



M- and L-cones in early infancy: I. VEP responses to receptor-isolating stimuli at 4- and 8-weeks of age

Kenneth Knoblauch^{a,*}, Michelle L. Bieber^b, John S. Werner^c

^a *Institut de l'Ingénierie de la Vision, Université Jean Monnet, Site GIAT Industries, 3 rue Javelin-Pagnon, BP 505, 42007 St. Etienne Cedex 01, France*

^b *INSERM Unité 371, Cerveau et Vision, Bron, France*

^c *Department of Psychology, University of Colorado, Boulder, CO 80309-0345, USA*

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Abstract

A silent-substitution technique combined with measures of the visually-evoked potential (VEP) was used to determine whether M- and L-cones are functional in early infancy. Data were successfully collected from twenty six infants in response to three receptor-isolation conditions (rod, M- and L-cone isolation) and a luminance-modulation condition. The efficacy of the receptor-isolation conditions was first verified by measuring VEP responses from both dichromatic and color-normal adults to each of the receptor-isolation conditions. Both 4- and 8-week-old infants demonstrated VEP responses to the M- and L-cone isolating stimuli, though the amplitude of the responses at 4-weeks were reduced compared to those at 8-weeks. These data suggest that the functioning of M- and L-cones can be differentiated as early as 4-weeks of age. © 1998 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The trichromacy of human vision is based on the activity of three univariant cone photoreceptor classes (short-wavelength (S), middle-wavelength (M) and long-wavelength (L) sensitive), and at least three subsequent neural pathways that encode different combinations of their outputs. The extent to which these receptors are functional at birth, and the rate at which they mature postnatally, is only partially understood.

The anatomical immaturity of the human retina at birth [1–3,57] has engendered many questions about the functional status of the visual system in early infancy. For instance, the immature photoreceptors found in the infant retina possess photopigment-bearing outer segments that are only a fraction of the length of their adult counterparts. This factor should have implications for the spectral sensitivity of these recep-

tors. In addition, the increased width of the photoreceptor inner segment versus the outer segment, diminish both the wave-guide properties and the packing density of the photoreceptors. One analysis has estimated that these and additional factors combined render the newborn visual system at least three hundred times less efficient in utilizing incident quanta than that of an adult [4].

Despite these anatomical immaturities, it has been suggested on the basis of behavioral and electrophysiological evidence that nearly all of the retinal components necessary for adult-like trichromatic vision are present as early as 12-weeks [5,56,58,59]. In previous studies it has been possible to measure the spectral sensitivities of the rods [6,7] and the S-cones [8] as early as 4-weeks. In addition, the similarity of infant and adult photopic spectral sensitivity [9–12] provides indirect evidence for the functioning of L-cones as early as 8-weeks. The functionality of M-cones, however, can not be inferred from these data as a result of the more poorly defined contribution of the M-cones versus L-cones to the luminosity curve [13–18].

* Corresponding author. Tel.: +33 477923030; fax: +33 477923039; e-mail: knoblauch@vision.univ-st-etienne.fr.

Hamer et al. [19] demonstrated that 8-week-old infants are capable of making Rayleigh discriminations, which under the proper conditions rely only on M- and L-cones. A subsequent study by Varner et al. [20] demonstrated that 5-week-old infants also possess the ability to make tritan discriminations, which under proper conditions rely only on S-cones. Thus, one might want to conclude that 5–8 week-old infants possess all the retinal components necessary for adult-like trichromatic vision (S-, M- and L-cones and the corresponding postreceptoral circuitry). An alternative hypothesis acknowledged by the authors themselves, however, is that rod signals combined with one functional cone type may explain at least some of the Rayleigh and/or tritan discriminations reported from infants. Moreover, the use of large fields, weakly controlled fixation and low luminance levels, inevitable characteristics of most infant studies, favor contributions from rod signals. Rods can contribute to color discrimination in infants and adults under such conditions [21–25]. For example, Clavedetche et al. [23] examined the ability of 3- and 7-week-old infants to make chromatic discriminations of short-wave spectral lights surrounded by green (547 nm) light. Most 3-week-old infants failed to discriminate these stimuli on the basis of wavelength alone, while nearly all infants at 7-weeks could discriminate them even when intensity cues were eliminated. They found, however, that the response minima at 7-weeks and the discrimination failures at 3-weeks correspond quite closely to the rod spectral sensitivity curve. These results suggest that over most of the intensity levels tested, discrimination at 3- and 7-weeks is probably mediated by rods, with a somewhat less mature chromatic mechanism mediating discrimination at the shallow minima at 7-weeks. Furthermore, we have shown in a preliminary study [18,25] that visually-evoked potentials (VEPs) arising from rod modulation in an adult dichromat do occur under stimulus conditions often used to test infants. Thus, it is certain that rods can contribute and possibly even mediate infant discrimination at moderate luminance levels (i.e. luminances that for adults are in the mesopic and low photopic range).

Spectral sensitivities measured in previous work leave little doubt that rods, S-cones, and the L-cones are functional in early infancy [6–12]. However, because of the significant overlap between the M- and L-cone spectral sensitivities with one another as well as with the rod spectral sensitivity, inferences about the functioning of M-cones from these studies are not certain. Thus, it remains unclear whether or not infants are trichromatic and further, whether or not their discriminations are mediated exclusively by cones or, alternatively, by rods in some combination with one or more classes of cones.

We have adopted a receptor-isolation technique, referred to as ‘silent-substitution’ [26], in an attempt to verify the functioning of both M- and L-cones in early infancy, while controlling for potential rod responses. This technique allows one to maintain a constant output from a set of photoreceptors, while driving the response of the remaining receptors. If the conditions are properly arranged, it is possible to drive a single receptor class in isolation. The principle results of the present study show that VEP responses can be driven by both M- and L-cone isolating stimuli in infants as young as 4-weeks of age.

2. Methods

2.1. Subjects

Forty-nine full-term infants were recruited from birth announcements in the local newspaper to participate in this study. Informed consent was obtained from a parent before testing commenced. Data were successfully collected from 15 4-week-olds (nine males and six females) and 16 8-week-olds (ten males and six females). Each infant participated in one to three testing sessions that lasted approximately 45–60 min. Testing was completed within ± 7 days of the infants 4- or 8-week birthday. The parents of four male infants reported a familial history of color vision deficiency on the maternal side. We were unable to collect data from 18 infants due to crying, inattention or sleeping.

Comparison data were also obtained from three adults (one female, 22 years; two males, 19 and 41 years). The female subject displayed normal color vision, while one of the males was classified as a protanope and the other a deuteranope, as determined by the Neitz anomaloscope, Farnsworth panel D-15, and the American Optical HRR pseudoisochromatic plates.

2.2. Stimuli

Because normal human vision is mediated by four different photoreceptor types (S-, M-, L-cones and rods), the task of modulating only one of these receptor classes in isolation, using the silent-substitution technique, requires that the radiances of at least four lights be varied independently. To simplify the stimulus, we worked in the Rayleigh region of the visible spectrum (≈ 540 –700 nm) where S-cone sensitivity is negligible. Given that there are only three functional photoreceptor types operating in the Rayleigh region (rods, M- and L-cones) a variable mixture of only three lights is needed to silence any two of them at a time. An additional advantage of so restricting the stimulus is that age-related and individual variations of the ocular media density due principally to the lens [7,27–29] and

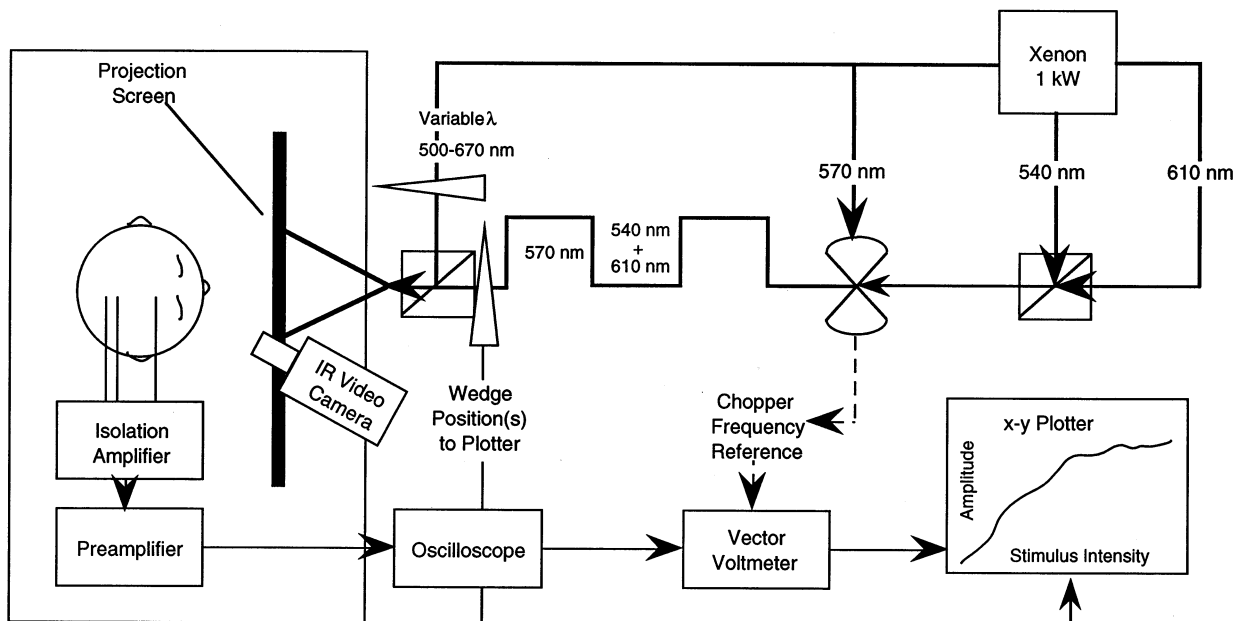


Fig. 1. Schematic of the optical and recording system.

the macular pigment [30] are minimized in this spectral region.

Our three primary receptor-isolating stimulus (presented as a 6° disk) consisted of a 570 nm light alternating at 7.5 Hz in square-wave counterphase with a mixture of 540 and 610 nm. To isolate the response of one receptor class, for example the L-cones, the ratio of the radiances of the 540/610 nm mixture in one field and the radiance of a 570 nm light in a second field were adjusted so that each field produced equal quantal absorptions in M-cones and rods, respectively. When presented successively to the same retinal area, the substitution of one field for the other should be silent for M-cones and rods; any response obtained would then be due to the modulation of only the L-cones. The same logic is used to isolate responses originating in M-cones and rods, in turn. The computational procedures for determining the isolation conditions are described in Appendix A.

While the number of possible trios of monochromatic light that are capable of isolating rods, M-cones or L-cones is virtually limitless (provided that each component wavelength is greater than 540 nm), there are particular trios that produce stronger responses from the three cone classes than others. The wavelength triplet used in the present experiment was determined such that the contrast that the triplet produced for the three receptor classes was optimized with respect to the amount of light available from the optical system. The calculated receptor contrasts for each of the receptor-isolating stimuli used are indicated in Appendix A.

2.3. Optical system

Fig. 1 shows a schematic of the optical system used to generate stimuli in all of the experiments described below. The light source was a 1000-W xenon arc lamp operated at 980 W with a d.c. power supply. Conventional optics were used to focus and collimate the light (via the use of doublet-achromat lenses) to create three independent light channels. Light from each channel was rendered monochromatic by use of narrow-band interference filters (Ditric) or holographic grating monochromators (Instruments SA, V-20), each of which produced half-height band passes of < 8 nm. The radiance of light coming from each channel was controlled by the use of calibrated neutral density filters and neutral density wedges, which were placed in collimated and focused beams, respectively. Beams from channels 1, 2 and 3 were brought to a common focus at a sectored mirror that was used to produce square-wave counterphase alternation (7.5 Hz) between a mixture of light from channels 1 and 2 (combined by a beamsplitter) with that from channel 3. After passing through a common field-stop, the light was focused onto a computer-driven neutral density wedge (≈ 2 log unit range, with spectrally-flat absorbance over the range of wavelengths used). The light from all three channels was then rear projected onto a screen inside a dark, electrically-shielded chamber where the subject was seated. During testing, subjects were continuously viewed with an infrared illuminating and video camera system so that recording could be temporarily interrupted if the subject moved, blinked, or turned away from the stimulus.

2.4. Calibrations

Radiometric calibrations were made with a P-I-N-10 silicon photodiode and linear readout system (United Detector Technology, Optometer 81) which itself was calibrated by standards traceable to the National Institute of Standards and Technology. This photodiode was also connected to an oscilloscope to calibrate flicker frequency. Spectroradiometric calibrations were obtained with a spectroradiometer (Photo Research, Model PR703-A) that was also used to confirm photometric measurements ordinarily made with a Minolta luminance meter (LS-100).

2.5. Recording system

Fig. 1 shows a schematic of the recording system used in the present experiments. Bipolar VEPs were recorded with conventional EEG electrodes placed 1 and 3 cm above theinion for infants and 2 and 8 cm above theinion for adults. A ground electrode was placed either on the forehead or the earlobe. Inter-electrode impedance, measured with a Grass Impedance Meter (model EZM5B), was $\leq 5000 \Omega$ for the adults and $\leq 15000 \Omega$ for the infants, typical levels for adults and infants [9,11]. The EEG signal was led first through an isolation amplifier (Analog Devices, Model 273K) and then into a battery-powered pre-amplifier (Princeton Applied Research, Model 113). The nominal gain of the pre-amplifier was 10000 and the low and high frequency roll-offs were set at 3 and 10 Hz, respectively. Both the isolation and the pre-amplifier were in the shielded chamber with the subject. The amplified and filtered EEG signal was monitored on an oscilloscope and passed to a 2-phase lock-in amplifier or vector voltmeter (Ithaco, Model 393). The vector voltmeter [31] was synchronized to the sectored-mirror that provided the 7.5 Hz counterphase flickering stimulus so that the phase-independent amplitude of the VEP could be extracted (4 s time constant) at frequencies of interest. Because the vector voltmeter extracts the VEP in real time, continuous changes in amplitude could be plotted as a function of the mean luminance of the stimulus as shown by the X - Y plot in Fig. 1. Preliminary measurements¹ demonstrated that the amplitude of the fundamental component of the VEP was greater than the second harmonic, under our testing conditions.

¹ In a separate group of subjects, measurements were obtained at 7.5, 8.25 and 15 Hz (the fundamental, 10% above and the second harmonic, respectively) in response to the 7.5 Hz L-cone isolation stimulus. The signals generated in bands other than that centered at the fundamental were small and uncorrelated with the stimulus luminance level. The amplitude in a band centered at 7.5 Hz in response to a ramped increase of a 570 nm light with no flicker over a 30-s period also yielded responses that were no greater than the level of noise.

2.6. Procedure

No dark adaptation period was used. After the electrodes were positioned, the infants were seated on a parent's lap 32 cm from the stimulus in the shielded chamber, as shown by Fig. 1. Adult subjects were positioned 32 cm away from the stimulus via a chin and forehead rest. For both infants and adults, VEPs were measured in response to three different receptor-isolating stimuli (rod, M- and L-cone isolation). The amplitude of the fundamental component of the VEP was measured while continuously increasing the mean luminance of the receptor isolating-stimulus for 30 s, over a 2 log unit range. For all subjects tested, VEP responses were obtained for a luminance-modulation condition (570/dark flicker) to insure that a VEP flicker response could be obtained.

Digitized VEP records obtained from each subject in response to each condition were individually averaged to generate a mean response function to each receptor-isolating condition for each subject. Incomplete VEP records were not included in the means. An incomplete record was obtained if the infant looked away from the stimulus for periods exceeding 5–10 s. As previously mentioned, if the observer looked away or closed his/her eyes, recording could be temporarily interrupted. If the infant regained fixation within a relatively short period of time, recording was resumed when the VEP amplitude reached the pre-interruption level. For each adult observer, four to six individual VEP records were obtained for each receptor-isolating condition, while one to seven individual VEP records were obtained from each infant.

3. Results

Fig. 2 shows mean VEP data obtained from three adult subjects. VEP amplitude (in microvolts) is plotted as a function of the mean luminance of the receptor-isolating stimulus. Panels A, B and C show mean functions collected from a color-normal female, a male protanope, and a male deutanope, respectively. Also shown are the mean functions of each observer in response to the luminance-modulation condition (solid-diamond function). It should be noted that all three of these observers demonstrated reliable responses to pure luminance modulation.

The color-normal individual (panel A) demonstrates strong VEP responses to each of the receptor-isolating stimuli, as expected given that this individual possesses all receptor classes tested. The saturation of the VEP response at higher intensities is due to instrument saturation. The protanope, on the other hand, produces a strong response to M-cone isolation and a somewhat smaller response to the rod-isolation condition with

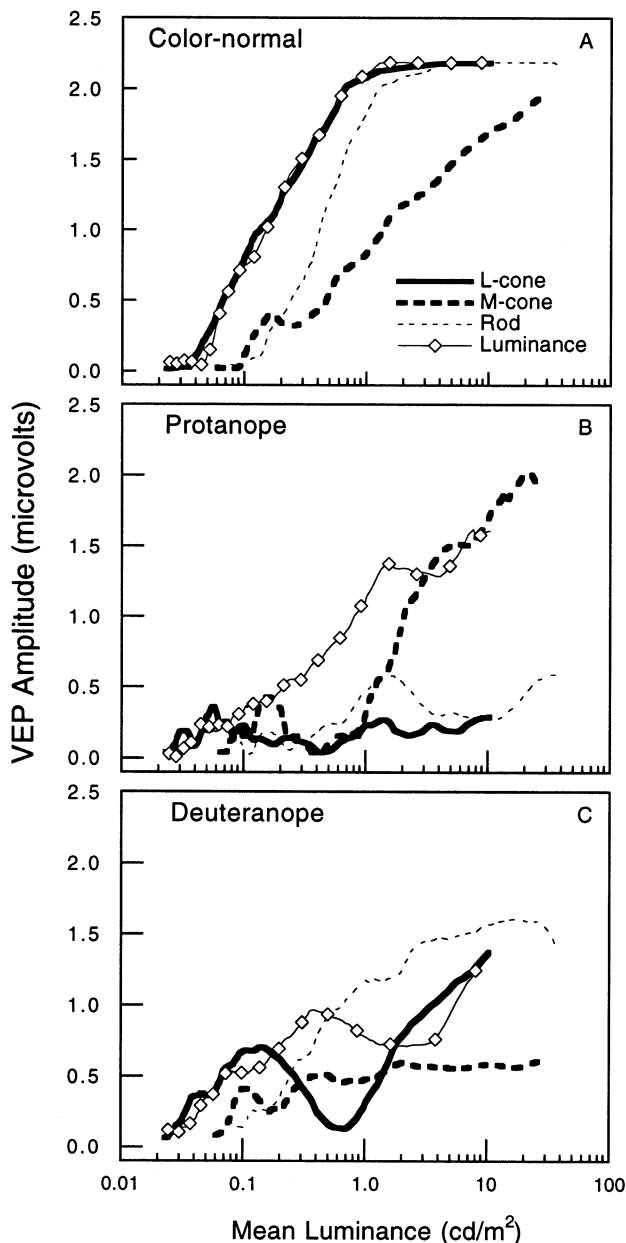


Fig. 2. VEP amplitude (microvolts) is plotted as a function of the mean luminance of the L- (bold line), M-cone (bold dashed line), rod isolating stimuli (dashed line) and a luminance-control condition (diamonds). Panel A shows data obtained from a color-normal adult; panel B shows data from a protanope; panel C shows data from a deuteranope.

little or no response to the L-cone isolation condition². Conversely, the deuteranope produces a relatively strong response to L-cone isolation and to rod-isolation

² For this observer, the response to the luminance condition is quite strong at luminance levels for which there is no response under the other receptor-isolation conditions. This is likely due to the fact that the luminance condition provided 100% contrast modulation while the other receptor-isolation conditions provided modulation at < 40% contrast.

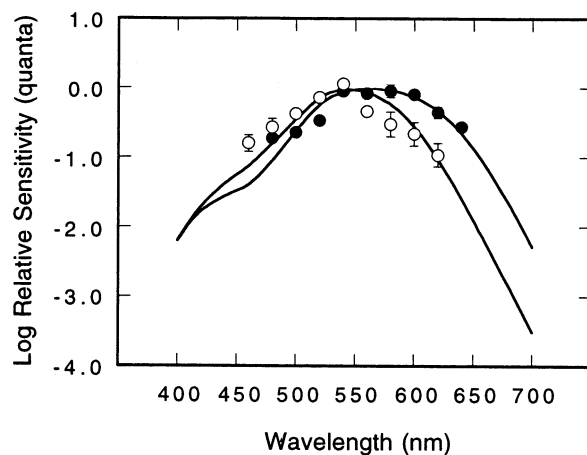


Fig. 3. Log relative sensitivity (quanta) is plotted as a function of wavelength (nm). This figure shows psychophysically derived action spectra obtained from a color-normal female adult under conditions of M- (open circles) and L-cone (filled circles) isolation, normalized to the Smith and Pokorny [38] M- and L-cone spectral sensitivity curves, respectively. Error bars denote the standard deviation of the mean.

with a shallower response to the M-cone condition. This observer's VEP response under M-cone isolation is not completely flat as might be expected from an individual who possesses no M-cones. One possible explanation is that this observer is not a complete deuteranope even though by standard tests he appears to be. Another possible explanation is that we have not perfectly silenced his L-cones due to a potential photopigment polymorphism [32,33].

These data help to confirm that the stimuli do in fact isolate the responses of the M-, L-cone and rod receptors. Throughout testing, a large degree of variation was noticed in the amplitudes generated to rod-isolation. This might be explained by the observation that the VEP response is generally dominated by signals originating in the fovea [34–36]. A second possible factor might be that rod modulation is not in the optimal range for rod excitation. For instance, the rod-isolation condition generates a contrast of 0.38, while under optimal conditions the Weber fraction for the rods is no smaller than 0.2 [37]. Third, dark adaptation was not controlled in the present study. Lastly, rod responses may be saturating with increases in the luminance of the stimulus.

As an additional test for the efficacy of our receptor-isolation conditions, psychophysically-derived action spectra were collected from the color-normal adult observer in response to the M- and L-cone isolating stimuli. The observer's task involved adjusting the intensity of a superposed monochromatic field (500–660 nm) to just eliminate flicker in the receptor-isolating field. These data are shown in Fig. 3. Log relative sensitivity is plotted as a function of wavelength. Data obtained under conditions of M-cone (open circles) and

L-cone (filled circles) isolation are normalized, using a least-squares criterion, to the Smith and Pokorny M- and L-cone fundamentals [38]. These data are in good agreement with standard M- and L-cone fundamentals, consistent with a previously reported study using similar methods [39].

3.1. Infant VEPs

Fig. 4 shows individual (dashed functions) and mean (bold function) VEP response functions obtained from one 8-week-old subject for luminance modulation. VEP amplitude (microvolts) is plotted as a function of the mean luminance of the stimulus. Individual responses in this case vary by about a factor of two in their position along the abscissa. In general, the response to luminance modulation is quite strong for all subjects as would be expected given that the luminance condition modulates all receptor classes operating in the Rayleigh region, at a high contrast. After the VEP response to luminance modulation was obtained, VEP response functions were obtained for each of the receptor-isolating stimuli. Panels A, B and C of Fig. 5 show individual (dashed functions) and mean (bold function) response functions from the same 8-week-old infant for the L-cone, M-cone and rod-isolating stimuli, respectively. VEP amplitude is plotted as a function of the mean luminance of the receptor-isolating stimuli. On average, this infant demonstrates an increase in VEP amplitude as the intensity of the receptor-isolating stimulus is increased. Assuming that the VEP response is driven by an individual receptor type (all others properly silenced), increases in VEP amplitude indicate the presence of the given receptor class.

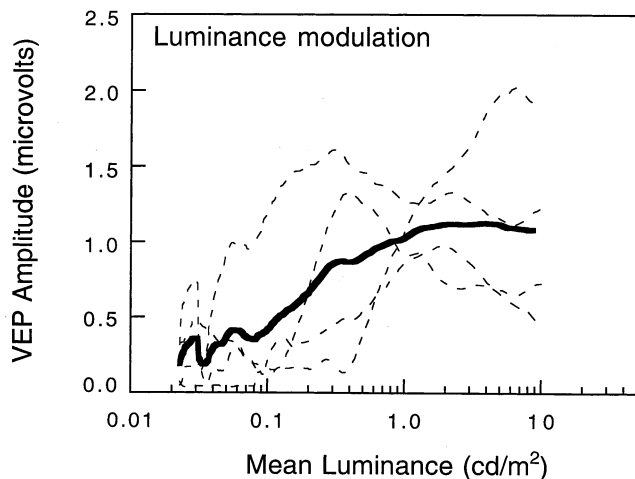


Fig. 4. Individual (dashed lines) and mean (bold line) response functions obtained from a 2-month-old infant in response to the luminance modulation condition are plotted. VEP amplitude (μV) is plotted as a function of the mean luminance of the stimulus.

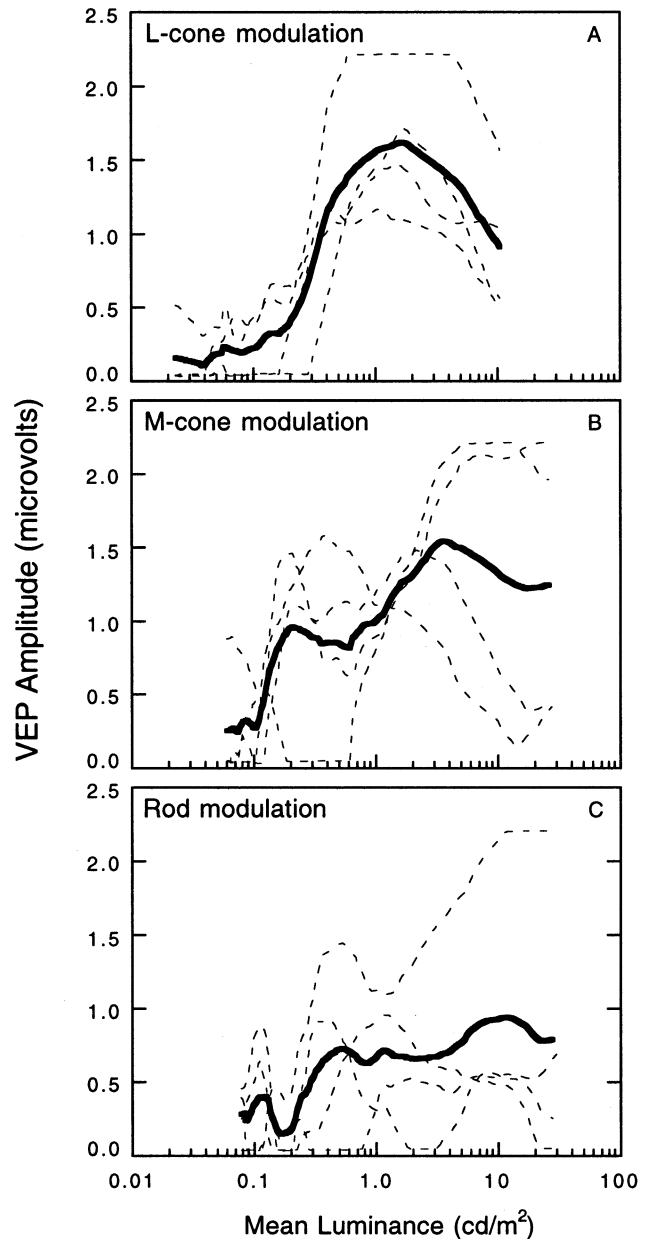


Fig. 5. Individual (dashed lines) and mean (bold line) VEP response functions obtained from a 2-month old infant in response to the L-cone, M-cone and rod-modulating stimuli are plotted in panels A, B and C, respectively. VEP amplitude (μV) is plotted as a function of the mean luminance of the stimulus.

Fig. 6 shows mean VEP data obtained from 12 8-week-old infants in response to each of the receptor-isolating stimuli. VEP amplitude is plotted as a function of the mean luminance of the L-, M- and rod-isolating conditions in panels A, B and C, respectively. Each thin function represents the mean response from an individual infant to the corresponding isolation condition. The solid bold function represents the mean VEP response obtained from the adult trichromat (Panel A, Fig. 3) under the respective conditions. In nearly all cases, the mean VEP amplitude in infants increases as the mean

luminance of the stimulus increases. Notice, however, that there is considerable variability between observers in the absolute amplitude of their VEP response, as expected from previous research. These data suggest that both M- and L-cones are functioning as early as 8-weeks.

Fig. 7 shows data obtained from 11 4-week-old infants. Panels on the left represent the mean VEP amplitude functions (microvolts) obtained from a color

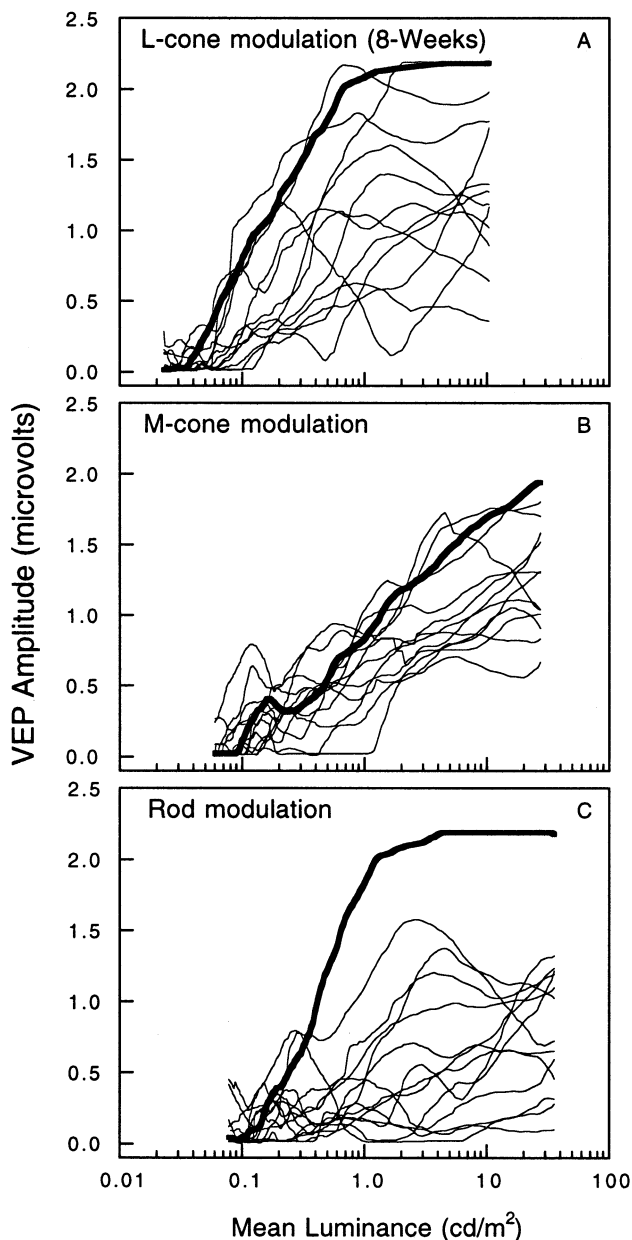


Fig. 6. VEP amplitude (μV) is plotted as a function of the mean luminance of the M-cone (Panel A), L-cone (Panel B) and rod-isolating stimuli (Panel C) for 12 8-week-old infants. Thin functions represent mean VEP data obtained from individual infants while the bold function represents the mean VEP response function obtained from a color-normal adult (see also Fig. 3, panel A) in response to each of the respective receptor-isolating stimuli.

normal (bold function), 2 dichromatic adults (dashed functions; protanope and deuteranope in the L- and M-cone isolation panels, respectively) and individual infants (thin functions). As presented here, infants at 4-weeks display no obvious increases in amplitude with increases in stimulus luminance. Their responses are no greater than those obtained from dichromatic adults. Such a result could arise if the M- and L-cones were not yet functional at this age. An alternate possibility is that the visual signals are simply smaller at this age, compared with older infants. To test this latter hypothesis, we supposed that if there is simply an overall reduction in response, then normalization by the response to luminance will render the infant and normal adult curves more similar. However, if one or both cone classes are not present, then such a normalization should produce curves that vary randomly about a mean value, as do the functions obtained from adult color defectives for the cone class each lacks. Normalization was performed by calculating each observer's mean voltage response to the luminance modulation condition and then dividing each of their mean functions for L-, M-cone and rod modulation by this factor. The normalization factor obtained indicates that, on average, the responses of 4-week-old infants are about four times less than 8-week-old infants. As shown on the right in Fig. 7, normalized VEP amplitude increases as the mean luminance of the receptor-isolating stimulus increases for both the color-normal adult and the infant subjects, but not for the dichromatic observers. On this scale, these data resemble those obtained at 8-weeks and in adults, suggesting that M- and L-cones are functional as early as 4-weeks.

Fig. 8 compares the mean of the normalized responses of the 4-week and 8-week-olds with the color normal and dichromatic adult responses. On average, the 4- and 8-week responses covary with intensity in the same fashion as a color-normal adult and not as a dichromat. Surprisingly, after normalization, 4-week-old infants show somewhat stronger responses to the rod-isolation condition than do the 8-week-old infants. This might be explained by differences in fixation behavior with age, with younger infants having poorer fixation abilities, and thus giving rise to more peripheral stimulation.

4. Discussion

One potential criticism of this study is that it is difficult to know for certain, based on the present results, whether or not individual receptor classes were indeed being isolated in infants. A critical assumption of the silent-substitution technique is that the spectral sensitivities of the photoreceptors/mechanisms held constant be known. If the stimuli are not properly

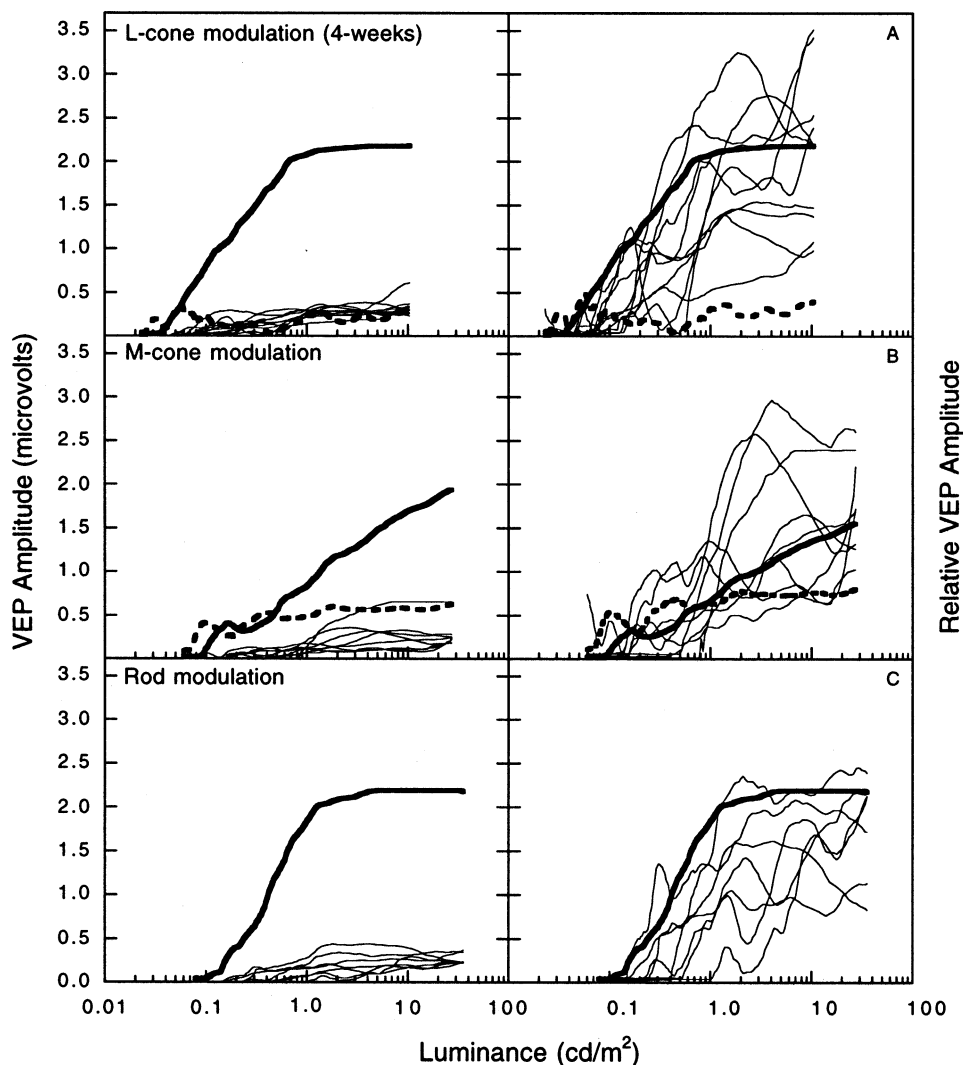


Fig. 7. In the left panels, VEP amplitude (μV) is plotted as a function of the mean luminance of the M-cone (Panel A), L-cone (Panel B) and rod-isolating stimuli (Panel C) for 9 4-week-old infants. Each thin function represents mean VEP responses obtained from individual infants. The bold functions represent the mean VEP response functions obtained from a color-normal adult (solid function), a protanope (dashed; panel A) and a deutanope (dashed; panel B) in response to each of the respective receptor-isolating stimuli. The right panels show the relative VEP amplitude (for both infants and adults) normalized by each subject's mean response to luminance modulation.

equated for these photoreceptors, the interpretation that the receptor class probed is functional will be compromised. For example, if infants expressed a set of juvenile photopigments unlike those of a normal adult, as in some species of fish [40,41] then none of the conditions derived from standard adult curves would be accurate. More subtly, recently identified polymorphisms in the M- and L-cone photopigments may challenge the utility of the silent-substitution technique for use in color vision research as they imply that the spectral sensitivities of some individual observers are likely to differ systematically from average normal curves [32,42]. The extent to which these factors may disrupt receptor-isolation conditions based on average curves is not known. To resolve this issue definitively will require the measurement of action spectra for each

of the receptor-isolating stimuli in order to verify that its spectral sensitivity is not inconsistent with that from an adult. We pursue this issue in a subsequent paper [43].

We have attempted to verify the efficacy of our receptor-isolation conditions by showing that protanopic and deuteranopic observers do not demonstrate VEP responses under the isolation conditions that correspond to the receptor classes that they are missing. We have also shown that a color-normal observer demonstrates strong responses to all the receptor-isolation conditions, as they should. The results obtained from these three observers suggest, at least for adult observers, that we have succeeded in modulating the responses of individual receptor classes. Additional support for the efficacy of the receptor-isolating conditions

with infants comes from data obtained from four male infants, not presented here [44,45], who were classified as suspects for a color deficiency based on weak responses to only one of the cone-isolating stimuli, as well as subsequent verbal reports from their parents of a maternal history of color deficiency. If the technique can successfully identify and distinguish infants who lack M- or L-cones from normal infants, then the conclusion that the signals recorded do arise from modulation of individual receptor classes is strengthened.

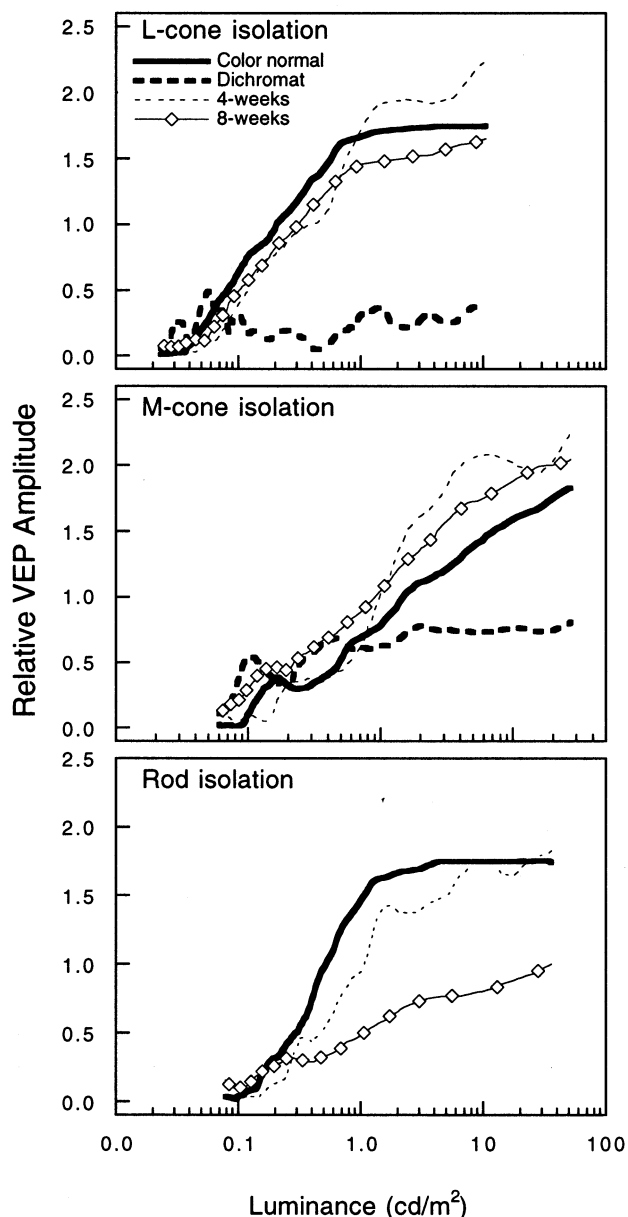


Fig. 8. Means of the normalized VEP amplitudes are plotted as a function of the mean luminance of the L-cone (upper), M-cone (middle) and rod-isolating stimuli (lower) for a color normal (solid/bold), a protanope (dashed/bold; upper), a deuteranope (dashed/bold; middle), all 4-week-olds (dashed) and all 8-week-olds (diamonds). The normalization procedure is described in the text.

While it may be reasonable to assume that the photopigments mediating infant vision are the same as those mediating adult vision (within the normal range of variation), it is not reasonable to assume that the spectral sensitivities of infant photoreceptors are the same as an adult. For example, preretinal screening and photopigment optical density differences between infants and adults affect the sensitivity of the photoreceptors. The reduced lens density [7,28,29] and macular pigment density [46–48] of the infant eye elevate short-wave sensitivity relative to adults. For wavelengths greater than 540 nm, however, these age-related effects are minimal, and thus, not likely to upset the efficacy of our receptor-isolation conditions.

In addition to preretinal filtering differences between infants and adults, the outer segments of foveal cones in the newborn are much shorter than those in an adult [1], presumably resulting in reduced photopigment optical density. The spectral sensitivity of the photoreceptors depends, in part, on photopigment optical density. If photon absorption in the cones is assumed to follow the Beer-Lambert Law [37], then the shorter outer segments of infant cones will result in a narrowing of the photopigment absorption spectrum, as a result of reduced self-screening. If one assumes that infant receptor sensitivity is equivalent to the extinction spectrum of adult foveal cones then one can evaluate the potential residual contrast that may be generated in putatively nulled receptor classes in the infant eye. We have made such calculations elsewhere [25] and concluded that if the difference between the peak optical densities of infant and adult photoreceptors exceeds 0.2, then such residual receptor contrasts should be detectable. Observations by a dichromatic observer in the same study confirmed that mismatches of this magnitude produced visible flicker. Nevertheless, the same mismatched stimuli did not produce sufficient contrast to generate a VEP signal that increased systematically with the mean luminance. In other words, the observer would not have been mislabeled as a trichromat. It may be that the noise level of the technique used here renders it insensitive to mismatches of this magnitude.

Two additional criticisms can be leveled at the technique that we used. First, since the pupil size is uncontrolled over the course of the luminance sweep during which the VEP is measured, differential spectral absorption by the lens and the wavelength dependence of the Stiles-Crawford effect [49] could upset the silent-substitution conditions. As already remarked, the differential effects of the lens are likely to be small with the wavelength triples used. If a component of the Stiles-Crawford effect due to the rods or one class of cones produced a detectable mismatch of the lights, we would have seen a significant response from the protanope and deuteranopic observers to L- and M-cone isolation conditions, respectively. We did not, however,

suggesting that such an effect, if present, is small. Second, no dark adaptation period was used and we did not systematically wait for a full recovery after each luminance sweep of the stimulus. The final luminance levels of each condition were not sufficiently high so as to bleach significantly the photopigments and change their spectral sensitivities. Thus, any differential adaptation of photoreceptors or post-receptor systems could not upset the quantum matches of the flickering stimuli for two of the three receptors. The effects of adaptation would then be limited to rendering the observers less sensitive overall to the lights or reducing their maximal response. We saw no tendency for the peak responses to be diminished on successive runs. Furthermore, no part of the analyses depends on the absolute position of the curves along the luminance axis.

From previous discrimination studies [19,20], we can surmise that two, and probably three, photoreceptor mechanisms are functional in early infancy. The ability of infants to make Rayleigh discriminations limits the identities of two of these mechanisms to the long wavelength part of the spectrum (M-cones, L-cones or rods) while tritan discriminations are suggestive of either rods or S-cones. Further interpretation of these data, however, is not possible principally because rod involvement was not definitively excluded. Moreover, the results of Clavedetche et al. [23] explicitly show that rod responses are not only possible, but likely to contribute to discrimination under stimulus conditions often used in testing infants.

In the present experiments, we tried to control possible rod contributions so as to make inferences specifically about M- and L-cone functioning. The results of the present experiment suggest that the signals generated by M- and L-cones are sufficiently strong to reach the cortex as early as 4 weeks of age. Nevertheless, the signals at 4 weeks appear about four times weaker than those at 8 weeks. This difference in response might be associated with the 2–3-fold increase in temporal contrast sensitivity observed between 1 and 3 months of age [50,51]. It is interesting to note that several studies of chromatic VEPs have failed to detect VEP signals to chromatic stimuli before 5–8 weeks [52–55].

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Appendix A. Calculation of the silent-substitution conditions

Let the relative spectral sensitivities of the i th photopigment ($i \in \{L, M, \text{rod}\}$) at each of the three wave-

lengths $\lambda = 540, 570$ and 610 nm be a vector $\sigma_i = (\sigma_{i,540}, \sigma_{i,570}, \sigma_{i,610})$, and let $\mathbf{r} = (r_{540}, r_{570}, r_{610})$ be a vector of radiances corresponding to the peak amplitudes of the three components of a square-wave temporal modulating field composed of a mixture of three wavelengths. Here, a negative component indicates a 180° phase shift of a component. The net excitation, ϕ_i , of the i th photoreceptor by the temporal modulation corresponding to a radiance vector, \mathbf{r} , at these three wavelengths is:

$$\phi_i(\mathbf{r}) = \sigma_i \cdot \mathbf{r} = \sigma_{i,540}r_{540} + \sigma_{i,570}r_{570} + \sigma_{i,610}r_{610}$$

We seek radiance vectors, r_j ($j = 1, 3$), such that:

$$\phi_i(r_j) = \begin{cases} 1; & i = j \\ 0; & i \neq j \end{cases} \quad (1)$$

i.e. that yield a net output of zero from two photoreceptors and not the third. Such combinations of radiances modulate the output of the i th photoreceptor in isolation. By making each vector of spectral sensitivities the row of a matrix, \mathbf{S} , it is easy to see that the columns of its inverse, \mathbf{S}^{-1} , satisfy condition (1) and thus give the relative radiances for each of the receptor-isolating conditions. The Smith and Pokorny fundamentals were used for the values of the M- and L-cone spectral sensitivities and the CIE V'_λ curve, tabled by Wyszecki and Stiles [37], as an estimate for the rods. The receptor modulation, m_i , of the i th photoreceptor produced by its isolation condition is calculated to be:

$$m_i = \frac{\sum_{\lambda} \sigma_{i\lambda} r_{i\lambda}}{\sum_{\lambda} |\sigma_{i\lambda} r_{i\lambda}|}$$

which yields values of 0.23, 0.37 and 0.38 for M-cones, L-cones and rods, respectively.

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