

Ambystomatid salamanders can see ultraviolet (UV) light: evidence for UV photo-receptors in the eyes of axolotls

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The presence of ultraviolet (UV) retinal photoreceptors has been demonstrated in all the major classes of jawed vertebrates; however, there have been relatively few studies investigating the UV visual capabilities of amphibians (for review see Goldsmith 1994, Jacobs 1992). To date, only four physiological experiments have investigated UV photoreception in

the amphibian retina. One study on the frog *Rana temporaria* suggests that at least this species of frog does not possess UV photoreceptors (Govardovskii and Zueva 1974). In contrast, two experiments have provided evidence for the presence of UV photoreceptors in the retina of larval tiger salamanders *Ambystoma tigrinum* (Perry and MacNaughton 1991, Harosi 1994). In addition, we have recently shown that a population of UV photoreceptors is also present in the retina of the neotenic mexican salamander *Ambystoma mexicanum* (Deutschlander and Phillips 1995). In this article we have presented a summary of our experiments as well a discussion of their implications for research concerning the visual system of urodeles. We have attempted to present a simplified version of our techniques so that those unfamiliar with visual physiology can gain an understanding of the experiments.

Electroretinogram (ERG) Recording Apparatus

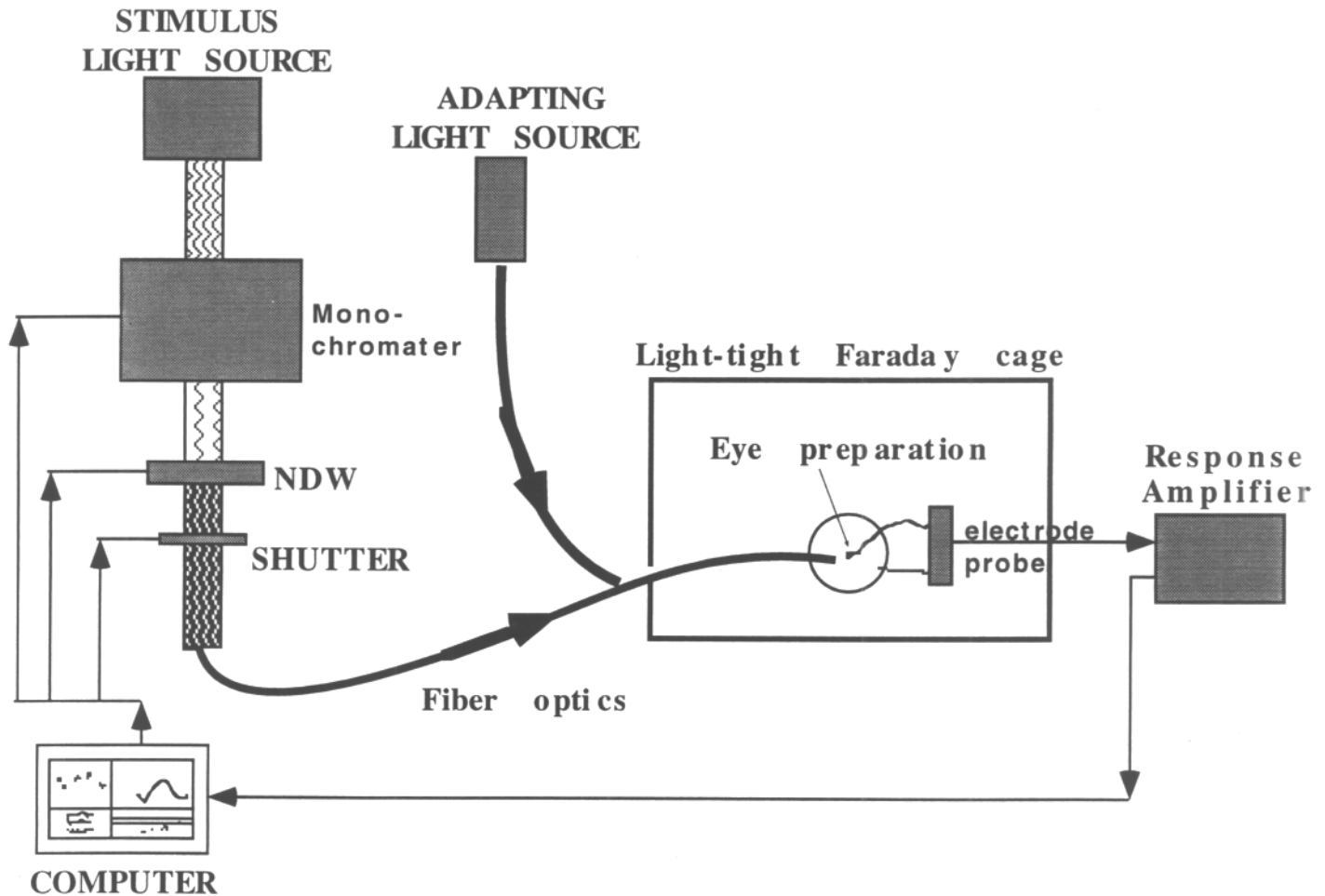


Figure 1. Schematic of the apparatus used to record ERGs (see text for description).

In order to determine if UV photoreceptors were present in the eyes of axolotls, we measured a response from the eye known as the electroretinogram (ERG). An ERG is a measure of the voltage change due to the mass response of the whole eye to a light stimulus (Goldsmith 1986). The response of the photoreceptors as well as any second order cells subsequently excited by the photoreceptors contribute to the ERG waveform. ERGs are commonly used to determine the sensitivity of the eyes of animals to various colors across the visual spectrum (known as the spectral sensitivity of the eye). By comparing the ERG response of the eye to various narrow-bandwidth light stimuli (i.e., to specific colors of light), the relative sensitivity of the eye to different colors of light can be determined. In some cases, the contribution of a single class of photoreceptor to the ERG waveform can be determined by using an adapting, or background, light of sufficient color to "adapt out" the response of other classes of photoreceptors. This technique is known as selective chromatic adaptation, an example of which will be presented below.

A schematic of the apparatus used to measure ERGs is shown in Figure 1. A stimulus light of narrow bandwidth is created by passing a light through a monochromator. A monochromator uses a diffraction grating, analogous to a prism, to "break white light up" into its spectral components. Then the light is passed out a small opening (e.g., 10 nm bandwidth) so that only a narrow band of the spectrum is contained in the resulting beam of light. The intensity of the stimulus can be adjusted by passing the light through a neutral density wedge (NDW), which attenuates the light. The light is then passed through a fiber optic to the eye preparation, which is contained in a light-sealed Faraday cage, to provide electrical shielding and keep stray light from reaching the eye. The eye can be exposed to a background adapting light via a fiber optic as well. An ambient, full-spectrum (i.e., white) adapting light can be used to determine the sensitivity of the eye under daylight, or "photopic," conditions (where the ERG response is due primarily to cone photoreceptors). A colored-adapting light can also be used to selectively adapt specific classes of photoreceptors so that they no longer contribute to the ERG response to the stimulus light (e.g., a red light would primarily adapt the response of long-wavelength photoreceptors).

ERGs can be recorded from anaesthetized

live animals, or, in cold-blooded animals, the whole eye can be easily removed from a sacrificed animal in order to record from it. When removing the whole eye the cornea and lens can be removed so that recordings can be made directly from the surface of the retina (known as the open eye-cup technique) yielding a much stronger, less noisy recording. To record an electroretinogram from an open eye cup a wire electrode is placed on the surface of the retina and a reference electrode is placed in a Ringer's bath behind the eye. The electrode output is amplified and displayed for analysis on a computer using appropriate software (Fig. 1). A more detailed description of the materials, methods, and data analysis can be found in Deutschlander and Phillips (1995).

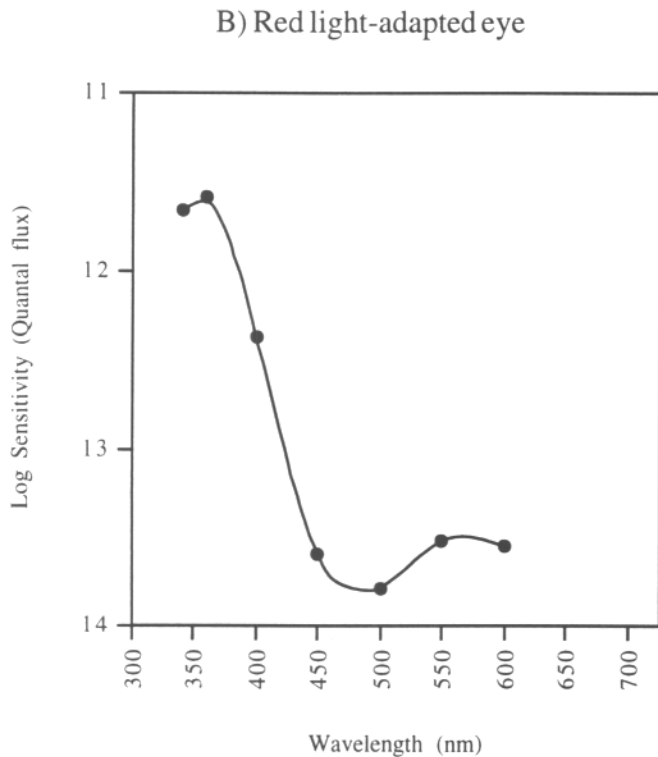
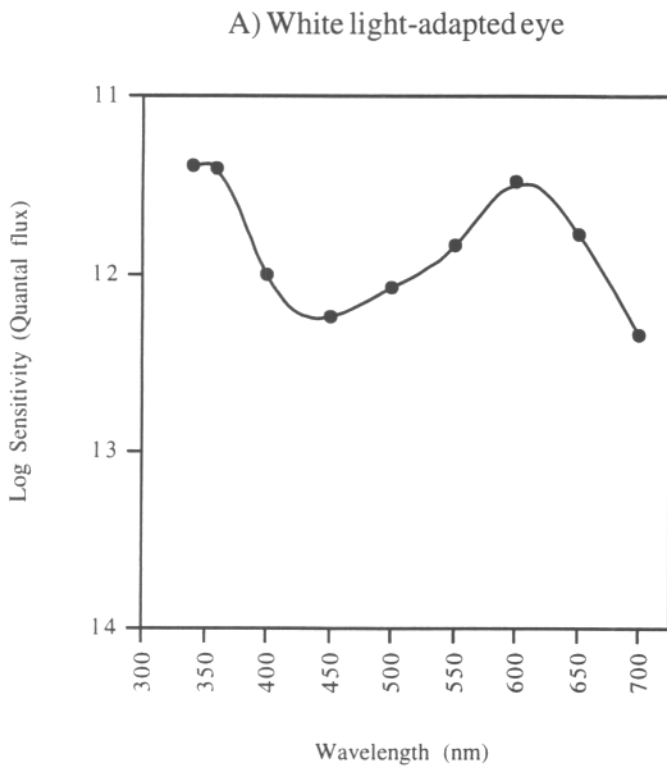
The spectral sensitivity curves obtained under two different adapting light conditions (i.e., white light-adapted and long-wavelength chromatically adapted) are shown for one open eye cup preparation from an individual axolotl (Fig. 2). The spectral sensitivity of the eye cup under full-spectrum, white light indicates that the eye is most sensitive to red light (around 600 nm) and UV light (around 360 nm) under daylight conditions. The peak at 600 nm indicates the presence of a cone photoreceptor with an absorption peak around 600 nm. In theory, three different mechanisms could produce a UV peak of sensitivity in the ERG (Hawryshyn and Beauchamp 1985, Jacobs 1992).

(1) UV sensitivity could result from absorption by a secondary-absorption peak of the long-wavelength cone pigment. Vertebrate photopigments typically have two peaks of absorption. The more sensitive, primary peak is at longer wavelengths than the less sensitive, secondary peak. So the photopigment responsible for the long-wavelength peak at 600 nm in Figure 2a is expected to have a secondary peak in the near-UV (i.e., 340-390 nm).

(2) UV sensitivity could result from UV-generated fluorescence from other ocular components (e.g., the remaining vitreous humor in the open eye cup). UV-generated fluorescence would result in the production of long-wavelength light that could subsequently be absorbed by a long-wavelength pigment (Jacobs 1992).

(3) Finally, UV sensitivity could result from a photoreceptor with a primary absorption peak in the UV.

In order to rule out mechanisms 1 and 2,



the retina was subjected to selective chromatic adaptation with long-wavelength (i.e., red) light (Fig. 2a). This treatment has been shown to be effective in spectrally-isolating UV receptors in birds and fish by decreasing the contribution of the longer-wavelength cones to the electroretinogram (Chen *et. al.* 1984, Hawryshyn and Beauchamp 1985). Long-wavelength adaptation of a long-wavelength sensitive photopigment causes a proportional reduction in the sensitivity of both the primary and secondary absorption peaks of the photopigment. Therefore, if the UV sensitivity in Figure 2a is due to the secondary absorption peak of the long-wavelength photopigment (i.e., the pigment responsible for the peak in sensitivity at 600 nm), long-wavelength chromatic adaptation would cause a proportional decrease in both the long-wavelength and UV peaks (Goldsmith 1986). Moreover, if long-wavelength sensitivity is reduced, a response to UV-stimulated fluorescent emission should also exhibit a proportional reduction in sensitivity (Jacobs 1992).

The spectral sensitivity of a single preparation following selective chromatic adaptation with red light (>610 nm) reveals a persistent UV peak in sensitivity while the long-wavelength sensitivity has dramatically decreased by about 1000-fold (Fig. 2b). The average spectral sensitivity of 6 retinas exposed to long-wavelength (orange-red) chromatic adaptation demonstrated that the peak in sensitivity is around 360 nm (data not shown; see Deutschlander and Phillips 1995). These data clearly show that axolotls possess a class of photoreceptor that is selectively sensitive to UV light. In addition, these data support the existing evidence for the presence of UV cones in salamanders of the genus *Ambystoma* (Perry and McNaughton 1991, Harosi 1994).

The evidence presented thus far for UV photoreception in amphibians has been collected in larval tiger salamanders (Perry and McNaughton 1991, Harosi 1994) and neotenic mexican salamanders (Deutschlander and Phillips 1995), both of which belong to the Ambystomatid family. Two

Figure 2. Spectral sensitivity of a single eye under various ambient light conditions. a) White light-adaptation of the open eye cup preparation resulted in a spectral sensitivity curve with a peak around 600 nm and a peak in the UV. b) Long-wavelength chromatic adaptation of the light-adapted retina revealed the contribution of UV photoreceptors (x = no response).

obvious questions are raised by these studies.

(1) Are UV photoreceptors present in adult terrestrial salamanders? In salmonid fishes, UV photoreceptors are present in juveniles but are lost in the transition to adulthood [for example, brown trout *Salmo trutta* (Bowmaker and Kunz 1987) and rainbow trout *Oncorhynchus mykiss* (Hawryshyn *et. al.* 1989)]. Thyroxin treatment has been shown to cause a precocial loss of the UV sensitivity in the rainbow trout (Browman and Hawryshyn 1992) and may be the regulatory mechanism for the normal developmental loss of UV sensitivity. We are currently collecting data from adult tiger salamanders which demonstrate that the UV photoreceptors persist in individuals that have metamorphosed into the terrestrial "adult" form. Therefore, the increase in thyroxin associated with amphibian metamorphosis does not appear to cause a loss of UV photoreceptors as it does in salmonid fish.

(2) Are UV photoreceptors present in other families of salamanders and, for that matter, other orders of amphibians? The available evidence for frogs, albeit sparse, suggest that ranid frogs do not possess UV photoreceptors in the retina. However, ranid frogs do possess a UV-sensitive photoreceptor in the frontal organ of the pineal gland (Dodt and Heerd 1962). No information is currently available from other families of frogs. We are currently surveying other groups of salamanders to determine whether UV photoreceptors are present in the retina. So far we have found that aquatic, adult eastern red-spotted newts (*Notophthalmus viridescens*) in the family Salamandridae also possess UV photoreceptors, and we are planning to conduct experiments on other salamanders in the families Salamandridae and Plethodontidae this fall.

In addition to these developmental and phylogenetic questions, the functional significance of UV cones in *Ambystoma* is still not known. Color vision in hymenopteran insects (such as bees), birds, reptiles, and fish (rev. in Jacobs 1992, Goldsmith 1994) has been shown to involve UV photoreceptors. Whether or not *Ambystoma* possess color vision or chromatic discrimination capabilities which involve a UV photoreception mechanism is unknown. Salamanders in the genus *Triturus*, however, have been shown to exhibit color-dependent responses to prey and mates (Himstedt 1972, 1979). The European fire salamander, *Salamandra salamandra*, has been demonstrated to possess trichromatic

color vision (Przyrembel *et. al.* 1995), but no evidence was found for the involvement of UV photoreceptors. Therefore, even if *Ambystoma* do possess true color vision, the mechanism might not involve the UV photoreceptors.

In addition to color discrimination, UV photoreceptors may play a "specialized" role in the amphibian visual system. For example, UV photoreceptors may be especially for detection of polarized light. In juvenile rainbow trout orientation with respect to polarized light is mediated by UV-sensitive cones in the retina (Hawryshyn 1992). Adult tiger salamanders have been shown to orient with respect to plane polarized light (Taylor and Adler 1973), although the response appears to be mediated by the pineal complex in *Ambystoma* and may not involve retinal input (Taylor and Adler 1978). Salamanders have also been shown to possess a magnetic "compass" sense (Phillips 1977, 1986). Recent evidence indicates that salamanders sense the earth's magnetic field via a light-dependent mechanism (Phillips and Borland 1992). These data implicate the involvement of the visual system in magneto-reception. If so, it may be that UV photoreceptors play a specialized role in magneto-reception.

Evidence for the presence of retinal UV photoreceptors in larval and neotenic *Ambystoma* raises a number of important questions about the phylogeny, development, and behavioral significance of UV photoreceptors in the amphibian visual system. Although these questions have been addressed in other taxons, we are only beginning to investigate the taxonomic distribution, developmental trajectory, and adaptive significance of UV vision in amphibians.

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