Homogeneity and diversity of color-opponent horizontal cells in the turtle retina: Consequences for potential wavelength discrimination

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Color information processing in fish and turtles starts with the transformation of the tetra-chromatic cone system into two types of color-opponent horizontal cells (C-type). Few studies reported on large variability between C-type horizontal cells of the same class, suggesting it might improve color vision. However, such variability is contradictory with the tight coupling between horizontal cells that tends to average intercellular differences. We addressed this apparent discrepancy, and studied the spectral properties of C-type horizontal cells in the turtle retina. Photoresponses were recorded in the eyecup preparation, using light stimuli of different wavelengths and intensities. The spectral properties of each cell were defined by the neutral points (wavelengths at which response polarity reversed), which were derived from sensitivity data and from large-amplitude photoresponses. For each C-type horizontal cell, a linear relationship between log stimulus intensity needed for polarity reversal and wavelength was found. With this definition, homologous C-type horizontal cells from the same retina were practically identical in their spectral properties, indicating that the averaging effects of the horizontal cell syncytium eliminated any intercellular variability. In contrast, C-type horizontal cells of the same class exhibited large inter-retina variability. We tested the potential for wavelength discrimination by applying the line element theory to the action spectra of the two chromatic (Red/Green &Yellow/Blue) horizontal cell channels, and found good agreement with behavioral data from a similar species of turtles.

Keywords: turtle, retina, horizontal cells, color vision, spectral variability, cellular coupling, wavelength discrimination

Introduction
In cold-blooded vertebrates (e.g., fish and turtle), color-opponency is found already in horizontal cells, 2nd order neurons that receive direct inputs from cone photoreceptors (Kamermans & Spekreijse, 1995; Piccolino, 1995; Svaetichin & MacNichol, 1958; Twig, Levy, & Perlman, 2003a). Color-opponency in the chromaticity (C-type) horizontal cells is expressed in photoresponses of opposite polarity to light stimuli of different wavelengths. C-type horizontal cells are grouped into distinct classes according to the number of times response polarity reverses (biphasic and triphasic), and the wavelength at which polarity reversal occurs. Several studies in fish demonstrated large variability among C-type horizontal cells belonging to the same class (homologous cells). The wavelength at which response polarity reversed was reported to vary by a range of 50-70 nm (Burkhardt & Hassan, 1983; Djamgoz & Greenstreet, 1996; Kamermans & Spekreijse, 1995) or even 100 nm (Djamgoz, 1984). In fact, a few studies suggested that the wavelength at which response polarity reversed could occur throughout the entire visible spectrum (Govardovskii, Byzov, Zueva, Polisczuk, & Baburina, 1991; Mitarai, Asano, & Miyake, 1974; Tamura & Niwa, 1967). If color-coding of horizontal cells is eventually conveyed to the brain via discrete routes (ganglion cells), such variability may be beneficial for wavelength discrimination (Zrenner, 1985).

Physiological variability within a given class of C-type horizontal cells is inconsistent with the tight electrical coupling between homologous horizontal cells (Pottek & Weiler, 2000; Teranishi, Negishi, & Kato, 1984). With such tight coupling, the averaging effect of the syncytium is expected to abolish any variability between individual cells. Under conditions of reduced cellular coupling between horizontal cells, such as occurring during background illumination (Baldridge & Ball, 1991; Dong & McReynolds, 1991, 1992; Myhr, Dong, & McReynolds, 1994; Shimematsu & Yamada, 1988; Umino, Lee, & Dowling, 1991), spectral variability among C-type horizontal cells may increase.

The turtle is another example where the tetra-chromatic cone system (four different cone visual pigments) is transformed into two classes of color-opponent horizontal cells (Fuortes & Simon, 1974; Piccolino, 1995; Twig et al., 2003a). Spectral variability among individual C-type horizontal cells is expected due to variability in retinal wir-
ings and/or due to differences in the topographical distribution of L, M, S- and UV-cones (Kolb & Jones, 1987). However, the tight coupling between homologous horizontal cells (Ammermüller & Kolb, 1996; Ammermüller, Möckel, Perlman & Röhrenbeck, 1966) acts to eliminate such variability. Here we studied the spectral properties of C-type horizontal cells in the turtle retina and tested their inter- and intra-retina variability under different states of adaptation. Then the spectral properties of horizontal cells were used to predict the potential wavelength discrimination function using the line element analysis.

Methods

Preparation

The membrane potential and photoresponses of C-type horizontal cells were recorded intracellularly in the everted eyecup preparation (Normann, Perlman, & Daly, 1986) of the turtle Mauremys caspica. The turtle was decapitated with a guillotine in accordance with institutional guidelines for the use of laboratory animals. One eye was enucleated, hemisected, and the vitreous humor was removed. The posterior eyecup was everted over a balza wood dome and transferred to the recording chamber. The dissection procedure was conducted in room light. The eyecup was superfused with normal Ringer solution of the following composition (in mM): NaCl 110, KCl 2.6, CaCl₂ 2, MgCl₂ 2, Na-HCO₃ 20, and D-glucose 10. The solution was continuously bubbled with a mixture of 95% air/5% CO₂ to maintain the pH around 7.4.

Data acquisition and photostimulation

Microelectrodes were pulled (Sutter Instruments, Novato, CA, USA) from capillary tubing and filled with 3M-KAc solution (resistance was in the range of 100-200 MΩ). The signal of the microelectrode was amplified (Almost Perfect Electronics, Basel, Switzerland), and continuously digitized at a rate of 1kHz by a computer equipped with a data acquisition board (National Instruments, Austin TX, USA). In most of the experiments, we did not apply a band-pass filter to the microelectrode signal, and therefore 50-Hz noise from the power line can be evident, especially in small amplitude photoresponses.

The photostimulation system consisted of two light beams originating from a single light source (250-W tungsten halogen filament). One beam served for background illumination and the other for light stimuli. The wavelength and intensity of each beam were independently controlled by sets of narrow-band interference and neutral density filters. The intensity of the light was measured with a PIN 10 photodiode (United Detector Technology, Baltimore, MD, USA), and was calculated in photons × s⁻¹ × μm⁻².

Procedure

The microelectrode was advanced through the retina from the vitreal side, while flashing dim white light stimuli (500 ms in duration) at 3-s intervals. C-type horizontal cells were identified according to physiological criteria that had been previously described (Asi & Perlman, 1998; Fuortes & Simon, 1974; Perlman, Itzhaki, Malik, & Alpern, 1994; Piccolino, 1995). They all had homogeneous receptive field of at least 3-mm diameter. Red/Green horizontal (RGH) cells were characterized by reversal of response polarity in the range of 600-620 nm, whereas the photoresponses of Yellow/Blue horizontal (YBH) cells reversed in the range of 540-560 nm. In all experiments reported here, large diameter light stimuli (4.2 mm) and backgrounds (5.8 mm) were used to illuminate the entire receptive fields of the horizontal cells (Itzhaki & Perlman, 1984; Lamb, 1976; Perlman & Ammermüller, 1994; Twig et al., 2002).

Analysis

Flash sensitivities were calculated from small amplitude (< 1 mV) photoresponses that were within the linear range of the horizontal cells. To improve signal-to-noise ratio for sensitivity measurement, 20 responses elicited by identical flashes (50 ms in duration), delivered at a rate of 1Hz, were averaged. Light sensitivities for 14 different wavelengths (700, 680, 650, 620, 600, 580, 560, 540, 520, 500, 480, 450, 430, and 400 nm) were calculated by dividing response amplitude by the quartant content of the light stimulus, and are given in μV × photon⁻¹ × μm².

Response-intensity curves were constructed from the initial peak of the photoresponses that were elicited by light stimuli of fixed wavelength but different intensities. For complex response waveforms that contained a mixture of depolarizing and hyperpolarizing waves, we used a MATLAB routine that calculated the first derivative of the photoresponse as a function of time to identify the occurrence of the initial peak.

Results

Inter-retina variability of C-type horizontal cells

The most common approach to describe the spectral properties of a neuron in the visual system is to derive its action spectrum from sensitivity measurements at different wavelengths. Figure 1 shows dark-adapted action spectra of 26 RGH cells and 15 YBH cells that were studied in different eyecup preparations of the turtle Mauremys caspica (A and B, respectively). The action spectra are plotted with depolarizing photoresponses as positive values and hyperpolarizing ones as negative values. To avoid variability due to quality of recording, status of preparation and other technical factors, the action spectrum of each cell was normalized to its maximum hyperpolarizing sensitivity. In both
classes of C-type horizontal cells, the normalized action spectra vary in depolarizing to hyperpolarizing sensitivity ratio.

The wavelength-dependency of the action spectra seems similar within each type of cells. The wavelength at which light sensitivity reversed from positive to negative values (the neutral point, assigned here the symbol $\zeta$) was calculated for each cell by interpolation between the sensitivity of the longest wavelength that elicited a hyperpolarizing photoreponse and the sensitivity of the shortest wavelength that elicited a depolarizing photoreponse. The average (± SD) values of $\zeta$ for all RGH cells ($N = 26$) and for all YBH cells ($N = 15$) were 594.8 nm (± 2.5) and 530.0 nm (± 4.0), respectively. This variability is compatible with a model assuming that the sensitivity action spectrum of a C-type horizontal cell can be constructed by a linear combination of inputs from different spectral types of cones (Asi & Perlman, 1998; Burkhardt & Hassin, 1983). In our sample of C-type horizontal cells (Figure 1), the variability in depolarizing to hyperpolarizing sensitivity ratio is relatively small compared to previous findings from the walleye retina (Burkhardt & Hassin, 1983). It ranges from 0.4 to 0.7 for 80% of the RGH cells, and from 0.2 to 0.6 for 80% of the YBH cells. When applying the linear model (Burkhardt & Hassin, 1983) to the RGH cells, this range of depolarizing to hyperpolarizing sensitivity ratio corresponds to 10-15 nm range in the neutral points ($\zeta$). For the YBH cells, the model is more complex to calculate because all four spectral types of cones (L$_2$, M$_2$, S$_r$, and UV-cones) contribute inputs. We estimate that the measured range of depolarizing to hyperpolarizing sensitivity ratio in YBH cells corresponds roughly to a 10-25 nm range in the neutral points.

Variability between homologous C-type horizontal cells that were studied in different preparations increased when large amplitude photoresponses were elicited by bright monochromatic light stimuli. Figure 2 compares the photoresponses of two RGH cells (A) and two YBH cells (B) that were studied in four different eyecup preparations. The cells were stimulated with wavelength within the zone where response polarity reversed: 600-620 nm for the RGH cells and 540-560 nm for the YBH cells. The neutral points ($\zeta$) that were determined from the cells’ action spectra were very similar: 593.7 nm and 594.8 nm for the RGH cells (eye #1 and eye #2, respectively), whereas those of the two YBH cells were 529.4 nm and 530.6 nm (eye #3 and eye #4, respectively). Despite the similarity in the neutral points, pronounced intercellular variability within each spectral type of horizontal cells is seen when large amplitude photoresponses are considered (Figure 2). This is most apparent with the photoresponses to light stimuli of 600 nm for RGH cells and of 540 nm for YBH cells (Figures 2A and 2B, respectively). With these light stimuli, one cell in each spectral class responded mainly with depolarization, whereas the other responded with hyperpolarization. These data indicate that a comparison between two C-type horizontal cells cannot be limited to linear range responses (action spectra), and should rely on a more generalized description of their spectral properties.

![Figure 1. Dark-adapted action spectra of 26 Red/Green (RGH) and 15 Yellow/Blue (YBH) horizontal cells that were recorded in different eyecup preparations of the turtle Mauremys caspica (A and B, respectively). The action spectrum of each cell was normalized to its peak hyperpolarizing sensitivity.](https://jov.arvojournals.org/pdfaccess.ashx?url=/data/journals/jov/932832/)

![Figure 2. Inter-retina variability of homologous C-type horizontal cells, using bright light stimuli. The photoresponses of two RGH cells (A) and two YBH cells (B) that were studied in different eyes are compared. The responses were elicited with wavelengths in the spectral region where reversal of response polarity occurs. Responses within each row in either part were elicited by a fixed wavelength and intensity whose values are given to the left of each photoresponse in Log photon $\times$ s$^{-1}$ $\mu$m$^{-2}$.](https://jov.arvojournals.org/pdfaccess.ashx?url=/data/journals/jov/932832/)
The photoresponses in Figure 2 show a change in response waveform as a function of intensity as was demonstrated previously in fish (Burkhardt & Hassin, 1983; Kamermans, Kraaij, & Spekreijse, 2001; Naka & Rushton, 1966a, 1966b) and turtle (Fuortes & Simon, 1974; Twig et al., 2002). To quantify this relationship, we recorded the photoresponses of C-type horizontal cells using light stimuli of different wavelengths (within the depolarizing range) and intensities, as shown in Figure 3 for one RGH cell. Representative photoresponses that were elicited by four different wavelengths and different intensities are shown in Figure 3A. The waveforms of these photoresponses clearly depend on intensity. Dim light stimuli are characterized by a pronounced depolarizing pattern, but as light stimulus is made brighter, the hyperpolarizing component becomes dominant. Therefore, for each wavelength, an intensity can be found for which the initial wave is of 0 mV.

We measured the amplitude of the initial wave, as described in Methods, and constructed the response-intensity curve for each wavelength (Figure 3B). The log light intensity needed for reversal of response polarity was defined by interpolating the response-intensity curve between the data points (arrow heads marked a-d in Figure 3B), and are plotted as a function of wavelength (Figure 3C). The relationship between log intensity needed for null response and wavelength (termed here the $\lambda_{null}$ function) can be accurately described by a linear function ($R > 0.99$). We also measured the action spectra of this RGH cell, and calculated the neutral point ($\zeta$) to be 593.2 nm. The intensity $I_0$, that is associated with the neutral point, was derived by extrapolating (dashed line) the linear $\lambda_{null}$ function to wavelength $\zeta$ (Figure 3C). It is apparent that the neutral point, derived from the action spectrum, is the shortest wavelength at which response polarity reversal can be obtained. When large responses are considered, the neutral point shifts to longer wavelengths. It is important to note that $I_0$ is not a measurable parameter, but a derived one that can be used for normalization purposes, because it depends on the absolute sensitivity of the cell and the relative contributions of the hyperpolarizing and depolarizing inputs.

The interactions of depolarizing and hyperpolarizing inputs in C-type horizontal cells is linear for dim light stimuli, but become highly nonlinear when bright lights are used (Burkhardt & Hassin, 1983; Gottesman & Burkhardt,

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**Figure 3.** Deriving the neutral point function ($\lambda_{null}$ function). (A). Photoresponses of one RGH cell that were elicited by light stimuli of different wavelengths and intensities (given in log photon $\times$ s$^{-1} \times \mu$m$^{-2}$ to the right of each response). An arrowhead denotes the initial peak in each response. (B). Response-intensity curves of the initial peak of the photoresponses. The intersections of the curves with zero amplitude (dotted horizontal line) are indicated (arrowheads, a-d). (C). The relationship between log stimulus intensity (photon $\times$ s$^{-1} \times \mu$m$^{-2}$) that was needed to elicit reversal in response polarity and wavelength was fitted by a linear regression procedure ($R > 0.99$). $I_0$ is the intensity of the light stimulus that elicits a zero response when wavelength $\zeta$ (neutral point of the action spectrum) is used.
variability between homologous cells may become more apparent when large amplitude photoresponses are considered. In this respect, using the $\lambda_{\text{null}}$-function is advantageous for detecting spectral variability among C-type horizontal cells because (i) it describes the spectral properties of a given C-type horizontal cell throughout a wide range of intensities, and (ii) it is derived from the steepest part of the response-intensity curve where small changes in the relative contributions of the antagonistic inputs manifest as large changes in amplitude. Therefore, we used this function to further investigate variability between homologous C-type horizontal cells.

The $\lambda_{\text{null}}$-function of 20 RGH cells (continuous lines) and 10 YBH cells (dotted lines), studied in different eyecup preparations, are compared in Figure 4A. The $\lambda_{\text{null}}$-functions of homologous C-type horizontal cells vary considerably (Figure 4A). Part of this variability can be accounted for by differences in sensitivity to light between cells. Because we measured the action spectra in all these cells and derived the neutral point ($\xi$), we normalized the $\lambda_{\text{null}}$-function by setting the stimulus intensity at the shortest wavelength where response polarity reversed ($I_0 = 0$), and re-plotted in Figure 4B. The normalized $\lambda_{\text{null}}$-functions of each class of C-type horizontal cells originate from a common region; 585-595 nm for the RGH cells and 520-535 nm for the YBH cells. Thus, for very dim intensities (linear range of the cells), the range of the neutral point, within a class of C-type horizontal cells, is relatively narrow. However, the $\lambda_{\text{null}}$-function of different cells in a class vary in slope, and therefore marked variability in the neutral point is expected for bright light stimuli. For instance, for a constant quantal flux of 1 Log relative intensity, the range of wavelengths for reversal of response polarity is about 30 nm and 15 nm for the RGH cells and YBH cells, respectively. For 3.0 Log relative intensity units, the range of the neutral point for both RGH cells and YBH cells exceeds 50 nm. Thus, different intensity criteria that are used to define the neutral point can lead to different conclusions regarding the spectral diversity of C-type horizontal cells.

Intra-retina variability of C-type horizontal cells: Dark-adapted state

We have shown that variability between homologous C-type horizontal cells, which are studied in different preparations, increases when light stimuli of bright intensities are used to elicit large amplitude photoresponses. This can be a general property of C-type horizontal cells or can reflect inter-retina diversity.

Figure 5A shows the photoresponses of three RGH cells that were studied in the same eyecup preparation during separate microelectrode penetrations. Each cell was located in a different retinal area, keeping a minimal distance of 1.5 mm between adjacent recording sites. Monochromatic (620 nm) light stimuli of different intensities were used to elicit these photoresponses. Though the amplitudes of the photoresponses to a given stimulus vary considerably among the three RGH cells, their waveforms appear similar. To emphasize this similarity, the photoresponses were normalized to their corresponding maximal amplitude. For a given stimulus intensity, the normalized photoresponses of the three RGH cells are nearly identical (Figure 5A, 4th column). The wavelengths $\xi$ for these three RGH cells, derived from their action spectra, were very close: 594.1 nm, 594.8 nm, and 594.4 nm, respectively. Furthermore, the $\lambda_{\text{null}}$-functions that were determined from response-intensity curves were nearly identical even without normalization as shown in Figure 5B (open symbols, dashed lines). Similar findings were obtained in seven additional experiments in which at least two RGH cells were recorded in each retina. In each eyecup, the $\lambda_{\text{null}}$-functions of the cells were very similar, but could differ significantly from those of another retina as illustrated in Figure 5B (filled symbols, continuous lines). Thus, C-type horizontal cells of the same spectral type that are studied in the same retina have identical spectral properties when dim as well as bright light stimuli are used.

This homogeneity disappears when the area stimulated by the light is reduced as shown in Figure 6. The photoresponses of three different YBH cells were recorded in the same retina (minimal distance between adjacent cells of 1.5 mm) using the same wavelength (540 nm) and intensity (Log$_{\text{stim}} = 6.1$ photon $\times$ s$^{-1}$ $\times$ mm$^{-2}$), but different spatial patterns. The responses to large-field (4.2-mm diameter) stimuli (upper row) are hyperpolarizing having different amplitude, but similar waveform as indicated by normalization (4th column). In two of the three cells, the waveform of the response, elicited by a small spot (0.55-mm diameter), is characterized by a depolarizing component (Figure 6, bottom row), reflecting the dependency of the spatial properties on wavelength (Twig et al., 2002). The photoresponses of the three YBH cells to small spotlight stimuli clearly dif-

![Figure 4. Inter-retina variability of C-type horizontal cells. (A). Dark-adapted $\lambda_{\text{null}}$-functions of 20 RGH cells and 10 YBH cells (continuous and dotted lines, respectively) that were studied in different eyes. Because these functions exhibited excellent fit to a linear function ($R > 0.98$), only the fitted lines are plotted. Intensity is given in photon $\times$ s$^{-1}$ $\times$ mm$^{-2}$. (B). The $\lambda_{\text{null}}$-functions, shown in (A), were normalized to the neutral points of the action spectra ($\xi$) by subtracting from each curve its calculated value of $I_0$, as described in the text.](https://jov.arvojournals.org/pdfaccess.ashx?url=/data/journals/jov/932832/)
Figure 5. Intra-retina homogeneity of RGH cells in the dark-adapted state. (A). The photoresponses of three RGH cells that were studied in the same dark-adapted retina (eye #1) but in different loci (separated by a minimal distance of 1.5 mm). The photoresponses in each row were elicited by 620-nm light stimuli of different intensities given in Log photon $\times s^{-1} \times \mu m^{-2}$ to the left of each row. For comparison of waveforms, the photoresponses were normalized to their maximal hyperpolarizing amplitude (right column). (B). The $\lambda_{null}$-functions of the RGH cells in (A) (open symbols, dashed lines) and of two other RGH cells (cells #4 and #5) that were studied in a different preparation (eye #2) (filled symbols, continuous lines) are compared.

Intra-retina variability of C-type horizontal cells: Light-adapted state

Adaptation to background light decreases coupling between horizontal cells in certain species (Baldridge & Ball, 1991; Dong & McReynolds, 1991; Umino et al., 1991; Weiler, Pottek, He, & Vaney, 2000). Because uncoupling reduces the averaging effect of the horizontal cell syncytium, it may increase spectral diversity within a given class of C-type horizontal cells.

Figure 7 shows photoresponses of three RGH cells that were studied in the same eyecup preparation during continuous illumination with a bright green (540 nm) light ($\text{LogBg}_{540nm} = 5.3 \text{ photon} \times s^{-1} \times \mu m^{-2}$). The experiment started in the dark-adapted retina. After a successful impalement of the first RGH cell (cell #1), it was studied in the dark-adapted state, and again after 5 min of continuous illumination with the green (540 nm) background light. As expected, the green background shifted the chromatic balance toward the long-wavelength depolarizing input, and therefore light stimuli of 580 nm that elicited pure hyperpolarizing photoresponses in the dark-adapted state evoked a pure depolarizing photoresponse (Figure 7A). The green background light was maintained throughout the entire duration of the experiment (~3 hr), allowing sufficient time for complete activation of all possible light adaptation mechanisms. RGH cells #2 and #3 were studied 80 min and 160 min, respectively, after RGH cell #1, and should have been fully affected by any background-induced effect. The minimal distance between each pair of cells was 1.5 mm.

The photoresponses of these RGH cells to 580-nm (three cells) and 600-nm (two cells) light stimuli of different intensities are similar in waveform, but different in amplitude (Figure 7B). This is better illustrated by normalizing the photoresponses to their respective peak amplitude as shown in Figure 7B (right column). The waveforms of the
Figure 7. Intra-retina homogeneity of RGH cells in the light-adapted state. (A). Effects of green (540 nm) background light (LogBg=5.3 photon s^{-1} × μm^{-2}) on the photoresponses of one RGH cell. The wavelength and intensity (log photon s^{-1} × μm^{-2}) of the light stimuli are given above each column. (B). Photoresponses of three RGH cells that were studied in the same eyecup during the same green background light. Cells #1, #2, and #3 were studied at different time intervals (5, 80, and 160 min) after background onset. For comparison of waveforms, the photoresponses were normalized to their respective peaks 160 min) after background onset. For comparison of waveforms, the photoresponses were normalized to their respective peaks.

Photoresponses are nearly identical for each of the light stimuli. Similar results were observed in five eyecup preparations that were studied under green (N = 3) or red (N = 2) background illumination. These results indicate that despite the change in the balance between the antagonistic inputs to RGH cells, the cells remained sufficiently coupled to present similar photoresponses.

**Discussion**

**Spectral properties of chromaticity horizontal cells**

The responsiveness to light of color-opponent horizontal cells in cold-blooded vertebrate is a complex function of wavelength and intensity. Therefore, a single neutral point is not sufficient to describe their behavior. Rather, a function relating wavelength and intensity for response reversal is needed (λnull-function). Such a relationship, between log intensity needed for response polarity reversal and wavelength, was derived here for the turtle retina (Figure 3). This relationship could be accurately described by a linear function, a property that can be accounted for by the neurocircuitry of the distal turtle retina (Fuortes & Simon, 1974; Piccolino, 1995; Twig, Levy, & Perlman, 2001) and the action spectra of the cone photoreceptors. With sufficiently bright long-wavelength light, the depolarizing inputs to the C-type horizontal cells reach saturation, and become independent of intensity. The opposing hyperpolarizing inputs to the C-type horizontal cells continue to grow, when intensity is further increased, until the polarity of the photoresponse is reversed. The long-wavelength portion of the action spectra of M- and S-cones exhibit a linear reduction in log sensitivity as the wavelength is increased (Baylor & Hodgkin, 1973; Itzhaki, Malik, & Perlman, 1992; Perlman et al., 1994; Schneeweis & Green, 1995). Because M-cones and S-cones supply the hyperpolarizing inputs to RGH cells and S-cones to YBH cells (Asi & Perlman, 1998; Fuortes & Simon, 1974), the λnull-functions of both RGH cells and YBH cells are expected to be linear.

**Inter- and intra-retina variability**

The λnull-functions of homologous C-type horizontal cells indicate large inter-retina variability (Figure 4), and very small intra-retina variability (Figure 5). The intra-retina homogeneity of homologous C-type horizontal cells can be disrupted by reducing the diameter of the light stimuli (Figure 6). This indicates that the relative contributions of the antagonistic inputs vary among individual homologous horizontal cells within a given preparation. The basis for variability may simply reflect random wiring within the retina and/or topographical variability in the distribution of L-, M-, S- and UV-cones.

We recalculated from Kolb and Jones (1987) the ratio of spectral cones supplying depolarizing and hyperpolarizing inputs to the C-type horizontal cells for different retinal loci. For a given retina of the turtle Pseudemys scripta elegans, the [L-cones] to [M-cones + Scones] ratio (antagonistic inputs to RGH cells) ranges from 0.8 to 1.5, and the [L-cones + M-cones] to [S-cones + UV-cones] ratio (antagonistic inputs to YBH cells) ranges from 2.5 to 8. Because the retinal anatomy of Pseudemys is very similar to the Mauremys (Kolb, Perlman, & Normann, 1988), we tend to attribute intra-retina variability between individual C-type horizontal cells, as revealed by small-spot stimulation (Figure 6), to topographical variability in the distribution of different spectral types of cones. Topographical variability in the distribution of L-cones and M-cones were also used to explain variability in the L-cone to M-cone contrast gain of H1-horizontal cells (Dacey, Diller, Verweij, & Williams, 2000; Deeb, Diller, Williams, & Dacey, 2000) and ganglion cells (Diller, Packer, Verweij, McMahon, Williams, & Da-
ceny, 2004) in the primate retina. In turtle, intra-retina variability between homologous C-type horizontal cells is abolished by the averaging effect of cellular coupling (Ammermüller & Kolb, 1996; Ammermüller et al., 1996).

With these considerations, inter-retina variability within a class of C-type horizontal cells can reflect biological variability in retinal circuitry and/or inter-retinal differences in the L-cones to M-cones to S-cones ratio. Although no such studies were done in the turtle retina, abundant evidence supports considerable variability in L to M-cones ratio in the primate (Dacey et al., 2000; Deeb et al., 2000) and human retinas (Carroll, Neitz, & Neitz, 2002; Roorda & Williams, 1999).

Our findings of small intra-retina variability were independent of the state of adaptation. The wavelength and intensity of the ambient illumination altered the balance between the cone inputs, and therefore changed the spectral properties of C-type horizontal cells (Figure 7A). However, homologous C-type horizontal cells that were studied in the same retina during prolonged background illumination exhibited identical spectral properties (Figure 7B), indicating that the averaging effect of the horizontal cell syncytium was sufficiently strong. This is supported by previous findings on the effects of prolonged background illumination (several minutes) on receptive field size of turtle L-type horizontal cells (Perlman & Ammermüller, 1994). We also measured receptive field sizes of few RGH cells and YBH cells during prolonged background illumination (over 30 min), and found no significant change compared to the dark-adapted state (data not shown).

These observations (Figure 7) indicate fundamental differences between fish and turtle retinas. Both species are characterized by tetra-chromatic cone system (L, M, S, and UV-cones) that is transformed into two color-opponent channels in the horizontal cells. In both species, a chromatic background changes the spectral properties of the horizontal cells by altering the balance between the antagonistic inputs (Fuortes & Simon, 1974; Kamermans, van Dick, & Spekreijse, 1991; Tomita, 1965; Twig et al., 2001; Twig, Levy, Weiner, & Perlman, 2003b; Yazulla, 1976). However, in fish horizontal cells, additional long-term mechanisms (e.g., spinule formation) develop (Kirsch, Wagner, & Djamgoz, 1991; Wagner, 1980; Weiler, Schultz, Pottek, & Tieding, 1998; Weiler & Wagner, 1984), adding color-opponent channels and increasing diversity in spectral properties of the opponent channels (Djamgoz, Downing, Kirsch, Prince, & Wagner, 1988; Negishi, Salas, Parthe, & Drujan, 1988; Wagner & Djamgoz, 1993; Weiler et al., 1998; Weiler & Wagner, 1984). Turtle horizontal cells do not exhibit such morphological changes as a function of ambient illumination (Ammermüller & Kolb, 1996; Wagner, 1980).

**Implications to wavelength discrimination**

Wavelength discrimination is a basic feature of color vision. It can be measured using behavioral experiments, and can be calculated from the spectral properties of the chromatic channels (Arnold & Neumeyer, 1987; Neumeyer, 1986). The spectral properties of the cone system provide the basis for wavelength discrimination ability, but neuronal interactions may modify this feature of color vision. In goldfish, the cone system alone could not account for behavioral wavelength discrimination and neuronal interactions had to be postulated to account for the data (Neumeyer, 1986). In the turtle *Pseudemys scripta elegans*, behavioral wavelength discrimination could be explained by modifying the absorption spectral of the cone pigments using the properties of the oil droplets (Arnold & Neumeyer, 1987). However, additional neuronal interactions, such as those leading to the genesis of color opponency, may further modify the potential wavelength discrimination.

The horizontal cells are not part of the direct route for visual signals flowing from the photoreceptors toward the brain, but they shape the responsiveness of bipolar cells and contribute to the surround component of their receptive field (Dowling, 1987; Marchiafava, 1978; Toyoda & Kujiroka, 1982), and therefore also of ganglion cells (Mangel, 1991). Color-opponency in bipolar cells (Haverkamp, Möckel, & Ammermüller, 1999; Kamermans & Spekreijse, 1995; Twig et al., 2003a) and ganglion cells (Marchiafava & Wagner, 1981; Raynauld et al., 1979) of cold-blooded vertebrates, especially for surround illumination, have been suggested to involve mechanisms of color-coding in the horizontal cells. Regardless of the exact contribution of C-type horizontal cells to color-coded output of the retina, these cells represent the first chromatic stage of the visual system in cold-blooded vertebrates, and therefore, the first site that can potentially affect color vision of the turtle.

To assess potential wavelength discrimination of turtle C-type horizontal cells, we adopted an analytical approach, similar to that previously used in the goldfish (Neumeyer, 1986). With two chromatic channels, a given wavelength can be represented by a two-dimensional vector whose coordinates are determined by the relative excitations of these channels. The angle (θ) between any two such vectors can be used to quantify the difference between the two wavelengths that they represent. The larger is this angle, the better is the potential to separate between the two wavelengths. The angle (θ) between two vectors was calculated from the cosine sentence

$$\cos \theta_{A1-A2} = (\Delta A_1^2 + \Delta A_2^2 - r^2)/(2 \times \Delta A_1 \times \Delta A_2)$$

where \(\Delta A_1\) is the length of the vector representing wavelength \(A_1\), whose coordinates (\(RG_{A1}\) and \(YB_{A1}\)) are determined by the relative excitations of the RGH and YBH channels, respectively. \(\Delta A_2\) is the length of the vector representing wavelength \(A_2\) at coordinates \(RG_{A2}\) and \(YB_{A2}\), and \(r\) is the distance between the vectors. We used the average dark-adapted action spectra of 26 RGH cells and 15 YBH cells to calculate the angle (θ) between vectors representing
two wavelengths that were separated by 0.5 nm, and plotted it in Figure 8A (continuous curve) as a function of wavelength. This curve exhibits two prominent peaks: one around 580 nm and the other around 530 nm.

To calculate potential wavelength discrimination by the two chromatic horizontal cell channels, we needed to transform our theoretical curve from the angle ($\theta$) domain to the spectral domain. This was done using the principle of the line element theory (Wyszecki & Stiles, 1982). This theory assumes that the angle between two vectors representing two different wavelengths that are “just-discriminated” is of a fixed value (assigned the letter R). Namely, any two wavelengths whose vectors are separated by an angle equal to R will also be just discriminated. Arnold and Neumeyer (1987) measured behaviorally wavelength discrimination in the turtle Pseudemys scripta elegans. Although these are two different species, a previous study indicated that the action spectra of their C-type horizontal cells were very similar (Ammermüller, Itzhaki, Weiler, & Perlman, 1998).

In two Pseudemys turtles, a minimum of 11 nm was needed to discriminate between 501 nm and 512 nm (Arnold & Neumeyer, 1987). Assuming this was the just-discriminated difference in the spectral space of the chromaticity horizontal cells, we calculated from Figure 8A (continuous curve) a value for R of 0.195 rad. For each wavelength $\lambda$, we calculated the longer ($\lambda_{s}$) and shorter ($\lambda_{l}$) wavelengths that could be just discriminated ($\theta = 0.195$), and plotted in Figure 8B (continuous curve) the mean wavelength discrimination function ($(\Delta \lambda_{s} + \Delta \lambda_{l})/2$) as a function of wavelength. It should be noted that the theoretical wavelength discrimination curve is limited to the range 470-640 nm, because for this range only could we calculate wavelength discrimination for both shorter and longer wavelengths. For $\lambda < 470$ nm, shorter wavelengths could be discriminated only going below 400 nm, and for $\lambda > 640$ nm, longer wavelengths to be discriminated were above 700 nm.

The theoretical wavelength discrimination function is compared to experimental curves (Figure 8B, red curves) that were measured behaviorally in two Pseudemys turtles (Arnold & Neumeyer, 1987). The two curves are similar for wavelengths between 470 nm and 600 nm, but deviate for wavelengths longer than 600 nm. One possible explanation for this discrepancy is the participation of the cone luminosity channel (L-type horizontal cells). To test this possibility, we also derived the wavelength discrimination function, as described above, using two chromatic and one achromatic channels (Figure 8, dashed curves). The major difference is seen in the long-wavelength range of the spectrum where the L-type horizontal cells are most sensitive (Asi & Perlman, 1998; Fuortes & Simon, 1974). With the three channels, the theoretical wavelength discrimination function better fits the behavioral data for the range 600-620 nm. However, a large discrepancy between the predicted and measured wavelength discrimination appears for $\lambda > 620$ nm. This suggests that the two color-opponent horizontal cells are sufficient to account for behavioral wavelength discrimination in the turtle.

The agreement between the wavelength discrimination curves, measured behaviorally in Pseudemys scripta elegans, and the theoretical one, derived from data of the Mauremys caspica turtle, is very good considering: (1) the difference in variety and color of the oil droplets in the cone photoreceptors (Kolb et al., 1988). (2) The behavioral tests were performed under conditions of background light, while the theoretical wavelength discrimination curve was derived from the dark-adapted action spectra. (3) Our analysis did not consider a UV input to the YBH cells (Ammermüller et al., 1998) that could account for the peak discrimination in the behavioral data for wavelengths in the blue range of the spectrum (shorter than 450 nm). Despite these limitations, our analysis indicates that two homologous color-opponent channels with spectral properties similar to those of RGH cells and YBH cells can account for wavelength discrimination ability of turtles.
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