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ULTRAVIOLET COLOUR OPPONENCY IN THE TURTLE RETINA

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Summary

We have examined the functional architecture of the turtle *Pseudemys scripta elegans* retina with respect to colour processing, extending spectral stimulation into the ultraviolet, which has not been studied previously in the inner retina. We addressed two questions. (i) Is it possible to deduce the ultraviolet cone spectral sensitivity function through horizontal cell responses? (ii) Is there evidence for tetrachromatic neural mechanisms, i.e. UV/S response opponency? Using a constant response methodology we have isolated the ultraviolet cone input into the S/LM horizontal cell type and described it in fine detail.

Monophasic (luminosity), biphasic L/M (red-green) and triphasic S/LM (yellow-blue) horizontal cells responded strongly to ultraviolet light. The blue-adapted spectral sensitivity function of a S/LM cell peaked in the ultraviolet and could be fitted to a porphyropsin cone template with a peak at 372 nm.

In the inner retina eight different combinations of spectral opponency were found in the centre of the receptive field of ganglion cells. Among amacrine cells the only types found were UVSM–L+ and its reverse. One amacrine and four ganglion cells were also opponent in the receptive field surround.

UV/S opponency, seen in three different types of ganglion cell, provides a neural basis for discrimination of ultraviolet colours. In conclusion, the results strongly suggest that there is an ultraviolet channel and a neural basis for tetrachromacy in the turtle retina.

Key words: ultraviolet vision, spectral sensitivity, colour vision, turtle, retina, intracellular recording.

Introduction

The turtle retina has been used extensively as a model for the study of the neural mechanisms of chromatic processing. Turtle photoreceptor pigments were among the first to be described by microspectrophotometry (Liebman, 1972; Liebman and Granda, 1971; Lipetz and MacNichol, 1982; Lipetz and MacNichol, 1983; Lipetz, 1985; Lipetz and MacNichol, 1990) as were their associated oil droplets (Liebman, 1972; Liebman and Granda, 1971; Fujimoto et al., 1957; Ohtsuka, 1984). Later, intracellular recordings of single cones were performed, and their dynamic properties have also been studied electrophysiologically (Baylor and Fuortes, 1970; Baylor et al., 1971; Fuortes et al., 1973; Granda and Dvorak, 1977; Ohtsuka, 1985a; Ohtsuka, 1985b).

Explorations of the outer retina have revealed four types of horizontal cells according to their response to different wavelengths: a monophasic type with hyperpolarizing responses to all wavelengths (Fuortes and Simon, 1974; Simon, 1973; Yazulla, 1976); two biphasic types, one with hyperpolarizing responses to short (S) and middle (M) wavelengths, from 400 to 600 nm, and depolarising responses to long (L) wavelengths above 600 nm (Fuortes and Simon, 1974; Yazulla, 1976; Asi and Perlman, 1998); the other with polarity reversal at approximately 540 nm (Fuortes and Simon, 1974; Asi and Perlman, 1998); and a triphasic type with hyperpolarizing responses to short and long wavelengths and depolarising responses to the middle range, from approximately 520 to 640 nm (Ohtsuka, 1985a; Ohtsuka and Kouyama, 1985; Yazulla, 1976). The monophasic type is also referred to as a luminosity horizontal cell, while the other types have been termed chromaticity horizontal cells. The biphasic horizontal cell with polarity reversal at 600 nm is called an L/M or red-green horizontal cell, whereas the biphasic cell with polarity reversal at 540 nm and the triphasic horizontal cell are both referred to as S/LM or yellow-blue chromaticity cells.

The electrophysiology of the inner retina showed a much more complex picture, with 37 morphological types of amacrine cells and 24 morphological types of ganglion cells associated with different response patterns (Ammermüller and Kolb, 1995; Ammermüller et al., 1995; Ammermüller and Weiler, 1988; Weiler and Ammermüller, 1986).

Until 1997, none of the studies of the physiology of the turtle retina included stimulation in the ultraviolet range. Thus all existing data on retinal processing of chromatic stimuli in the turtle are incomplete. There are a few studies in the outer retina

that include the ultraviolet range (for a review, see Ventura et al., 1999) but, to our knowledge, there is no account of retinal processing in the inner retina that includes the ultraviolet range in any vertebrate species.

Among the pioneering studies of ultraviolet vision in vertebrates was the discovery of both sensitivity and discrimination in the ultraviolet range in turtles using a behavioral training technique (Arnold and Neumeyer, 1987). The technique consisted of training the turtles to swim underwater to a panel where two lights, the training and comparison stimuli, were presented side by side. Reward was given when the training wavelength was approached. The procedure was repeated for several wavelengths throughout the spectrum. Two turtles were tested. The authors found best discrimination at 400 nm, 510 nm and at 570 nm for one turtle or 600 nm for the other. The high discrimination ability at 400 nm was explained by assuming an ultraviolet photoreceptor. Using a variation of the same technique, the authors also determined the spectral sensitivity function at short wavelengths for one individual, and found maximum sensitivity at 370–380 nm. The turtle could be sensitive to ultraviolet light through activation of β bands of the L, M and S opsins, as its ocular media are transparent to ultraviolet (Ammermüller et al., 1998). But discrimination necessarily implies the existence of an ultraviolet-sensitive cone. Other indirect evidence for an ultraviolet sensitive cone was obtained in electrophysiological recordings from turtle retinal neurons (Ammermüller et al., 1998; Ventura et al., 1999).

To date, there is no direct evidence for an ultraviolet sensitive cone in turtle, but in addition to Arnold and Neumeyer's behavioral data (Arnold and Neumeyer, 1987), other findings are compatible with its existence. Approximately 5 % of the photoreceptors contain ultraviolet transmitting oil droplets (Ohtsuka, 1985a; Kolb and Jones, 1987). These cones are morphologically different from those known to be red-, green- and blue-absorbing cones (Kolb and Jones, 1987; Goede and Kolb, 1994). If the turtle indeed possesses four types of single photoreceptors, and double cones and rods, its vision could well be tetrachromatic provided that its visual system includes mechanisms for comparing the four chromatic channels. In other words, there would have to be spectrally opponent neurons.

What types of opponent neurons in the inner retina could be expected in a tetrachromatic species if the ultraviolet range were included? The number of combinations of excitation and inhibition that are possible with three cones is eight (2^3) and this rises to 16 (2^4) with four cones. In each case, there are two combinations that are non-opponent excitations or inhibitions in response to all wavelengths. Therefore, the possible number of opponent neurons is six for three cones and 14 for four cones. In half of the eight possible new combinations, there is opponency between ultraviolet and blue. The only description of opponency between ultraviolet and blue is at the level of the outer retina in cyprinid fish (Hashimoto et al., 1988). All the results from the turtle retina published to date show responses in the ultraviolet range with the same polarity as those in the blue (outer retina, Ammermüller et al., 1998; outer and inner retinas, Ventura et al., 1999).

In birds, as in mammals, the evolution of opsins begins with M and S pigments. Both the L at one end and either the violet or the ultraviolet at the other end, called VS by some authors, were later acquisitions (Bowmaker et al., 1991). In the turtle, all possibilities of opponency with three cones, i.e. six types, were found (Marchiafava and Wagner, 1981; Ammermüller et al., 1995; Weiler and Ammermüller, 1986), but these investigations did not include the ultraviolet range.

In the last few years, we have made intracellular recordings in the turtle retina with the objective of determining (i) whether an ultraviolet channel is present and, more recently, (ii) what types of colour-opponent neurons are present. We provide evidence here of responses to ultraviolet light that must come from an ultraviolet-sensitive cone and cannot be explained by absorption in the beta band of longer-wavelength-sensitive photopigments. Moreover, the existence of an ultraviolet-sensitive cone is indirectly confirmed by chromatic adaptation in horizontal cell responses, as demonstrated previously (Ammermüller et al., 1998) and there is a neural substrate for tetrachromacy in the inner retina.

Materials and methods

Preparation

Turtles of the species *Pseudemys scripta elegans*, measuring 23–38 cm, were used. They were sacrificed by decapitation, and one of the eyes was immediately enucleated and hemisected to prepare for recording; the head with the remaining eye was kept in the refrigerator for recordings to be performed the next day. The vitreous was carefully removed with paper strips. The resulting eyecup was everted and bathed continuously with turtle physiological solution. Intracellular recording electrodes were made of borosilicate glass and had resistances in the range of 100–200 MΩ when filled with 3 mol l^{-1} KCl, and 200–500 MΩ when filled with 3 mol l^{-1} KCl and Neurobiotin.

Stimulation and recording

The preparation was kept in a shielded case with no light other than the stimulating spots and annuli focussed onto the retina. Stimulus sizes are indicated in the figure legends. Recordings were performed either with no background or with a blue background (Schott DIL 457 nm; 1.9×10^{12} quanta s^{-1} cm^{-2}).

The stimuli were provided by a double-beam all-quartz optical system each. Each beam had a Xenon light source (75 W) and a motor-driven grating monochromator (Bausch and Lomb 38-86-79 and Amko LTi 01-001) used, respectively, to produce a spot and an annulus at the level of the retina, with a demagnification of 20×. In the beam used for the spot stimulus (beam 1), the intensity was controlled by a motor-driven quartz circular neutral-density wedge (Starna) and the spot diameter by a motor-driven total-closure iris (Rolyn

Optics). In beam 2, used either to present background light or an annulus (produced by blocking the central image area), a motorized iris (Rolyn Optics) controlled the outer diameter of the annulus and quartz neutral density filters (Schott) set the intensity. In both beams, the stimulus duration and inter-stimulus intervals were controlled by electromechanical shutters. Stimulus parameters in beam 1 (wavelength, intensity, diameter and temporal parameters) and in beam 2 (wavelength, diameter and temporal parameters) were under computer control. The computer also controlled pre-programmed sequences of flashes, and recorded the onset and offset times of each flash and monitored the corresponding membrane voltages, to check the cell's physiological condition. The same setup has been used by us in previous work (Ventura et al., 1999).

The setup included a fast, automated procedure for the measurement of spectral sensitivity [$S(\lambda)$], the Dynamic Constant Response Method (de Souza et al., 1996; DeVoe et al., 1997), which was used to obtain $S(\lambda)$ functions in horizontal cells. It consisted of recording the intensities of a flickering stimulus that adjusted the intensities to produce a constant response amplitude at each wavelength throughout the spectrum. The spectral scans, run in steps of 4 nm from 300–700 and *vice versa*, took about 2 min each way. At the end of each scan, the spectral sensitivity was plotted on-line as the reciprocal of the intensity at each wavelength used to keep the response amplitude at a preset criterion level (1–3 mV). A consequence of this method is that a constant state of adaptation is maintained (de Souza et al., 1996).

In amacrine and ganglion cells, we screened $S(\lambda)$ with equal-quanta flashed spots at four or more wavelengths spaced throughout the spectrum (usually at 370, 450, 540 and 640 nm). For the shortest testing sequence, the wavelengths tested above 400 nm were the same as or close to those used by other authors (e.g. Ammermüller et al., 1995), to allow comparison with their data. Responses to stimuli in the ultraviolet region had not been tested previously in the inner retina. The choice of 370 nm was based on behavioural data: this was the peak of the spectral sensitivity function obtained by Arnold and Neumeyer (Arnold and Neumeyer, 1987). A control spot of fixed intensity and wavelength was presented at the end of each sequence to monitor the stability of the cell's response.

The wavelength sequences of spot stimuli were repeated at different intensities. Annuli were also tested at the same wavelengths. If the recording was stable, sequences with more wavelengths were tested. Cells in the inner retina usually lasted only a few minutes, frequently not longer than 10 min. In the outer retina, monophasic and biphasic cells lasted around 30 min or more, but triphasic cells were rare and lasted a short time, about 5–10 min.

Since the setup was entirely automated and the experiments were pre-programmed, maximum use of the brief recording time available could be made.

Whenever possible, we injected Neurobiotin into the cell to identify it morphologically for comparison with cells whose morphology and physiology had been previously described. For this purpose, Neurobiotin was injected iontophoretically for 2–5 min at the end of the recordings, with positive current pulses of 2 mA at a frequency of 1 Hz. However, on many occasions, the cells were lost before they could be injected.

After fixation and dehydration, the retina was incubated in Streptavidin–CY3, and stained cells were viewed in a confocal microscope (Zeiss model LSM3/4).

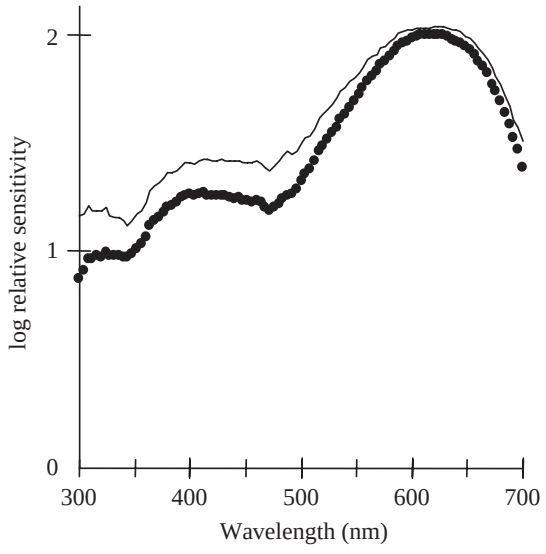

Fig. 1. Spectral sensitivity, $S(\lambda)$, functions of horizontal cells measured using the constant-response method, with no background. The figure shows an average $S(\lambda)$ function of 38 dark-adapted luminosity horizontal cells (filled circles) and the corresponding standard deviations (thin line). (Modified from Fig. 4 in Ventura et al., 1999, with permission of *Visual Neuroscience*.)

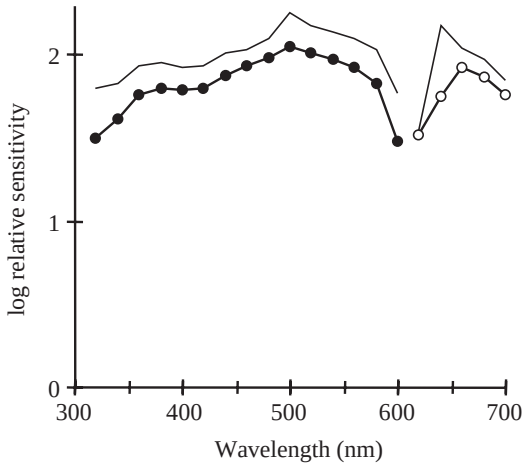

Fig. 2. Average spectral sensitivity $S(\lambda)$ functions of three L/M biphasic cells measured with the flash method (filled circles, hyperpolarizing responses; open circles, depolarising responses; thin line, standard deviation).

Results

Outer retina

We recorded from 61 horizontal cells. The classic non-opponent (N=47) and spectrally opponent L/M (N=10) and S/LM (N=4) horizontal cell types were found, using either equal-quanta flashes or the constant-response method. The addition of the ultraviolet range did not reveal a tetraphasic horizontal cell type with depolarising responses in the ultraviolet, as reported in cyprinid fish (Hashimoto et al., 1988). For all three types, responses in the ultraviolet region were always hyperpolarizing.

In the monophasic horizontal cells the response does not change polarity and the sensitivity reaches a peak at approximately 600 nm (Fig. 1). In the biphasic, L/M cell type, the $S(\lambda)$ function shows a minimum at about 600 nm (Fig. 2). At this point, sensitivity decreases greatly. Where the $S(\lambda)$ dips, the voltage response reverses polarity (open circles in Fig. 2).

The triphasic, S/LM cell type, $S(\lambda)$ measured by the constant-response method (N=2) had three peaks, two in the violet (410 nm) and green (520 nm) regions, where the responses were hyperpolarizing, and a third in the red (640 nm), where the responses were depolarizing (Fig. 3A). This cell's responses to flashes from 340 to 680 nm confirmed that it was an S/LM horizontal cell.

In $S(\lambda)$ functions obtained with a blue background (Schott DIL 457 nm filter), the relative sensitivity to short wavelengths increased considerably (Fig. 3B) and peaked in the ultraviolet region. A porphyropsin cone template (Stavenga et al., 1993) with a peak at 372 nm fitted the resulting function closely.

Inner retina

Recordings were made from 73 amacrine and ganglion cells, most of which were non-opponent. Spectral opponency was found in three amacrine and 12 ganglion cells. Five non-opponent amacrine and six ganglion cells had wavelength-dependent response components, that is, components that were present in response to certain wavelengths but not to others. Responses of many of the cells in the inner retina showed a very intricate picture with a variety of response types and a potential for complex processing of chromatic stimuli.

An example (Fig. 4) of this complexity is shown in the recordings from a spectrally opponent ganglion cell identified morphologically as G15 (Ventura et al., 1999). This ganglion cell responded with depolarisation (excitation) to ultraviolet (370 nm) and blue (450 nm) stimuli and with hyperpolarisation (inhibition) to green (540 nm) and red (640 nm) stimuli, with a reversal point at about 520 nm, which is typical of S/LM cells. This is shown in the responses to a series of equal quanta flashes from 300 to 700 nm (upper trace). Responses to another series of flashes are shown at a faster time scale in the lower trace. This spectral opponency was lost at higher intensities, where hyperpolarizing responses were seen at all wavelengths. The same happened when a large red surround (Schott OG590 filter) was flashed simultaneously with the stimulus (see Figs 10 and 11, Ventura et al., 1999). In contrast, with a red background, opponency was also lost, but the responses became depolarising (excitatory) to all wavelengths (see Fig. 11 in Ventura et al., 1999).

Another ganglion cell, which was found to respond with excitation to ultraviolet, blue and green and inhibition to red centre spots, had the opposite responses to stimuli in the periphery of its receptive field (Fig. 5). Thus, it was a double opponent cell: a UVSM+L− type in the centre and UVSM−L+ in the periphery.

Responses to ultraviolet light, shown in the two cells described, were found in all ganglion and amacrine cells. In

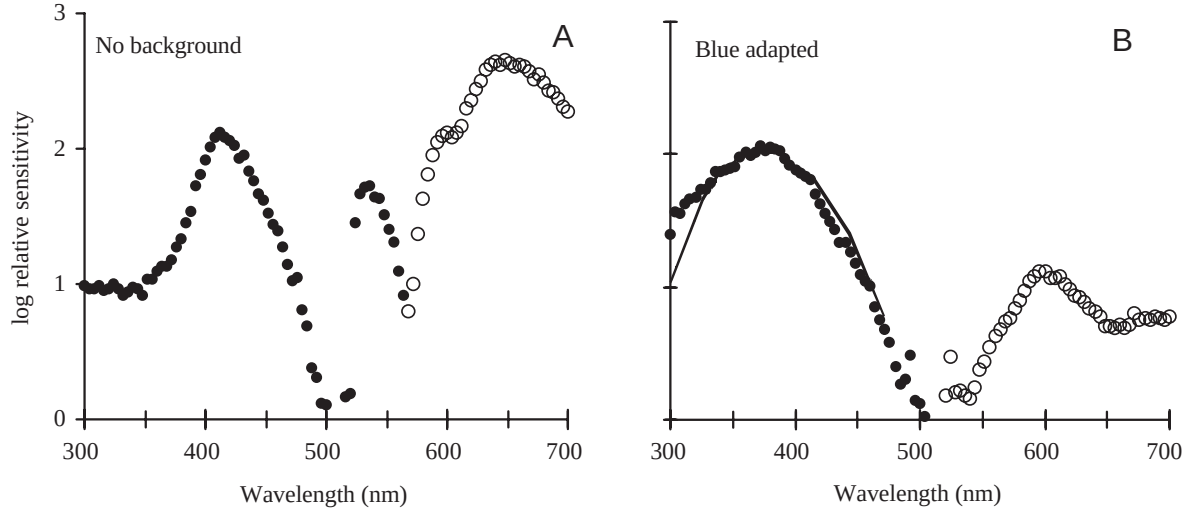

Fig. 3. Spectral sensitivity $S(\lambda)$ measurements of an S/LM cell with no background (A) and under blue adaptation (B). All curves are averages of four $S(\lambda)$ scans, collected alternately from 300 to 700 nm and in the opposite direction. The polarisation reversal was detected by a phase change in the response to the flickering stimulus. (A) $S(\lambda)$ function recorded in the absence of background light (filled circles, hyperpolarizing responses, open circles, depolarising responses). (B) The $S(\lambda)$ function of the same cell under a bright blue background (Schott DIL 457 nm; 1.9×10^{12} quanta s^{-1} cm^{-2}). The solid line is the cone porphyropsin template (Stavenga et al., 1993) with a peak at 372 nm.

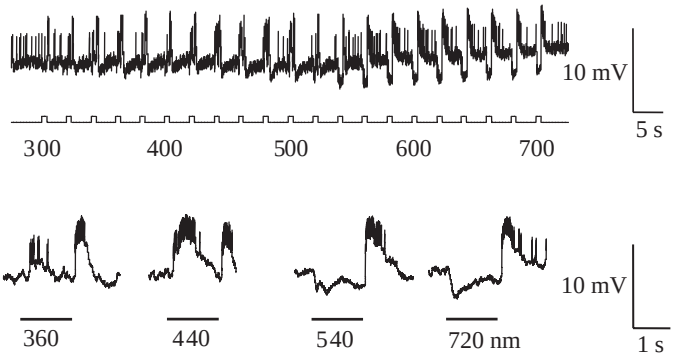

Fig. 4. Intracellular recordings obtained in a spectrally opponent cell (G51L31C1), stained with Neurobiotin and identified as a G15 ganglion cell. Upper trace: the response to a large spot stimulus at the receptive field centre (radius, ϕ=1250 μm) depolarises from 300 to 500 nm and hyperpolarizes from 520 to 700 nm. Lower trace: responses to equal quanta flashes at four wavelengths. (Modified from Fig. 10 in Ventura et al., 1999 with permission of *Visual Neuroscience*.)

most cells responses were of the same polarity as those elicited by blue stimuli, but we also found five cells with opponency between the ultraviolet and the blue. These were of the following types: UV− SML+; UVL−SM+; UVML−S+.

In the ganglion cell of Fig. 6 there is spectral opponency between UV and S channels, in addition to L/M opponency. Its centre responded with hyperpolarisation to the extreme spectral regions, ultraviolet and red, and with depolarisation and firing of action potentials to blue and green. This was a UVL−SM+ cell type in the receptive field center. It was also spectrally opponent in the peripheral receptive field, with opponency between ultraviolet and the rest of the spectrum (UV−SML+): the responses to a red spot and to a red annulus had opposite signs. Therefore it was an incompletely double opponent cell, since it was spatially opponent in the red, but not in the other spectral regions.

A summary of the findings is presented in Table 1, which lists all possible combinations of excitation and inhibition that could be produced by four cone systems. The first and last rows of the table correspond to non-opponent neurons. These were the most frequently found types. The most frequent type of opponent neuron hyperpolarised (or its spike frequency decreased) in response to ultraviolet, blue and green stimuli and depolarised (or its spike frequency increased) in response to red stimuli (UVSM−L+). This neuron type corresponds to that classified previously as a L/M cell. Its reverse, UVSM+ L−, was also found. Another type found was UVS+ML− and its reverse UVS−ML+. Five cells with opponency between ultraviolet and blue were found: two examples of each of the types UV−SML+ and UVL-SM+ and one of type UVML−S+.

One of the ganglion cells was a complete double-opponent cell with a UVS+LM− centre and a UVS−LM+ surround (Fig. 5). Another was incompletely double opponent (Fig. 6), with spatial opponency restricted to red stimuli.

In summary, eight different combinations of spectral opponency were found in the centre of the receptive field of ganglion cells. Among amacrine cells the only types found were UVSM−L+ and its reverse. UV/S opponency was found in five ganglion cells of three spectrally opponent types. One amacrine and four ganglion cell types were also opponent in the receptive field surround.

Discussion

Outer retina

We confirm that non-opponent and opponent horizontal cells in the turtle retina respond with hyperpolarisation to ultraviolet light (Figs 1–3) and that L/M cells lose spectral opponency in response to high-intensity flashes (Fig. 5 in Ventura et al., 1999), as also reported by others (Ohtsuka and Kouyama, 1985; Ohtsuka and Kouyama, 1986; Ammermüller et al., 1995). This has been confirmed in biphasic S/LM cells by Ohtsuka and Kouyama (Ohtsuka, 1985b; Ohtsuka and Kouyama, 1986), but not in triphasic S/LM cells (Ammermüller et al., 1995).

The present results offer a detailed functional description of the ultraviolet-sensitive cone $S(\lambda)$ function, obtained indirectly through chromatic adaptation in a S/LM horizontal cell. A porphyropsin cone template (Stavenga et al., 1993) with a peak at 372 nm fits this function (Fig. 3B). This is consistent with the hypothesis that there is an ultraviolet-sensitive cone in the turtle. A porphyropsin template also matched the absorption

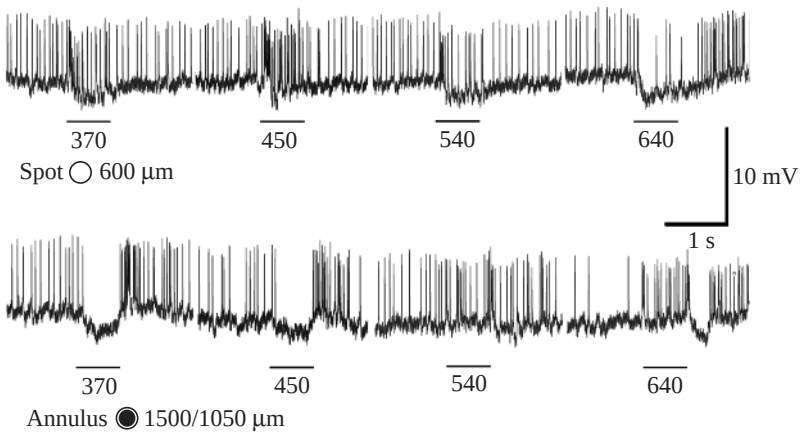

Fig. 5. Intracellular recordings from a double opponent ganglion cell (G86R1C1). Upper trace: responses to a spot stimulus (radius, ϕ=600 μm) at the centre of the receptive field are excitatory to ultraviolet (370 nm), blue (450 nm) and green (540 nm) flashes and inhibitory to red (640 nm) flashes. Lower trace: responses to an annulus (ϕ_{ext}=1500 mm, ϕ_{int}=1050 mm) reversed the excitation and inhibition seen at the centre, at all wavelengths. Note that at 540 nm, where the crossover occurs, only transient responses, at the stimulus onset and offset, are reversed.

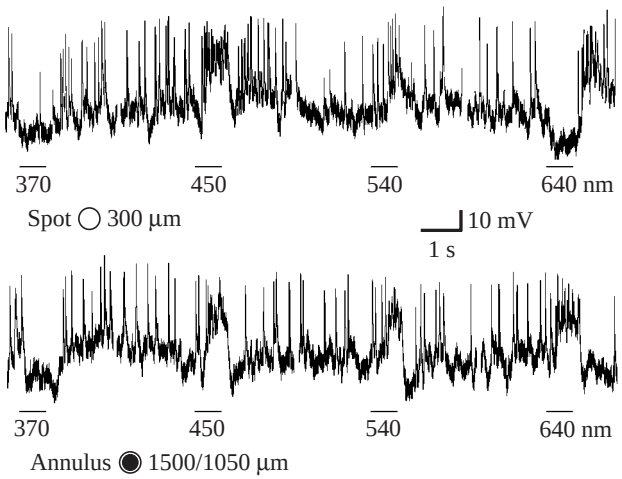

Fig. 6. Intracellular recordings from a spectrally opponent ganglion cell (G81L3C1) that showed opponency between ultraviolet and blue. Upper trace: responses to a spot stimulus at the receptive field centre (radius, ϕ=300 μm) were hyperpolarizing to ultraviolet (370 nm) and red (640 nm) and depolarising to blue (450 nm) and green (540 nm) flashes. Lower trace: responses to an annulus (ϕ_{ext}=1500 μm, ϕ_{int}=1050 μm) reversed the polarity to red flashes, relative to the centre responses, but not to ultraviolet, blue and green flashes. Therefore, the cell presented spatial opponency only to red stimuli.

curve of an ultraviolet cone pigment determined by microspectrophotometry in goldfish (Bowmaker et al., 1991) and the $S(\lambda)$ function obtained by recording membrane photocurrents of isolated goldfish cones (Palacios et al., 1998).

Since all cone pigments have a secondary beta-band absorption peak in the ultraviolet, responses to ultraviolet light could have been attributed to beta-band activation of the L, M or S cones. The wavelength peak of the beta band is a function of the maximum wavelength of the alpha band ($\lambda_{max\beta}$ =0.3947$\lambda_{max\alpha}$+138.95; from Palacios et al., 1996). Applying this function to the turtle, L ($\lambda_{max\alpha}$=620 nm), M ($\lambda_{max\alpha}$=518 nm) and S ($\lambda_{max\alpha}$=450 nm) cones (Liebman and Granda, 1971) would have beta-band peaks, respectively, at 384 nm, 343 nm and 317 nm. The 384 nm beta-band of the L receptor is closest to that of the 372 nm ultraviolet peak and could be responsible for the 372 nm ultraviolet peak with S adaptation.

The present results rule out this possibility as the sole explanation for responses in the ultraviolet. In addition to behavioral data showing sensitivity and discrimination in the UV (Arnold and Neumeyer, 1987), there are several arguments that strongly suggest the presence of an ultraviolet-sensitive cone. The first concerns filtering by the oil droplets. Oil droplets act as long- and medium-wavelength high-pass filters, blocking short wavelengths (Liebman, 1972). Absorption of ultraviolet light by L, M and S cones is already very small, since beta-band absorption is not greater than about 20 % relative to the alpha band (Palacios et al., 1998) and filtering by oil droplets further reduces absorptions of ultraviolet wavelengths.

A second argument is that there is an oil droplet type, first found in the turtle *Geoclemys reversii*, that does not block the ultraviolet light: the pale oil droplet (Fujimoto et al., 1957). This oil droplet is associated with a cone type morphologically different from the S cone proposed as a putative ultraviolet cone by Kolb and collaborators (Kolb and Jones, 1987; Goede and Kolb, 1994).

Table 1. *Summary of spectral response types of turtle amacrine and ganglion cells*

Receptive field centre	Spectral response UV	S	M	L	Receptive field surround
G77R2C2[a]					
G111L2C1 (New)					
G115L1C3 (New)	–	–	–	–	G75R2C2
G111L3C1 (G14)					
G118R1C1 (G18)					
	+	–	–	–	
G112L3C11[b]	–	+	–	–	
G51L2C1 (G15)2[c]	+	+	–	–	
G111L4C1 (New)	–	–	+	–	
	+	–	+	–	
A82R1C4; A87L1C13					
G88R1C21;G87L1C14	–	–	–	+	A82R1C4
G119L1C1 (G21)					
	+	–	–	+	
G81L3C1; G88R1C24	–	+	+	–	
A119R1C1 (A22)	+	+	+	–	G75R1C1
G86R1C1					
	–	+	–	+	
	+	+	–	+	
G75R1C1	–	–	+	+	G86R1C1
	+	–	+	+	
G87L1C12;	–	+	+	+	G81L3C1
G119L1C1 (G21)					G119L1C1
G75R5C5 (G23)					
G62R2C1 (G8)	+	+	+	+	G77R2C2; G75R1C1
A113R2C3 (New)					G87L1C12; A87L1C13

All combinations of excitation (+) and inhibition (–) for the four photoreceptor types are indicated in the middle columns of the table. Cells whose receptive field center corresponded to a given line of the table are shown in the column labelled Receptive field centre. Similarly, the column labelled Receptive field surround indicates the cell whose receptive field surround corresponds to a given combination of excitations and inhibitions.

[a]Cell identification: first letter, A, amacrine; G, ganglion. The first number identifies the turtle, letter L or R corresponds to left or right eye, the second number identifies the order of track penetration (1st, 2nd, etc), and the following letter C and number correspond to the cell number in a given track.

[b]Shaded rows indicate cells with opponency between UV and blue.

[c]Bold type indicates morphologically identified cells. Classification (according to Ammermüller and Kolb, 1995 and Ammermüller et al., 1995) is indicated in parentheses after the cell identification. 'New' indicates a type not described previously.

Thirdly, responses in the ultraviolet and red ends of the spectrum are of opposite polarity in opponent horizontal cells (Fig. 2, Fig. 3). This is also found in some ganglion cells (Fig. 4, Fig. 6).

The present demonstration of ultraviolet cone input to an S/LM horizontal cell (Fig. 3B) is also in agreement with these arguments and confirms a previous report using a different method (Ammermüller et al., 1998). The fact that the other two types of horizontal cells, monophasic and biphasic L/M horizontal cells, do not present this input reinforces this argument.

The confirmation of an ultraviolet cone input into a horizontal cell type is in line with the behavioral demonstration of tetrachromacy in this species (Arnold and Neumeyer, 1987).

Inner retina

The ultraviolet cone input seen in horizontal cells adds a new channel to the known L, M and S channels. Here we consider what role this channel plays in the chromatic vision of the turtle.

If the turtle UV channel is in fact involved in colour vision, the number of possible combinations of excitation and inhibition would rise to 16 (2^4), rather than eight in trichromates, with two non-opponent and 14 opponent possibilities. Which of the 14 possible colour opponent cell types are actually present in the turtle inner retina?

So far, we have identified eight of these types. Several new spectrally opponent types of cells exist in addition to the L/M and S/LM types. With the inclusion of ultraviolet stimuli, it is possible to show that some cells previously described as non-opponent (Ammermüller et al., 1995) are now found to be opponent, with responses to ultraviolet wavelengths of opposite sign to the responses to the rest of the spectrum. Also, we have found triphasic cells that would have previously been described as biphasic. Tetraphasic cells, such as those described in cyprinid fish (Hashimoto et al., 1988), were never found by us or by other authors. Spectral opponency between ultraviolet and blue stimuli in the inner retina of the turtle is demonstated here for the first time in the retina of a vertebrate, but is restricted to ganglion cells. We found no UV/SLM opponent amacrine cells.

Ammermüller et al. had already identified all six possible combinations of excitation and inhibition (Ammermüller et al., 1995). There are two types among these that we did not record from, M+SL− and M−SL+. If we consider the ultraviolet range, there could be at least two, maybe four, more types, depending on how they respond to ultraviolet light.

The existence of UV/L spectral opponency (Fig. 4, Fig. 5; Fig. 6, lower trace) in turtle ganglion cells further confirms that ultraviolet responses are not driven by the red cone through stimulation of its beta band. In addition, we have found a monophasic ganglion cell with maximum response in the ultraviolet region of the spectrum (Y. Zana, D. F. Ventura, J. M. de Souza, R. D. DeVoe, manuscript in preparation), also suggesting a specific ultraviolet channel in the inner retina.

The function of color vision in this species is open to speculation. Kolb and Lipetz (Kolb and Lipetz, 1990) argued that the muddy water habitat where turtles live might be responsible for the great proportion of L receptors in the turtle retina, and additionally, double cones with red pigment in both principal and accessory members but an oil droplet only in the principal member. In keeping with this, Granda and Fulbrook (Granda and Fulbrook, 1989) reported that most ganglion cell responses were L-cone dominated; however, we have found several other ganglion cell types. Vision in the ultraviolet region of the spectrum might contribute to improved contrast, by allowing better discrimination of objects under water against the light of the sky, and to the identification of other species (e.g. Losey et al., 1999; Cronin et al., 1994). A thorough investigation of the chromatic characteristics of the habitat of the turtle is needed for a more complete understanding of its colour vision.

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