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Pigmentation predicts the shift in the line of decussation in humans with albinism

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Abstract

In albinism a large proportion of nerve fibres originating in temporal retina cross the midline at the chiasm and project to the contralateral hemisphere. Studies in rodents with albinism have suggested that the extent of this misrouting at the chiasm is inversely related to pigmentation levels. Here, we examine whether there is evidence for a similar relationship in humans with albinism. Functional MRI was performed on 18 subjects with albinism, 17 control subjects and six controls with nystagmus as they underwent hemifield visual stimulation of nasal or temporal retina. Functional activation in 16 coronal slices beginning at the posterior occipital lobes were analysed and the extent of hemispheric response lateralization at each slice position was determined. During temporal retina stimulation, the control response was lateralized to the hemisphere ipsilateral to the stimulated eye for all slices. In albinos, the response in posterior slices was predominantly in the contralateral hemisphere, consistent with misrouting of temporal retina fibres. However, as slice location became progressively anterior, response lateralization reverted to the ipsilateral hemisphere. The slice location at which the transition from contra- to ipsilateralization occurred provided an estimate of the extent of fibre misrouting in the individual. The slice transition location correlated negatively with pigmentation level, providing the first evidence for a relationship between pigmentation and the extent of misrouting in humans with albinism.

Introduction

Albinism is a genetic disorder characterized by an abnormal decussation pattern at the optic chiasm. In humans the line of decussation normally lies along the nasotemporal divide through the fovea, such that fibres originating in nasal retina cross the midline and project to the contralateral hemisphere and fibres originating in temporal retina remain uncrossed at the chiasm and project ipsilaterally. In albinism there is a shift in the line of decussation into temporal retina, which results in an abnormally large contralateral projection from each eye (Lund, 1965). Normal retinotopic organization in visual cortex is thus disrupted, with each cortical hemisphere receiving abnormal input from the ipsilateral hemifield in addition to the normal contralateral hemifield representation (Hubel & Wiesel, 1971; Kaas & Guillery, 1973; Creel *et al*., 1981; Guillery *et al*., 1984; Leventhal & Creel, 1985; Hoffmann *et al*., 2003).

Although at least five different genes which give rise to albinism have been identified in humans, the enzymes or proteins encoded by these genes all adversely affect melanin production (Tomita *et al*., 1989; Boissy *et al*., 1996; Manga *et al*., 1997; Puri *et al*., 2000; Newton *et al*., 2001; Rinchik *et al*., 1993), and it is the reduction in or complete absence of melanin during development of the eye which causes a significant proportion of ganglion cell nerve fibres originating in temporal retina to project to the contralateral hemisphere (Jeffery *et al*., 1994; Jeffery, 1997). Hypopigmentation also results in an underdeveloped fovea in individuals with albinism (Elschnig, 1913). Despite the shift in the line of decussation, not all fibres originating in temporal retina project contralaterally. Fibres originating in more peripheral temporal retina revert to the normal projection pattern to the ipsilateral hemisphere (Creel *et al*., 1981; Hoffmann *et al*., 2003, 2005).

The size of the abnormal projection from temporal retina has been shown to vary across different strains of hypopigmented animals. A comparison of Siamese and albino cats reveals that the shift into temporal retina of the line of decussation is more severe in tyrosinase-negative albino cats than in the hypopigmented Siamese variety (Creel *et al*., 1982; Leventhal & Creel, 1985; Ault *et al*., 1995), resulting in a much larger contralateral projection in the albino cat. Similar observations have been made regarding the extent of misrouting in different strains of albino mice (Balkema & Drager, 1990; LaVail *et al*., 1978) and mink (Sanderson *et al*., 1974), where albino strains exhibiting higher pigmentation levels had proportionately smaller abnormal projections from temporal retina. Thus, it appears that albino mutations associated with more severe deficits in melanin, and hence lower pigmentation levels, cause a greater shift in the line of decussation into temporal retina.

Although such a relationship between pigmentation and the severity of misrouting remains to be determined in humans with albinism, retinotopic mapping techniques (Hoffmann *et al*., 2003) and visual evoked potential studies (Hoffmann *et al*., 2005) have shown that the size of the abnormal contralateral projection also varies amongst humans with albinism, with the shift in the line of decussation into temporal retina ranging between 2 and 15° of visual field. These techniques afford a precise estimate of the shift but have some limitations: retinotopic mapping is only appropriate for use in subjects with nystagmus less than a few degrees (Baseler *et al*., 2002;

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Hoffmann *et al*., 2003) and both require selective stimulation of the visual field.

A recent functional magnetic resonance imaging (fMRI) study (Schmitz *et al*., 2004) used hemispheric lateralization of activity, elicited by monocular full-field visual stimulation, to quantify the extent of misrouting in 16 individuals with albinism. Although the hemispheric lateralization varied markedly across subjects and correlated with some clinical features of albinism, it did not correlate with pigmentation. We build on that study by evaluating hemispheric lateralization of activity, but restrict the analysis to the early visual areas of the brain rather than the full hemispheres. This overcomes the problem of significant unilateral activations that were presumed to result from abnormal oculomotor behaviour in albinos (Schmitz *et al*., 2004). Such an effect would reduce the accuracy of hemispheric lateralization as a measure of the routing of visual fibres. We also take advantage of the topographic mapping of the retina onto the cortex, which results in central and peripheral retinal locations being represented at posterior and anterior parts of the occipital lobe, respectively. Measuring hemispheric lateralization as a function of the axial distance from the occipital pole therefore provides a useful gauge of where on the retina the line of decussation exists. In taking the two steps briefly described above we have been able to assess the shift of the line of decussation and relate it to other features of albinism. We have been able to show, for the first time, that pigmentation predicts the shift in the nasotemporal division in humans with albinism.

Materials and methods

Eighteen subjects with albinism (mean age 42, range 18–64 years; 14 females) were recruited for this study. It is not possible to apply the histological assessment of ocular pigmentation used in previous work on the relationship between pigmentation and misrouting in animal models to our human subjects. Instead, pigmentation levels were determined by visual assessment of hair and skin pigmentation and classified on a numeric scale from 1 to 6, with 1 indicating no pigmentation in hair or skin and 6 indicating dark hair and good skin tanning (modified from the scales used by Schmitz *et al*., 2003). Pigmentation assessment was performed by the authors prior to scanning. Further details of the patients are listed in Table 1.

In addition, 17 control subjects (mean age 39, range 18–64 years; 10 female) and six control subjects with nystagmus (mean age 33, range 18–54 years; three female) were tested. The nystagmus amplitude range in these six subjects was ±1 to ±4°.

All subjects gave their informed consent. The study was approved by the Royal Holloway Ethics Committee and conformed with the Declaration of Helsinki.

Subjects underwent fMRI whilst observing a visual stimulus. The visual stimulus consisted of a left or right hemifield high-contrast chequerboard with seven concentric half-annuli, each with 12 checks extending to an eccentricity of ±15° from a central fixation cross. All visual stimulation was performed monocularly on the subject's preferred eye for viewing. An eyepatch was used to cover the other eye and to ensure viewing of the stimulus was monocular. Hemifield stimuli were presented in a blocked design with seven 36-s cycles, each consisting of 18 s of stimulus presentation and 18 s of uniform grey background, with a central fixation cross throughout. The pattern stimulus was presented as a pattern-reversal paradigm with a frequency of 1 Hz.

T2* images were acquired throughout using a multislice gradient-echo EPI pulse sequence (TE = 52 ms, TR = 3 s, 128 × 128 acquisition matrix, FOV 24 cm, slice thickness 4 mm) on a Siemens 3T Trio. A set of 16 coronal slices positioned over the back of the head ensured full coverage of the occipital lobe of both hemispheres. A total of 84 temporal samples were obtained. In addition to the functional data, high-resolution MP-RAGE image volumes were acquired (TI = 1100 ms, TR = 11.4 ms, 176 sagittal slices, 256 × 256, slice thickness 1 mm).

Functional data were Fourier-analysed to determine the correlation of each voxel time series with the fundamental frequency of stimulation (Engel *et al*., 1997). Functional scans were aligned with each subject's high-resolution anatomical scan (Stanford VISTA software, http://white.stanford.edu/software) and the latter's Talairach transform was determined (Talairach & Tournoux, 1988) using the same software package. A set of anatomically defined volumes in Talairach coordinates from the Talairach atlas (Talairach & Tournoux, 1988) covering early visual areas were then transformed onto each individual's anatomical scan and from there, using the transformation determined through the alignment process, into the functional

TABLE 1. Details of the subjects who took part in the study

Subject	Gender	Age (years)	Classification of albinism	Level of pigmentation*	Visual acuity (preferred eye)	Nystagmus (± deg)
AHa	F	38	OCA	3	0.25	3.2
ALM	F	34	OCA	4	0.10	6.0
AnC	F	34	OCA2	4	0.50	1.5
BE	M	60	OCA	2	0.33	1.5
CE	F	47	OCA2	3	0.21	–
CH	F	58	OCA1a	2	0.27	4.0
CLH	F	21	OCA1	1	0.10	5.0
DH	M	19	OCA1a	1	0.13	8.0
ES	F	32	OCA	2	0.50	1.5
FM	F	65	OCA1b	2	0.17	2.7
JK	F	25	OCA1	2	0.20	2.0
JM	M	34	OA	5	0.10	4.1
LS	F	34	OCA	2	0.17	4.5
MG	F	54	OCA1a	1	0.17	5.0
RMN	M	64	OCA	3	0.33	4.0
SU	F	52	OCA1a	1	0.12	1.8
TM	F	55	OCA	3	0.17	7.0
VP	F	35	OCA	4	0.10	6.0

*Pigmentation level scale: 1, completely white hair, white skin; 2, yellowish-white hair, white skin with possible tanning; 3, pale blonde hair, pale skin with some tanning; 4, blonde hair, pale skin w with visible tanning; 5, dark blonde or light brown hair, good skin tanning; 6, brown, dark brown or black hair, good skin tanning.

inplanes. The predefined anatomical areas used as regions of interest (ROI) were the left and right inferior occipital lobes, the left and right cuneus and the left and right lingual gyri. These areas covered an extensive part of the occipital lobe, ensuring inclusion of multiple visual areas. The ROIs for each hemisphere were then combined to form a single ROI on a slice-by-slice basis, such that any overlap between the predefined anatomical regions was removed. As not all subjects' anatomical slices were placed in the same anatomical location, slice position was 'normalised' such that slice 1 in all subjects represented the most posterior slice containing part of the ROI. In following the approach described we arrived at unbiased automated selections of ROIs for each individual within which activity in response to visual stimulation could be examined. Such unbiased and automated selections of cortex are essential, given that we had a knowledge of levels of pigmentation of the individual patients which could have biased any manual selection procedures. Therefore, although more precise information may have been derived by manually selecting an ROI for each subject, using anatomical landmarks such as the banks and fissure of calcarine cortex, we felt it could make the study vulnerable to bias and to low signal strength over a far smaller region of cortex.

We considered assessing the activity in a flattened representation of the cortical surface, as is commonplace in retinotopic mapping procedures (e.g. Engel *et al*., 1997). This confers the advantage of being able to specify dimensions and ROIs in surface-based coordinates. However, given that we had no topographic landmark that could be derived from functional activity, we felt that the advantage gained by a surface-based approach would have been outweighed by the reduction in the objective and automated manner in which the ROI could be defined.

An asymmetry index, AI, was defined to quantify the degree of response lateralization within the ROIs in each coronal slice, based on a Michelson contrast ratio and normalized for ROI size:

$$AI = (N_C N_{IT} - N_I N_{CT})/(N_C N_{IT} + N_I N_{CT}) \quad (1)$$

where N_C and N_I are the number of significant voxels at a correlation threshold of 0.3 ($P < 0.01$) in the contralateral and ipsilateral hemisphere ROIs of a given slice, and N_{CT} and N_{IT} are the total number of voxels in the respective ROIs. The AI therefore ranges from −1 to +1, where a value of 0 indicates equal distribution of signal across both hemispheres, −1 indicates a response lateralized to the right hemisphere for a given slice, and +1 a response lateralized to the left hemisphere. Where there is no response in either hemisphere, the asymmetry index is undefined as this results in division by 0.

Although it is well known that increasingly eccentric visual field locations are normally represented at increasingly anterior cortical locations in the occipital lobe, we wished to determine whether such a relationship held for individuals with albinism. The slice-by-slice analysis we propose would only be effective in assessing the shift in the line of decussation if the posterior to anterior cortical mapping of central to peripheral retinal locations were to hold. We investigated the issue by assessing, for four albinos, retinotopic data that have been previously published (Hoffmann *et al*., 2003). Using the same ROIs defined above, we evaluated the average phase of the cortical response to an expanding-rings stimulus in these ROIs on a slice-by-slice basis. In the case of an expanding-rings stimulus, phase is an indicator of visual field eccentricity as more peripheral visual field locations are stimulated at later times in the stimulus cycle. Figure 1 plots the average eccentricity on a slice-by-slice basis in the four albinos presented in the Hoffmann *et al*. (2003) study. In all subjects, increasing slice numbers were associated with larger phase shifts and hence higher eccentricities, indicating that slice location is a good predictor of retinal eccentricity. It would appear, therefore, that visual areas that do not represent increasing eccentricity in a posterior–anterior fashion and representations of central visual field that lie anterior to the occipital pole do not disrupt the mapping relationship we predicted. However, this may well be due to the ROI we selected, which principally selects regions in and around the calcarine sulcus, where the relationship is strong.

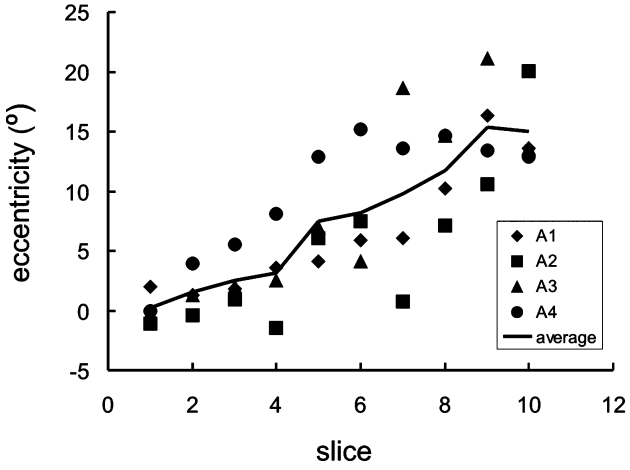

FIG. 1. Eccentricity as a function of slice number for the expanding rings retinotopy data from Hoffmann *et al*. (2003). The solid line represents the average across all four subjects. Greater eccentricities, i.e. greater phase delays, are apparent in more posterior slices, indicating that slice location is a good estimator of visual field location.

Results

The cortical response to nasal and temporal retina pattern-reversal stimulation in a control subject and in an albino is shown in Fig. 2. Both the control and the albino displayed contralateral response lateralization to stimulation of nasal retina. When temporal retina was stimulated, the control response was lateralized to the ipsilateral hemisphere but the albino response was predominantly contralateral. This lateralization became increasingly ipsilateral in more anterior visual cortex. Thus, central visual field locations were abnormally represented in the contralateral hemisphere whereas more peripheral visual field representations reverted to the ipsilateralization expected from temporal retina stimulation in the absence of any abnormality.

Before assessing the lateralization of cortical activity with the asymmetry index analysis, we examined the overall number of voxels that exceeded the correlation threshold for differences between each group and stimulation condition (Fig. 3). The distribution of activation across the hemispheres differed markedly between the control groups and the albinos. The control subjects exhibited strong contralateralization for stimulation of nasal retina, as demonstrated by much larger voxel counts, and similarly strong ipsilateralization in response to temporal retina stimulation. The same pattern of activation was apparent in the nystagmus control group although the lateralization was not quite as marked. For the albino group, the distribution of voxels across the left and right hemispheres was very similar to those of the control groups when nasal retina was stimulated. However, the distribution of activated voxels during stimulation of temporal retina in albinos was abnormal, with more active voxels in the hemisphere contralateral to the eye for posterior slices ($P < 0.05$ by ANOVA for slices 1–5). In more anterior slices the number of voxels in the

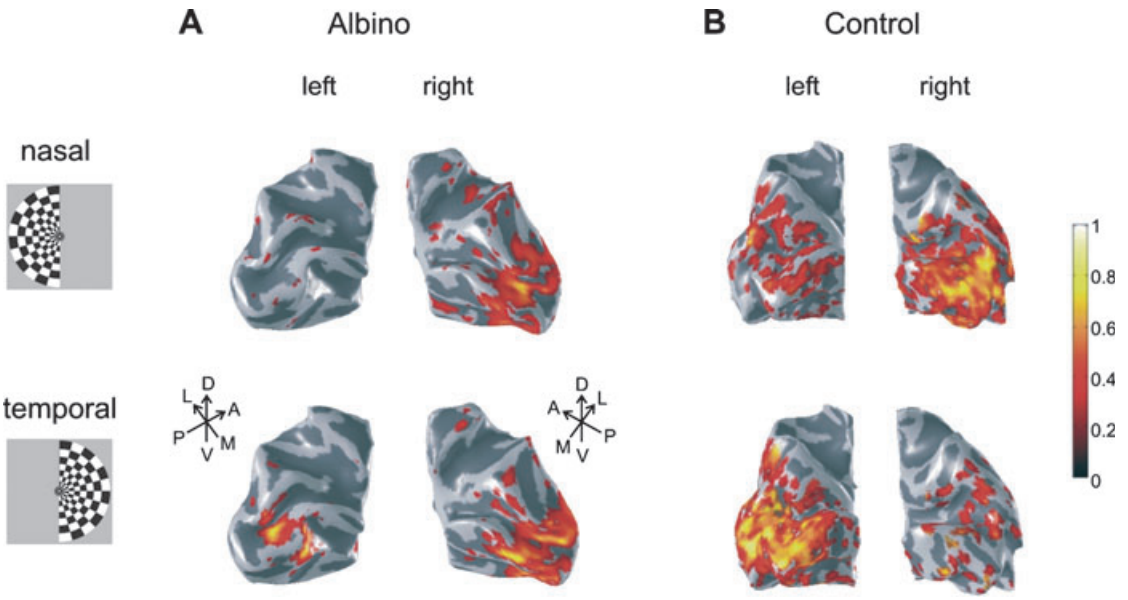

FIG. 2. Cortical response to left and right hemifield pattern-reversal stimulation of nasal and temporal retina (top and bottom row, respectively) in (A) an albino and (B) a control subject for stimulation of the left eye. The cortical response is overlaid on the subjects' rendered and inflated left and right occipital lobes. Nasal retina stimulation (left visual hemifield, top row) results in a response lateralized to the hemisphere contralateral to the stimulated eye in both the albino and the control subjects. When temporal retina was stimulated (right visual hemifield, bottom row) the control response was lateralized to the hemisphere ipsilateral to the stimulated eye. The albino response to temporal retina stimulation was contralateral to the stimulated eye for posterior parts of the occipital lobe, representing central visual field locations, but reverted to the 'normal' ipsilateralization for more peripheral visual field representations. The colour bar refers to the correlation coefficient, thresholded at 0.3 ($P < 0.01$).

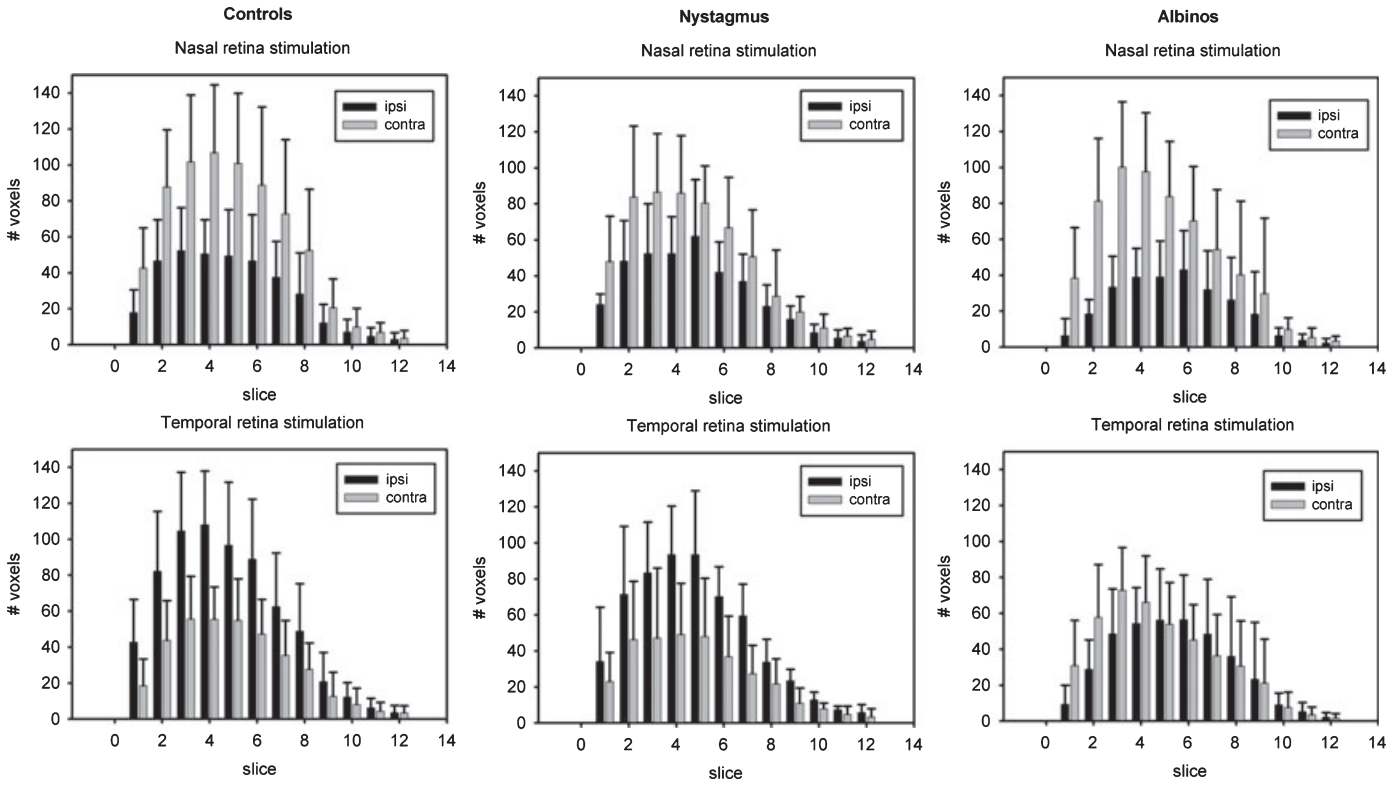

FIG. 3. The number of active voxels in the ipsilateral vs. contralateral hemisphere for each slice is plotted for controls, controls with nystagmus, and albinos.

hemisphere ipsilateral to the eye exceeded the number in the contralateral hemisphere; that is, the pattern reverted to normal. Total voxel counts for each stimulus condition were similar in both controls and albinos (data not shown) and showed no significant differences ($P = 0.36$ and $P = 0.56$ for stimulation of temporal and nasal retina, respectively).

A feature common to all groups was the variation of active voxel numbers with slice number, which peaked around slice 4. At anterior

slices the number of voxels decreased to low values; this was most evident in subjects with nystagmus (both control and albino). As it is important that any effect of reduced voxel numbers on the asymmetry index, and hence lateralization, is properly accounted for, we examined the data obtained from the groups to establish a criterion for the minimum number of voxels that should contribute to the calculation of the index for individuals. We found that there was no longer a significant difference between ipsilateral and contralateral voxel counts for the stimulation of temporal retina in subjects with nystagmus where the total number of active voxels in the slice was < 50 ($P > 0.05$, by ANOVA). Thus, the predicted ipsilateralization was not evident when the mean voxel count for the group was < 50. We therefore used this criterion, being the most stringent one, as a threshold for all subjects when calculating asymmetry indices.

Figure 4 depicts asymmetry indices as a function of coronal slice number averaged across each subject group. In response to nasal retina stimulation all groups displayed positive asymmetry indices, indicating a visual response lateralized to the contralateral hemisphere; analysis with a general linear model indicated that overall stimulation of nasal retina resulted in no significant group difference ($P = 0.124$). Interestingly, a significant difference ($P < 0.01$ for group × slice interaction for nasal retina) in the asymmetry index was evident at the most posterior slices (slices 1 and 2, $P < 0.001$), where the index for the albino group exceeded those for the controls groups. This is probably a result of the bilateral representation of the vertical meridians and very central visual field in the control group subjects and the absence of these bilateral representations in the albinos. When temporal retina was stimulated, the albino group displayed a markedly different response pattern from the control groups overall ($P < 0.001$). While both the controls and the controls with nystagmus had a response that was ipsilateral across all slices, the albino group response was lateralized to the contralateral hemisphere in posterior slices and only became ipsilateral as slice location became progressively anterior ($P < 0.001$ for group × slice interaction for temporal retina). These results emphasize that for more peripheral visual field locations the projection pattern reverted to the ipsilateral hemisphere.

The asymmetry index as a function of slice number is plotted for each albino individually in Fig. 5A and B. Accompanying the plots for albinos are individual data for controls with nystagmus (Fig. 5C). Although there was some variability across subjects the asymmetry index was predominantly positive for stimulation of the nasal retina, indicating a lateralization to the hemisphere contralateral to the eye (Fig. 5A). The variability observed in these data only resulted in two negative values of the asymmetry index, which would indicate an ipsilateral projection. The same plots of asymmetry index for the subjects with nystagmus also resulted in some erroneous lateralization (one value for nasal and one for temporal stimulation conditions), so the effect was not group-specific and probably resulted from random noise. When temporal retina was stimulated (Fig. 5B), the albino response showed significant intersubject variability. In posterior slices (lower slice numbers), all subjects initially exhibited a response that was lateralized to the contralateral hemisphere, as demonstrated by a positive asymmetry index. As slice location became progressively anterior the lateralization gradually reverted to the ipsilateral hemisphere; however, the location at which this transition occurred varied from one albino subject to another. The strong change of asymmetry index with slice number was not a systematic feature evident in the individual plots for subjects with nystagmus when stimulated in either hemifield (Fig. 5C). Although the individual plots of asymmetry index against slice number exhibited some noise, the systematic change from contra- to ipsilateralization in albinos indicates that the slice at which this transition occurs allows us to quantify the severity of the shift of the line of decussation in individuals with albinism.

The slice number at which the transition from contralateral to ipsilateralization occurred in each albino is plotted in Fig. 6 as a function of pigmentation level. Where the transition occurred between slices, data was interpolated and the fractional slice value is plotted for that subject. Based on the criterion established from the nystagmus control group, subjects who displayed no transition to a predominantly ipsilateral response before voxel counts dropped below 50 voxels within the slice were assigned the first slice falling below this threshold as their 'transition' slice. It should be noted that voxel counts did not drop below 50 before slice 8 in any of these subjects. A Spearman rank correlation analysis revealed a significant correlation ($r = -0.58$, $P < 0.02$) between the slice number at which lateralization became ipsilateral and individual pigmentation levels. The analysis was repeated with the ocular albino excluded because in this

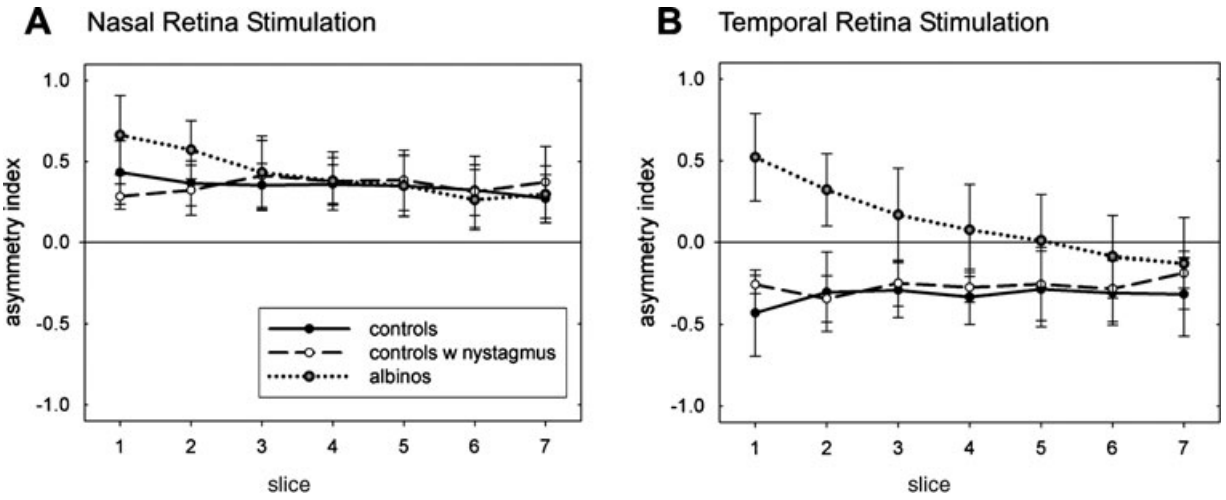

FIG. 4. Asymmetry index as a function of coronal slice number in response to (A) nasal retina stimulation and (B) temporal retina stimulation, averaged across the three subject groups. The asymmetry index was positive for stimulation of nasal retina for all subject groups, indicating response lateralization to the contralateral hemisphere. When temporal retina was stimulated (B), visual response was lateralized to the ipsilateral hemisphere for the control groups. The albino group response, however, was contralateral for posterior slices and became increasingly ipsilateral as slice position becomes more anterior. Error bars represent the SD.

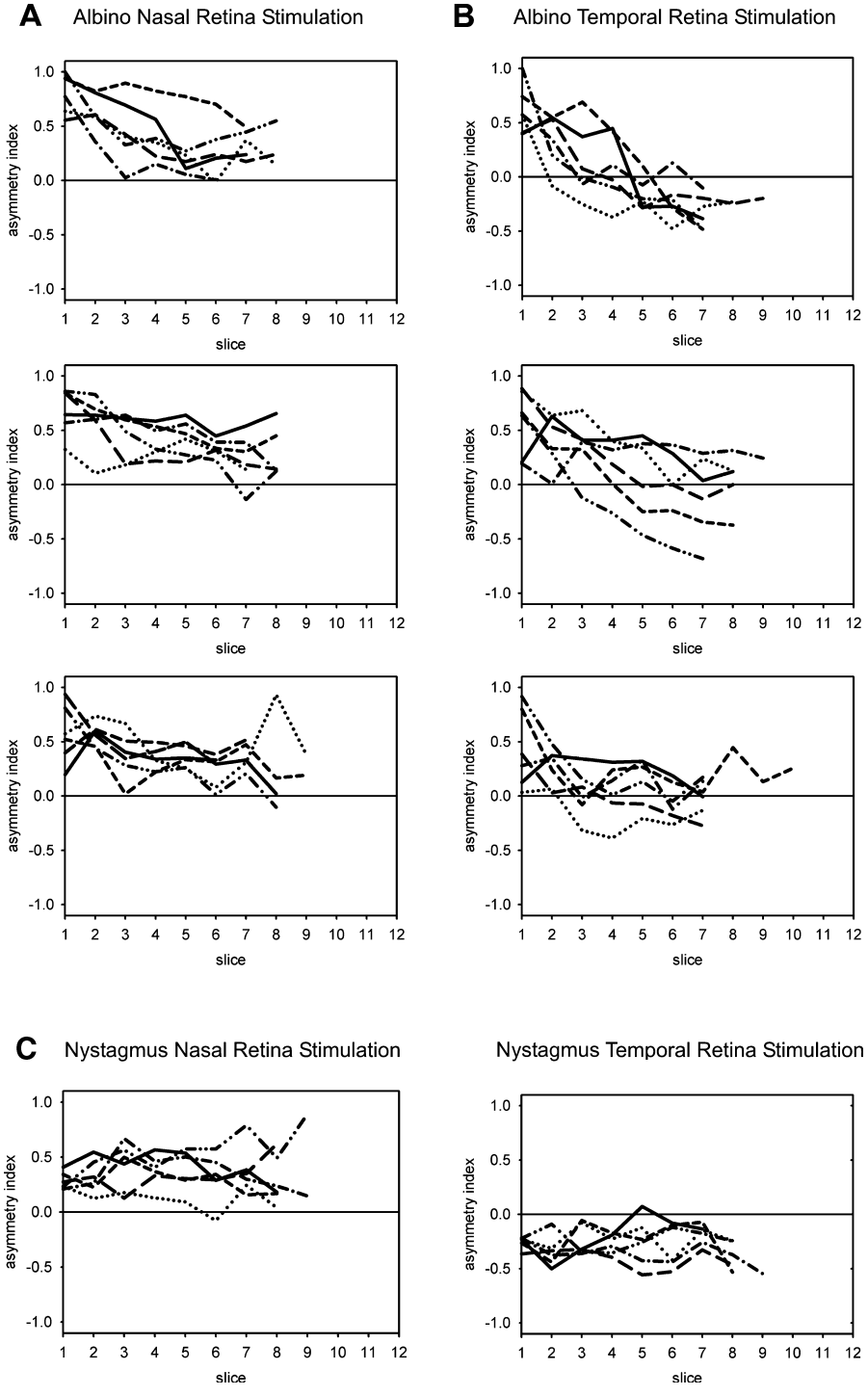

FIG. 5. Asymmetry index as a function of coronal slice number in response to (A) nasal and (B) temporal retina stimulation in all subjects with albinism. Subjects are plotted individually across three panels for clarity. (C) The control subjects with nystagmus are plotted individually in the lower panel. Positive values for the asymmetry index indicate a visual response lateralized to the contralateral hemisphere. (A) When nasal retina was stimulated, all subjects with albinism displayed contralateralization of the visual response. (B) When temporal retina was stimulated, the albino response was contralaterally lateralized for posterior slice locations but, as slice location became more anterior, the albino response became increasingly ipsilateral. (C) In control subjects with nystagmus, nasal retina stimulation resulted in contralateralization of response whereas temporal retina stimulation resulted in an overwhelmingly ipsilateral response. A threshold of 50 active voxels was applied to the data such that anterior slices with <50 voxels are not plotted here.

case the pigmentation of the skin and hair is unlikely to give an accurate indication of the ocular pigmentation. Reassuringly, the correlation increased and remained highly significant ($r = –0.61$, $P < 0.01$). The correlation was also performed when the anterior–posterior dimension of the occipital lobe (defined as the distance from the pole to the junction of the calcarine sulcus and parieto-occipital sulcus) was used to normalize the slice thickness, thereby accounting for variations in size of the occipital lobe. The increased correlation under these conditions was $r = -0.59$, and supports the findings of the original analysis.

Although we acquired data for patients with nystagmus to act as controls it is possible that, in addition to nystagmus, subjects with albinism might have had eccentric fixation, which could have had some bearing on our results. We undertook experiments on a normally sighted control subject to simulate the effects of eccentric fixation on the pattern of asymmetry index as a function of slice number. The results for this series of experiments are presented in Fig. 7. The open symbols in the top row of panels indicate that a variety of patterns of asymmetry vs. slice number can be produced with different fixation positions after stimulation of ‘temporal’ retina. Two of the fixation

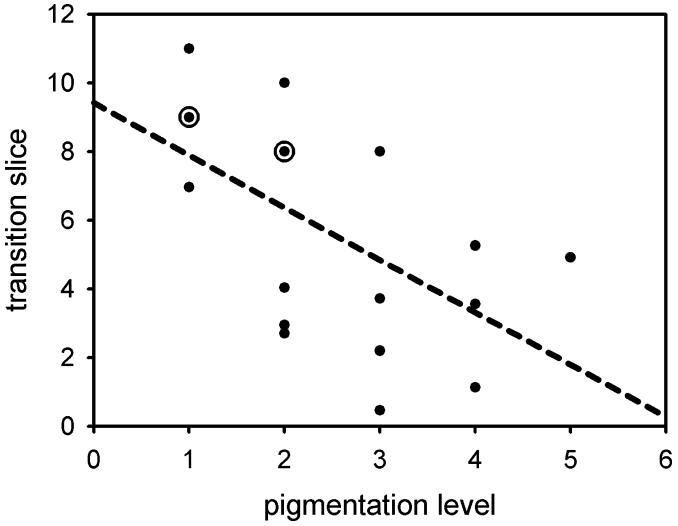

FIG. 6. The slice number at which the abnormal contralateralization of visual response to temporal retina stimulation reverted to the normal ipsilateralization is plotted for each albino subject as a function of their pigmentation level, where 1 indicates little or no pigmentation of the skin and hair and 6 indicates high levels of pigmentation. The dotted line represents the line of best fit through the data. There was a significant negative correlation ($P < 0.02$) between pigmentation level and the slice transition number, such that lower levels of pigmentation were associated with a much larger abnormal projection from temporal retina. The unfilled circles represent overlapping data points.

positions, 5 and 10°, produced patterns that were qualitatively similar to those of the albino sample as demonstrated by the *z*-scores and associated *P*-values in the lower panels. At these values of eccentric fixation, stimulation is provided to both hemifields and therefore there is little asymmetry in the response because both hemispheres are activated.

However, for all the eccentric gaze locations the pattern of asymmetry for stimulation of the 'nasal' retina was not consistent with the data obtained from the albino subjects. For the smallest eccentric fixation value of 5°, the asymmetry index for nasal stimulation was abnormally high. At the larger eccentric gaze locations of 10 and 15° the abnormally high contralateralization was maintained for anterior slices but an abnormally low index was present at posterior slice locations. Under none of the conditions were we able to observe responses that captured the abnormality evident in the subjects with albinism. If responses to temporal retinal stimulation shared some similarity with those of albinos, the complementary response to nasal stimulation did not. It is also noteworthy that we simulated only the direction of eccentric fixation displacement that would have the potential to explain the results we derived for albinos.

Although not the primary goal of this study, it is valuable to determine whether nystagmus amplitude and visual acuity are predicted by either pigmentation levels or extent of misrouting. The correlation analysis revealed that neither pigmentation levels nor the extent of misrouting were significant predictors of nystagmus

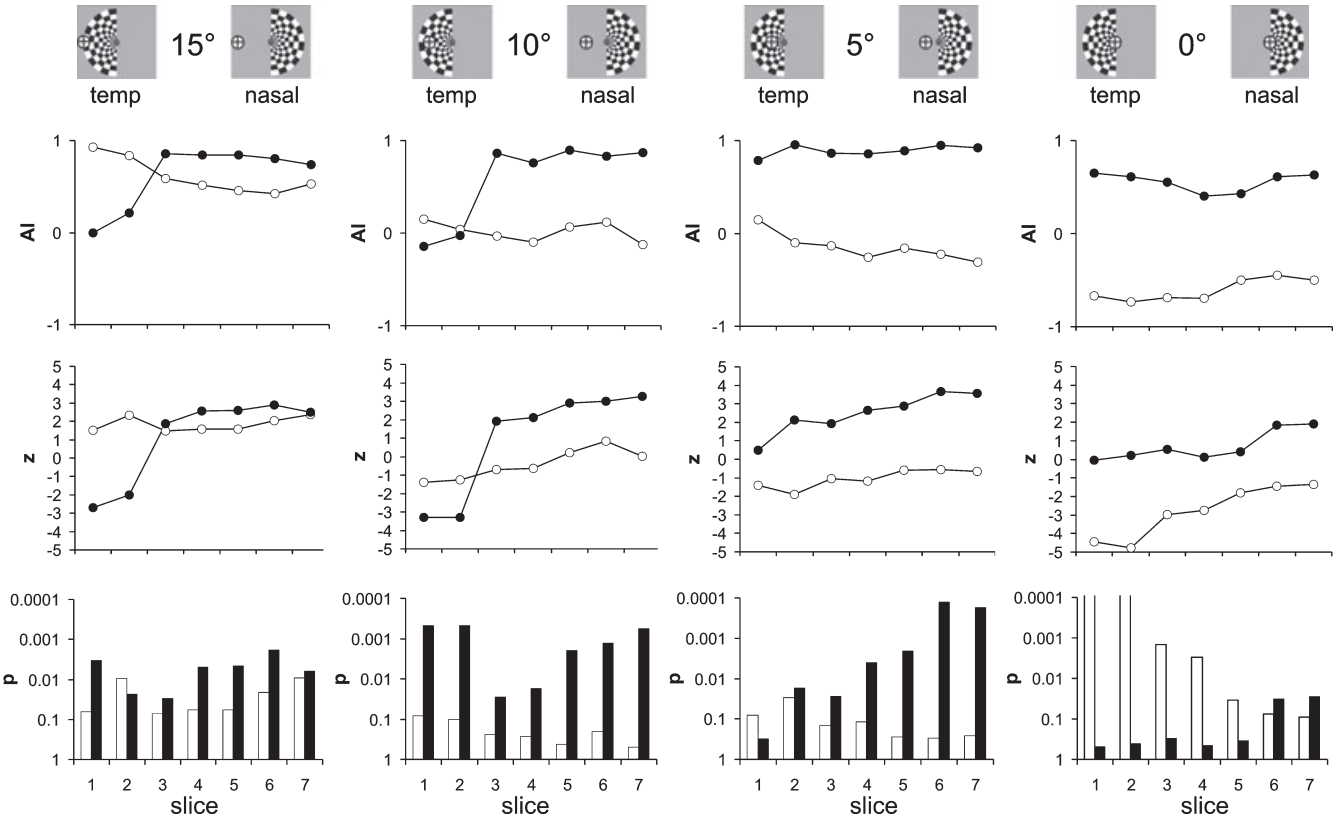

FIG. 7. The upper panel represents asymmetry indices as a function of slice number in a normal control subject at various eccentric fixation positions. Solid circles represent the data for nasal retina stimulation and open circles represent data in response to temporal retina stimulation. The lower two panels represent the *z*-score and *P*-values when these data were compared to the albino response to temporal (open bars) and nasal (solid bars) retina stimulation. The difference between the control asymmetry at large eccentric fixation positions during nasal retina stimulation differed significantly from the albino group response to nasal retina stimulation.

amplitude or visual acuity. However, acuity and nystagmus were themselves significantly correlated ($r = –0.612$, $P < 0.01$) such that more severe nystagmus in an individual was associated with poorer visual acuity.

Discussion

We are aware of only four studies that have attempted to assess the extent to which visual fibres project erroneously in humans with albinism (Creel *et al*., 1981; Hoffmann *et al*., 2003, 2005; Schmitz *et al*., 2004). Two studies have employed visual evoked potentials and have revealed variability in the assessed erroneous projection within the groups of albinos tested (Creel *et al*., 1981; Hoffmann *et al*., 2005). In each case, specific field locations were stimulated because the visual evoked potential is a poor localizer of cortical activity. Because a reasonably precise localization of the stimulus on the retina is key to the method, this approach may not be of sufficient precision because of the nystagmus suffered by albinos. Our previous work on human albinism using fMRI (Hoffmann *et al*., 2003) was able to reveal explicitly that the shift in the nasotemporal division was markedly different across individuals. However, our work could only derive such information for four subjects with reasonable ocular stability, a feature that is rarely observed in albinos. Another fMRI study on a larger group of subjects assessed the hemispheric lateralization of cortical responses elicited by full-field visual stimulation and used it as a measure of the extent of misrouting in albinism (Schmitz *et al*., 2004). In common with other previous work, considerable variation in the chosen measure of the erroneous projection was observed and was related to certain clinical measures of albinism.

We have extended the approach of Schmitz *et al*. (2004) in several ways: we used a hemifield stimulus, we restricted our analysis to early visual areas and we measured hemispheric lateralization in coronal slices forward from the posterior poles. These developments confer advantages over previous measurements in assessing misrouting. Firstly, by using a hemifield stimulus we isolated the hemispheric response arising from temporal retina projections from those arising in nasal retina, and *vice versa*. Secondly, lateralized activity outside of early visual areas that may be specific to the subject groups does not contribute to the measure of misrouting, a problem identified by Schmitz *et al*. (2004). Finally, the location in cortex at which the projection reverts to normal from abnormal was identified, which provides an estimate of the retinal location at which the nasotemporal division occurs based on the topographic representation of the visual field in cortex. This overcomes issues associated with ocular instability because the line of decussation is fixed in retinal coordinates and will therefore have a fixed cortical locus.

We tested the underlying assumptions of our approach by assessing the representation of the visual field as a function of slice number (distance from the occipital pole). Furthermore, we accounted for effects of eccentric fixation on our measurements and tested a control group with ocular instabilities, which were similar to our albino group. The method was therefore scrutinised for vulnerability to errors arising from (i) a potentially false assumption underlying the methodology, and (ii) oculomotor features that can arise in albinism.

Group effects

The overall group effects were very robust and indicated abnormal lateralization of activity in the albino group for stimuli presented to temporal retina. Stimulation of the nasal retina resulted in a lateralization pattern consistent with normal visual projections. Interestingly, however, activity was more strongly contra-lateralized than normal at the occipital pole, a probable consequence of bilateral representations of the very central visual field in normal control subjects but not albinos. Our group results build on preliminary investigations of albinism with fMRI (Hedera *et al*., 1994; Morland *et al*., 2002) and complement the retinotopic mapping data we acquired on four individuals with albinism (Hoffmann *et al*., 2003) and the previous measurements of hemispheric lateralization of fMRI activity in albinism (Schmitz *et al*., 2004). There is now therefore a significant body of evidence indicating that fMRI can successfully capture the fundamental misrouting present in human albinism. It may be fruitful to compare this approach with the standard diagnostic tool that is the visual evoked potential (e.g. Apkarian *et al*., 1983).

The variation of lateralization with distance from the occipital pole when the temporal retina was stimulated in albinos indicates that the abnormal routing present in albinism is not complete and reverts to a normal projection in the peripheral retina. Our principal aim was to examine whether variations in misrouting were related to the level of pigmentation exhibited in our subjects. Previous research in animal models has attempted to determine whether the shift in the line of decussation is related to the extent of hypopigmentation. Key findings that support the link between pigmentation and misrouting have been reported in cat (Creel *et al*., 1982; Leventhal & Creel, 1985; Ault *et al*., 1995), mice (Balkema & Drager, 1990; LaVail *et al*., 1978) and mink (Sanderson *et al*., 1974). Moreover, manipulations of melanin production during visual development result in predictable changes in the routing of retinal fibres (Lavado *et al*., 2006). Armed with a sensitive measure of the shift in the nasotemporal division, we have been able to document a strong relationship between the level of pigmentation and the extent of misrouting in a group of 18 human albinos. Previous fMRI work has highlighted a related result: iris translucency is correlated with hemispheric lateralization under full-field stimulation (Schmitz *et al*., 2004). However, the same study was not able to demonstrate a relationship between skin and hair pigmentation and the lateralization measure they employed. Our work is the first to demonstrate a relationship between pigmentation and the misrouting of visual fibres in humans that parallels the one found in other species.

The relationships between clinical features of albinism and pigmentation have previously received examination (Abadi & Pascal, 1991). It might be expected that pigmentation would predict the extent of the anatomical foveal deficit in humans with albinism. Acuity is ordinarily used to assess foveal function but, for subjects with albinism, acuity will measure not only the retinal deficit but also the effect of nystagmus, residual refractive error and amblyopia (see Schmitz *et al*. (2004)). Although previous work indicates an increase in nystagmus amplitude and decrease in acuity for phenotypes for which less pigment is expressed (Abadi & Pascal, 1991), it is unclear whether this demonstrates a strong link between an underlying retinal deficit and level of pigmentation. The finding that acuity is indeed strongly related to nystagmus (the present study and Abadi & Pascal, 1991) underlines the need for caution when relating acuity alone, as a measure of retinal function, to pigmentation levels and may also explain why we were unable to document a relationship between acuity and pigmentation.

If pigmentation were to determine both the extent of foveal hypoplasia and misrouting, then a correlation between acuity and misrouting is predicted. Indeed, the previous fMRI study that quantified misrouting for a group of 16 albinos reports such a relationship (Schmitz *et al*., 2004). Our present study does not support

this result, but this may be a consequence of acuity being a poor assessment of underlying foveal function for our albino subjects with nystagmus as discussed above. Our subject group had a tight negative coupling between nystagmus amplitude and acuity. Thus, for our subjects, acuity was strongly associated with ocular instability, which would mask the ability of acuity to indicate an underlying foveal deficit. Perhaps the previous study was fortunate in having a sample of albinos for whom acuity and nystagmus were dissociable in which case the link between the two anatomical abnormalities, foveal hypoplasia and misrouting, could be inferred by the correlation of hemispheric lateralization and acuity that was reported (Schmitz *et al*., 2004).

Conclusion

We conclude that the shift in the line of decussation varies amongst humans with albinism and that the variation is strongly correlated with pigmentation. There is also a growing body of evidence that now indicates that fMRI is a useful tool in characterising the misrouting of visual information that is exhibited in human albinism.

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Abbreviations

fMRI, functional magnetic resonance imaging; ROI, region of interest.

References

Abadi, R.V. & Pascal, E. (1991) Visual resolution limits in human albinism. *Vision Res.*, **31**, 1445–1447.

Apkarian, P., Reits, D., Spekreijse, H. & Van Dorp, D. (1983) A decisive electrophysiological test for human albinism. *Electroencephalogr. Clin. Neurophysiol.*, **55**, 513–531.

Ault, S.J., Leventhal, A.G., Vitek, D.J. & Creel, D.J. (1995) Abnormal ipsilateral visual field representation in areas 17 and 18 of hypopigmented cats. *J. Comp. Neurol.*, **354**, 181–192.

Balkema, G.W. & Drager, U.C. (1990) Origins of uncrossed retinofugal projections in normal and hypopigmented mice. *Vis. Neurosci.*, **4**, 595–604.

Baseler, H.A., Brewer, A.A., Sharpe, L.T., Morland, A.B., Jagle, H. & Wandell, B.A. (2002) Reorganization of human cortical maps caused by inherited photoreceptor abnormalities. *Nat. Neurosci.*, **5**, 364–370.

Boissy, R.E., Zhao, H., Oetting, W.S., Austin, L.M., Wildenberg, S.C., Boissy, Y.L., Zhao, Y., Sturm, R.A., Hearing, V.J., King, R.A. & Nordlund, J.J. (1996) Mutation in and lack of expression of tyrosinase-related protein-1 (TRP-1) in melanocytes from an individual with brown oculocutaneous albinism: a new subtype of albinism classified as 'OCA3'. *Am. J. Hum. Genet.*, **58**, 1145–1156.

Creel, D., Hendrickson, A.E. & Leventhal, A.G. (1982) Retinal projections in tyrosinase-negative albino cats. *J. Neurosci.*, **2**, 907–911.

Creel, D., Spekreijse, H. & Reits, D. (1981) Evoked potentials in albinos: efficacy of pattern stimuli in detecting misrouted optic fibers. *Electroencephalogr. Clin. Neurophysiol.*, **52**, 595–603.

Elschnig, A. (1913) Zur Anatomie des menschlichen Albinoauges. *Graefe's Arch. Clin. Exp. Ophthalmol.*, **84**, 401–419.

Engel, S.A., Glover, G.H. & Wandell, B.A. (1997) Retinotopic organization in human visual cortex and the spatial precision of functional MRI. *Cereb. Cortex*, **7**, 181–192.

Guillery, R.W., Hickey, T.L., Kaas, J.H., Felleman, D.J., Debruyn, E.J. & Sparks, D.L. (1984) Abnormal central visual pathways in the brain of an albino green monkey (Cercopithecus aethiops). *J. Comp. Neurol.*, **226**, 165–183.

Hedera, P., Lai, S., Haacke, E.M., Lerner, A.J., Hopkins, A.L., Lewin, J.S. & Friedland, R.P. (1994) Abnormal connectivity of the visual pathways in human albinos demonstrated by susceptibility-sensitized MRI. *Neurology*, **44**, 1921–1926.

Hoffmann, M.B., Lorenz, B., Morland, A.B. & Schmidtborn, L.C. (2005) Misrouting of the optic nerves in albinism: estimation of the extent with visual evoked potentials. *Invest. Ophthalmol. Vis. Sci.*, **46**, 3892–3898.

Hoffmann, M.B., Tolhurst, D.J., Moore, A.T. & Morland, A.B. (2003) Organization of the visual cortex in human albinism. *J. Neurosci.*, **23**, 8921–8930.

Hubel, D.H. & Wiesel, T.N. (1971) Aberrant visual projections in the Siamese cat. *J. Physiol. (Lond.)*, **218**, 33–62.

Jeffery, G. (1997) The albino retina: an abnormality that provides insight into normal retinal development. *Trends Neurosci.*, **20**, 165–169.

Jeffery, G., Darling, K. & Whitmore, A. (1994) Melanin and the regulation of mammalian photoreceptor topography. *Eur. J. Neurosci.*, **6**, 657–667.

Kaas, J.H. & Guillery, R.W. (1973) The transfer of abnormal visual field representations from the dorsal lateral geniculate nucleus to the visual cortex in Siamese cats. *Brain Res.*, **59**, 61–95.

LaVail, J.H., Nixon, R.A. & Sidman, R.L. (1978) Genetic control of retinal ganglion cell projections. *J. Comp. Neurol.*, **182**, 399–421.

Lavado, A., Jeffery, G., Tovar, V., de la Villa, P. & Montoliu, L. (2006) Ectopic expression of tyrosine hydroxylase in the pigmented epithelium rescues the retinal abnormalities and visual function common in albinos in the absence of melanin. *J. Neurochem.*, **96**, 1201–1211.

Leventhal, A.G. & Creel, D.J. (1985) Retinal projections and functional architecture of cortical areas 17 and 18 in the tyrosinase-negative albino cat. *J. Neurosci.*, **5**, 795–807.

Lund, R.D. (1965) Uncrossed visual pathways of hooded and albino rats. *Science*, **149**, 1506–1507.

Manga, P., Kromberg, J.G., Box, N.F., Sturm, R.A., Jenkins, T. & Ramsay, M. (1997) Rufous oculocutaneous albinism in southern African Blacks is caused by mutations in the TYRP1 gene. *Am. J. Hum. Genet.*, **61**, 1095–1101.

Morland, A.B., Hoffmann, M.B., Neveu, M. & Holder, G.E. (2002) Abnormal visual projection in a human albino studied with functional magnetic resonance imaging and visual evoked potentials. *J. Neurol. Neurosurg. Psychiatry*, **72**, 523–526.

Newton, J.M., Cohen-Barak, O., Hagiwara, N., Gardner, J.M., Davisson, M.T., King, R.A. & Brilliant, M.H. (2001) Mutations in the human orthologue of the mouse underwhite gene (uw) underlie a new form of oculocutaneous albinism, OCA4. *Am. J. Hum. Genet.*, **69**, 981–988.

Puri, N., Gardner, J.M. & Brilliant, M.H. (2000) Aberrant pH of melanosomes in pink-eyed dilution (p) mutant melanocytes. *J. Invest. Dermatol.*, **115**, 607–613.

Rinchik, E.M., Bultman, S.J., Horsthemke, B., Lee, S.T., Strunk, K.M., Spritz, R.A., Avidano, K.M., Jong, M.T. & Nicholls, R.D. (1993) A gene for the mouse pink-eyed dilution locus and for human type II oculocutaneous albinism. *Nature*, **361**, 72–76.

Sanderson, K.J., Guillery, R.W. & Shackelford, R.M. (1974) Congenitally abnormal visual pathways in mink (Mustela vision) with reduced retinal pigment. *J. Comp. Neurol.*, **154**, 225–248.

Schmitz, B., Kasmann-Kellner, B., Schafer, T., Krick, C.M., Gron, G., Backens, M. & Reith, W. (2004) Monocular visual activation patterns in albinism as revealed by functional magnetic resonance imaging. *Hum. Brain Mapp.*, **23**, 40–52.

Schmitz, B., Schaefer, T., Krick, C.M., Reith, W., Backens, M. & Kasmann-Kellner, B. (2003) Configuration of the optic chiasm in humans with albinism as revealed by magnetic resonance imaging. *Invest. Ophthalmol. Vis. Sci.*, **44**, 16–21.

Talairach, J. & Tournoux, P. (1988) *Co-Planar Stereotaxic Atlas of the Human Brain*. Thieme, Stuttgart.

Tomita, Y., Takeda, A., Okinaga, S., Tagami, H. & Shibahara, S. (1989) Human oculocutaneous albinism caused by single base insertion in the tyrosinase gene. *Biochem. Biophys. Res. Commun.*, **164**, 990–996.