

29. Detecting colour vision deficiency in 4- and 8-week-old human infants

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Abstract

Using silent substitution we have measured visually evoked potentials (VEPs) from 4- and 8-week-old infants to L and M cone-isolating stimuli, while the rod response was nulled. Data are shown from six male infants, four of whom were classified as candidates for defective colour vision. These results demonstrate that infants as young as 4 weeks show clear responses to L and M cone-isolating stimuli. This technique may be useful in identifying infants with sex-linked colour deficiencies.

Introduction

It is generally accepted that infants as young as 2 months of age possess some ability to discriminate stimuli on the basis of wavelength. For example, previous studies have demonstrated the ability of infants as young as 2 months to make both tritan (Varner *et al.*, 1985) and Rayleigh discriminations (Hamer *et al.*, 1982; Packer *et al.*, 1984). Based on the results of these studies, one might conclude that all three cone types (S, M and L cones) and the post-receptoral circuitry necessary for wavelength discrimination are functional by this age. However, under the conditions in which infants are often tested, it is difficult to be certain which mechanisms are involved. The use of large fields, poorly controlled fixation, and low luminance levels all favour contributions from rod signals. Rod signals combined with one functional cone type may explain some of the Rayleigh discriminations reported from infants.

In an attempt to verify the functioning of both L and M cones in early infancy, we used 'silent substitution' (Estévez and Spekreijse, 1982) which allows one to isolate the response of a single receptor type while maintaining a constant output from the other receptors. If this method is valid it should allow one to examine the ontogenesis of both normal and abnormal visual mechanisms. We have collected data from three adults and 22 infants to M and L cone-isolating stimuli. The data presented here are from six male infants, four of whom yielded weak responses to only one of the cone-isolating stimuli; these were subsequently classified as candidates for defective colour vision, based on

verbal reports from their parents of a familial history of colour deficiency on the maternal side of the family.

Methods

Three adults (1 with normal colour vision, 1 protanope, and 1 deuteranope) and six full-term infants (three 4-week-olds and three 8-week-olds) served as subjects. Each participated in 1–4 testing sessions that each lasted approximately 45–60 min.

To simplify the stimulus, we worked in the Rayleigh region of the visible spectrum (wavelengths ≥ 540 nm), thereby minimizing any S cone contribution. Assuming that there are three functional photoreceptor types (rods, L and M cones) operating in the Rayleigh region, a variable mixture of three lights is needed to silence any two of them at a time. Our three-primary stimulus, presented in a 6° disc, consisted of a 570 nm light alternating in square-wave counterphase at 7.5 Hz with a mixture of 610 and 540 nm. The intensity of the 570 nm light and the ratio of the 610/540 nm mixture can be set so as to modulate only the L or M receptors. For example, L cone isolation results when the number of quantal absorptions produced by the 570 nm light is the same as that produced by the 610/540 nm mixture for the rods and M cones, but not for the L cones. In two separate conditions (L and M 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 sec, over a 2 log unit range.

Electrodes were placed over the occipital cortex with a ground on the forehead. The EEG signal was amplified and filtered by passing it through an isolation amplifier and then into a battery-powered pre-amplifier. The amplified and filtered EEG signal was then fed into a vector voltmeter, which selectively amplifies a particular frequency component of the EEG signal. Our vector voltmeter was synchronized to the 7.5 Hz modulation of our stimulus, in order to selectively amplify the phase-locked amplitude of the fundamental component (7.5 Hz) of the VEP signal. Because the vector voltmeter extracts the VEP in real time, continuous changes in amplitude of the 7.5 Hz VEP response could be plotted as a function of the intensity of the receptor-isolating stimulus.

To estimate the level of background noise and artefacts during testing, separate VEP measurements were made at a frequency 10% above the fundamental (8.25 Hz) and at the second harmonic in 15 full-term infants between 1 and 2 months of age. The amplitude of the VEP response was maximal at the fundamental frequency.

Results

Figure 1 shows mean VEP data obtained using L and M cone-isolating stimuli

from two 4-week-old infants and two 8-week-old infants. VEP functions represent the mean of 2–8 VEP records (the total number of VEP records collected from each infant under each condition). Mean VEP data have been collected under both L and M cone isolation from 18 candidate colour-normal infants (see Knoblauch *et al.*, 1994; Werner *et al.*, 1995). Candidates for normal colour vision were classified based on strong responses to both L and the M cone isolation and no reported family history of colour deficiency. The data for two of these infants are presented in the upper panels of Figure 1. Data from two infants who were classified as candidate protans are shown in the lower panels. Both these infants showed a stronger response to the M cone-isolating than to the L cone-isolating stimulus. For both of these infants the VEP responses obtained using the L cone-isolating stimulus were no greater than

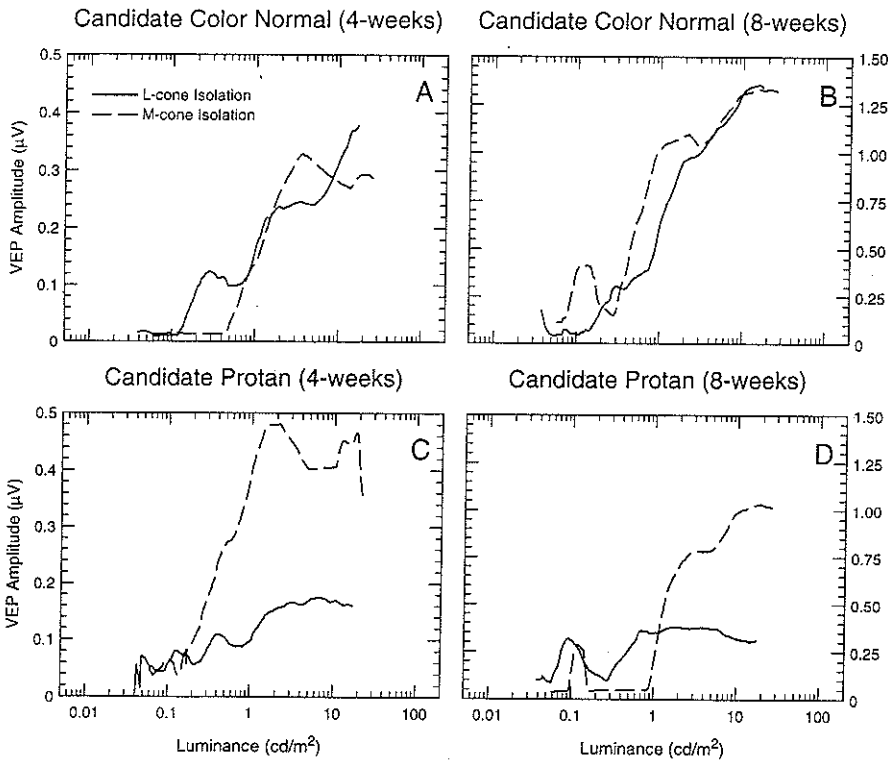


Fig. 1. Mean VEP records obtained from two 4-week-old (A, C) and two 8-week-old (B, D) infants. The upper and lower panels present data from candidate colour-normal infants and candidate protans, respectively. Solid and dashed functions represent mean VEP functions obtained using L and M cone-isolating stimuli, respectively. Amplitude (μV) of the VEP response is plotted as a function of the luminance (cd/m^2) of the receptor-isolating stimulus. Note that the amplitudes measured from 4- and 8-week-old infants are scaled differently.

the noise level. Two other infants (one 4-week and one 8-week, data not shown) were classified as candidate deuterans on the basis of a strong response to the L cone-isolating stimulus with little or no response to the M cone-isolating stimulus. The parents of all four of these infants subsequently reported a familial history of colour deficiency on the maternal side. In addition, red/green flicker photometric data were obtained from the mother of the 4-week-old candidate protan. Females, heterozygous for a protan deficiency, often demonstrate Schmidt's sign, a reduced sensitivity at longer wavelengths (Schmidt, 1955). A 530 nm standard was alternated at 15 Hz in square-wave counterphase with a 650 nm test field, in a 2° disk. The intensity of the test field was varied, using the method of adjustment, to minimize flicker. Consistent with Schmidt's sign, the sensitivity of the mother of the 4-week-old protan suspect fell between the normal and protan luminosity function. Her flicker minimum required more than twice as much light at 650 nm as two presumably non-heterozygous females tested at the same time.

Discussion

One necessary assumption of this approach is that the spectral sensitivities of the photoreceptors are known. It is reasonable to assume that the spectral sensitivities of infant photoreceptors are the same as those of the adult in the Rayleigh region, with the exception of differences that arise from variations in photopigment optical density and pre-retinal absorption. The shorter outer segments of infant cones and consequent reduced photopigment optical density manifests itself as a narrowing of the photopigment absorption spectrum. However, we have shown earlier (Knoblauch *et al.*, 1996), that over a range of cone optical densities that might be reasonably assumed for human vision, an incorrect assumption is not likely to upset the receptor isolation conditions enough to produce a false-positive VEP response.

In order to validate further our receptor isolation conditions we performed several control experiments using both colour-normal and dichromatic adult observers. Individuals with normal colour vision should demonstrate a strong VEP response to both the L and M cone-isolating stimuli, whereas dichromats should demonstrate a strong response to only one of the isolating stimuli, giving a negligible response to the isolated mechanism that they lack (i.e. protanopes should not respond to L cone isolation and deuteranopes should show no response to M cone isolation). Figure 2 shows mean VEP data collected from a colour-normal observer to L cone and M cone-isolating stimuli. VEP data from a protanope and a deuteranope are also shown. VEP functions represent the mean of 2–7 records. The colour-normal adult showed a strong response to both the L and M cone-isolating stimuli. The protanope, on the other hand, produced a strong response only to the M cone isolation condition, and the deuteranope produced a strong response only to the L cone isolation condition.

While the measurements made with dichromatic individuals provide strong

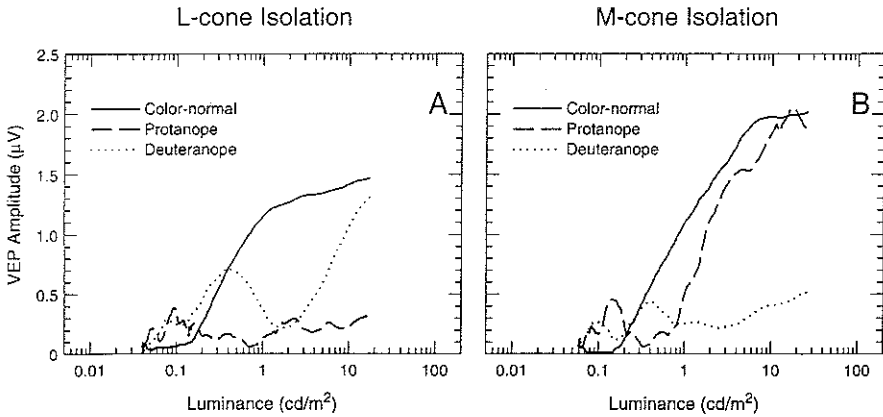


Fig. 2. Mean VEP records obtained from an adult with normal colour vision (bold function), deuteranope (dotted function) and protanope (dashed function) for L (A) and M (B) cone-isolating stimuli, respectively. Amplitude (μV) of the VEP response is plotted as a function of the luminance (cd/m^2) of the receptor-isolating stimulus.

support for our isolation conditions, a more direct test would be to measure the action spectra of isolated mechanisms. For example, the action spectrum measured under L cone isolation should correspond to the L cone fundamental and that measured under M cone isolation should correspond to the M cone fundamental. Psychophysically derived action spectra, not shown here, collected from two colour-normal adults using L cone- and M cone-isolating stimuli are in good agreement with the Smith and Pokorny L and M cone fundamentals, consistent with a previously reported study using similar methods (Estévez *et al.*, 1975).

Compared with previous methods used to study the ontogeny of visual mechanisms, the silent substitution technique shows considerable promise. The VEP data obtained using this technique demonstrate that infants (with the exception of those classified as candidate colour defectives) as young as 4 to 8 weeks of age show clear responses to L and M cone-isolating stimuli. These results are consistent with previous studies using different methods (Hamer *et al.*, 1982; Packer *et al.*, 1984). However, because these previous studies did not adequately control for rod response, it is difficult to be sure which mechanisms are functional in early infancy. While holding rod output constant we have demonstrated that L and M cones are functional and that their signals can be measured in the cortex as early as 4 weeks of age. In addition, this technique appears to be able to identify sex-linked colour deficiencies in early infancy.

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