Optic Nerve Projections in Patients with Primary Ciliary Dyskinesia

Michael B. Hoffmann,[1] *Barbara Wolynski,*[1] *Michael Bach,*[2] *Synke Meltendorf,*[1] *Wolfgang Behrens-Baumann,*[1] *and Franziska Golla*[1]

Purpose. Recently, it has been suggested that misprojections of the temporal retina to the contralateral hemisphere might not be specific for patients with albinism and might also be associated with the Kartagener syndrome (i.e., with situs inversus totalis in the presence of primary ciliary dyskinesia [PCD]). The authors tested whether such projection abnormalities are associated with PCD and situs inversus.

Methods. In 10 patients with PCD (five with situs inversus totalis) and in 10 age- and sex-matched controls, visual evoked potentials (VEPs) were recorded monocularly, as follows: conventional pattern-onset VEPs (cVEPs) and multifocal VEPs (mfVEPs) for 60 locations constituting a visual field of 44° diameter. cVEPs from 13 albinotic subjects were included as a reference. For each eye, interhemispheric difference potentials were calculated and correlated with each other to assess the lateralization of the responses: positive and negative correlation coefficients indicated lateralizations on same or opposite hemispheres, respectively. Misrouted optic nerves are expected to yield negative interocular correlations.

Results. For both cVEPs and mfVEPs, the distribution of the correlation coefficients in the PCD patients yielded largely positive values and did not differ from that of the controls. Consequently, neither large- nor small-scale lateralization abnormalities were observed in PCD. Further, the optic nerve projection did not depend on the presence of situs inversus.

Conclusions. The absence of evidence for projection abnormalities in a cohort of 10 subjects with PCD, five of whom had Kartagener syndrome, underscores that misrouting of the optic nerves is not a common trait of these subjects. (*Invest Ophthalmol Vis Sci.* 2011;52:4617–4625) DOI:10.1167/iovs.11-7194

In humans, the nasal retina normally projects to the contralateral and the temporal retina to the ipsilateral hemisphere. The line of decussation that divides crossed from uncrossed fibers thus coincides with the vertical meridian through the fovea. This projection of retinal fibers is altered in albinism, where the line of decussation is shifted into the temporal retina. As a result, a great number of fibers from the temporal retina cross the chiasmal midline and project contralaterally,[1–7] such that the visual cortex receives substantially abnormal input from the ipsilateral visual field for visual processing.[8–10] This projection abnormality is regarded as specific for the albinotic phenotype,[11–14] and its detection serves as a valuable diagnostic aid to identify albinism in subjects with only mild pigmentation deficits.[15–17] Given this diagnostic characteristic of misrouting, it is particularly topical that albino-type misrouting of the optic nerves has recently been reported in the absence of albinism,[18–20] which questions the decisive nature of the test for misrouting for the diagnosis of albinism. Misrouting was demonstrated in some patients with congenital stationary night blindness (CSNB),[18–20] and van Genderen et al.[20] described misrouting of the optic nerves for a subject with Kartagener syndrome,[21] a condition that is associated with primary ciliary dyskinesia.[22,23]

Primary ciliary dyskinesia (PCD) is a rare, genetically caused disorder affecting 1 in 16,000 subjects.[24] It is characterized by recurrent airway infections among other symptoms that are related to abnormal ciliary structure and function. If the ciliary defect is associated with the classical Kartagener triad—situs inversus totalis, bronchiectasis, and sinusitis—the condition is referred to as the Kartagener syndrome (KS).[25,26] The mechanism underlying the association of this syndrome with abnormal optic nerve projections described is only speculative. It might be a side effect of the general lateralization abnormality observed in KS patients (situs inversus totalis), which has been suggested to partially affect brain asymmetries,[27,28] on optic nerve routing. Alternatively, it might be related to the PCD encountered in these patients, perhaps mediated by an altered functionality of ciliary proteins involved in axonal transport[29] that might influence nerve fiber path finding. Finally, it might be a fortuitous finding in an isolated case and therefore not be directly associated with PCD or KS. As a consequence, studies testing a greater sample of patients with PCD for misrouted optic nerves are needed to determine whether this finding is typically associated with PCD or KS.

Visual evoked potentials (VEPs) are a valuable tool for identifying albino-like misrouted optic nerves in humans.[16] For this purpose, the interhemispheric VEP differences obtained for left and right eye stimulation are compared. As detailed in Subjects and Methods, positively correlated VEP differences indicate normal optic nerves, and negatively correlated VEP differences indicate misrouted optic nerves. This paradigm can be combined with conventional VEPs (cVEPs) or with multifocal VEPs (mfVEPs). Although conventional VEPs to pattern-onset stimulation are a routine tool for the detection of albinotic misrouting,[15] they might not be sensitive enough to detect local abnormalities. In cVEP recordings, responses are pooled across a large expanse of the visual field such that small, local representation abnormalities are likely to be masked by the residual normal representation. In contrast, multifocal VEPs enable us to record cortical responses from a great number of

From the [1]Department of Ophthalmology, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany; and the [2]Department of Ophthalmology, Albert-Ludwigs-University Freiburg, Freiburg, Germany.

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Corresponding author: Michael B. Hoffmann, Universitäts-Augenklinik, Visual Processing Laboratory, Leipziger Strasse 44, 39120 Magdeburg, Germany; michael.hoffmann@med.ovgu.de.

distinct visual field locations[30–33] and thus support the identification of small representation abnormalities.[12]

A recent report of misrouted optic nerves in a patient with KS not only questions the validity of an incontrovertible identification of albinism by detecting misrouted optic nerves, it also suggests a mechanism causing misrouted optic nerves that is independent of melanin-related mechanisms in albinism. Here we studied, in a sample of 10 patients using cVEPs and mfVEPs, whether misprojections of the optic nerves are a general trait of PCD or KS patients.

Subjects and Methods

Subjects

Ten patients with PCD (age range, 18–63 years; 6 men, 5 with situs inversus totalis, 1 with situs inversus abdominalis) and two reference groups (10 sex- and age-matched visually normal control subjects; age range, 18–60 years [±3 years]; decimal visual acuity ≥1.0) (13 albinotic subjects; age range, 24–47 years; 6 male; 12 participants of a previous study[9]) participated in the study. cVEPs were recorded for all subjects, and mfVEPs were recorded only for the PCD patients and the control group. The procedures followed the tenets of the Declaration of Helsinki,[34] and the protocol was approved by the ethics committee of the Otto-von-Guericke-University of Magdeburg. All subjects gave their informed written consent before the study.

Patients were referred after the clinical diagnosis of PCD. No abnormalities of fundus, macula, iris, or optic nerve head were observed during an ophthalmological examination. Visual field sensitivities as determined with static white-on-white perimetry (glaucoma-2 program, Octopus 101; Fa. Haag-Streit, Wedel, Germany) were normal, and no nystagmus was evident. To exclude early signs of retinitis pigmentosa, which can be associated with PCD,[24] ERGs were recorded according to the ISCEV standard[35] in the patient cohort (Retiport; Roland Consult, Brandenburg, Germany), and normal scotopic and photopic ERG responses were obtained. Refraction-corrected monocular decimal visual acuities, binocular visual function (Lang test, TNO test), and other patient details are given in Table 1.

VEP Investigations

Rationale of VEP Detection of Albinotic Misrouting of the Optic Nerves. In albinism, each eye projects predominantly to its contralateral hemisphere. Monocular stimulation of the central visual field is, therefore, expected to elicit greater VEPs on the hemisphere contralateral to the stimulated eye than on the ipsilateral hemisphere. As a consequence, the polarity of the interhemispheric VEP difference is inverted for left compared with right eye stimulation in subjects with albinism. In contrast, for controls the polarity does not depend on the eye stimulated.[11] Supplementing this paradigm with a correlation analysis simplifies the approach and enhances its objectivity. In albinism, the interhemispheric activation differences obtained for right and left eye stimulation are, because of the polarity inversion of the traces, likely to be negatively correlated. In contrast, for control subjects (i.e., in the absence of such a polarity inversion), they are likely to be positively correlated.[17,18,36,37] This correlation approach supports an objective analysis even of small signals. To obtain a greater spatial resolution when sampling the visual field for representation abnormalities, the VEP paradigm for the detection of misrouted optic nerves can be combined with the multifocal stimulation technique.[12]

Procedure. In a dimly lit room, cVEPs and mfVEPs were recorded in successive sessions separated by a break. The entire recording session, including preparation and breaks, took approximately 2.5 hours. Left and right eyes were stimulated in separate blocks while the respective fellow eye was patched. The blocks were presented in a balanced interleaved sequence (a-b-b-a scheme).

cVEP Recording. The electroencephalogram (EEG) was recorded with gold-cup electrodes at Oz, OL, and OR (4 cm left and right from Oz, respectively), referenced to Fz.[38] The ground electrode was attached to Fpz. The EEG was amplified with a physiological amplifier (50,000×; Grass Instruments, Quincy, MA), analog filtered in the range of 0.3 to 100 Hz, and digitized at a rate of 1 kHz with 12-bit resolution. Stimulation (frame rate, 75 Hz) and recording used the EP2000 Evoked Potentials System[39] running on a G4 Power Macintosh. This program presented the stimuli while stepping through the check size sequence, acquired the signals, displayed them online, checked for and discarded artifacts (using an amplitude window of generally ±50 μV and repeating sweeps where this was exceeded), displayed online averages, and saved the records for off-line processing. To ensure subject alertness, random digits from 0 to 9 appeared in random intervals at the center of the screen and were reported by the subjects.

For visual stimulation, black-and-white checkerboard patterns were presented monocularly at a viewing distance of 114 cm in pattern-onset-offset mode (40 ms on, 440 ms off).[40] The central visual field (19° × 15°) was stimulated with a checkerboard using three different check sizes that were presented in an interleaved manner (2.0°, 1.0°, and 0.5°). A total of 160 responses per condition were obtained. The subjects were instructed to maintain fixation at a central target (3° diameter) and wore optimal refractive correction. The recordings were performed for 98% stimulus contrast twice for each eye in an interleaved sequence and then repeated at 20% stimulus contrast, again twice for each eye. The stimulus had a mean luminance of 45 cd/m^2 and, because of a change of setup, of 110 cd/m^2 for four albinotic patients, for four PCD patients (P1, P2, P3, P9) and their matched controls. In pilot experiments this luminance difference had been demonstrated to be of no consequence for general trace shapes and response lateralizations and for the corresponding interocular correla-

Table 1. Characteristics of the PCD Patients

Subject	Sex	Age	Situs Inversus	Visual Acuity		Visual Field	Stereo Tests			Strabismus	Dominant Eye	ERG
				OD	OS		Lang	Titmus	TNO			
1	M	35	+	1.3	1.6	+	+	40″	30	−	OD	+
2	F	48	+	0.8	1.3	+	−	−	−	S. conv. OD	OS	+
3	M	18	(+)	1.0	1.0	+	+	30″	40″	−	OD	+
4	M	36	−	1.0	1.0	+	+	40″	60″	−	OS	+
5	F	45	+	1.0	1.0	+	+	100″	120″	−	OS	+
6	F	58	+	1.3	1.3	+	+	40″	60″	−	OD	+
7	M	28	−	1.3	1.3	+	+	40″	60″	−	OS	+
8	M	63	+	1.3	1.8	+	+	50″	240″	−	OD	+
9	M	24	−	1.3	1.3	+	+	40″	15″	−	OD	+
10	W	38	−	1.3	1.3	+	+	40″	60″	−	OS	+

−, negative or absent; +, normal, positive or present; (+), situs inversus abdominalis; S. conv., strabismus convergens.

tion coefficients of the interhemispheric activation differences determined as described below.

cVEP Analysis. The offline analysis was performed using technical graphing and data analysis software (IGOR 5.0; WaveMetrics, Inc., Lake Oswego, OR). The difference VEPs (OL minus OR) for each eye were digitally low-pass filtered (40 Hz cutoff in accordance with Hoffmann et al. 2005[17]) and correlated with each other to obtain Pearson's correlation coefficient (r; ranging between −1 and 1). In accordance with a previous study,[17] a time window from 50 to 250 ms was used for this correlation. The correlation allows for the distinction of normal and abnormal projections of the optic nerves. Positively correlated traces indicate that both eyes project to the same cortical regions, whereas negatively correlated traces indicate that both eyes project to opposite hemispheres.[17,36]

mfVEP Recording. mfVEPs were recorded from six gold cup electrodes referenced to the inion. Electrodes were placed at OL and OR, as defined, and 8 cm left and right to the location 1 cm above the inion (lateral occipital sites) and 5 cm left and right to POz (lateral parietal sites[38]). The EEG was amplified with a physiological amplifier (100,000×; Grass Instruments), band-pass filtered (low- and high-frequency cutoffs 3 and 100 Hz), and digitized at 1200 Hz. Stimulus delivery and electrophysiological recordings were performed (VERIS 5.01.10X; EDI, San Mateo, CA). Supported by a chin rest, subjects viewed the stimuli that were presented at a distance of 36 cm on a computer monitor driven with a frame rate of 75 Hz. They were asked to fixate the center of a central black cross of 3° diameter. The stimulus display, a circular dartboard pattern (diameter, 44°; mean luminance, 64 cd/m^2; contrast, 98%) was subdivided into individual fields, each comprising a checkerboard of 4 × 4 checks. The radial extent of the fields was scaled with eccentricity from 1.5° in the center to 7° in the periphery. The fields were stimulated independently with an m-sequence (m-sequences consist of a pseudorandom succession of 0 and 1 states). For the pattern-reversal stimulation[12,41] applied, these two states were represented by two contrast-inverted checkerboard fields. The minimal duration of one state lasted one frame (13.3 ms). Stimuli were presented monocularly in two separate blocks for either eye, yielding a total of four blocks of mfVEP recording. A single block of pattern-reversal stimulation lasting 7 minutes consisted of an m-sequence with $2^{15} - 1$ (i.e., 32,767) elements. The blocks were divided into 16 overlapping segments, each lasting approximately 27 seconds.

mfVEP Analysis. First-order kernels were extracted (VERIS 5.01; EDI). Spatial smoothing and artifact rejection features available in VERIS were not used. All subsequent analyses were performed with technical graphing and data analysis software (IGOR 5.0; WaveMetrics, Inc.). The traces were, in accordance with previous studies,[12,13] digitally low-pass filtered with a high-frequency cutoff of 30 Hz. To assess the lateralization of the responses, we calculated the difference potentials between each of the three electrodes on one hemisphere and its corresponding electrode on the other hemisphere. These difference potentials entered the further analysis.

To assess signal presence we evaluated the signal-to-noise ratio (SNR*) as described by Zhang et al.[42] using a mean noise-window SNR. First, the records from the two blocks for each stimulus were averaged. Then the SNR* for each i-th sector (of the $n = 60$ total sectors) of subject j was defined as

$$\mathrm{SNR}^*_{ij} = \frac{\mathrm{RMS}_{ij}(45\text{–}150\ \mathrm{ms})}{\left[\sum_i \mathrm{RMS}_{ij}(325\text{–}430\ \mathrm{ms})/n\right]} - 1 \qquad (1)$$

The denominator in equation 1 is the average of the individual RMS values of $n = 60$ sectors in the noise window (325–430 ms after stimulus onset). It should be noted that this SNR definition deviates from the standard-SNR definition because of the subtraction of 1 and is therefore termed SNR*. An estimate of false-positive rates was obtained from the distribution of SNR* values in the noise window for each i-th sector, j-th subject, m-th electrode pair, and q-th condition following to Hood et al.[43]:

$$\mathrm{SNR}^*_{ijmq} = \frac{\mathrm{RMS}_{ijmq}(325\text{–}430\ \mathrm{ms})}{\left[\sum_i \mathrm{RMS}_{ijmq}(325\text{–}430\ \mathrm{ms})/n\right]} - 1 \qquad (2)$$

Thus, we calculated $i \times j \times m \times q$ SNR* values (i.e., 2400 values; $i = 60$ locations; $j = 20$ subjects; $m = 1$ [one electrode pair with maximal noise root-mean-square (RMS) of 3 electrode pairs]; $q = 2$ conditions [left and right eye stimulation]). Analysis of the distribution of these SNR* values showed that the probability of SNR* ≥0.75 to be part of the noise distribution is smaller than 5.4%. Therefore,, we applied an SNR* threshold of 0.75 to exclude "silent" visual field locations (without recordable signals) from our analyses. In our quantitative analyses, we compared two stimulus conditions (left and right eye stimulations). Each stimulus location was required to evoke suprathreshold responses in at least one of the two conditions to enter the analysis (logical OR-operator). Thus, a bias of the results to one of these two conditions because of the thresholding procedure can be avoided. For example, an AND operator would lead to an *exclusion* of stimulus locations that are suppressed below the SNR* threshold in only one of the 2 stimulus conditions and would, as a consequence, cause an underestimation of possible interocular differences of the responses.

For further analysis we selected for each visual field location the difference potential for the pair of electrodes on opposing hemispheres that yielded the greatest SNR* during stimulation of either eye.[43] This ensured that the same electrode pair was selected for left and right eye stimulation. Next, similar to the analysis of the cVEPs, the difference VEPs obtained for each eye were correlated with each other to obtain Pearson's correlation coefficient (r). For this correlation the signal time window (45–150 ms) was used in accordance with previous studies.[12,13] It should be noted that the correlation approach is a more objective approach than a single peak analysis and is particularly useful for dealing with small signal amplitudes.

To determine whether the mfVEP responses at recording sites on the left and right hemisphere were greater for stimulation in the contralateral hemifield (i.e., for stimulation in the right and left hemifields, respectively), the following analysis was performed: (1) For each hemifield, the maximal suprathreshold SNR* value (SNR* > 0.75) was extracted for each visual field location from the three recording sites on the left hemisphere (OL, left lateral occipital and left lateral parietal sites versus inion, and, separately, for the corresponding recording sites on the right hemisphere). Thus, one set of the maximal suprathreshold SNR* values was obtained for left hemisphere recordings and another for right hemisphere recordings. (2) For each set, these SNR* values were averaged across the left and across the right hemifield in separation, yielding an average SNR* for the right and another for the left hemifield. (3) The difference between these SNR* values was determined, yielding the interhemifield SNR* difference for each left and right hemisphere recording. As both responses for stimulation of either eye were processed separately, a total of four interhemifield SNR* differences were obtained for each subject (recording sites on 2 hemispheres × 2 stimulated eyes). To assess systematically whether there was an effect across hemispheres (left and right), subject groups (controls, PCD-patient without and with situs inversus totalis), and eye stimulated (left and right), a three-way ANOVA was conducted on the interhemifield SNR* difference averages (factors hemisphere, subjects group, and eye stimulated).

Results

Conventional VEPs

The interhemispheric activation differences were assessed using the difference cVEPs, OL minus OR, as described in Subjects and Methods. The difference traces of the PCD patients

and the matched controls are given in Figure 1 for a stimulus contrast of 98% (similar traces are obtained for 20% stimulus contrast; data not shown). From the juxtaposition of the difference traces obtained for left and right eye stimulation, it is evident that the traces are roughly parallel (i.e., positively correlated) for both the PCD patients and the controls. This feature is analyzed more formally by correlating the responses for both eyes with each other obtaining the correlation coefficient, *r*, as described in Patients and Methods. For a normal projection pattern, responses to stimulation of the right and left eyes are lateralized similarly. As a result, the obtained cVEP difference traces are positively correlated. In contrast, for an albino-like representation, abnormality responses to stimulation of the right and left eyes are to some degree represented on opposite hemispheres. Consequently, the difference traces obtained for the two eyes are expected to be negatively correlated.[12] Sample traces for three albinotic subjects are depicted in Supplementary Figure S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7194/-/DCSupplemental. The median *r* values from all 13 albinotic subjects were nega-

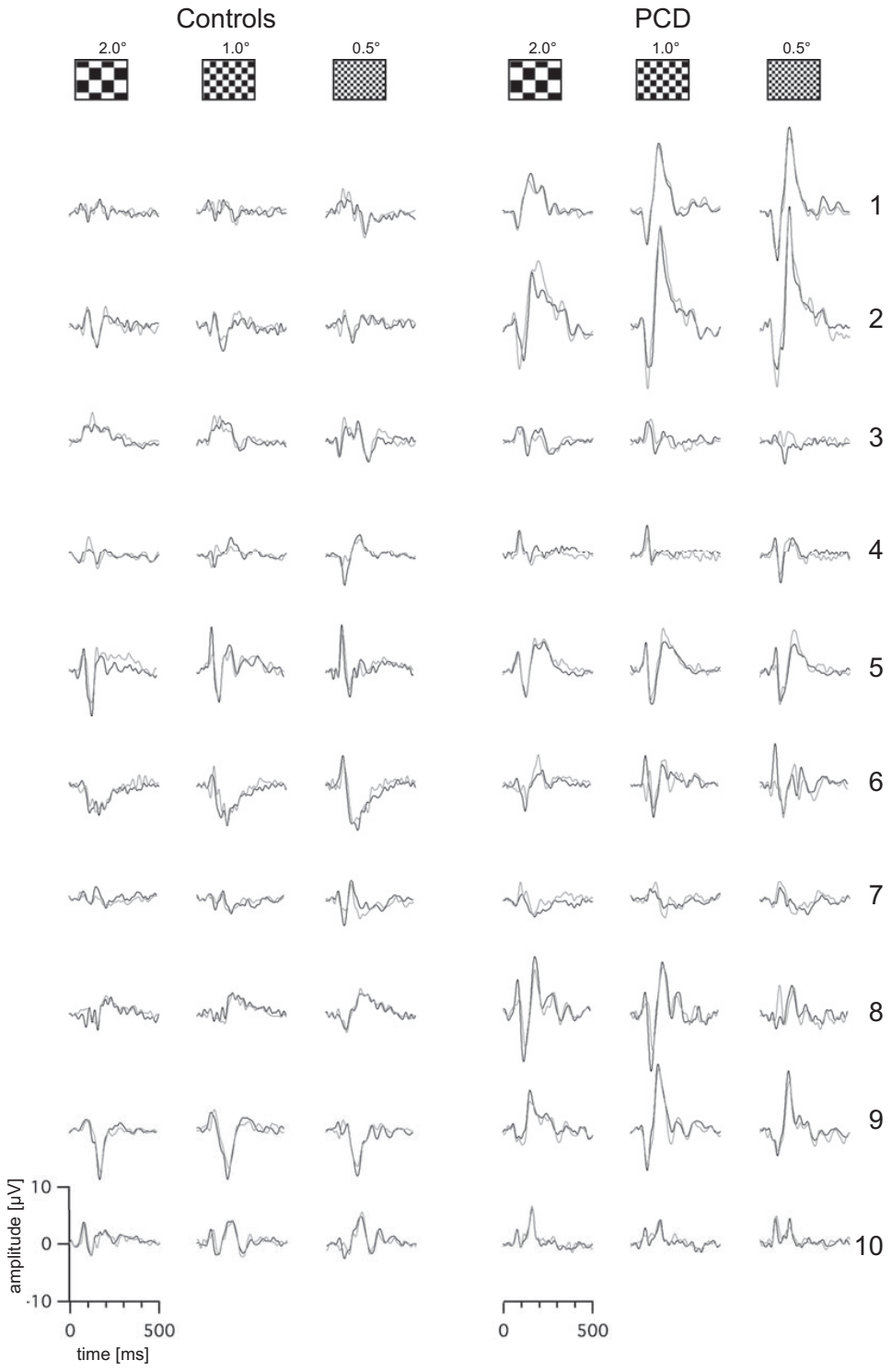

Figure 1. Individual interhemispheric activation-difference cVEPs for matched controls and PCD patients for three check sizes (2.0°, 1.0°, and 0.5°, as indicated by the icons) and 98% stimulus contrast, as obtained for the right eye (*black traces*) and the left eye (*gray traces*). Parallel traces are evident for all subjects.

TABLE 2. *r* Values for Interocular Correlation of the Interhemispheric Activation Difference Measured with the cVEP

Condition		Controls (n = 10)			PCD (n = 10)			Albinism (n = 13)		
Contrast	cs	Median	LQ	UQ	Median	LQ	UQ	Median	LQ	UQ
C98	0.5	0.84	0.79	0.90	0.78	0.48	0.95	−0.89	−0.91	−0.77
	1.0	0.79	0.71	0.88	0.85	0.74	0.96	−0.87	−0.91	−0.76
	2.0	0.74	0.66	0.89	0.88	0.78	0.94	−0.75	−0.80	−0.67
C20	0.5	0.89	0.84	0.94	0.87	0.70	0.97	−0.68	−0.88	−0.18
	1.0	0.79	0.75	0.85	0.75	0.56	0.97	−0.80	−0.84	−0.33
	2.0	0.73	0.69	0.85	0.91	0.75	0.93	−0.61	−0.75	−0.35

cs, check size (°); LQ, lower quartile; UQ, upper quartile; C98 and C20, 98% and 20% stimulus contrast, respectively.

tive for all conditions (albinism [n = 13]; r values for check size of 0.5°, 1.0°, and 2.0° for 98% contrast: −0.89, −0.87, −0.75; for 20% contrast: −0.68, −0.80, −0.61). For the controls and for PCD patients, the median r values were positively correlated for all conditions (controls [n = 10]: r values for check size of 0.5°, 1.0°, and 2.0° for 98% contrast: 0.84, 0.79, and 0.74 for 20% contrast: 0.89, 0.79, and 0.73; PCD [n = 10]; r values for check size of 0.5°, 1.0°, and 2.0° for 98% contrast: 0.78, 0.85, and 0.88; for 20% contrast: 0.87, 0.75, 0.91). A more detailed description of the distribution of the r values is given in Table 2. Although the comparison of the groups indicated that normal optic nerve projections were evident in the PCD patients, a subject-by-subject account is given in Figure 2. Here the obtained r values are depicted for each subject and stimulus condition. For albinism, 6 of 78 (13 subjects × 6 stimulus conditions) r values were positive, five of which were obtained from a subject with particularly small misrouting as confirmed by fMRI,[9] thus highlighting the specificity of the cVEP to identify misrouting. PCD patients and controls yielded greater r values than any albinotic subjects for each stimulus condition, with only one exception, namely for 20% stimulus contrast and 0.5° check size (this condition had the least accuracy in the detection of albinotic misrouting because it yielded a positive r value for one albinotic subject who had negative r values for each of the other stimulus conditions; see Fig. 2).

Further, for one condition (0.5° check size at 98% contrast), the r values were smaller in three PCD patients (one PCD patient with situs inversus abdominalis [P3] and two with situs inversus totalis [P6 and P8]) than in the controls. Even here, the r values are not negative; the only slightly negative r value is observed for P3 for small check size stimuli (0.5°) and 20% contrast. These results might suggest a potential subtle abnormality for three PCD patients, which is clearly less pronounced than the abnormalities commonly observed in albinism. This could imply that the abnormality is restricted to small parts of the visual field, a hypothesis that was tested with mfVEPs.

mfVEP

To assess the interhemispheric activation differences for specific visual field locations, mfVEPs were recorded and analyzed in a way similar to that for cVEP analysis. Difference traces of the mfVEPs recorded at symmetrical electrode sites on the left and right side of the scalp were calculated. An example of such difference traces from control subjects is given in Supplementary Figure S2, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7194/-/DCSupplemental. For each eye the signals were characterized by a great variability of signal strength and shape across the visual field, which is a well-known feature of mfVEPs and is related to the cortical convolution.[33,44] In contrast, the responses obtained for the two eyes at a particular visual field location resembled each other, which indicates that they were, as expected, similarly affected by the cortical convolution. As a consequence, correlation of these traces yielded predominantly positive r values for the controls, as is demonstrated for two subjects in Supplementary Figure S2, http://

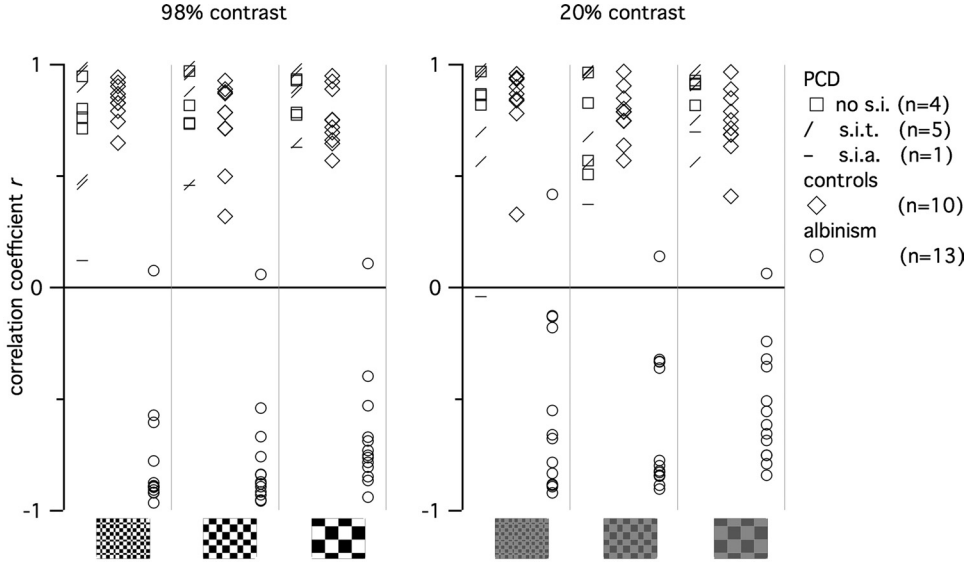

FIGURE 2. Individual interocular correlation coefficients of interhemispheric activation differences given in separate columns for PCD patients with different expressions of situs inversus, for controls, and for albinotic subjects for each stimulus condition (for three check sizes [0.5°, 1.0°, and 2.0°] and two stimulus contrasts [98% and 20%] as indicated by the icons). In contrast to the albinotic subjects, none of the PCD patients and controls had consistently negative correlation coefficients across conditions. s.i.t./a., situs inversus totalis/abdominalis.

www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7194/-/DCSupplemental. In Figure 3, data are given for three patients: two PCD patients (P8 and P2) with the highest frequency of negative *r* values for the mfVEP difference traces (percentages of $r < 0$, 14.0 and 11.7, respectively) and for the only PCD patient (P3) with negative *r* values for the cVEP difference traces for some stimulus conditions (percentage of $r < 0$ for mfVEPs, 1.8). It should be noted that especially for P8, the frequency of suprathreshold responses, i.e., with an SNR* > 0.75, was comparatively small, indicating that the data were confounded by low SNR*. Accordingly, excursions of the traces with negative *r* values were comparatively small for both subjects (corresponding to small SNR*). These are only weak indicators, if any, of abnormal visual field representations. Taken together, although the selection of the subjects contributing to Figure 3 was deliberately biased to those with partic-

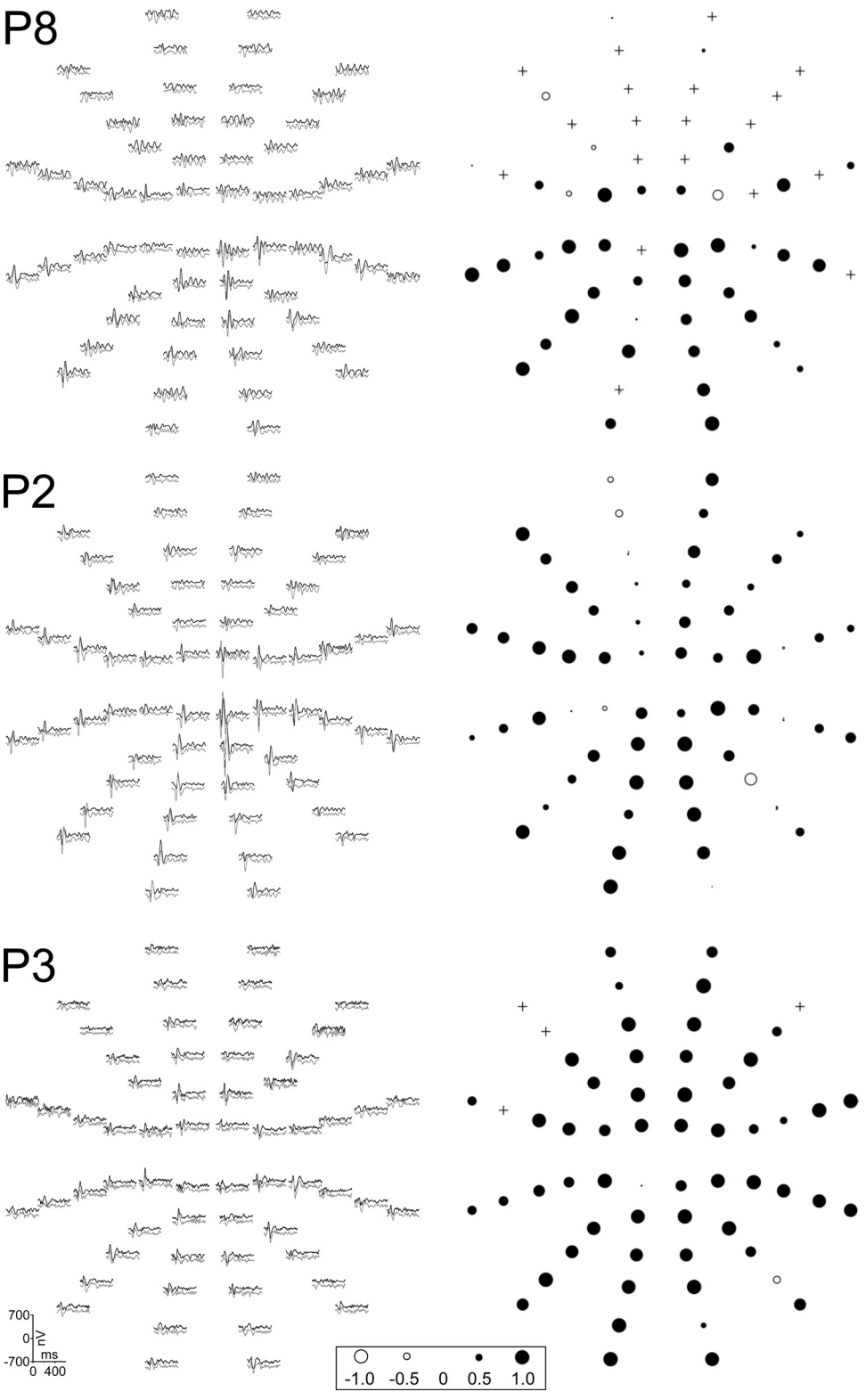

FIGURE 3. Comparison of right and left eye mfVEP responses to pattern-reversal stimulation for the two PCD patients (P8 and P2) with the highest incidence of negative correlation coefficients and the only PCD patient with negative *r* values for some cVEPs (P3). Traces and symbols are arranged according to the spatial layout of the visual field locations that evoked them; traces and symbols from different eccentricities are arranged in an equidistant manner, whereas the actual stimulus layout is approximately m-scaled. *Left*: interhemispheric mfVEP difference traces after the depiction of right (*black traces*) and left (*gray traces*) eye stimulation. As for the controls, the responses varied across the visual field, whereas for a particular visual field location, similar traces were obtained for both eyes. *Right*: correlations of the interhemispheric mfVEP differences in stimulation of the left and right eyes are depicted. The strength of the correlation is indicated by the diameters of the circles. The diameters of the circles scale linearly with the absolute correlation coefficient obtained; the resultant diameters for correlation coefficients of ± 1.0 and ± 0.5 are given in the legend. *Filled symbols*: positive correlation (normal projection pattern). *Open symbols*: negative correlation (abnormal projection pattern). *Plus signs*: subthreshold responses (SNR* < 0.75). As for the controls, for most suprathreshold visual field locations, positively correlated responses were obtained. For a few locations, the *r* value was negative, which is indicative of antiparallel traces for the corresponding traces.

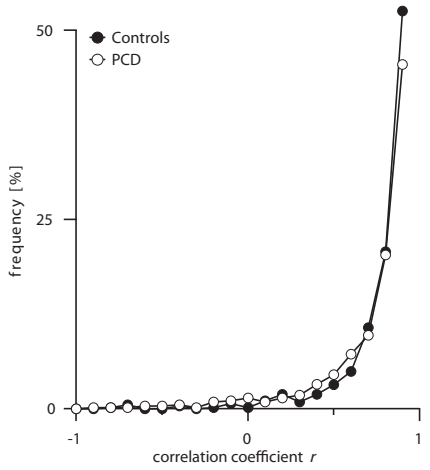

FIGURE 4. Frequency distribution of the *r* values obtained in controls (*top*) and PCD patients (*bottom*) for interocular correlations of interhemispheric mfVEP differences. Although similar gross distributions were obtained for both groups, there was a slight tendency for the PCD patient cohort to a higher frequency of smaller *r* values.

ularly negative *r* values, most responses yielded positive *r* values.

For a quantitative assessment of the data, we compared the results of the controls and the PCD patients. We obtained similar percentages of suprathreshold (SNR* $\geq$ 0.75) visual field locations for controls and PCD patients for pattern reversal responses (mean $\pm$ SD: 94.8% $\pm$ 3.0% and 92.7% $\pm$ 9.6%, respectively). To assess whether in the PCD patients there are some visual field locations that follow the abnormal representation pattern evident in patients with albinism, the frequency distributions as a function of the *r* values were determined for the control group and the group of PCD patients (Fig. 4). Similar distributions of correlation coefficients were evident for both groups, with a slight trend to smaller *r* values in that of the PCD patients (controls and PCD, proportion of $r < 0.0$: 1.9% and 4.0%, respectively). An analysis on a subject-by-subject basis is depicted in Figure 5. For three patients (P2, P4, and P8), two with situs inversus, the interhemispheric activation differences were less correlated for the two eyes than for any of the control subjects. It should be noted, however, that the deviation from the normal interocular correlation of the interhemispheric activation differences is a very subtle feature, resulting in only few clearly negative correlations ($r < -0.5$). Further, it was tested whether there was a correlation in the frequency of negative *r* values in the mfVEP recordings with the *r* values obtained for the different cVEP conditions. None of the correlations reached significance for the controls or for the PCD patients, underscoring the absence of a consistent representation abnormality.

These analyses demonstrated that the responses in the visual cortex were similarly lateralized for stimulation of left and right eyes for both the controls and the PCD patients tested. Finally, it was of interest to assess whether the right and left hemifields were indeed represented on the respective contralateral hemispheres in the PCD patients, as is normally the case. Although farfetched, situs inversus might also be associated with an inversion of this general lateralization pattern entailing a lateralization of visual responses on the visual cortex ipsilateral to the stimulated hemifield for both eyes. This feature would go unnoticed in the analyses described here. Consequently, we sought to determine whether the mfVEP responses at recording sites on the left and the right hemisphere were greater for stimulation in the contralateral hemifield (i.e., for stimulation in the right and left hemifield, respectively). As detailed in Subjects and Methods, the SNR* differences between stimulation in the left and the right hemifield, the so-called interhemifield SNR* differences, were determined for the recording sites on the left and, separately, for those on the right hemisphere for each subject and stimulation condition. Thus, a total of four interhemifield SNR* differences were obtained for each subject (recording sites on 2 hemispheres $\times$ 2 stimulated eyes). In case of a normal lateralization pattern, it should be expected that the interhemifield SNR* differences ($SNR^*_{\text{left hemifield}} - SNR^*_{\text{right hemifield}}$) are positive for right hemisphere recordings and negative for left hemisphere recordings. Indeed, this normal lateralization pattern is evident from Supplementary Figure S3, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7194/-/DCSupplemental, for all subject groups (for controls, for PCD patients without situs inversus totalis, and, though least pronounced, for PCD patients with situs inversus totalis). To assess systematically whether there was an effect across hemispheres (left and right), subject groups (controls, PCD patients without situs inversus totalis, and PCD patients with situs inversus totalis), and eye stimulated (left and right), a three-way ANOVA was conducted on the interhemifield SNR* difference averages (factors hemisphere, subject group, and eye stimulated). A significant effect was obtained only for the factor hemisphere ($P < 0.0001$), indicating that the lateralization of the mfVEPs depended on the hemifield stimulated. Importantly, there was no significant interaction of subject group $\times$ hemisphere, which underscores that the lateralization of the responses did not differ significantly between the controls and either patient group. In summary, for the controls and for the PCD patients with and without situs inversus totalis, the mfVEPs were dominant at recording sites contralateral to the stimulated hemifield.

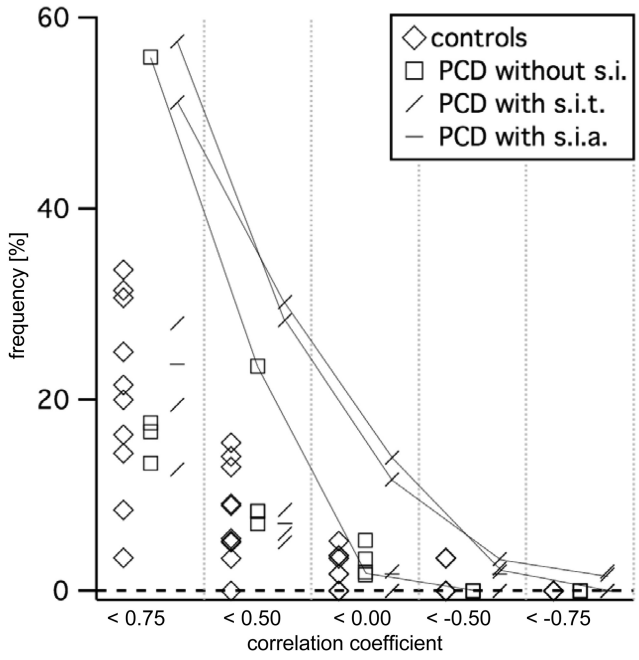

FIGURE 5. Comparison of individual *r* value distributions. The frequency of *r* values smaller than 0.75, 0.50, 0.00, -0.50, and -0.75 is given for controls, PCD patients without situs inversus, and PCD patients with situs inversus. *Connected symbols*: three PCD patients, two with situs inversus totalis (P8, P2) and one without situs inversus (P4) stand out from the control cohort. s.i.t., situs inversus totalis.

Discussion

We conclude from the results of the present study that the visual pathways in PCD are normal. Neither PCD nor the profound lateralization abnormality situs inversus itself was associated with an abnormal lateralization of the optic nerve projections typically observed in albinism. Studying 10 PCD patients, five with situs inversus totalis and one with situs inversus abdominalis, no consistent indications of abnormally lateralized cortical visual field representations were evident, either at a large scale as reflected by cVEPs or at a small scale as reflected by mfVEPs. This is taken as evidence that a previous report of misrouting in a PCD patient with situs inversus was a singular finding. General lateralization abnormalities associated with situs inversus do not disturb the normal projection pattern of the optic nerves that is mandatory for partial decussation at the optic chiasm. Similarly, defects in ciliary proteins leading to PCD in the investigated cohort are not related to and causal for such projection abnormalities.

Functional Characteristics of PCD Patients

For the patients examined in the present study, there was no electrophysiological evidence of abnormal optic nerve projections, either for cVEPs or for mfVEP, approaches, which are known for their sensitivity in detecting such abnormalities. For the cVEP-paradigm devised by Apkarian et al.,[11] many studies[11,17,36,45] demonstrated a high accuracy of up to 100%. Similarly, high accuracies were estimated for the corresponding mfVEP paradigm with a specificity of approximately 95% and a sensitivity of 75%.[12,13] Importantly, the electrophysiological findings in the PCD patients were corroborated by their functional and ophthalmological characteristics, such as normal fundi and iridae, largely normal visual acuity, absence of nystagmus, and, with the exception of one patient (P2), normal ocular alignment and stereoscopic vision. Thus, traits typically associated with abnormally crossing optic nerves were absent in the patients in the present study. In contrast, the only patient with PCD to be reported to have misrouted optic nerves[20] had foveal hypoplasia and functional deficits such as reduced visual acuity, nystagmus, and an absence of stereo vision. These are features of the visual system that are not typical for PCD, underscoring that the respective patient was an exceptional case not only in terms of the optic nerve projections but also in terms of other ocular symptoms.

Incidence of Abnormal Crossing of Optic Nerve Fibers

Misrouted optic nerves have long been considered to be specific for albinism.[16] In fact, the abnormality is absent in human carriers of albinism (see Ref. 11 for review) and patients without albinism but with some ocular symptoms observed in albinism, such as foveal hypoplasia,[14] dissociated vertical deviation and missing stereopsis,[45–47] and congenital nystagmus.[48,49] Further, normal monocular projections were observed in patients with unilateral anophthalmia or severe microphthalmia.[50] As a consequence, the projection abnormality has long been considered to be a pathognomonic sign of albinism and has since served as a diagnostic aid. Recent reports did question this view, as they indicated that markedly abnormal crossed projections at the optic chiasm might be evident in the absence of the albinotic phenotype. They suggested that the abnormality might be associated with other diseases, namely PCD and CSNB.[18–20] Although the present study highlights that abnormal optic nerve projections are not typical for PCD or Kartagener syndrome, they appear, at least in a proportion of the patients, to be associated with X-linked CSNB.[18–20] It is at present not fully resolved whether the occurrence of misrouting in these CSNB patients might be related to mild forms of albinism, in particular ocular albinism OA1, which is also associated with an X-linked locus.[18–20] It should be noted here that X-linked OA1 (a female with parents reported to be "healthy") cannot cause the misrouting in the Kartagener patient described by van Genderen.[20] Taken together, crossed visual pathways are typical and indicative of albinism, and some incidental findings might be associated with mild expressions of albinism. Further studies in these subjects are needed to incontrovertibly clarify whether there might be a mechanism to induce the pathway abnormality that is entirely independent of albinism. In particular, the explicit exclusion of an OA1 genotype in apparently nonalbinotic subjects with misrouting would be compulsory in such studies.

Correlation-based interocular comparisons of interhemispheric cVEP and mfVEP differences allow for the detection of abnormally lateralized visual field representations that are a consequence of misrouted optic nerves typical for albinism. In the present study, this approach was applied to 10 PCD patients and normal response patterns were obtained. In conclusion, abnormal cortical lateralization patterns indicative of misrouting of the optic nerves do not appear to be a common trait of PCD patients.

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