Latency Measures of Pattern-Reversal VEP in Adults and Infants: Different Information from Transient P1 Response and Steady-State Phase

Jin Lee, Deirdre Birtles, John Wattam-Bell, Janette Atkinson, and Oliver Braddick

PURPOSE. Temporal properties such as the peak latency of pattern-reversal (PR) visual evoked potentials (VEPs) have been found to be a sensitive indicator of visual development. Latency can be assessed from the slope of a plot of phase against temporal frequency (TF) for steady state VEP measurements as well as from the transient P1 peak. This study aimed to discover whether the two methods provide different information regarding early visual development.

METHODS. Developmental changes of the transient peak latency were tracked using low TFs of one to four reversals per second (r/s) and a spatial frequency (SF) of 0.24 cycles per degree (cpd) in comparison with latencies calculated from the phase versus TF gradient in the range of 1 to 19 r/s. PR-VEP responses were recorded from 81 adults and 137 infants (ages 3.6–79 weeks).

RESULTS. Values of the calculated and transient peak latencies were similar in adults, but the calculated latency was statistically longer than transient peak latency in younger infants. Moreover, while the transient peak latency asymptoted to an adult value of 104 ms at approximately 15 weeks of age, the calculated latency did not asymptote until after 30 weeks.

CONCLUSIONS. In this study, the effectiveness of the phase-based method to calculate latency was confirmed. In infants, the rapid decrease of P1 latency may be due to the progressive maturation of conduction time in the afferent visual pathways, with the development of adult levels of phase-based calculated latency being due to the maturation of later cortical processing in infants. (Invest Ophtalmol Vis Sci. 2012;53:1306–1314) DOI:10.1167/iovs.11-7631

Visual evoked potentials (VEPs) are neural responses recorded from the surface of the scalp, synchronized with transitions in repeated presentations of a visual stimulus. Several studies have shown that peak latency is less variable than amplitude measures, both within and between subjects.1–6 Peak latency is also a sensitive indicator of visual development,7 which has been used for clinical evaluation of vision,8 and to study changes in attention,9 binocularity,9 acuity,10,11 spatial frequency (SF) sensitivity,12,13 and cortical aging.14 Latency has also been used for clinical evaluation of vision.15 Pattern or phase reversal (PR) stimulus presentation is commonly used to test responses to contrast changes. The stimulus is typically a checkerboard pattern or a sine or square wave in which the luminance of adjacent checks or stripes is periodically interchanged (180° phase change in a grating).16 A typical response has a positive peak, P1 (typically approximately 100 ms in adults), and two negative deflections—N1 and N2. P1 is reported to be generated in the occipital cortex,17,18 and is dependent on stimulus luminance, contrast,19 orientation, and spatial frequency.20

Previous studies on infant visual development have reported that the P1 transient peak latency for large checks decreases from 260 ms at birth to adult-like values approaching 100 ms at approximately 4 months.21–23 This rapid change may be attributed to various factors: retinal development, especially the cone photoreceptors24; progressive myelination of the optic nerve and radiation17,24; rapid cortical synaptogenesis; and maturation of synaptic transmission of the various parts of the visual pathways.8 It should be noted that the PR stimulus generates responses at the retinal level from on and off responses in the ganglion cells.26 PR-VEP is useful in demonstrating that contrast information has arrived at the cortex, but need not reflect any processing within the visual cortex.

Most studies measuring latency have been based on the time needed to produce the first prominent positive peak in the waveform—the ‘transient peak latency.’ However, this may be a problematic measure in developmental studies, given that the shape, number, and latency of peaks vary with age.14,21 To provide an alternative measure, the present study also measured latency indirectly. The phase of the steady state (SS)VEP was analyzed at two or more different temporal frequencies (TFs), giving a phase versus TF plot, whose slope provides a calculated value of apparent latency.10,26,27

Phase has been found to be a reliable measure between1,14 and within subjects,15 and is linearly related to peak latency8,11 and to the stimulus TF.14,21,26 However, because phase values cycle every 360°, a measurement of 75° can also correspond to 75° + 360° = 435°, and so on.1,10,28 To “unwrap” the phase, the sum of multiples of 360° must be subtracted from the subsequent phase, changing the slope and the calculated latency. Current literature offers no rigorous method for resolving this ambiguity. In previous studies multiples of 360° were subtracted to “sort [phase] over the whole TF range,”29 and “produce maximum orderliness,”15,16 or minimize the distance to the preceding point.1 The criteria used in the present study are described in the Materials and Methods section.

It should be noted that the gradient of phase with TF corresponds to the peak latency only if the temporal dynamics of the response can be modeled by a pure delay.1 Unlike P1 (or any other individual peak), the phase measurements reflect the entire time course of the VEP response. Thus an early component such as P1 will primarily reflect the arrival time at the cortex of the barrage of impulses, depending on the latency of retinal events, the transmission time from retina to cortex, and perhaps the...
initial dynamics of the cortical activation by this barrage. The phase-based measure can be expected to have an additional contribution from preceding and subsequent components of cortical processing. Comparison of the two measures, particularly in development, should reveal any differences in the maturation between different levels of processing in the visual pathway.

The present study investigates: the efficacy of the slope method to calculate a measure of VEP latency in adults and infants; the relation between calculated latency and P1 peak latency of the transient VEP; and the developmental courses of PR latency measures using both approaches.

MATERIALS AND METHODS

We used two types of VEP recordings: transient VEP and SS-VEP. In transient VEP, the brain's response returns to the resting state before the next stimulus, consequently producing a waveform with distinct VEP components. A TF of 2 Hz (4 reversals per second 1/r/s) or lower is used, allowing the brain's response to return to the resting state. Latency was determined from the P1 peak. In SS-VEP recording, the stimulus rapidly alternates at frequencies ≥4 r/s between two states. This generates a periodic neural response at the stimulus frequency and its harmonics, from which signal amplitude and phase at the stimulus frequency were computed using Fourier analysis.

Participants

Eighty-one adults were tested (median age, 21 years; range, 16–43 years) with normal or corrected to normal vision. Healthy full-term infants born within 14 days of their due date were recruited. One hundred thirty-seven infants (3.6–79.0 weeks) were tested (see Table 1). Twenty-five of these infants were tested at two different ages, six infants were tested at three ages, and five infants were tested at four different ages. The longitudinal data of the repeated sessions were analyzed separately from the cross-sectional analysis.

Stimulus

The stimulus was a sine wave grating, with a spatial frequency (SF) of 0.24 cycles per degree (cpd) (comparable to 81° of arc checks), oriented at 45°, and alternated with periodic 180° phase shifts (mean luminance 51 cd/m² contrast 0.95). To serve as a better comparison between pattern and orientation-VEP in subsequent studies, a sine wave grating was chosen rather than a checkerboard pattern. The grating was generated using the Lua scripting language (ver. 5.1; available at http://www.lua.org) running on a PC (Windows XP; Microsoft Corp, Seattle, WA), and presented on a CRT monitor (800 × 600 pixels, viewable area 323 × 240 mm [18.4° × 13.7°] at a 40 cm viewing distance) at 100 Hz frame rate. The display computer was coupled to a recording computer, PC (Windows XP).

VEP Recording

Transient VEP and SS-VEP. Three gold cup electrodes were used: one on the vertex, one 1 cm above the inion, and a ground electrode positioned high on the forehead, and signals recorded using a computer-based acquisition system (Espion; Diagnosys LLC, Cambridge, United Kingdom). Impedance was measured with an applied voltage at 1000 Hz and electrodes were adjusted until this was <10 kΩ. Signals were amplified (20,000×), band pass filtered between 0.5 and 30 Hz, and sampled at 1000 Hz. For each recording, 100 sweeps (two reversals per sweep) were averaged on the computer. Any sweeps containing signals >200 μV in amplitude were automatically rejected from the signal averaging as artifacts. To minimize onset effects, recording began a few seconds after the stimulus appeared. The order of TF used in testing was randomized to minimize any systematic adaptation effects.

In infants, a small noisy toy was shaken in front of the center of the computer screen to attract the infant’s attention. Recording was temporarily interrupted when subjects became inattentive or looked away.

Bandpass filtering potentially introduces phase changes in the recorded VEP signals. We empirically measured the phase response to an input signal and found that in the range 2–19.2 r/s, the phase shift was always <5°. At 1 r/s (a frequency used only with adult participants) the shift was 10°. We verified that an adjustment to the phase values at this frequency did not make any significant change to the calculated latency values. A photocell measurement of the monitor revealed a systematic software delay of 45 ms between the stimulus event at the middle of the computer screen and the recording cycle. Latency values presented have been corrected to take this into account.

Transient VEP. For adults, four TFs were tested: 1, 2, 3, and 4 r/s. For infants, three TFs were tested: 2, 3, and 4 r/s. The recording time required for 1 r/s proved generally too long to obtain statistically reliable results within the attention span of the infant group. Because each recording contained two complete cycles, the total recording epoch is 2 seconds for 1 r/s, 1 second for 2 r/s, 0.5 second for 4 r/s, and so on.

SS-VEP. For adults, SS-VEPs were recorded at each of the 12 different TFs: 1, 2, 3, 4, 6, 8, 9.6, 10.7, 12, 13.7, 16, and 19.2 r/s. For infants, SS-VEPs were recorded at each of the 7 different TFs: 2, 3, 4, 6, 8, 12, and 16 r/s. Fewer TFs were used due to infants’ attention span limiting available recording time.

VEP Analysis

The component of the response at the reversal frequency was extracted using Fourier analysis. The presence of a statistically significant response at a particular TF was tested using Moore’s test for the distribution of vector data, which yields the Mann-Whitney U statistic. This test determines the presence of a statistically significant response with a consistent phase across the run, as a whole, by taking the amplitude and phase measured at the reversal frequency within each sweep as a sample. The signal-noise ratio was calculated based on measurements of noise power in a band 1 Hz either side of the stimulus frequency. Any runs with P > 0.05 on the Mann-Whitney U test and/or a signal-noise ratio (SNR) <1.5 were discarded.

Both transient and steady state latency measures were corrected for the 45 ms software delay. ANOVA (multivariate and repeated measure [RM]) were then performed using statistical software (SPSS 14.0; IBM, New York, NY). All the following analyses were performed on infants with single visits only (one data point per child, unless indicated otherwise).

Transient Peak Latency

For the transient VEP latency, the time of the initial maximum positive peak was selected manually by placing a cursor on the most prominent positive peak for the low TF recordings (adults: 1, 2, 3, and 4 r/s;

<table>
<thead>
<tr>
<th>Age (wk) Tested</th>
<th>Transient</th>
<th>Calculated</th>
<th>Transient and Calculated</th>
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<tr>
<td>3.6–4.9</td>
<td>5</td>
<td>4</td>
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<tr>
<td>5–9.9</td>
<td>19</td>
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<td>13</td>
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<td>4</td>
</tr>
<tr>
<td>70–79.9</td>
<td>6</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
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<td>137</td>
<td>101</td>
<td>85</td>
</tr>
<tr>
<td>Adults total</td>
<td>81</td>
<td>74</td>
<td>78</td>
</tr>
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infants: 2, 3, and 4 r/s). Because each recording contained two complete cycles, two peaks were selected for each infant, and the average was used for subsequent analysis (see Fig. 1).

**Phase-Based Calculated Latency**

Phase values of the averaged signal components at the reversal frequency were measured, in the range 0° to 360°. As discussed above, an infinite series of phase values 360° apart are compatible with such a measurement. To choose the appropriate phase value, the difference between phase values at two adjacent frequencies was calculated. If the difference was positive, multiple(s) of 360° were subtracted from the phase value of the higher frequency until it became negative. Another 360° was then subtracted from the phase value of the next higher frequency.1,13 These ‘unwrapped’

![Graphs showing PR-VEP waveforms at different frequencies](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933464/)
Two Latency Measures of PR-VEP in Adults and Infants

RESULTS

Proportion of Participants Giving Significant VEP Responses

Among the 81 adults tested, significant transient responses were obtained from 75 individuals (92.6%), calculated latencies were obtained from all 81 (100%), and data for both transient and calculated latencies were obtained for 71 individuals (87%). Among the 137 infants aged 3.6–79.0 weeks, significant transient responses were obtained from 101 (73.7%), calculated latencies from 85 (62.0%), and data for both were obtained from 75 individuals (92.6%). Among the 81 adults tested, significant transient responses were available versus more than two TFs indicated no significant difference between the two methods \( F_{1,112} = 0.1; P > 0.1 \) nor any significant interaction between age and method \( F_{1,112} = 0.3, P > 0.1 \). Given the consistency of those values with the data set as a whole, as shown in Figure 3A, we conclude that appropriate latency values can be achieved from as few as two TFs. Significant responses at the second harmonic were also observed in some instances at all TFs tested for both infants and adults.

Adult versus Infant Latencies

Among the 81 adults tested, the range of transient peak latency ± SE (104.6 ± 1.7 ms; 95% confidence interval [CI], 100.8–106.9 ms) and calculated latency (103.6 ± 3.0 ms; 95% CI, 100.7–107.3 ms) were similar to the range (100–115 ms) found in the literature.7,13,15,19 Similar to the findings of Tobimatsu et al.,13 no significant difference between the two latency methods was found, using RM-ANOVA \( F_{1,69} = 0.4; P > 0.1 \) (Fig. 4A).

Infants. Compared with adults, the overall response waveform for young infants is prolonged (Fig. 1). In the latency of the first positive peak, we found similar age trends to other published studies.6,7,10,11,19,21 with a steep decrease over the first few months of life (Figs. 5A and 3B). Longitudinal data from four infants who had four repeated sessions at different ages (Figs. 5A and 5B) showed a similar developmental trend to the overall cross-sectional data (Figs. 3A and 3B).

The individual infants (without any repeated sessions) were divided into 10 age groups (Table 1). RM-ANOVA using all the age groups as a between-subjects factor confirmed a significant overall difference between the two latency methods \( F_{1,64} = 4.5; P = 0.04 \), and a significant interaction effect of method and age groups \( F_{1,64} = 2.8; P = 0.01 \). Both P1 and calculated latencies decreased with age, from mean latency of appropriate age.

Transient versus Calculated Latencies

Transient Peak Latency. VEP waveforms showed classical PR responses with prominent, easily identifiable P1 peaks (Fig. 1). One-way ANOVA showed that the mean differences among the latency values for this peak at low TFs in adults were not significant \( F_{3,81} = 2.2, P > 0.1 \). In infants, a two-way ANOVA was performed with age treated as a between-subject factor. The peak latency differences at low TFs were tested with 7 different TFs from 1 to 19.2 r/s. The 4-week-old had \( R^2 = 0.98 \), slope = -101.6, latency = 237.3 ms. The 15-week-old had \( R^2 = 0.99 \), slope = -54.9, latency = 107.5 ms.

Phase values were plotted as a function of TF, and a slope of the linear regression was calculated. Finally, the slope was converted into apparent latency using the formula: latency (ms) = \(-\Delta \) phase/\( \Delta \)TF \times 1000 ms/360°, providing a single latency value for each individual subject. As many closely spaced TF values as possible were chosen for this study to minimize the risk of any data point being misplaced by 360°.15

When the calculated latency derived from the whole slope was a potential outlier (>3 SD) from the mean slope of the entire sample, a cycle of 360° was subtracted from the last phase point to get the best-fit linear regression based on the \( R^2 \) value. If the final calculated latency remained 3 standard deviations (SD) above the mean latency, the outlier was eliminated from the data pool.

Phase-Based Calculated Latency. In both adults and infants, the phase-based slope method proved effective in calculating an apparent latency value (Fig. 2). The absence of any clear split in the slope between the upper and lower part of the TF range11,20 suggested that any difference between transient and calculated latencies was not simply due to the different TF ranges used.

Although an increased number of TFs will enhance the accuracy of the calculated latency, a latency value can be derived from as few as two TFs. In infants, ANOVA (with age as a covariate) between the calculated latency derived where only two TFs were available versus more than two TFs indicated no significant difference between the two methods \( F_{1,112} = 0.1; P > 0.1 \) nor any significant interaction between age and method \( F_{1,112} = 0.3, P > 0.1 \). Given the consistency of those values with the data set as a whole, as shown in Figure 3A, we conclude that appropriate latency values can be achieved from as few as two TFs. Significant responses at the second harmonic were also observed in some instances at all TFs tested for both infants and adults.
proximately 215 ms at 3.6 weeks to 86 ms at 80 weeks of age. The interaction appears to reflect P1 latency decreasing initially at a faster rate. Post-hoc analysis (Games-Howell) indicated that the two latencies merged by 50 weeks ($P > 0.1$) (Fig. 3B).

**Comparison between Adults and Infants.** Calculated PR latency was found to be significantly longer than transient latency in infants but not in adults. Infant data showed overall higher variance than adults, especially in the infants phase-based calculated latency.

Latencies were significantly longer for younger infants than for adults. Post hoc analysis (Games-Howell) revealed that the infant transient peak latency was significantly longer than adult values before 15 weeks of age ($P < 0.001$). Infants’ phase-based calculated latency was significantly longer than adult values before 30 weeks of age ($P < 0.001$) (Fig. 3B).

As the latency of the transient VEP in infants is not significantly longer than in adults after 15 weeks of age, linear regression was fitted between latency and age over the age range from 3.6 to 14.4 weeks. This is in line with other published practices ($r = 0.8$, $F_{(1,23)} = 41.4; P < 0.001$; latency = $-11.6 \times$ age + 261.8) and calculated latency ($r = 0.5$, $F_{(1,25)} = 7.9; P = 0.01$; latency = $-7.6 \times$ age + 252.1). While the transient latency decreased at 11.6 ms per week, the calculated latency decreased at 7.6 ms per week for the first 15 weeks of life.

**DISCUSSION**

We obtained response latencies for PR-VEPs in both adults and infants using two methods: transient peak latency for the first positive peak in the waveform and phase-based calculated latency from relative phase measurements.

The traditional P1 latency from transient VEP reflects the arrival time of the visual stimulus at the visual cortex from the eye. The elapsed time represents initial retinal processing of contrast; transmission through optic nerve, tract, and radiation; and sufficient activation of visual cortical cells in the feedforward pathway to generate postsynaptic currents for a large-scale synchronization to be detected at the scalp. While studies have found P1 to arise from area V1 and its surroundings, the precise area of origin of adult P1 within the occipital lobe is not fully resolved.

Similar to others, our data could be well fitted with a single regression line. Some published data were fitted by different gradients in the low versus high TF ranges: in infants, in older adults (mean age 72 years), and in rats. However, the
discontinuities seemed to occur around 20 to 30 r/s, above the TF range (1–19.2 r/s) used in this study. Moreover, careful examination revealed that some ‘split slopes’ in the cited studies were so close that the full range could also be well fitted by a single regression line. This suggests that across the range 1 to 19 r/s, our measured VEPs are most likely to be driven by a similar population of neurons.

Latency Development

Similar to the findings of Moskowitz and Sokol (1983), VEP morphology in our data develops from a single late positive peak at birth to an adult-like double peak-and-trough complex. While the transient latency asymptoted to the adult value at approximately 15 weeks, the calculated latency did not reach adult values until approximately 30 weeks. The progression of the transient latency merged with the calculated latency at approximately 50 weeks of age (Fig. 3B).

While both transient and calculated methods yielded latency approaching 100 ms in adults, the calculated measure was significantly longer in comparison with the transient value in infants. Linear regression for the first 15 weeks showed that the calculated latency decreases at approximately 7.6 ms per week and transient peak latency decreases at approximately 11.6 ms per week.

Changes in transient latency may be influenced by the coincident synaptic maturation in retina, lateral geniculate nucleus (LGN), and occipital cortex, but the most widely cited factor is the progressive myelination of the visual pathways. Dubois et al. tested 15 infants (5.6–17 weeks) with diffusion tensor imaging of cerebral white matter and VEP to face stimuli. They found that the P1 latency was significantly correlated with infants’ age and with structural changes in optic radiation, but not with the global maturation of white matter. Similarly, Friendly suggested that the myelination of the LGN pathway was completed by 4 months, which is in accord with our results that adult P1 latency is reached by 20 to 30 r/s, above the TF range (1–19.2 r/s) used in this study. Moreover, careful examination revealed that some ‘split slopes’ in the cited studies were so close that the full range could also be well fitted by a single regression line. This suggests that across the range 1 to 19 r/s, our measured VEPs are most likely to be driven by a similar population of neurons.

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approximately 15 weeks of age. Others have shown continuation of myelin maturation for the first two years of life.\textsuperscript{44,45} Because myelination varies among different fibers during development,\textsuperscript{24,46} this variation will degrade phase coherence of neural transmission to the cortex, and would be expected to result in degraded amplitude and delayed latency in the VEP responses. The inhomogeneity of timing may contribute to the higher latency variance seen in the infant group.

In addition to incomplete myelination, there are many ways in which visual cortical processing is immature during the first 4 to 6 months.\textsuperscript{50} Burkhalter et al.\textsuperscript{47} found that long-range horizontal cells within cortical layer 2/3 become adult-like at approximately 16 weeks; this may underlie some aspects of functional cortical development. We propose that immaturity of cortical processing, reflected in infants’ simpler VEP waveforms, is responsible for the additional developmental delays seen in the latency calculated from relative phase compared with transient peak latency.

Factors Affecting Latency

Latency of PR-VEP is affected by many physical and physiological variables. Conduction speed in the visual pathways is influenced by external factors such as temperature, and structural physiological factors such as myelin thickness, axon diameter, and length.\textsuperscript{46} The latency values investigated in the present study may depend on some or all of the following physiological factors: sustained action potential reaching the scalp;\textsuperscript{77} large scale synchronization-temporally and spatially;\textsuperscript{30} feed-forward connections in response to the optimal visual stimuli;\textsuperscript{31,41}; additional delay from neural feedback that includes horizontal connections, inhibition and recurrent loops;\textsuperscript{49}, attention level of the participant;\textsuperscript{48,19}; and maturation rate of the different classes of neurons that generate SS-VEP and transient VEP signals.\textsuperscript{36} The differences seen between development of calculated and transient latencies in infants might reflect contributions from different classes of afferent neurons. However, it should be noted that the two measures were derived from overlapping TF ranges and so are unlikely to relate in any simple way to the magno/parvo or similar distinctions.

Calculated latency may be more affected by attention than the transient latency. Attention to the spatial frequency of a stimulus has been shown to affect transient VEP components at 150 ms and beyond, but not at 100 ms or earlier.\textsuperscript{50–52} Such attentional effects on later waveform components may contribute to the larger variance seen in the calculated latency. Our results show that calculating latency from relative phase in SS-VEP can yield usable results even with just two TFs. This is especially helpful for future studies on infants or patients where recording time is limited and sustained attention is poor.

Development of latency to contrast changes is strongly dependent on the specific stimuli used. From studying 439 children ages 1 month to 5 years, Moskowitz & Sokol (1983)\textsuperscript{21} found that P1 peak latency becomes adult-like by 1 year for large checks (30–240\textdegree), and beyond age 5 years for small checks (7.5 and 15\textdegree). McCulloch and Skarf (1991)\textsuperscript{22} found that infants’ peak latency lies within 1 SD of adult value by 4 to 5 months for 120’ and 60’ checks (consistent with our results) but is still not adult-like by 2 years for smaller checks (30’, 15’, and 7.5’). The precise ending of the period of latency development is difficult to determine and is certainly stimulus-dependent. It would be of interest to examine the calculated and peak latencies for high SF stimuli.

Conclusions

Our results showed that a single linear slope fitting a phase versus temporal frequency plot is an effective approach for calculating apparent latency in both adults and infants. This means that the method can be used in future studies to assess maturation of the visual pathway in infants with perinatal brain damage, and in addition, to gauge the success of early intervention in the first months of life. From the P1 peak latency comparisons, infants showed two types of functional changes in development that are reflected in the temporal properties of the PR-VEP. First, the dramatic reduction in the transient peak latency during the first 4 months of life can be attributed to the progressive development of conduction time in the afferent visual pathways and concurrent maturation of synaptic transmission within the visual pathway and cortex. The adult value is reached at approximately 15 weeks of age for low spatial frequencies. Second, the maturation of later cortical processing (including feedback loops, recurrent processing, and horizontal connections) that contribute to the overall VEP waveform, and hence to the latency calculated for relative phase, has a slower developmental rate. For this latter measure, the adult value is reached at approximately 30 weeks of age. Transient latency merged with the calculated latency at approximately 50 weeks of age. However, the similarity of calculated and transient latency in adults implies that in the mature system, the timing of the cortical response may be mostly dominated...
by transmission delays that determine the timing of the initial transient. Better understanding of the factors determining latency measures of the PR-VEP during development will help to interpret the relation between normative baselines and individuals’ results in future clinical evaluations.

References


