Slow Optical Changes in Human Photoreceptors Induced by Light

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Purpose. The basic assumption of fundus reflection densitometry is that changes in reflectance are solely determined by photolysis and regenerating visual pigments. This study was undertaken to investigate small but systematic deviations from this rule.

Methods. Spectral reflectance changes (450–740 nm) of the fovea were measured during light and dark adaptation over a period of 66 minutes in five healthy subjects. The directional properties of the fundus reflection were examined with a custom-built scanning laser ophthalmoscope (SLO) at 514, 633, and 790 nm. The same instrument was also used to find the spatial distribution of the reflectance changes.

Results. In addition to fast changes consistent with visual pigment, slower reflectance changes (lasting 10–20 minutes) were observed at all wavelengths including 740 nm. Because visual pigment does not absorb at 740 nm, a second mechanism must be involved. Factor analysis generated two factors (i.e., spectral curves) that explained more than 97% of the variations in the time course of the spectral reflectance. Total reflectance was modeled by means of an existing model for fundus reflection, and it was found that the first factor strongly resembled the rapid changes in absorption of the cone pigments. The second factor seems linked to slow changes in cone reflectance. Measurements with the SLO showed a clear increase in directionally dependent reflectance from 6 to 30 minutes in the dark. This was observed only in the central 6° of the retina.

Conclusions. The characteristics of the slow reflectance changes all point to the cone photoreceptors as the origin. Most likely, alterations in the index of refraction between the interphotoreceptor matrix and photoreceptors lie at the base of this hitherto unknown phenomenon. (*Invest Ophthalmol Vis Sci.* 2000;41:282–289)

Light reflectance of the human fundus has been studied since the invention of the ophthalmoscope by Helmholtz.[1] Quantitative assessment of fundus reflectance began more than 40 years ago[2,3] with the main purpose of measuring the density of visual pigments in vivo. This was achieved by taking the log of the ratio of the reflectance in two conditions: the first, adapted to a strong light that bleached away all visual pigments, and the second in a fully dark-adapted state with all pigments regenerated. For foveal cones, the reflectance in the light-adapted condition is measured at least 1 to 2 minutes after a bright light is turned on. The reflectance in the dark is generally measured 5 to 8 minutes after switching off a bright light. When a strong, bleaching light is switched off, reflectance of the fovea decreases because of the increase in absorption of the regenerating pigments. It is generally assumed that the reflectance changes are solely due to visual pigments. Yet, we occasionally noted slight but systematic increases in reflectance after 6 minutes in the dark. Such an increase was difficult to understand in terms of the visual pigment kinetics of cone photoreceptors.

Another phenomenon originating in the photoreceptors, which may modulate fundus reflection, is the optical Stiles–Crawford effect (SCE).[4–10] The optical SCE is the phenomenon that light reflected from the fundus is more intense near the center of the pupil than at the pupil's edges. The optical SCE is most pronounced in the central 6° of the retina. Wave-guiding is responsible for the directionality (i.e., SCE) of the photoreceptors. Wave-guiding characteristics have been shown to be influenced by the amount of unbleached visual pigment present in the photoreceptor.[11–13] Theoretical studies indicate that any change in dimensions or index of refraction may influence the wave-guiding characteristics of the cones.[14] Changes in the composition of the interphotoreceptor matrix (IPM) as a consequence of exposure to light have been described. Changes in chemical composition of the IPM in chicks, cats, and frogs have been observed as a result of exposure to light.[15–17]

We decided to explore reflectance changes systematically at much longer times than required for foveal cone pigments to completely bleach or regenerate. The slow reflectance changes described below can be explained by alterations in cone reflectance. Optical SCE measurements corroborate the idea that these slow reflectance changes originate in or near the foveal photoreceptors. Our findings undermine the basic assumption in densitometry that all pigments and reflectors in the fovea are photostable, except for the visual pigments contained in the photoreceptors.

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Materials and Methods

Spectral fundus reflectance was obtained with the Utrecht single-spot densitometer.[18] The entrance beam had a spot size of 2.7° on the retina. The intensity was 3.1 log td. In the entrance beam a rotating wheel (14 revolutions per second) offered a sequence of 12 interference filters in the range of 450 to 740 nm (half bandwidth, 7 nm) to enable a quasisimultaneous measurement of reflectance across the visual spectrum. Light reflected from the fovea was detected in a retinal field of 2.4° (Fig. 1) concentric to the entrance beam spot.

Light adaptation was attained using a yellow light (OG495 filter; Schott Glasswerke, Mainz, Germany) from a second channel of 30° with a maximum of 5.7 log td, which bleached more than 95% of the available photopigments.[19] In the dark period, neutral density filters were inserted lowering the intensity of the yellow light to 2.5 log td. The subject was asked to fixate on a set of cross hairs during the complete run. The combined intensity of the measuring beam and the yellow light resulted in a level of 3.2 log td.

All subjects gave their informed consent, after the nature and possible consequences of the study were explained. The research followed the tenets of the Declaration of Helsinki, and the study was approved by the local ethics committee. Of five healthy subjects (age, 21–30 years; mean, 24) one eye was tested. Each subject had a best corrected Snellen visual acuity of 1.0 or better, no ophthalmologic problems, no history of ingesting drugs, no diabetes or neurologic abnormalities, and a negative family history of retinal degeneration. During all tests, a maximum pupil size was ensured by dilating the pupils with 1 or 2 drops of tropicamide 1% once every hour. A bite board with a dental compound and two forehead rests ensured proper fixation of the subject's head. The subjects were asked to gaze at the middle of a cross to reduce eye movements.

Densitometer measuring sessions lasted 66 minutes. Before the run was started, the entrance beam was aligned in the subject's pupil plane so that the foveal reflectance was at its highest (i.e., the peak of the SCE). Next, subjects were adapted for 30 minutes to complete darkness. During the runs, subjects interrupted the measurement at preset times to relax, alleviating the strain of the long and careful fixation. A run involved a 15-minute baseline in the dark, followed by 16 minutes of light, and finally 35 minutes in the dark (Fig. 2A). The densitometer enabled the examiner to view the fundus (during bleaching) in a 30° field while reflectance was recorded. Thus, we monitored the quasispecular reflections of the inner limiting membrane, because these are very sensitive to changes in pupil position in relation to the entrance beam. If these reflections changed position or shape during a run, the data were discarded. In our subjects these reflections were at least 2° from the edge of the 2.4° detection field (Fig. 1).

Because light seemed to trigger slow reflectance changes, variations in adapting light intensity were explored. The intensity was regulated by inserting neutral density filters.

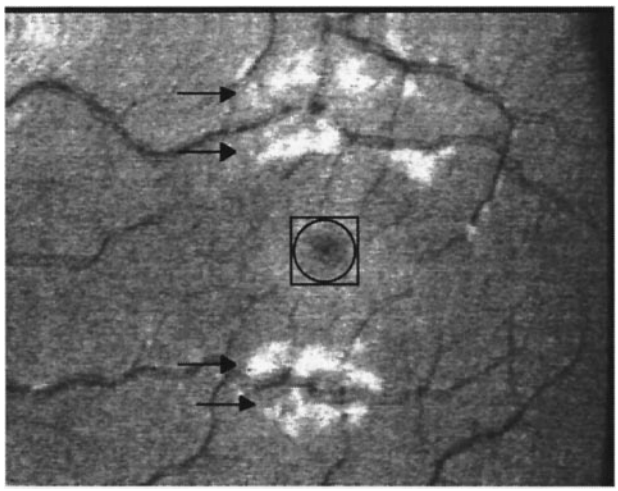

Figure 1. Fundus image (23° × 18°) acquired with the custom-built SLO at 514 nm. The *black circle* indicates the retinal detection spot size (2.4°) of the densitometer and the *black square* indicates the region (2.5° × 2.5°) used for the optical SCE in SLO measurements. *Arrows*: areas with quasispecular reflections from the inner limiting membrane (i.e., perimacular reflection).

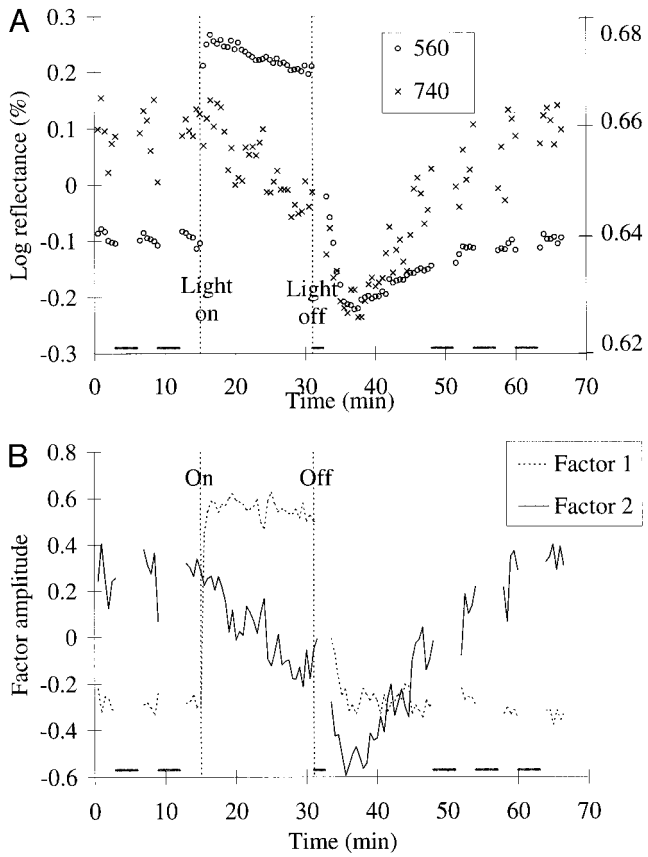

Figure 2. **(A)** Each data point represents the mean log reflectance of the fovea at two typical wavelengths for 30-second intervals measured with the densitometer. Reflectance data were acquired over a 66-minute period in five subjects. The *left* vertical axis refers to 560 nm and the *right* vertical axis to 740 nm. The fastest and largest changes in reflectance are observed at 560 nm at lights-on, and shortly after lights-off. These are related to changes in absorption of the cone visual pigments. A similar pattern of smaller and slower reflectance changes can be seen at both 560 and 740 nm. *Short horizontal bars* indicate periods when subjects relaxed by leaving the bite bar. **(B)** Time course of factors 1 and 2 explaining more than 97% of the variance in the log reflectance of the spectral (12 wavelengths; 450–740 nm) time course. **(A)** Two of the 12 wavelengths. Factor 1 probably represents changes in absorption of visual pigments and factor 2 slow reflectance changes of another origin.

The optical SCE[5–10] was measured with a custom-built scanning laser ophthalmoscope (SLO),[13,20] in three conditions: 1) light adapted for 12 minutes at 5.9 log td at 514 nm, 2) after 6 minutes in the dark, and 3) after 30 minutes in the dark. Fundus images were acquired at 514, 633, and 790 nm. Before the first series, the entrance beam was aligned in the subject's pupil plane so that the foveal reflectance was at its highest, ensuring optimum vertical positioning. Entrance and exit pupil moved jointly. The size of the retinal detection spot was 0.43°. A series of 15 to 30 fundus images was made by acquiring an image roughly every 0.25 mm of the horizontal meridian, from the nasal to the temporal pupil edge. The precise position of each image was recorded by the computer using a digital slide ruler (accuracy, ±0.01 mm) attached to the horizontal adjustment. Between series the subject was allowed to sit back and relax. Because the flash (0.04 seconds) of each image bleached up to 0.5% of the available visual pigment in a completely dark-adapted state,[19] the number of images was kept to a maximum of 15 in case of 514- or 633-nm wavelength. Because bleaching visual pigment is not a concern at 790 nm, we acquired up to 30 images in a single series at that wavelength. During the optical SCE measurement, fundus images with fixation deviations of more than 2° were discarded. The remaining images with slight eye movements were aligned to a common reference point, usually a retinal blood vessel intersection. From each image a mean background image was subtracted. To improve signal-to-noise ratio, the reflectance was averaged over the central 2.5° × 2.5° (Fig. 1). To quantify these results we fitted the reflectance percentage (least χ^2 method) with a model for the optical SCE $f(x)$[8]:

$$f(x) = A \cdot 10^{-\rho(x-x_0)^2} + B \qquad (1)$$

with A representing directionally dependent light, B nondirectionally dependent (stray) light, ρ curve peakedness, x horizontal pupil position, and x_0 the pupil position at which reflectance is at its maximum.

Reflectance was calibrated at 1% for both instruments by measuring reflectance of a white diffuser (a surface painted with Eastman 6080 white; Kodak, Rochester, NY) at 220 mm from the pupil plane of each instrument, assuming a focal length of the eye of 22 mm and Lambertian reflectance.

Results

Experiment 1: Time Course of Fundus Reflectance Measured with the Densitometer

Spectral fundus reflectance in the visual spectrum (450–740 nm) was recorded for 66 minutes with the densitometer in the central 2.4° of the visual field. Figure 2A shows the mean reflectance of five subjects at 2 of the 12 wavelengths, 560 and 740 nm. Each data point is the mean of 30 seconds of recording. The recording began with a dark-adapted, 15-minute baseline, alternating 3 minutes of recording at a background level of 3.2 log td with 3 minutes of relaxation. This dark-adapted reflectance was stable for all runs. At $t = 15$ minutes the light intensity was raised to 5.7 log td. In the first minutes when the bleaching light was turned on, the reflectance increased by approximately 0.3 log unit at 560 nm, in a short time of approximately 1 minute or less. This was the bleaching of visual pigments. However, the increase of reflectance was not maintained. When the bleaching light was turned off, reflectance should have decreased quickly as the pigment returned. This was the case, but the reflectance became less than at the beginning of the experiment (i.e., less than anticipated). More than 30 minutes was required for the reflectance to recover to the level measured before the bleaching. This result implied that more than the bleaching and regeneration of visual pigment occurred, and at 740 nm, when the absorption of cone pigments was very small, similar, although relatively smaller, changes in reflectance occurred.

From the 16-minute light period shown in Figure 2A it seems that the reflectance may have continued to decrease beyond 16 minutes. Because most subjects were not able to maintain adequate fixation for longer periods (relaxation periods are not possible during light), we had to restrict the light period to a maximum of 16 minutes. Limited traces obtained at longer times (up to 25 minutes; not shown) indicated very little or no further changes beyond 16 minutes.

Analysis of Experiment 1

The changes in the log of the mean reflectance spectrum (12 wavelengths) of five subjects during the light and dark periods (100 spectra per session) were decomposed by factor analysis (SPSS ver. 7.5; SPSS, Chicago, IL). This statistical method generates a number of spectra and their respective amplitudes to fit the variance in the data. The analysis yielded two factors (factor 1 variance, 82%; and factor 2 variance, 15%) with eigenvalues higher than 1, accounting for more than 97% of the total variation. The remaining factors were discarded.

The time courses of factors 1 and 2 are shown in Figure 2B. Changes in factor 1 occurred only in the first minute of light and in the first 6 minutes of dark. The time trace at 560 nm shown in Figure 2A seems to follow the same pattern as factor 1, but they differ in some parts. The time trace at 560 nm decreased from 16 to 31 minutes and increased from 37 to 66 minutes, whereas factor 1 was unchanged over these periods. Changes in factor 2 took place during 16 minutes of light and in the first 25 minutes of the second dark period. The time course of factor 2 closely resembled the time trace at 740 nm in Figure 2A. The spectral characteristics of factors 1 and 2 are given in Figure 3.

With the help of a recent model for fundus reflectance,[22] we tried to find the physiological meaning of factors 1 and 2. The model uses known ocular absorbers (such as lens pigment, macular pigment, blood, melanin, medium- and long-wave–sensitive visual pigments), combined with reflections at different interfaces (cornea, inner limiting membrane, photoreceptors, and sclera) inside the eye to explain the pathways of light. The total of 14 parameters are fitted simultaneously with the Marquardt–Levenberg fitting algorithm.[22] In measurements for the original model[1] a 1.6° retinal field was used instead of the present 2.4°. Macular pigment, melanin, blood, and cone reflectance all change with eccentricity. Therefore, the model was first adjusted to accommodate these changes. To explain the nature of factor 1 and factor 2 we varied each of the parameters independently. Factor 1 is well described by a change in visual pigment density. A change in cone reflectance explains the spectral characteristics of factor 2 very well. Because a change in a single parameter offered a satisfactory explanation for both factors, we did not perform a simultaneous fitting approach as was performed to model the reflec-

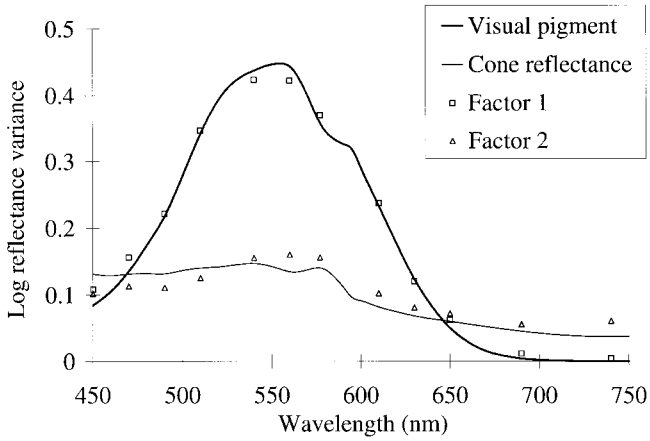

FIGURE 3. Mean spectral fundus reflectance variance of five subjects obtained by analyzing the raw spectral reflectance at each 30-second interval resulted in two principal factors (eigenvalues >1) over the time periods described in Figure 2. Results of the factor analysis are expressed as variance of log reflectance. Fit results are shown of a fundus reflectance model modulating respectively cone visual pigments and cone reflectance.

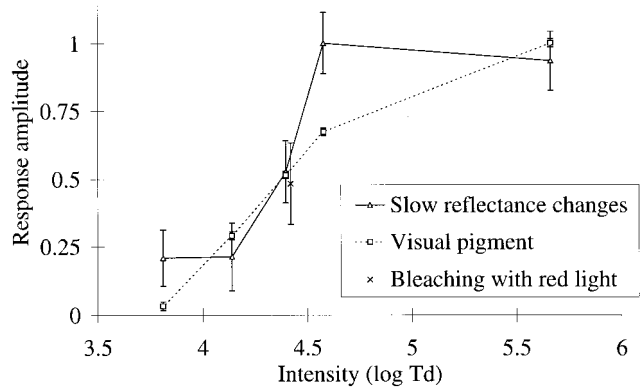

FIGURE 4. Mean response amplitude of five subjects at 740 nm as a function of light intensity (i.e., slow reflectance changes; response was defined as log $R_{34\ \text{minutes dark}}$ − log $R_{6\ \text{minutes dark}}$) and at 560 nm (i.e., visual pigment; response was defined as log $R_{15\ \text{minutes light}}$ − log $R_{6\ \text{minutes dark}}$). Responses at 560 nm and 740 nm were scaled to the same maximum. ×, response amplitude of the slow reflectance changes at 740 nm to a red light (average of three subjects). Error bars, SE.

tance spectra. Furthermore, we allowed separate modulation of lipofuscin,[23] melanin,[24] or blood[25] (as if these substances were present in the cones), using the model of van de Kraats et al.[1] For none of these pigments were acceptable fits of the spectrum of the slow reflectance changes found (see Fig. 8).

Experiment 2: Exploration of Characteristics of the Slow Reflectance Changes

The run shown in Figure 2A was repeated at different light intensities for five subjects, thus bleaching different fractions of the available visual pigments.[19] The response amplitude of the slow reflectance changes was defined as the log of the reflectance ratio between 6 minutes in the dark (t = 37 minutes) and 34 minutes in the dark (t = 65 minutes) at 740 nm. For visual pigment density it was defined as the log of the reflectance ratio between 15 minutes in the light (t = 30 minutes) and 6 minutes in the dark (t = 37 minutes) at 560 nm. Reflectance was averaged over 2-minute periods around the respective time points. The responses of the slow reflectance changes and visual pigment relative to the maximum response are plotted in Figure 4. The light intensity range within which both modulate reflectance showed some overlap. However, the response amplitude of the slow reflectance changes and visual pigment differs significantly at 4.6 log td.

For three subjects we checked whether a red light (OG590 filter) at 4.4 log td was equally effective in evoking slow reflectance changes as the yellow light at 4.4 log td (i.e., photopically equated bleach stimuli). This provided an indication whether absorption of light by cone visual pigments was the prime trigger. Light and dark periods were the same as described earlier. Figure 4 indicates that the yellow and red bleaching lights elicited the same response for the slow reflectance changes.

Two experiments were performed on three subjects to see whether control of reflectance changes was local. The fellow eye was adapted to yellow light (I = 5.3 log td), and dark adapted (I < 2.0 log td) while reflectance was measured in the other eye. In another experiment, a concentric retinal field stop (diameter 7.5°) was placed over the 2.4° measuring spot, so that only the surrounding retina was light adapted. Both experiments showed no foveal reflectance changes.

Experiment 3: Independent Test of the Origin of the Slow Reflectance Changes

To probe the possibility that the slow changes originated in the receptor layer, we studied changes in the directionally dependent reflectance.[7–10,13,19] Such information can be obtained with the optical SCE.

Measuring the optical SCE allows distinction between directional (A) and non-directional (B) reflectance.[13] In Figure 5

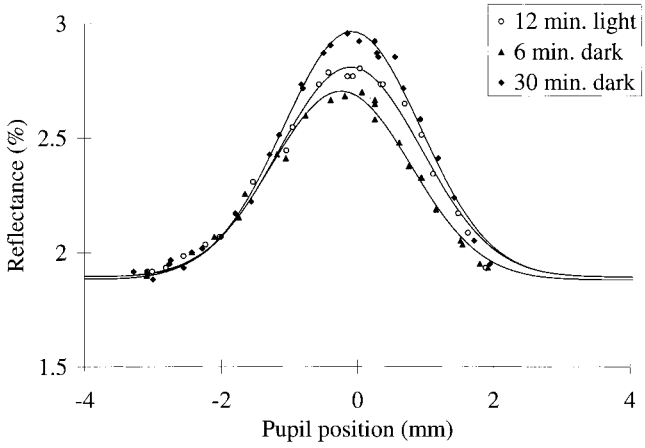

FIGURE 5. The optical SCE of subject 1 measured with an SLO at 790 nm. The mean reflectance of the central fovea, 2.5° × 2.5° (Fig. 1), was determined for three conditions: 12 minutes of light, 6 minutes of dark, and 30 minutes of dark. There was an increase in the directionally dependent reflectance from 6 to 30 minutes in the dark, whereas directionally independent reflectance remained the same. Reflectance data were fitted to a model (*continuous lines*) with four parameters, A (directionally dependent light), B (non-directionally dependent light), ρ (curve peakedness), and x_o (peak position of the curve).

Table 1. Mean Optical SCE Fit Parameters for 5 Healthy Volunteers

	Wavelength (nm)	12-Minute Light Adaptation	7-Minute Dark Adaptation (*A*)	30-Minute Dark Adaptation (*B*)	B − A	*P** (B − A)
514	*A* (%)	1.03 ± 0.21	0.25 ± 0.06	0.36 ± 0.07	0.11	<0.001†
	B (%)	0.34 ± 0.08	0.32 ± 0.07	0.32 ± 0.06	0.00	0.534
	ρ	0.20 ± 0.06	0.14 ± 0.03	0.17 ± 0.03	0.04	0.029†
	x_o (mm)	−0.15 ± 0.49	−0.17 ± 0.38	−0.26 ± 0.29	−0.09	0.305
633	*A* (%)	1.38 ± 0.09	0.70 ± 0.14	1.07 ± 0.23	0.37	0.002†
	B (%)	0.74 ± 0.13	0.72 ± 0.15	0.74 ± 0.12	0.02	0.366
	ρ	0.20 ± 0.02	0.17 ± 0.01	0.21 ± 0.01	0.04	0.039†
	x_o (mm)	0.01 ± 0.21	−0.05 ± 0.17	0.13 ± 0.43	0.18	0.189
790	*A* (%)	1.22 ± 0.20	0.96 ± 0.17	1.28 ± 0.20	0.32	<0.001†
	B (%)	1.47 ± 0.22	1.45 ± 0.15	1.48 ± 0.22	0.03	0.45
	ρ	0.21 ± 0.05	0.19 ± 0.03	0.23 ± 0.07	0.04	0.093
	x_o (mm)	−0.21 ± 0.23	−0.18 ± 0.25	−0.16 ± 0.22	0.02	0.715

All fit parameters were left free to fit with a least χ^2 method. Data are means ± SD.
* Paired *t*-test, two-sample for means: two-tailed.
† Significant ($P < 0.05$).

the optical SCE curves, obtained with a custom-built SLO,[20] of subject 1 are shown for 12 minutes in the light and 6 and 30 minutes in the dark at 790 nm. Because there is negligible influence of visual pigments at 790 nm, we choose this wavelength to demonstrate the effect of slow reflectance changes on the optical SCE. Directionally dependent reflectance decreased from 12 minutes in the light to 6 minutes in the dark. This was followed by an increase from 6 minutes in the dark to 30 minutes in the dark. The reflectance changes only concerned the curved part of the fitted line; the horizontal part remained at the same level for all three conditions. This means that for the period from 6 minutes to 30 minutes in the dark, the nondirectional reflectance remained constant. Small changes in peak position (0.15 mm as shown in Fig. 5) were noted within individual sessions resulting in nonsuperimposing parts of SCE curves. On the question of whether the SCE peak may shift itself, we tested for this possibility. We found no indication for either horizontal or vertical shift of the optical SCE. Moreover, we compared all the individual SCE sessions and concluded that these shifts were random. Small shifts may have been caused by movements of the subject's eyes or head between acquiring SLO images at the three different times. The optical SCE was also determined over the same light and dark periods at 514 and 633 nm. Changes in directional reflectance for five subjects were highly significant (Table 1). For the same period an increase in peakedness (ρ) was also found, but it was only significant at 514 and 633 nm.

Experiment 4: Spatial Distribution of the Slow Reflectance Changes

The spatial distributions of the slow reflectance changes were obtained by subtracting SLO images (i.e., reflectance) acquired at 6 minutes in the dark from images acquired at 30 minutes in the dark at the peak of the optical SCE for each subject. The mean (in five subjects) spatial pattern of the slow reflectance changes at 790 nm are shown in Figure 6. Reflectance increase was low at 0.5° eccentricity, rose to a peak at 1°, and decreased again toward the periphery. The spatial patterns at 514 and 633 nm were very similar to that of 790 nm. Such a pattern is also found for the peakedness (ρ) of the optical SCE.[13] Reflectance changes in rod-dominated areas beyond 6° eccentricity did not show an increase in reflectance in five subjects at 790 nm.

Discussion

We found slow reflectance changes (i.e., factor 2 in Figs. 2B and 3) in the fovea during light and dark periods that cannot be explained by variations in the absorption of cone pigments. This observation in human subjects is, to our knowledge, the first of its kind in 40 years of fundus reflectometry. The results of our experiments provide firm evidence that the source of the newly found reflectance changes lies in or near the cone photoreceptors. First, the factor analysis indicated that there are two factors with different time characteristics. Spectral information of these two factors was fit satisfactorily to model curves of cone pigments (medium and long wave sensitive) and cone reflectance, respectively (Fig. 3). Second, the response versus intensity curves of both components had some similarity (i.e., comparable half-response intensity). This indicates cone pigments as the prime trigger. This was corroborated by the responses of the slow reflectance changes being similar to photopically equated red and yellow light (Fig. 4). However, the measuring light level (3.1 log td) inhibited us in

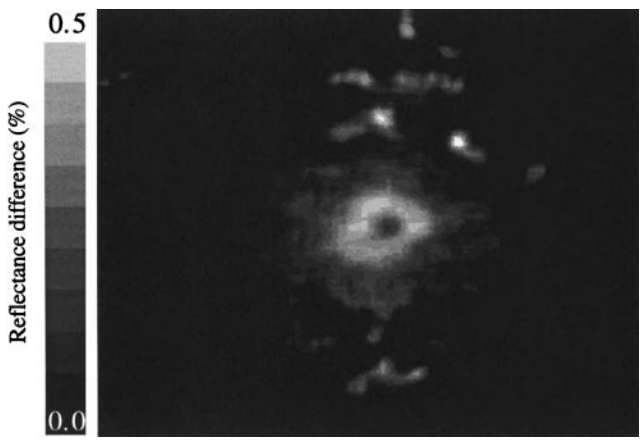

Figure 6. Reflectance difference between SLO images acquired (at the peak of the SCE) at 6 and 30 minutes in the dark. Resultant images from five subjects at 790 nm were averaged. The image shows a 23° × 18° fundus region with the fovea in the center. The highest reflectance increase is seen at around 1° eccentricity. The *gray-scale bar* on the *left* indicates reflectance change percentage.

exploring slow reflectance changes at lower light intensities. Third, the optical SCE results showed that the directional component, *A*, increased from 6 minutes to 30 minutes in the dark with no change in the nondirectional component (Table 1). Fourth, the spatial distribution of the slow reflectance changes (Fig. 6) was consistent with the directionality of cone receptors. The smaller amplitude of slow reflectance changes in the center of the fovea was probably caused by photoreceptors distributing the directional light over a larger angle (lower ρ[13]), so that more of the light fell outside the small (2-mm^2) exit pupil of the SLO. Even smaller slow reflectance changes recorded at more peripheral eccentricities were possibly because of a decreasing cone number per unit area.

Other investigators have reported faster effects,[2,26–29] within 5 minutes after a bleaching light was turned off, which also could not be explained by straightforward bleaching or regeneration of visual pigments. We are convinced that the results from the present study concern a different localization. Ripps et al.[29] showed compelling evidence relating these changes to the phenomenon of a spreading depression. Spreading depression is the effect of a semitransparent milky wave moving across isolated retina preparation after application of KCl. They argued that the spreading depression is mainly localized in the inner plexiform layer, which is absent in the center of the fovea. Furthermore, these reports concerned fundus reflectance changes recorded in cats (i.e., rod-dominated retinas) under pathologic conditions, revealing different spectral attributes than those described in the present study. These faster effects in cats were attributed to an "edema" effect occurring in the inner retina. Subjects in the present study were healthy humans under more physiological conditions revealing foveal (i.e., cone) reflectance changes.

We propose that the slow reflectance changes were caused by alterations in the index of refraction between outer segment disks and the IPM. Cone disks have a close relationship with the IPM, because they are continuous with the cell membrane. Changes in chemical composition of the IPM in chicks, cats, and frogs have been observed as a result of exposure to light.[15–17] Such changes involve alterations in concentration of a number of ions in a time span of several minutes. In a histochemical study in rats,[31] it was demonstrated that the chief components of the IPM undergo a major shift in distribution or molecular conformation after a light–dark transition. The dark–light transition was fast (within 5 minutes), whereas the light–dark transition was slow (1–2 hours). This is also true in the present experiments, although on another time scale.

Rod-dominated areas in the SLO images at peripheral eccentricity (6°) did not reveal any slow reflectance increase at 790 nm (Fig. 6), possibly because of low directionality of the rods and low cone number per unit area. Low directionality is also found in the very central cones.[13,32] These cones exhibited less slow reflectance changes than the cones at 1° eccentricity. Conversely, rods may exhibit little or no slow reflectance changes, because their discs, being discontinuous with the cell membrane, have a less intimate relation with the IPM than do the cones.

Light-adapting fundus regions outside the measuring field, and even in the other eye, did not evoke any slow reflectance changes in the fovea. This indicates that the control of the changes is local.

Supposing that slow cone-reflectance changes occur in the outer segment, reflectance changes undergo absorption by visual pigment in the dark period, but not in the light period. The factor analysis most likely found some intermediate spectrum of these two conditions. A better result can be attained by separating cone-reflectance changes with visual pigment present from those without. The first was attained by calculating the log of the reflectance ratio between 5 minutes in the dark and 33 minutes in the dark, the latter by calculating the log of the reflectance ratio between 1 minute in the light and 14 minutes in the light. Reflectance was averaged over a period of 2 minutes starting at the indicated times. The results are presented in Figure 7. The cumulative χ^2 of the fit for factor 2 was 0.0043. The cumulative χ^2 for the fits in the dark and light periods was four times smaller (0.0010).

In principle, retinomotor responses,[33] specific alterations in photoreceptor dimensions with melanin granule migration, or photomechanical changes in general could also explain the slow reflectance changes. However, histologic studies on primate retinas have failed to show any clear structural differences between dark- and light-adapted photoreceptors.[28] Intraretinal microelectrode studies[15–17] also report alterations in IPM volume, which may induce changes in dimensions of the photoreceptors. These have been suggested to influence ρ profoundly.[14] However, changes in ρ seemed less apparent than reflectance changes (Table 1). The assumption that retinomotor activity influences only one aspect of wave-guide behavior in cones may be an unjustified oversimplification. Evidence from species other than humans[33] suggests that retinomotor activity may involve a combination of dimensional alterations in outer segment, myoid, and inner segment, combined with melanin migration. It seems impossible to predict the overall reflectance changes caused by retinomotor effects, based on the available wave-guide theories for photoreceptors.

Recently, Jordan and Mollon[34,35] described slow red–green color-matching changes after exposure to bright sunlight. They suggested lipofuscin and melanin as possible ab-

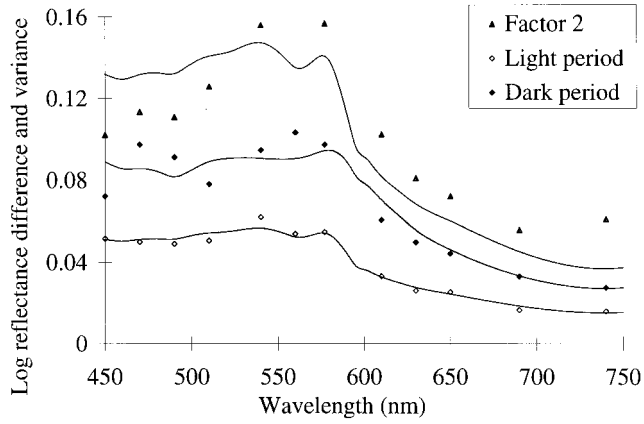

FIGURE 7. Slow reflectance changes expressed as log reflectance difference and variance with model fits.[22] *Filled triangles* indicate data gathered from the factor analysis described in Figure 3. *Filled* and *empty diamonds* indicate data log reflectance changes between 6 and 34 minutes in the dark and between 2 and 15 minutes in the light, respectively. Fits for the latter two were better than for the factor analysis.

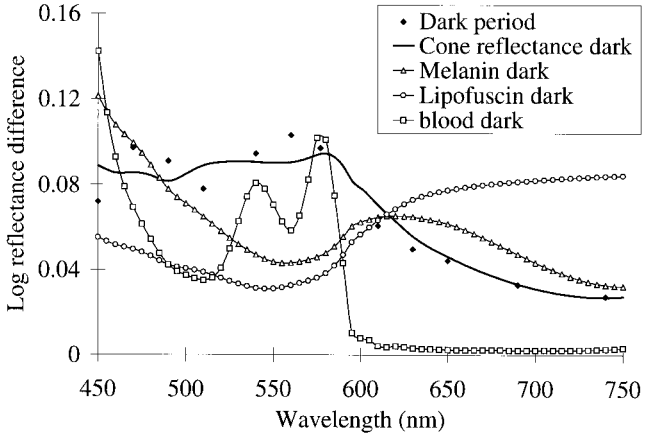

FIGURE 8. Influence of melanin, lipofuscin and blood on foveal reflectance compared with cone reflectance, determined by using an existing model.[22] Neither melanin, or lipofuscin, or blood seemed to have spectral characteristics for the slow reflectance changes.

sorption candidates to explain color-matching changes. By allowing separate modulation of lipofuscin[23] and melanin,[24] (as if these substances were present in the cones), by using the model of van de Kraats et al.,[22] we found that neither of these pigments acceptably fitted the spectrum of the slow reflectance changes from 10 to 30 minutes (Fig. 8). To find out whether there is a relation between the present data and psychophysics would require further study.

The present findings suggest that earlier estimates of the density of cone pigments may have been too high. Past reports usually mention light adaptation times far shorter than 16 minutes.[18,36–39] However, the time needed to align subjects may also have influenced the subsequent recordings. To get an idea of the contribution of the slow reflectance changes to density measurements in the past, we reanalyzed the data of the study of van de Kraats et al.[22] Changes at 740 nm were used to correct data at 560 nm, taking into account the spectral characteristics from Figure 3. This lowered the mean density from 0.46 to 0.41. With longer adaptation times, such as those used in the present study, visual pigment density in the fovea may have been overestimated by as much as 25%. A change of 0.45 density units in the peak absorbency of the cone pigments (560 nm) may be accompanied by a change of 0.15 density units in the cone reflectance at the same wavelength (Fig. 3).

The kinetics of cone visual pigments as studied with fundus reflectometry[36–41] also requires reanalysis. This may resolve ill-understood deviations from the single-compartment model.[38]

In recent years two extensive models for fundus reflectance have been proposed, which both needed an "unknown density" besides those of the visual pigments to explain the reflectance difference between dark- and light-adapted fundus. The "unknown density" was placed in the vitreous for lack of better understanding.[22,42] The present results indicate that the slow reflectance changes are the source of the unexplained effects.

The assumption in retinal densitometry that alterations in the foveal reflectance time course are solely due to visual pigments cannot be maintained. We conclude that fundus reflectometry may offer important insights in the dynamics of human photoreceptor optics.

References

1. Von Helmholtz H. *Beschreibung eines Augen-spiegels zur Untersuchung der netzhaut im lebenden Augen.* Berlin: Förtsner, 1851.
2. Weale RA. Photochemical reactions in the living cat's retina. *J Physiol.* 1953;121:322–331.
3. Rushton WAH, Campbell FW. The measurement of rhodopsin in the living human eye. *Nature.* 1954;174:1096–1097.
4. Stiles WS, Crawford BH. The luminous efficiency of rays entering the eye pupil at different points. *Proc Roy Soc Lond B.* 1933;112: 428–450.
5. Gorrand JM. Directional effects of the retina appearing in the aerial image. *Journal of Optics (Paris).* 1985;6:279–287.
6. van Blokland GJ. Directionality and alignment of the foveal receptors, assessed with light scattered from the human fundus in vivo. *Vision Res.* 1986;26:495–500.
7. Gorrand JM, Delori FC. A method for assessing the photoreceptor directionality. *Invest Ophthalmol Vis Sci.* 1990;31:425.
8. Gorrand JM, Delori F. A reflectometric technique for assessing photoreceptor alignment. *Vision Res.* 1995;35:999–1010.
9. Burns SA, Wu S, Elsner AE, Delori FC. Optical imaging of photoreceptor alignment. *Invest Ophthalmol Vis Sci.* 1994;35:1572.
10. Burns SA, Wu S, Delori FC Elsner AE. Direct measurement of human-cone-photoreceptor alignment. *J Opt Soc Am A.* 1995;12: 2329–2338.
11. Walraven PL. Recovery from the increase of the Stiles–Crawford effect after bleaching. *Nature.* 1996;210:311–312.
12. van Blokland GJ. Directionality and alignment of the foveal receptors assessed with light scattered from the human fundus in vivo. *Vision Res.* 1986;26:495–500.
13. DeLint PJ, Berendschot TTJM, van Norren D. Local photoreceptor alignment measured with a scanning laser ophthalmoscope. *Vision Res.* 1997;37:243–248.
14. Snyder AW, Pask C. The Stiles–Crawford effect: explanation and consequences. *Vision Res.* 1973;13:1115–1137.
15. Huang B, Karwoski CJ. Light-evoked expansion of the subretinal space volume in the retina of the frog. *J Neurosci.* 1992;12:4243–4252.
16. Li JD, Gallemore RP, Dmitriev A, Steinberg RH. Light-dependent hydration of the space surrounding photoreceptors in chick retina. *Invest Ophthalmol Vis Sci.* 1994;35:2700–2711.
17. Cao W, Govardovskii V, Li JD, Steinberg RH. Systematic hypoxia dehydrates the space surrounding photoreceptors in the cat retina. *Invest Ophthalmol Vis Sci.* 1996;37:586–596.
18. van Norren D, van de Kraats J. Retinal densitometer with the size of a fundus camera. *Vision Res.* 1989;29:369–374.
19. Wyszecki G, Stiles WS. *Color Science: Concepts and Methods, Quantitative Data and Formulae*. New York: Wiley; 1982.
20. van Norren D, van de Kraats J. Imaging retinal densitometry with a confocal scanning laser ophthalmoscope. *Vision Res.* 1989;29: 1825–1830.
21. Press WH, Flanney BP, Teukolsky SA, Vettering, WT. Numerical recipes in Pascal. New York: *Cambridge University Press;* 1989.
22. van de Kraats J, Berendschot TTJM, van Norren D. The pathways of light measured in fundus reflectometry. *Vision Res.* 1996;36: 2229–2247.
23. Boulton M, Docchio F, Dayhaw–Barker P, Ramponi R, Cubeddu R. Age-related changes in the morphology, absorption and fluorescence of melanosomes and lipofuscin granules of the retinal pigment epithelium. *Vision Res.* 1990;30:1291–1303.
24. Gabel VP, Birngruber R, Hillenkamp F, Visible and near infrared light absorption in pigment epithelium and choroid. In: Kyoto S, ed. *XXIII Concilium Ophthalmologium* (*Excerpta Medica*). Amsterdam: Elsevier; 1978.
25. Van Assendelft O. *Spectrophotometry of Haemoglobin Derivatives*. Assen, The Netherlands: Royal van Gorcum; 1970.
26. Weale RA. Slow and rapid regeneration in the living cat's retina (abstract). *J Physiol.* 1953;122:12P.

27. Hood C, Rushton WAH. The Florida retinal densitometer. *J Physiol.* 1971;217:213–229.
28. Bonds AB, MacLoed DIA. The bleaching and regeneration of rhodopsin in the cat. *J Physiol.* 1974;242:237–253.
29. Ripps H, Mehaffy L III, Siegel IM. Rhodopsin kinetics in the cat retina. *J Gen. Physiol*. 1981;77:317–334.
30. Gouras P, Carr RE. Primate retinal responses: slow changes during repetitive changes with light. *Science.* 1964;145:413–414.
31. Uehara F, Matthes MT, Yasumura D, LaVail MM. Light-evoked changes in the interphotoreceptor matrix. *Science.* 1990;248: 1633–1636.
32. Westheimer G. Dependence of the magnitude of the Stiles–Crawford effect on retinal location. *J Physiol.* 1967;192:309–315.
33. Burnside B, Nagle B. Retinomotor movement of photoreceptors and retinal pigment epithelium: mechanisms and regulation. In: Osborn N, Chader G, eds. *Progress in Retinal Research.* Oxford, UK: Pergamon; Vol.2. 1983:67–109.
34. Jordan G, Mollon JD. Adaptation of colour vision to sunlight. *Nature.* 1997;386:135–136.
35. Jordan G, Mollon JD. Shift in Rayleigh match after adaptation to monochromatic light of various intensities. *Vision Res.* 1998;38: 3253–3257.
36. Weale RA. Photolabile pigments in normal and cone-monochromatic foveae. *J Physiol.* 1959;148:47–48.
37. Rushton WAH, Henry GH. Bleaching and regeneration of cone pigments in man. *Vision Res.* 1968;8:617–631.
38. Smith VC, Pokorny J, van Norren D. Densitometric measurement of human cone pigment kinetics. *Vision Res.* 1983;23: 517–524.
39. Elsner AE, Burns SA, Webb RH. Mapping cone pigment optical density. *J Opt Soc Am A.* 1993;10:52–58.
40. Rushton WAH. Physical measurement of cone pigment in the living human eye. *Nature.* 1957;179:571–573.
41. Coolen ACC, van Norren D. Kinetics of human cone photopigments explained with a Rushton–Henry model. *Biol Cybern.* 1988; 58:123–128.
42. Delori FC, Pflibsen KP. Spectral reflectance of the human ocular fundus. *Appl Opt.* 1989;28:1061–1077.