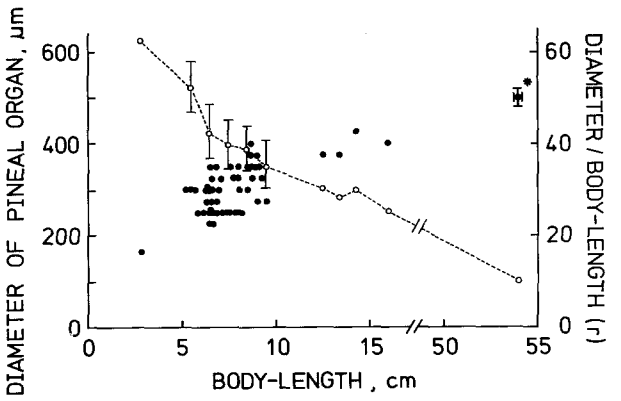

Fig. 2. Pineal diameter (filled circles) versus body-length of larval lampreys. Right ordinate, ratio (r) of pineal diameter/body-length (open circles with SD and broken line). The mean of 10 adults (*) is also shown

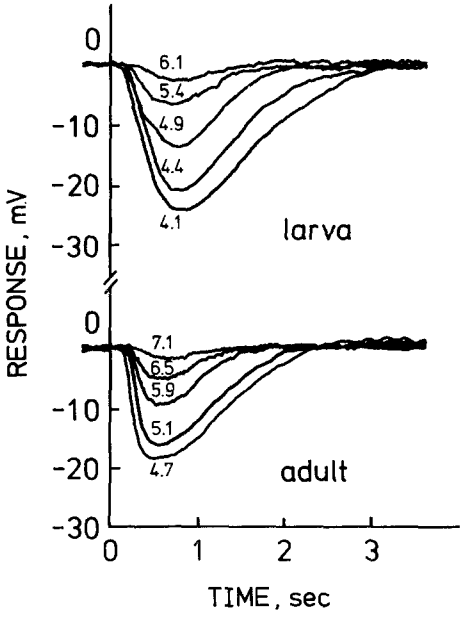

Fig. 3. Typical responses from single photoreceptors of a larva and an adult. Membrane potentials are always hyperpolarized by light stimuli. White light square pulse of 0.1 s was applied at 0 of abscissa. Numbers on the records are densities of ND filters. Responses of the larva and the adult saturated with stimuli of ND 4.1 and ND 4.7, respectively

becomes longer. In these cells responses saturated with stimuli of ND 4.1 in the larva and of ND 4.7 in the adult. The larval responses have a slower rise time to peak and longer duration than that of the adult animal in the case shown in Fig. 3. However the measurement of rise time and duration in 8 larvae and 6 adults revealed no statistically significant difference between the two groups.

The intracellular photoresponse of 20 mV recorded from the pineal of the smallest larva suggests that it is already functioning at this stage.

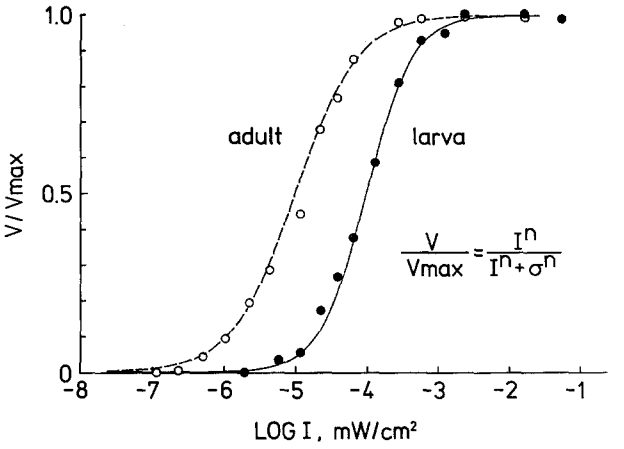

Fig. 4. Intensity-amplitude relationship of single photoreceptors from a larva (filled circles) and an adult (open circles). The ratio of responses to the maximum amplitude is plotted as a function of light stimulus intensity. Lines are calculated from Naka-Rushton's hyperbolic equation. Each measurement is fitted to the theoretical curve when σ is -4.0 log unit for the larva, -5.0 log unit for the adult, and n is 1.35 for the former, 1.0 for the latter

The intensity-amplitude relationship of the pineal photoresponse is plotted for a larva and for an adult in Fig. 4. Each line was calculated using Naka-Rushton's hyperbolic function $V/V_{max}=I^n/(I^n+\sigma^n)$, where V is the peak amplitude of the light-evoked response at intensity I, and V_{max} is the peak amplitude at the saturation of the response (Naka and Rushton 1966). σ is the light intensity required to produce half V_{max} and was -4.0 log unit for the larva, -5.0 log unit for the adult. The best fitted value for n was 1.35 for the larva and 1.0 for the adult. Some points deviated slightly from the theoretical curve.

In order to compare the response ranges of larvae and adults, the fitted theoretical curves for each individual set of measurements are plotted together, with the curves centered at the appropriate mean of σ (Fig. 5). Values of n and σ in each group are as follows:

n: 1.25 ± 0.26 (larvae), 0.95 ± 0.18 (adults);
σ: -3.95 ± 0.38 (larvae), -4.83 ± 0.48 (adults).

In adults σ is 0.88 log units, and n 0.3, less than in larvae. Statistical analysis revealed a significant difference in n between larva and adult ($P<0.01$). The thresholds for the just observable response were measured in both groups. They were 2.8×10^{-6} mW/cm^2 for larvae and 1.3×10^{-7} mW/cm^2 for adults.

The spectral sensitivities of the pineal photoreceptor cells are plotted in Fig. 6. The curves drawn

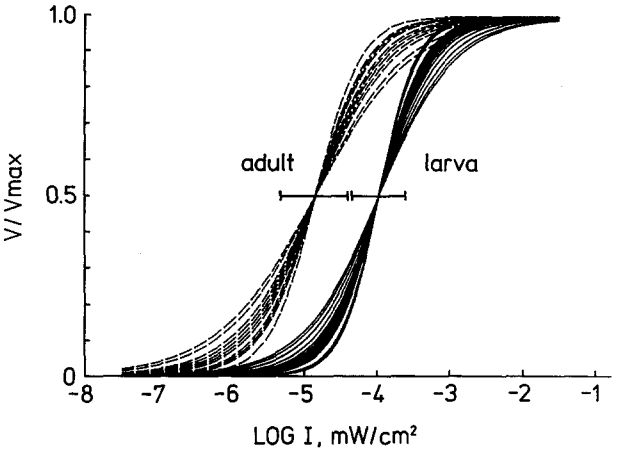

Fig. 5. Comparison of V-log I curves of larval and adult photoreceptors. The individual theoretical curves calculated from Naka-Rushton's equation which best fit experimental data are shifted horizontally to the mean of σ. The mean of σ is -3.95 ± 0.38 (SD) for larvae, -4.83 ± 0.48 for adults, and n is 1.25 ± 0.26 for the former, 0.95 ± 0.18 for the latter

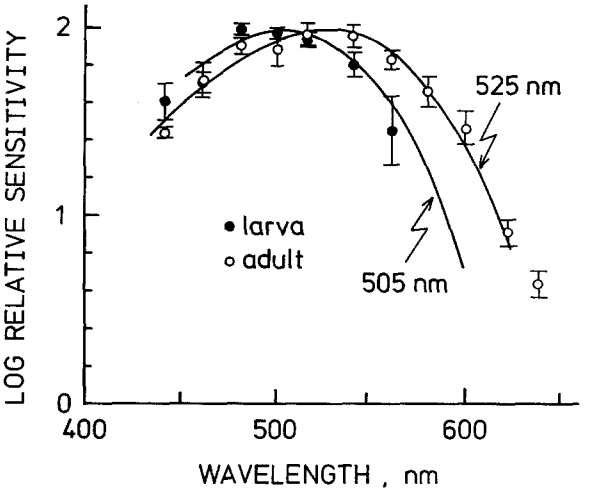

Fig. 6. Spectral sensitivities of larval and adult photoreceptors. Filled circles are means in larvae (8 cells) and open circles are from adults (10 cells). Data was shifted vertically to be compared at the same level. Vertical bars are SD. The curves drawn through the data points are Dartnall's nomograms centered at 505 and 525 nm (Dartnall 1953)

through the data points are Dartnall's nomograms (1953) centered at 505 nm and 525 nm, for larvae and adults respectively. Thus the adult spectral sensitivity curve is shifted about 20 nm toward longer wavelengths compared with larvae. Additionally, there is a slight shoulder in the 480-nm region of the spectrum in both adults and larvae.

Discussion

The role of the pineal organ and its relationship to photoreception have been investigated in the larvae of some species of lower vertebrate. The body skin of intact or eyeless larvae of amphibians becomes pale when they are placed in darkness. This body-lightening reaction is abolished by pinealectomy (Bagnara 1960; Charlton 1966). Ultrastructural (Ekström et al. 1983) and immunological (Veen et al. 1984) studies of the developing photoreceptors of the three-spined stickleback *Gasterosteus aculeatus* demonstrated that photoreceptor outer segments and visual pigments were found earlier in the pineal organ than in the retina of lateral eyes.

Larval lampreys display a diurnal rhythm of color change, which decreases in intensity as the larvae grow older. This color change rhythm is abolished in larvae by pinealectomy or hypophysectomy, and in adults either by removal of lateral eyes or hypophysectomy (Joss 1973). The lamprey metamorphoses after a larval period of several years, and pinealectomy prevents metamorphosis (Eddy 1969; Cole and Youson 1981). The pineal of lamprey must have important roles during the larval stage. This is also supported by the following observations: (a) Ammocoetes have no lateral eyes, whereas the pineal organ is already well developed (Fig. 1); (b) the ratio of the diameter of the pineal and the body-length was considerably higher in larvae than in adults (Fig. 2); (c) it was possible to record a receptor potential from the pineal photoreceptor cells even in the smallest larva (2.8 cm) used in this experiment.

The characteristics of the larval pineal photoreceptor are compared with that of adults, applying the Naka-Rushton's hyperbolic function. The mean value of σ for adults is 0.88 log units of intensity lower than in larvae, thus the adult pineal is more sensitive than the larval one. The threshold of photoreceptors in adult lampreys was 1.3 log units lower than in larvae. However, this threshold difference does not necessarily mean that larval pineals are less sensitive in intact animals under natural conditions. As described before, the tissue overlying the pineal is transparent in larvae, whereas the pineal window of adults is translucent (optical density 1.2) and diffuses the incident light. The difference of their actual response range is therefore not so large.

Statistical analysis indicated that the mean of n in adult pineal photoreceptors was lower than in larvae. This means that the same change in intensity of incident light affects the larval pineal more effectively than in adults. The pineal of larvae could therefore be more suitable for the shadow response. The actual role of the larval pineal in behavior is so far open for further investigation.

Spectral sensitivity measurements showed that the peak sensitivity of adult photoreceptors is shifted 20 nm toward longer wavelengths (Fig. 6). This suggests that their visual pigment is different from that of larvae.

The spectral sensitivity of the pineal photoreceptor of the larval sea lamprey *Petromyzon marinus* peaks between 540 and 560 nm (Pu and Dowling 1981). This differs from our result for the larva of *Lampetra japonica*, perhaps due to the species difference.

A small shoulder appeared in the 480 nm region of the spectrum (Fig. 6). Furthermore, a small difference between the theoretical curve of Naka-Rushton's equation and the experimental data was observed in Fig. 4. Since the equation originally holds for a system with a single visual pigment, these deviations might be due to a mixture of visual pigments. This hypothesis is supported by an analysis of the chromophore with high-pressure liquid chromatography in the same species of lamprey (Tamotsu and Morita in preparation).

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