The Detection Of Misrouting In Albinism: Evaluation of Different VEP Procedures in a Heterogeneous Cohort

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PURPOSE. To investigate the optimal procedures for multichannel visually evoked potentials (VEPs) to detect misrouting in albinism subjects.

METHODS. Investigations were done in a phenotypically heterogeneous group of 180 albinism subjects and 187 controls with and without ocular pathology. We retrospectively compared standard flash VEP (fVEP), high-frequency fVEP with a handheld device (hh fVEP), pattern-onset VEP (poVEP), and short-onset acuity sweep VEP. The diagnostic power of these stimuli were estimated by calculating the area under the curve (AUC). Subjects were divided in three age groups (<3, 3–6 [toddler], and ≥6 years). Subjects ≥6 years of age were further divided in two visual acuity groups (≤0.3 logMAR and >0.3 logMAR).

RESULTS. The optimal stimulus was hh fVEP, standard fVEP, and poVEP 60′ for subjects <3, 3–6, and ≥6 years of age, respectively. In subjects ≥6 years old with poor visual acuity, the area under the curve of fVEP was almost equal to that of poVEP 60′.

CONCLUSIONS. For the optimal detection of misrouting with multichannel VEP recordings, we recommend using a high-frequency hh fVEP in children <3 years of age, standard fVEP in toddlers, and poVEP 60′ in subjects ≥6 years of age. fVEP can also be used in the oldest age group for subjects with visual acuity of >0.3 logMAR. Remarkably, some albinism subjects showed misrouting on full-field stimulation but normal routing of the central retina, suggesting that not the whole line of decussation is shifted temporally.

Keywords: albinism, misrouting, pattern visual evoked potentials, flash visual evoked potentials, line of decussation

Albinism is characterized by a variety of abnormalities of the visual system. Ophthalmic characteristics of albinism include nonprogressive reduced visual acuity, delayed maturation of the visual system, nystagmus, iris translucency, foveal hypoplasia, fundus hypopigmentation, and an abnormal pattern of decussation at the optic chiasm, the so-called chiasmal misrouting. When all clinical signs are present, a diagnosis of albinism is evident. However, subjects may have a very mild phenotype, and on the other hand, some other ocular conditions may have overlapping features. Therefore, major and minor diagnostic criteria were recently introduced. Major criteria are ocular hypopigmentation, foveal hypoplasia grade 2 or more, and misrouting. Misrouting in albinism is characterized by a majority of the optic nerve fibers crossing at the chiasm and projecting to the contralateral hemisphere, resulting in a reduced or delayed signal in the ipsilateral hemisphere. This is in contrast to normal routing, where the visual information of each eye projects equally to both hemispheres. Misrouting can be detected by multichannel visually evoked potential (VEP) recordings. The interhemispheric difference potential has been quantified by use of an asymmetry index, Pearson’s correlate, or chiasm coefficient. Soong et al. showed that the Pearson’s correlate was more accurate than the asymmetry index in assessing misrouting. The Pearson’s correlate compared the interhemispheric difference potential waveforms from right eye (OD) and left eye (OS) stimulation measured in a window of 0–200 ms. Jansonius et al. introduced an improved correlate, the chiasm coefficient, that was calculated in a window of 60–300 ms. The difference between the chiasm coefficient and the Pearson’s correlate is that the calculation of the chiasm coefficient includes a high-pass filter to cope with drift. For both Pearson’s correlate and chiasm coefficient, a negative value indicates a predominantly anticorrelation between the interhemispheric difference potential waveform. This may be caused by an excessive amount of optic nerve fibers projecting to the contralateral hemisphere, as in albinism, or to the ipsilateral hemisphere, as seen in achromia. The correlations can take a value from −1 to +1; −1 indicates complete asymmetry and +1 complete symmetry. The more noise the signals contain, the more the value will shift toward 0. Figure 1 shows simulated examples of the effect on the level of correlation for offset, amplitude difference, drift, and noise on the chiasm coefficient and Pearson’s correlate. The chiasm coefficient appears less sensitive to drift, although with a nonlinear drift even the chiasm coefficient may deteriorate severely (Fig. 1g). Previous studies concluded that flash VEPs (IVEPs) should be used for the detection of misrouting in subjects under the age of 3 years, pattern-onset VEP (PoVEP) in subjects from 6 years of age onward, and both
fVEP and poVEP in toddlers between 3 and 6 years. Misrouting could be detected only sporadically with poVEP in subjects younger than 3 years of age. The pattern reversal and hemifield stimulation proved to be unreliable.\textsuperscript{2,4,8–12} Therefore, the International Society for Clinical Electrophysiology of Vision (ISCEV) guide recommends for multichannel VEP recordings to use fVEP in young children and poVEP in children $\geq$ 6 years and adults.\textsuperscript{12} Earlier studies compared phenotypically evident albinism subjects to controls without ocular pathology, except for idiopathic nystagmus.\textsuperscript{2,4,7–12,14}

The purpose of this study is to evaluate if the currently recommended VEP stimuli are still applicable for a more heterogeneous group of albinism subjects and controls and to investigate if the preferred stimulus may also be visual acuity (VA) dependent instead of only age dependent. Also, we investigated if new techniques, that is, monocular handheld Ganzfeld and short-onset acuity sweep, could improve the detection rate of misrouting.

METHODS

The study was approved by the Medical Ethics Committee of Leiden University Medical Center and adhered to the tenets of the Declaration of Helsinki.

Subjects

We retrospectively analyzed VEP recordings of 180 albinism subjects and 187 age-matched controls. All albinism subjects met the previously described diagnostic criteria.\textsuperscript{1} The diagnoses in the control group are described in Table 1. We divided albino subjects and controls in three age groups according to the recommendations of Apkarian:\textsuperscript{4} younger than 3 years (albinism, \(n = 55\); controls, \(n = 51\)), 3 to 6 years (toddlers) (albinism, \(n = 32\); controls, \(n = 34\)), and 6 years of age and older (albinism, \(n = 93\); controls, \(n = 102\)).

This last group was further divided in two VA groups: VA $< 0.3$ (albinism, \(n = 39\); controls, \(n = 83\)) and VA $> 0.3$ logMAR (albinism, \(n = 54\); controls, \(n = 18\)) to investigate the effect of VA on the used stimulus.

Apparatus, Misrouting Calculation, and Statistics

All VEPs were obtained with Espion E2 or E3 (Diagnosys LLC, Cambridge, UK). Tests were recorded with a central electrode (\(O_2\)), and one electrode on the left (\(O_L\)) and right (\(O_R\)) hemisphere referenced to \(F_{Z.O.L}\) and \(O_R\) were positioned at a distance of 5 cm from \(O_2\). A bandpass filter was set at 0.625 to 100 Hz. For quantification of the interhemispheric difference, we calculated the chiasm coefficient by using the differential signal (\(O_L-O_R\)) recorded from the OD and OS.

We used the chiasm coefficient instead of the Pearson’s correlate because this is an improved calculation that copes with drift.\textsuperscript{6,7} Jansonius et al.\textsuperscript{10} used a window of 60–300 ms for the chiasm coefficient. However, asymmetry in the pattern onset response occurs mainly in the first 125 ms, more specifically between the 80–110 ms. The response after this window is often similar to that of normal controls, and any noticeable asymmetry in albinism is more variable. Thus, asymmetry assessment after 125 ms is unreliable.\textsuperscript{10,15} For fVEP, according to Apkarian and Tijssen,\textsuperscript{12} latency regions of up to 200 ms are more than sufficient for the assessment of misrouting in neonates and can even be smaller in older subjects.\textsuperscript{12} Russell-Eggitt et al.\textsuperscript{9} concluded that an early fVEP asymmetry occurs at around 80 ms, independent of age, and a greater second asymmetry window occurs in a more variable window between 96–178 ms. Based on these studies, we calculated the chiasm coefficient in a 60–150 ms interval for poVEP and 60–200 ms for fVEP. This should be sufficient to capture all relevant asymmetry, and it copes with the slight delay caused by integration over the previously recorded 60 ms in the calculation of the chiasm coefficient. Figure 2 shows the misrouting in this poVEP window in a typical patient. We demonstrated the delay caused by the filter in the Supplementary Figure S1.\textsuperscript{6,7}

Statistical analyses were performed with IBM SPSS Statistics software version 22. We compared different VEP stimuli (see below) for their diagnostic ability to discriminate between the albino and control group. For each stimulus, we calculated the area under the curve (AUC) of the receiver operating characteristic to identify the optimal test stimulus. To discover

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1}
\caption{Simulated examples of the effect on the chiasm coefficient (CC) and Pearson’s correlate (PC) on (a) fully anticorrelated signals between OD (red lines) and OS (blue lines), (b) fully correlated signals, (c) partially anticorrelated and partially correlated signals, (d) anticorrelated signals with different amplitudes, (e) anticorrelated signals with drift, (f) anticorrelated signals with nonlinear drift, (g) only noise, (h) anticorrelated signals with noise, and (i) correlated signals with noise. The CCs and PCs are calculated in a window of 60 to 300 ms. Also, in the last three examples, the values for a window of 60 to 200 ms are calculated and shown in between brackets.}
\end{figure}

\begin{align}
\text{fVEP} & : \text{cc} = -1.00; \text{pc} = -1.00 \\
\text{poVEP} & : \text{cc} = +1.00; \text{pc} = +1.00 \\
\text{c} & : \text{cc} = -0.51; \text{pc} = -0.25 \\
\text{d} & : \text{cc} = -1.00; \text{pc} = -1.00 \\
\text{e} & : \text{cc} = -1.00; \text{pc} = -1.00 \\
\text{f} & : \text{cc} = -0.93; \text{pc} = -0.31 \\
\text{g} & : \text{cc} = +0.01; \text{pc} = +0.27 \\
\text{h} & : \text{cc} = -0.05 (0.26); \text{pc} = -0.03 (0.17) \\
\text{i} & : \text{cc} = -0.78 (0.89); \text{pc} = -0.67 (0.79) \\
\text{j} & : \text{cc} = +0.88 (0.95); \text{pc} = +0.62 (0.68)
\end{align}
any significant differences between the AUCs of stimuli, we did linear regression analyses, because subjects were tested with different stimuli resulting in paired data. We additionally investigated if combining the results of poVEP and fVEP increased sensitivity and specificity. To investigate if the preferred stimulus may be VA-dependent, the AUC of the different stimuli were compared between the two VA groups. Because these groups consist of different subjects, the data are unpaired and a nonoverlap of the 95% confidence intervals is used to detect any significant differences. To obtain representative results, we did not perform analyses on subgroups that contained fewer than 10 persons.

Besides the AUC we also determined a sensitivity and specificity for each VEP stimulus for each subgroup. The cut-off value for the chiasm coefficient to determine the sensitivity and specificity was based on the point closest to (0,1) on the receiver operating characteristic curve.16,17

Flash VEP. We used two different fVEP stimuli/apparatus, namely, standard (sfVEP) and handheld (hh fVEP). sfVEP was measured with a stimulus according to the ISCEV standard (Ganzfeld illumination, 3 cd/s/m², 1.09 Hz) by using a bowl (ColorDome) that covers both eyes at the same time. To stimulate one eye at a time, the other eye was patched with an amblyopia sticker and an eye cup.18 The hh fVEP used a small handheld bowl (Colorburst) (Ganzfeld, 3 cd/s/m², with a higher frequency of 2.5 Hz), which only covers the eye to be measured, while the other eye is not occluded, as in sfVEP.

For both standard and hh fVEPs, we calculated the chiasm coefficient in a window of 60–200 ms.12

Pattern-Onset VEP. We used two different kinds of poVEP stimuli. The first stimulus was according to the standard ISCEV protocol (check sizes 60′ and 15′; 200/400 ms).18 The second stimulus was a pattern short-onset acuity sweep VEP (check sizes 60′, 30′, 15′, and 7.5′; 40/260 ms). Both standard poVEP and short-onset acuity sweep VEP were measured with a field size of 15 degrees. A total of 15 albimnus subjects and 14 controls in the youngest age group were measured at a shorter distance of approximately 30 cm, resulting in a field size of 60 degrees.

For poVEPs, we calculated the chiasm coefficient in a window of 60–150 ms.10,15

We compared the chiasm coefficients of fVEP and poVEP calculated in windows of 60–200 ms and 60–150 ms, respectively, with the chiasm coefficients in the window of 60–300 ms used by Jansonius et al.6 Because chiasm coefficients were not normally distributed, we compared the coefficients with a Wilcoxon rank sum test.

Table 1. Different Disorders in Control Group

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Number of Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>No ocular pathology</td>
<td>69</td>
</tr>
<tr>
<td>Amblyopia</td>
<td>10</td>
</tr>
<tr>
<td>Idiopathic infantile nystagmus</td>
<td>40</td>
</tr>
<tr>
<td>Opticopathy</td>
<td>30</td>
</tr>
<tr>
<td>Retinal dystrophy</td>
<td>9</td>
</tr>
<tr>
<td>Cortical visual impairment</td>
<td>22</td>
</tr>
<tr>
<td>Other</td>
<td></td>
</tr>
<tr>
<td>Delayed visual maturation</td>
<td>4</td>
</tr>
<tr>
<td>Isolated foveal hypoplasia</td>
<td>1</td>
</tr>
<tr>
<td>PAX 6 mutations causing nystagmus and foveal hypoplasia</td>
<td>1</td>
</tr>
<tr>
<td>Proptosis with normal ocular function</td>
<td>1</td>
</tr>
</tbody>
</table>

Because generally toddlers are less cooperative than sleeping infants or older children, it may be more difficult to accurately place the electrodes at symmetrical positions. We calculated a second cutoff value by subtracting 0.5 of the differential signal measured with ODS (both eyes at the same time) from the differential signal of OD and from that of OS to correct for the possible asymmetry (Supplementary Fig. S2).

RESULTS

Table 2 shows the AUC, sensitivity, specificity, accuracy, and optimal cutoff values for the different procedures in the three age groups. For subjects under the age of 3 years, the optimal test for the detection of misrouting was hh fVEP (AUC, 0.95). For toddlers, poVEP 60′ had the highest AUC (0.84), but the AUC of sfVEP was almost similar (0.82). For subjects ≥6 years of age, poVEP 60′ was the optimal test with the highest AUC (0.92). With the current amount of subjects and controls, no statistically significant differences in the AUC for the different stimuli could be detected for each age group.

Table 2 shows that most cutoff values for the chiasm coefficients were around 0 (-0.17 to 0.00), except for poVEP in the toddler group (+0.31 and +0.39), acuity sweep VEP 7.5′ at the age of 6 and older (+0.20), and hh fVEP for all age groups (-0.49, -0.40, and -0.35).

Combining the outcome of fVEP (sfVEP or hh fVEP) and poVEP 60′ did not lead to better results. Supplementary Table S1 shows that if both tests had to confirm misrouting, this led to a drastic decrease in sensitivity compared to the most sensitive test. If only one of the two tests had to confirm misrouting, specificity strongly decreased.

In the albinus group, the chiasm coefficients of the poVEP and hh fVEP had significantly more frequently higher positive values in a window of 60–300 ms compared to the smaller windows that we used (all P values < 0.01). Figure 2 shows the
Subjects 3–6 y of age: 32 albinism subjects (median 4, IQR 2), 34 controls (median 4, IQR 1)

‡Subjects

VEP Stimulus AUC (n Albinism/n Controls) Sensitivity, % Specificity, % Accuracy, % Optimal CC cutoff

Subjects <3 y of age: 55 albinism subjects (median 0, IQR 1), 51 controls (median 1, IQR 1)

<table>
<thead>
<tr>
<th>VEP Stimulus</th>
<th>AUC (n Albinism/n Controls)</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Accuracy, %</th>
<th>Optimal CC cutoff</th>
</tr>
</thead>
<tbody>
<tr>
<td>poVEP 60'</td>
<td>0.72 (19/22) (CI 0.55–0.89)</td>
<td>68</td>
<td>68</td>
<td>68</td>
<td>−0.11</td>
</tr>
<tr>
<td>sfVEP</td>
<td>0.75 (32/32) (CI 0.62–0.87)</td>
<td>75</td>
<td>78</td>
<td>77</td>
<td>−0.17</td>
</tr>
<tr>
<td>hh IVEP</td>
<td>0.89 (41/41) (CI 0.90–0.99)</td>
<td>93</td>
<td>88</td>
<td>90</td>
<td>−0.49</td>
</tr>
</tbody>
</table>

Subjects 3–6 y of age: 32 albinism subjects (median 4, IQR 2), 34 controls (median 4, IQR 1)

<table>
<thead>
<tr>
<th>VEP Stimulus</th>
<th>AUC (n Albinism/n Controls)</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Accuracy, %</th>
<th>Optimal CC cutoff</th>
</tr>
</thead>
<tbody>
<tr>
<td>poVEP 60'</td>
<td>0.84 (23/22) (CI 0.73–0.95)</td>
<td>74</td>
<td>77</td>
<td>77</td>
<td>+0.31</td>
</tr>
<tr>
<td>sfVEP</td>
<td>0.77 (20/19) (CI 0.61–0.94)</td>
<td>95</td>
<td>68</td>
<td>82</td>
<td>+0.39</td>
</tr>
<tr>
<td>hh IVEP</td>
<td>0.82 (27/28) (CI 0.70–0.95)</td>
<td>82</td>
<td>75</td>
<td>78</td>
<td>−0.07</td>
</tr>
</tbody>
</table>

DISCUSSION

Recent studies showed that albinism is a much more heterogeneous disorder than previously thought. Accurate assessment of chiasmal misrouting may greatly aid in the diagnosis of mildly affected albinism subjects. The aim of our study was to compare multichannel VEPs of a phenotypically heterogeneous group of albinism subjects to controls with and without ocular pathology, in order to optimize VEP procedures and to investigate if previous recommendations are still appropriate for clinical use.

For both VA groups, the poVEP 60' had the highest AUC (Table 3).

We included seven subjects with fine stereopsis (≤60 arcseconds). Four of these subjects tested positive for misrouting and in three we failed to detect misrouting. The Detection of Misrouting in Albinism

TABLE 2. Different Stimuli for All Age Groups

<table>
<thead>
<tr>
<th>VEP Stimulus</th>
<th>AUC (n Albinism/n Controls)</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Accuracy, %</th>
<th>Optimal CC cutoff</th>
</tr>
</thead>
</table>
| Subjects >3 y of age: 93 albinism subjects (median 17, IQR 22), 102 controls (median 19, IQR 22)

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>VA of ≤0.3</th>
<th>VA of &gt;0.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>60' acuity</td>
<td>n albinism/n controls</td>
<td>32/60</td>
</tr>
<tr>
<td>AUC (CI)</td>
<td>0.84 (0.74–0.95)</td>
<td>0.85 (0.75–0.96)</td>
</tr>
<tr>
<td>30' acuity</td>
<td>n albinism/n controls</td>
<td>32/60</td>
</tr>
<tr>
<td>AUC (CI)</td>
<td>0.86 (0.77–0.95)</td>
<td>0.85 (0.74–0.96)</td>
</tr>
<tr>
<td>15' acuity</td>
<td>n albinism/n controls</td>
<td>32/60</td>
</tr>
<tr>
<td>AUC (CI)</td>
<td>0.82 (0.73–0.92)</td>
<td>0.71 (0.56–0.86)</td>
</tr>
<tr>
<td>7.5' acuity</td>
<td>n albinism/n controls</td>
<td>32/60</td>
</tr>
<tr>
<td>AUC (CI)</td>
<td>0.80 (0.68–0.89)</td>
<td>0.69 (0.50–0.88)</td>
</tr>
<tr>
<td>60' onset</td>
<td>n albinism/n controls</td>
<td>37/73</td>
</tr>
<tr>
<td>AUC (CI)</td>
<td>0.87 (0.79–0.95)</td>
<td>0.90 (0.80–1.00)</td>
</tr>
<tr>
<td>15' onset</td>
<td>n albinism/n controls</td>
<td>37/72</td>
</tr>
<tr>
<td>AUC (CI)</td>
<td>0.82 (0.72–0.91)</td>
<td>0.80 (0.62–0.98)</td>
</tr>
<tr>
<td>flash</td>
<td>n albinism/n controls</td>
<td>36/72</td>
</tr>
<tr>
<td>AUC (CI)</td>
<td>0.67 (0.54–0.79)</td>
<td>0.88 (0.79–0.97)</td>
</tr>
</tbody>
</table>

All subjects were 6 y of age or older to be certain of stable VA. VA, visual acuity.
noise dominating the signal in OL-OR. misrouting on poVEP and definite misrouting on fVEP. The poVEP was the four albino subjects in the oldest age group that had no signs of (OR) hemisphere recorded twice from the right eye (\(L_O\)) lacking these features.1 It may, therefore, very well be that also complete gene has been knocked out. For all other albinism than laboratory mice or other animal models, in which a are far more heterogeneous in their genotype and phenotype some albinism patients indeed have normal routing. Patients means that the absence of detection of misrouting does not subject has albinism, misrouting should be present and that in controls misrouting is always absent. However, our study as well as the majority of previous studies on albinism reported sensitivities around 80%.3–5,7,9–11

One cause of the failure to detect misrouting is a poor signal-to-noise (S/N) ratio. But, we also could not detect misrouting in several cooperative subjects with clear VEP signals. As with other signs of albinism, misrouting may be a gradual feature, with some patients having 90% misrouted fibers and 10% normal ipsilaterally routed fibers, whereas other patients may have a 60%:40% ratio of crossed and uncrossed fibers.22 The latter probably cannot be detected by VEP. This means that the absence of detection of misrouting does not mean that misrouting is absent. However, it is also possible that some albinism patients indeed have normal routing. Patients are far more heterogeneous in their genotype and phenotype than laboratory mice or other animal models, in which a complete gene has been knocked out. For all other albinism features, including foveal hypoplasia and ocular hypopigmentation, there are (genetically confirmed) albinism patients lacking these features.3 It may, therefore, very well be that also misrouting is absent in some patients. A further confirmation of normal routing proved to be the good stereoacuity that we measured in some of our patients. For instance, in Figure 3a, VEP recordings are shown of an 8-year-old albinism patient with a VA of 0.1 logMAR who probably does not have misrouting because he has good stereo acuity (TNO test 60').

To discriminate between the absence to detect misrouting (while it is present) and real absence of misrouting (i.e., normal routing) in albinism patients, it would be very interesting to investigate the projection to the visual cortex with functional magnetic resonance imaging, as described by Hoffmann et al.23

With regard to specificity, if subjects have a large difference between VA of OD and OS, for example due to unilateral deep amblyopia or optic nerve pathology, a discrepancy in latency may result in “pseudo” misrouting. A great discrepancy in the midline (\(Oz\)) between eyes or an asymmetrical brain could also lead to false-negative results. Thus, in the albinism group, there are probably some subjects without misrouting, and in the control group some subjects may show asymmetry due to pathology other than albinism. Consequently, an accuracy of around 85%, as we detect with the optimal stimulus in our study (Table 2), may be the highest achievable. Accuracy in the toddler group is probably slightly lower than 85% because VEPs in this age group may sometimes be difficult to record. In children <3 years of age, accuracy may be a little high due to selection bias. We included subjects only if they met the diagnostic criteria for albinism, which sometimes were difficult to assess because of poor compliance with clinical examinations. Therefore, in this age group more cases had to be excluded.

<3 Years of Age

In the youngest subjects, hh fVEP reached higher sensitivity, specificity, and accuracy rates than sVEP which may be due to S/N ratio. We noticed a smoother recorded signal with hh fVEP than sVEP. Sometimes, VEPs were made in sleeping infants, which is easier with hh fVEP than with sVEP because there is no need for occlusion with the risk of waking up the infant. This may result in less noise. The more negative cutoff value with hh fVEP also supports this theory of a better S/N ratio because noise causes a shift toward 0. However, because we also detected a more negative cutoff value for hh fVEP in the other age groups, the sleeping infant cannot be the sole reason. Another explanation could be that the higher stimulus frequency of 2.5 Hz leads to shorter recording time and possibly less blinking, eye movement, or fluctuations in pupil size, which, in turn, results in better recordings.

We were able to detect misrouting with poVEP in 68% of the youngest albino subjects, which is in contrast to earlier studies that poVEP under the age of 3 years shows no evidence of misrouting in the majority of cases.12 But, because hh fVEP is faster and easier to record in young subjects and has higher accuracy, we recommend hh fVEP in this age group.

3 to 6 Years of Age

This is the most difficult age group to measure. Toddlers are not asleep during recordings, and concentration can be very hard. Also, it can be difficult to put the electrodes at exact symmetrical positions and keep them at the right position during the whole test. Cooperation and concentration is especially important for poVEP. Probably in this group we had to deal with more asymmetry, which caused the cutoff value to be +0.31 and +0.39 for the pattern onset 60' and 15', respectively. When we corrected for possible asymmetry, chiasm coefficients became more negative and sVEP had the highest AUC instead of poVEP 60'.(Table 2). The literature recommends to use both IVEP and poVEP in this age group. When we required both tests to confirm misrouting, specificity
increased but sensitivity strongly decreased, and if we required only one test to confirm misrouting, the opposite was true (Supplementary Table S1). When time is limited, we recommend iVEP over poVEP because the measurements depend less on good central fixation and cooperation. The better AUC with sVEP than with hh iVEP may be explained by selection bias. In our clinic, it was protocol to start with sVEP in this age group. When misrouting was evident, hh iVEP was not routinely recorded as well, but if results were unreliable or if subjects were very uncooperative, we tried measuring iVEPs with the handheld device.

≥6 Years of Age
In our more heterogeneous group of albinism subjects, we confirmed the results of earlier studies, that is that poVEP is the optimal stimulus for the detection of misrouting in subjects ≥6 years. Surprisingly, although the sensitivity with poVEP (85%) in this patient group was higher than with sVEP, sVEP also had good sensitivity (70%) and specificity (85%). This is in contrast to earlier studies and the ISCEV guide, which state that iVEPs are usually normal in adults with albinism.13 Even if we consider only subjects ≥18 years of age, we detected misrouting with sVEP in 32/45 (71%). sVEP was also significantly better for the detection of misrouting in subjects with poorer VA. The AUC of 0.88 for subjects with VA of >0.3 logMAR was even better than that of subjects <3 years of age and the toddler group (0.72 and 0.80, respectively). These results suggest that the preferred stimulus depends less on age and more on VA.

Line of Decussation
In normally pigmented subjects, the line of decussation of the retinal optic fibers goes through the fovea. The fibers of the temporal half of the retina project to the ipsilateral hemisphere and the fibers of the nasal half to the contralateral hemisphere. Previous studies reported that in albinism this line is shifted temporally, resulting in temporal fibers projecting to the contralateral hemisphere as well. The extent of the shift is variable in individuals. Thus, the smaller the amount of misrouting, the closer the decussation line is to the foveal region.10,22 With IVeP, a greater part of the retina is stimulated than with poVEP. Consequently, if misrouting is easily visible with IVeP, one would expect to definitely detect misrouting with poVEP. However, we investigated four cooperative albino subjects in the oldest age group, who had no signs of misrouting on poVEP, but IVeP showed definite misrouting. This suggests that the temporal shift does not occur in a more or less straight line but may follow a more irregular path than previously thought. In these subjects, it seems that peripheral temporal fibers cross to the contralateral hemisphere (i.e., misrouting), whereas the macular region has normal routing. We demonstrate one of these subjects in Figure 3b. Furthermore, seven subjects in our study had stereo acuity of <60 seconds of arc; four of these subjects tested positive for misrouting with poVEP. In misrouting, an overlap exists in the routing of the central retina compared to the periphery may be associated with the different timing of the development of retinal ganglion cells, which occurs in two stages. During the first stage, cells in the central retina are produced with crossed and uncrossed projection. The second stage is the expansion to the peripheral retina, with cells originating from the temporal retina projecting to the ipsilateral hemisphere.26-31 If only the second stage is affected, this would lead to a normal development of the central retina and abnormal development of the periphery. It would be very interesting to further study patients with misrouting but with fine stereo acuity, for instance with functional magnetic resonance imaging, to understand the way in which the decussation line in albinism shifts exactly.22,25

Recommendations
In conclusion, we recommend the following VEP procedures for optimal detection of misrouting. The first two recommendations constitute a change from current ISCEV guidelines.

1. For quantification of the interhemispheric difference, to calculate the chiasm coefficient in a window of 60 to 200 ms for sVEP and hh iVEP and 60 to 150 ms for poVEP.
2. To record handheld iVEP in children younger than 3 years of age instead of standard iVEP.
3. Standard iVEP is preferred over poVEP for children between 3 and 6 years old.
4. Misrouting in subjects ≥6 years of age should be investigated with poVEP; however, a protocol that uses 60° only is sufficient. If VA is >0.3 logMAR, iVEP can be used as well, especially when time is limited.

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