Blue flicker modifies the subfoveal choroidal blood flow in the human eye

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Lovasik, John V., Hélène Kergoat, and Marcelo A. Wajsilber. Blue flicker modifies the subfoveal choroidal blood flow in the human eye. *Am J Physiol Heart Circ Physiol* 289: H683–H691, 2005. First published April 1, 2005; doi:10.1152/ajpheart.01187.2004.—The objective of the present study was to reveal an interaction between choroidal blood flow (ChBF) and light-induced photoreceptor activity, a physiological coupling that has been already demonstrated for retinal blood flow but rejected for ChBF. Ten healthy adults volunteered for this study. A real-time recording near-infrared laser-Doppler flowmetry was used to quantify the subfoveal ChBF while the luminance of blue flicker between 1 and 64 Hz was first increased and then decreased by 4.0 log units in 1.0-log unit steps between 0.0375 and 375 cd/m². In separate testing, flash electroretinograms (ERGs) provided electrophysiological indexes of the relative response of short-wave cones (s-cones) and rods to blue light stimulation. Group-averaged, normalized ChBF measurements revealed that it was modulated by ~9% by flicker frequency. Increasing the blue flicker luminance from low to high attenuated the subfoveal ChBF, volume, and velocity by ~32%, ~30%, and ~5%, respectively. Decreasing the luminance from high to low over the same range had no effect on the subfoveal choroidal hemodynamics. The markedly different effects of reversed directions of change in blue flicker luminance on the subfoveal ChBF were linked to transitions between rod-dominated and s-cone-dominated retinal responses. Collectively, these findings indicate that the blue light-induced photoreceptor response is associated with a differential distribution of the ChBF across the ocular fundus according to the degree and type of retinal photoreceptor stimulated.

blue-flash electroretinogram; choroidal blood flow; choroidal hemodynamics; laser-Doppler flowmetry; neural retinal activity

Experimental data pointing to the existence of vascular autoregulation (AR) in the human choroid have been reported only within the last 5 years or so. The principal purpose of choroidal AR is to keep choroidal blood flow (ChBF) constant throughout changes in the ocular perfusion pressure (OPP) such as occurs during exercise (19, 28). Vascular AR also adjusts blood flow to meet the changing metabolic demands of retinal neurons and to prevent rupture of fine capillary networks where the exchange of metabolites occurs.

The relatively recent adaptation of near-infrared (NIR) laser-Doppler flowmetry (LDF) (26) to the human eye has made possible several studies into choroidal hemodynamics including the discovery of blood flow regulation in the choroidal vasculature (19, 28). Unfortunately, the fundamental interaction between light-induced changes in neuroretinal activity and ChBF has remained largely unexplored.

In lower-order animal species, photic stimulation of the retina increases ChBF (8) through the Edinger Wespah nucleus reflex. In sharp contrast, diffuse flash stimulation of the human retina has been linked to changes in blood flow in the retinal vasculature and optic nerve head (7, 10, 13, 22, 24, 27) but not the choroid (13, 18). To our knowledge, only two other research groups have examined the interaction between light and ChBF in humans (13, 18). Longo et al. (18) were the first to investigate the influence of retinal light levels on ChBF. Specifically, they found that a 20-min transition from cone- to rod-dominated vision decreased the subfoveal ChBF by ~15%. The subsequent exposure of dark-adapted eyes to ambient room lighting restored the pretest ChBF level within 6 min (18). Inasmuch as the transition from light to dark altered the ChBF, it was surprising that stimulating the central 40° of the retina for 10 min with a bright green light (peak spectral emission at 524 nm and a 60-nm bandwidth at 50% peak transmission) failed to elicit significant changes in ChBF. However, these conflicting observations could be reconciled if different mechanisms controlled blood flow in the choroid during light-dark exposure of the retina and when the retina was stimulated by light in photopic conditions.

In a more recent study, Garhofer et al. (13) reported that stimulation of the central 30° of the retina with 8-Hz flickering light from a Grass PS2 stroboscope covered with a 550-nm low-pass filter failed to change the subfoveal ChBF but did increase blood flow in the optic nerve head by 30%. From these data, Garhofer et al. (13) concluded that a light-induced increase in retinal activity had no effect on ChBF and that ChBF was independent of changes in retinal metabolism. However, they acknowledged that the 550-nm low-pass flicker may have preferentially activated the dense cone population at the macula and that the resulting subfoveal choroidal hemodynamics may have differed from ChBF reactions in the greater retinal periphery.

These limited and inconclusive findings on the correlation between light-induced neural activity in the retina and blood flow changes in the choroid prompted us to further investigate this issue. Therefore, instead of using flashes with a broad spectral profile that unavoidably would differentially stimulate all macular cones to different degrees in the macular zone, as was the case in previous studies, we presented blue flashes over a broad luminance range to selectively stimulate rods and s-cones. By presenting blue flicker in a low-to-high luminance order, our goal was to elicit responses principally from the rhodopsin-bearing rods, then the rods and short-wave blue cones, and finally the s-cones with the brightest flicker.

We rationalized that narrow-band blue flash (473 nm) stimulation of the retina at very low luminance was more likely to induce changes at the subfoveal measurement site because of selective activation of rods populating the greater retinal periphery instead of exciting the cones and rods located closer to...
the fovea by high luminance flicker. Because the rod-to-cone ratio in the human retina is about 20:1 (5), we postulated that strong activation of rods by dim blue flashes would change the distribution of ChBF across the retina, and this would be reflected by changes in the subfoveal ChBF. It is likely that previous studies could not find ChBF changes at the fovea because they used either broad-band spectral stimuli or bright narrow-band light that stimulated all cone types in the macula, and these determined the metabolic need for ChBF in the foveomacular zone.

To determine which type of retinal photoreceptor and the degree to which it was activated by the blue flash luminance sequences, retinal responses were compared using the amplitude and temporal features of the flash-evoked retinal potential, the electroretinogram (ERG).

**MATERIALS AND METHODS**

**Subjects and Procedures**

Ten healthy adult volunteers, seven women and three men, with an average age of 25.4 yr (SD: ± 9.4) participated in this study. All participants were experienced research subjects with excellent fixation ability. The subjects’ range of refractive errors was +1 D to −5 D equivalent sphere, the intraocular pressure (IOP) was between 10 and 20 mmHg, and the systemic blood pressure (BP) was within normal limits. All testing protocols complied with the declaration of Helsinki and ethical guidelines for humans in research at the University of Montreal. A signed informed consent was obtained from each participant after the nature and purpose of the experiment had been explained to each volunteer.

**Measurement of ChBF**

Before being tested, each subject was familiarized with the experimental protocols. The pupil of the test eye was dilated with two drops of mydriacyl (1% tropicamide) and one drop of mydfrin (2.5% phenylephrine HCl). Choroidal hemodynamics were quantified with a noninvasive continuously recording NIR 784-nm laser-Doppler flow-meter (Ocuflux). All subjects adapted to the normal laboratory lighting for at least 20 min before any measurements of ocular blood flow were taken. Because the design and operation of the dynamic LDF used to measure the subfoveal ChBF have been reported previously (26), only a summary of its use is presented here. The LDF consisted of a fundus camera modified to deliver the narrow laser beam into the foveal avascular zone. The laser power at the cornea was 90–95 mW, which complied with ANSI 136.1 standards for laser safety (1). The laser power was kept constant throughout the entire experiment through a feedback power supply. The laser light back scattered by static tissue and moving blood cells in the subfoveal choriocapillaris was focused in the retinal image plane of the fundus camera, and a 90-µm-diameter fiber-optic sensor guided the laser light to an Avalanche photodiode. The photocurrent was sampled at 20 kHz and then processed by a dedicated program on a NeXT computer to provide real-time (about 22 points/s) measurements of blood flow parameters based on the Bonner and Nossal theory (3). The hemodynamic parameters included ChBF, choroidal blood velocity (Ch BV vel), and choroidal blood volume (Ch BV vol). The measured blood flow represents the product of blood cell velocity and the concentration of blood cells in the sampled tissue volume. A blood flow sensor clipped to the ear provided measurements of heart rate throughout the experiment. All LDF measurements were carried out in moderately reduced room lighting to minimize reflections of visible light into the light sensor for the back-scattered light. As a further precaution to eliminate stray light from contaminating blood flow measurements, a 50-mm dichroic filter ("cold" filter) sealed the aperture leading to the small chamber containing the fiber-optic sensor; thus only infrared light from the test eye was analyzed.

**Flicker Stimuli**

A cluster of five high-intensity blue light-emitting diodes (LEDs) with a peak spectral emission at 473 nm and a 22-nm bandwidth at half peak height was used as the flicker light source. The first experimental protocol involved increasing the luminance of blue flicker in 1.0-log unit steps, with neutral density filters, from 0.0375 to 375 cd/m². In the follow-up experiment, the order of step changes in flicker luminance was reversed.

The blue flicker frequency and stimulus duration were controlled by dedicated electronics. Light from both the LEDs and the NIR laser was projected onto the retina by the background illumination optics of the fundus camera. An auxiliary optical system directed the probing laser light into the center of the flicker field, which filled the entire field of view of the subject. Maxwellian optics in the fundus camera assured a bright uniform illumination of the retina.

**Effect of Increased Flicker Luminance on ChBF**

This phase of the study involved flicker stimulation of the retina at frequencies between 1 and 64 Hz in 24 steps. The duration of each flicker frequency was 10 s with no delay between frequencies. A complete ChBF stimulus-response profile involved continuous measurement of the subfoveal ChBF during five consecutive flicker-frequency series as described above and presented over a 4.0-log cd/m² luminance range. The luminance of the aerial image of the LED matrix formed by the fundus camera optics was 375 cd/m² (Minolta CS-100 Chromameter; Milton Keynes, UK).

In this test phase, flicker luminance started at the lowest level, 0.0375 cd/m², and was increased to the brightest one, 375 cd/m², in 1.0-log unit steps. The lowest flicker luminance, 4.0-log unit steps below 375 cd/m², was achieved by placing 4.0 ND Kodak Wratten in front of the LED light source. At the end of each flicker series, normal room lighting was restored for 1 min to ensure that the retina remained light adapted. During this interval, subjects maintained fixation of the probing laser. ChBF recordings were stored to computer memory, and the ND filters were changed to increase the flicker luminance by 1.0-log unit step. As such, each test eye underwent 20 min of flicker stimulation throughout this phase of the experiment.

Because the LDF system measured only relative changes in choroidal hemodynamics, the ChBF for all test conditions was recorded in one continuous session to be able to quantify absolute changes in ChBF as elicited by changes in flicker frequency and luminance.

**Effect of Decreased Flicker Luminance on ChBF**

Six of the original ten subjects participated in this follow-up study, where the order of flicker frequencies was maintained but the order of step changes in luminance was reversed, such that the first flicker frequency series was presented at a luminance of 375 cd/m² and the luminance of each subsequent flicker series was decreased by a 1.0-log unit step down to 0.0375 cd/m². It was hypothesized that any cumulative effect on the retina caused by diffuse blue flicker would be changed radically by a reversal in the step changes in flicker luminance. Conversely, if retinal activity and ChBF were unaffected by photic stimulation per se, then a reversal of the step changes in flicker luminance would also have no effect on retinal activity or choroidal perfusion, and, consequently, the ChBF response profile would be the same whether the flicker luminance was increased or decreased.

**Electroretinographic Index of Neuroretinal Responses to Light**

Blue flash (peak wavelength = 473 nm) ERGs were recorded to differentiate the contribution of rods and s-cones to changes in ChBF during blue flicker stimulation of the retina at different luminance levels. The ERGs were recorded with an Espion- (Diagnosys; Little-
ton, MA) evoked potential system respecting International Society for Clinical Electrophysiology of Vision guidelines for recording clinical ERGs (21). Each subject was positioned in the LDF system for ERG recordings. To record the ERG, a DTL fiber electrode (6) placed in the inferior sulcus of the test eye served as the reference electrode, whereas a second DTL electrode placed in the inferior sulcus of the contralateral light-occluded eye served as the reference electrode. That eye was occluded by a light-tight black patch. An Ag-AgCl electrode on the inner wrist formed the electrical ground. All ERGs were recorded in response to 1-Hz diffuse blue flashes delivered through the background channel of the fundus camera as during LDF recordings. Each retinal response to a flash was gated through a 0.15- to 100-Hz band-pass filter and amplified ×10,000. The average of ten 200-ms epochs synchronized to the onset of each flash comprised the ERG signal. This ERG was measured before and after each blue flicker series to quantify any changes in retinal responsiveness induced by the flicker. For the needs of this study, the ERG response was quantified by measuring the amplitude of the b wave. Changes in rod/cone contributions to the ERG b wave were derived from light-induced changes in the overall ERG waveform, including changes in the amplitude and temporal attributes of the principal ERG components with changes in blue flash intensity.

**Data Analyses**

The specific response parameters sought and the various methods used to extract the information from the large database are listed below.

Quantifying any changes in choroidal hemodynamics caused by flicker between 1 and 64 Hz and step increments in flicker luminance followed by flicker with step decrements in flicker luminance. The ChBF response for a particular flicker frequency was averaged over the corresponding 10-s flicker interval using an electronic averaging cursor in the LDF program (see Figs. 1 and 2). This process simultaneously yielded the time-averaged values for ChBF, ChBVol, and ChBVel, which were then normalized so that blood flow responses across subjects could be compared on a common scale. ChBF was normalized by assigning a 100% value to the 10 s of ChBF recorded in response to the first flicker stimulus (i.e., 1 Hz) and luminance level and expressing subsequent ChBF responses to incremental changes in flicker frequency as a percentage of the 100% reference value across all flicker luminance levels. The same data reduction was applied to ChBVol and ChBVel data for each subject for flicker of increasing and decreasing luminance. Any separation of consecutive flicker frequency response profiles would be due to step changes in flicker luminance.

Quantifying the effects of step increments in flicker luminance on ChBF. While the group averaging procedure was particularly effective for revealing the effect of flicker luminance on the frequency driven response profile, the interaction between flicker frequency per se and ChBF was much less evident (see Fig. 3). In fact, the first analysis somewhat obscured the frequency effects because ChBF peaked in narrow but different frequency ranges across subjects. Consequently, a second analysis of raw data was carried out and presented the modulation amplitude for ChBF as a function of the peak driving frequency.

Quantifying the interaction among ChBF, ChBVol, and ChBVel when flicker luminance was increased and then decreased. To better quantify the interactions among ChBF, ChBVol, and ChBVel, all individual normalized responses for ChBVol and ChBVel (n = 24 each) for each flicker luminance level (n = 5) were plotted against the corresponding normalized change in ChBF for flicker of increasing and decreasing luminance (see Fig. 4). Subsequently, curve-fitting functions helped define interactions between these hemodynamic parameters.

Quantifying changes in retinal sensitivity during repeated exposure to blue flashes of increasing and decreasing luminance. The ERG b wave peak time and amplitude were measured with electronic cursors (see Fig. 5). The most revealing and compelling information about the retinal sensitivity status came from a side-by-side comparison of the ERG profiles taken before and after each flicker series on the same scale and over flash luminance series that (1) increased luminance and (2) decreased luminance.

Correlating the neural and vascular responses to graded blue flashes. The existence of any physiological correlation was explored by simply plotting the normalized change in the subfoveal ChBF and the absolute ERG amplitude as a function of the blue flash luminance for flashes of increasing and decreasing intensity (see Fig. 6).

**Statistical Analyses**

ANOVA on the group-averaged changes in ChBF, ChBVol, and ChBVel were analyzed with flicker frequency, peak frequency, and luminance as repeated measures for an α-level of 5%. Tukey(a) post hoc comparisons were used where appropriate.

**RESULTS**

**Effects of Increased Blue Flicker Frequency and Step Increments in Luminance on ChBF**

The normalized, group-averaged subfoveal ChBF, ChBVol, and ChBVel are presented as a function of flicker frequency and flicker luminance in Fig. 1, A–C, respectively.

The flicker luminance values used in this phase of the study (0.0375 to 375 cd/m²) are presented adjacent to each ChBF response curve (Fig. 1). The vertical bar through each data point in Fig. 1 and all subsequent figures in this report represent mean ± SE values for a given data point. The curves drawn through the group-averaged data points for ChBF, ChBVol, and ChBVel were determined by fitting polynomial expressions for visible trends, first for points in the low flicker-frequency range (1–22 Hz) and then through remaining data points in the middle-to-high flicker-frequency range (22–64 Hz).

The ChBF results shown in Fig. 1A revealed two small peaks in the low-frequency range (small inverted arrows) and a more gradual positive peak in the high-frequency range at ~50 Hz, suggesting photic driving of ChBF. However, these group-averaged peaks did not achieve statistical significance (P > 0.05).

Even more visible than the small ChBF peaks within each of the flicker frequency series illustrated in Fig. 1A were the rather large downward shifts in successive flicker-frequency series. Each drop in the ChBF flicker-frequency response profile occurred after a 1.0-log unit step increase in flicker luminance. With each 1.0-log unit step increment in flicker luminance, ChBF was attenuated by about 8% except between steps 3 and 4. Overall, repeated blue flicker stimulation of the retina across five flicker-frequency series and four 1.0-log unit step increments in flicker luminance caused the subfoveal ChBF to decrease from its prestimulus value by ~32% (P = 0.0001).

A visual comparison of the population trends for ChBF, ChBVol, and ChBVel in Fig. 1 revealed that the overall pattern of change in ChBF was closely paralleled by the changes in ChBVol (P = 0.0001; Fig. 1B) but not in ChBVel (P > 0.05). This trend identified a drop in ChBVol as the dominant...
Effects of Increased Blue Flicker Frequency and Step Decrements in Luminance on ChBF

The normalized, group-averaged ChBF, ChBVol, and ChBVel response profiles to five consecutive flicker series coupled with four 1.0-log unit step decrements in luminance are presented in Fig. 2, A–C, respectively. A cursory examination of these data sets revealed that all parametric response curves were clustered more tightly than those of Fig. 1 and that the ChBVel response curves shown in Fig. 2C straddled the 100% reference line. Statistical analyses of the data trends in Fig. 2 revealed that none of the hemodynamic parameters changed during flicker-frequency testing that involved step...

Fig. 1. Graph illustrating normalized, group-averaged choroidal blood flow (ChBF; A), choroidal blood volume (ChBVol; B), and choroidal blood velocity (ChBVel; C) as a function of blue flicker frequency between 1 and 64 Hz and 1.0-log unit step increments in luminance. The vertical line through each data point in this figure and subsequent figures gives mean ± SE values for that data point. The numbers beside each curve give the flicker luminance value (in cd/m²).

Fig. 2. Graph illustrating the normalized, group-averaged ChBF (A), ChBVol (B), and ChBVel (C) as a function of blue flicker frequency between 1 and 64 Hz and 1.0-log unit step decrements in luminance. The numbers beside each curve give the flicker luminance value (in cd/m²).
decrements in flicker luminance ($P > 0.05$). Thus the reversed order of changes in flicker luminance did not show a large decrease in ChBF as seen in increased flicker luminance.

**Effects of Increased Flicker Frequency Per Se on Subfoveal ChBF**

Because raw LDF recordings for each subject showed considerably larger perturbations in ChBF than those indicated by the small group-averaged peaks at $\sim 5$, $\sim 17$, and $\sim 50$ Hz, another analysis procedure was used. Specifically, the raw data were reanalyzed and plotted to show the ChBF modulation amplitude for each subject as a function of the flicker frequency eliciting the largest change in the ChBF. The results of this secondary analysis are presented in Fig. 3 and show that ChBF was preferentially modulated by blue flicker at $\sim 7$, $\sim 22$, and $\sim 47$ Hz by amounts of $\sim 9\%$, $\sim 10\%$, and $\sim 8\%$, respectively.

The vertical and horizontal bars through each of the three data points in Fig. 3 represent means $\pm$ SE for the modulation amplitude and peak frequency driving the changes in ChBF amplitude, respectively. Statistical analyses showed that these amplitudes of modulation in ChBF were significant ($P \leq 0.011$) but that the ChBF modulation amplitudes did not differ across flicker frequencies ($P > 0.05$). It is noteworthy that the modulation amplitude identified by this second analytic approach was about three times greater than that shown in Fig. 1A and more closely reflected the amplitude of flicker-induced changes seen in ChBF recordings.

The differential effect of flicker frequency and flicker luminance on ChBF was particularly noteworthy. Figure 3 shows that blue flicker frequency between 1 and 64 Hz increased ChBF by a maximum of about 9%. In contrast, the data in Fig. 1A, as described above, show that an increase in the flicker luminance (from 0.0375 to 375 cd/m$^2$) continued to have an effect on the choroid over a much broader range of luminance than flicker per se, reducing the subfoveal ChBF over 4.0-log unit steps.

**Interaction Among ChBF, ChBVol, and ChBVel as Flicker Luminance Was Increased and Then Decreased**

Inasmuch as the subfoveal ChBF remained unchanged during repeated blue flicker stimulation of the retina when the luminance of flicker was decreased over four 1.0-log unit step decrements in the flicker luminance (Fig. 2), the reversal of the order of flicker luminance stimuli was hypothesized to be the reason for the relative constancy of the subfoveal choroidal hemodynamics.

The effect of the order of presentation of blue flicker with increasing versus decreasing luminance on choroidal hemodynamics is compared in the same subgroup of subjects in Fig. 4, A and B. Figure 4A shows the systematic reduction recorded in ChBVol when the flicker luminance was systematically increased over 4.0-log unit steps. In contrast, ChBVel initially decreased by just 4%, whereas ChBVol had decreased by about 10%. Thereafter, ChBVel formed a plateau with its remaining data points.

In contrast to this large decrease in ChBVol caused by blue flicker with increasing luminance, blue flicker with decreasing luminance produced very different response profiles for ChBVol and ChBVel data. Both ChBVol and ChBVel clustered tightly between 90% and 106% data intervals. A regression line drawn through the ChBVel data points showed a
slight positive trend, as did a regression line through the volume data. The large difference in the flicker luminance-induced changes in ChBVol and ChBVel parameters for ChBF seen in Fig. 4, A and B, was attributed to the difference in the order of luminance presentation.

Empirical evidence that the same photic stimuli presented in a forward (dim to bright) and reversed (bright to dim) order to the retina can elicit different retinal responses was verified experimentally through objective measurements of light-evoked retinal activity. The results of diffuse 1-Hz flash ERGs acquired in response to blue flashes of increasing and decreasing intensity (A vs. B) are presented in Fig. 5. These objective indexes of neural responses of the retina indicate that the same blue flash intensities when presented in the forward versus reverse direction elicited b waves with significantly different amplitudes. This evidence suggests a mechanism whereby blood flow at the fovea may be attenuated in favor of supporting more active photoreceptors in the retinal periphery during repeated blue flicker stimulation of the retina. In a subsequent analysis, the ERG b waves were measured and plotted as a function of blue flash luminance for test conditions where the luminance increases from dim to bright (Fig. 6A) and decreases from bright to dim (Fig. 6B). These were then matched with the simultaneously measured changes in the subfoveal ChBF. This latter analysis showed that increasing flicker luminance elicited increased amplitude ERGs and a decrease in the subfoveal ChBF. When the flicker luminance was presented from bright to dim, the same flicker luminance elicited different ERG b wave amplitudes before and after the flicker-frequency series. The subfoveal ChBF did not change with flicker luminance.

When the flicker luminance was increased from 0.0375 to 37.5 cd/m², the ERG b wave increased in amplitude and its peak time shortened (Fig. 5A). This type of change in the ERG b wave is characteristic of a rod-dominated retinal response. When the flash intensity was increased further to 375 cd/m², the amplitude of the b wave was abruptly reduced and its implicit time was shortened, indicating that the rod-dominated response had shifted to a cone-dominated ERG. The overlapping of the waveforms in Fig. 5A revealed that ERGs measured before and after each flicker series were identical, and thus no change in retinal sensitivity was induced by the preceding flicker-frequency series.

Figure 5B shows the ERGs before (“b”) and after (“a”) each flicker series where the blue flash luminance was decreased from 375 to 0.0375 cd/m² in 1.0-log unit steps. The before and
after flickers of the ERGs were similar for all flash luminances except for the 3.75- and 0.375-cd/m² flashes, where both retinal potentials were about 50% smaller than the ERGs elicited by the same flicker luminance but presented in reverse order. The small oblique arrows identify the global retinal response just after the eye had been adapted to room lighting and before the blue flicker-frequency series was initiated. This light exposure would have reduced the rod contribution to the ERG. However, after the retina was stimulated by the blue flicker-frequency series, the ERG response increased, thereby indicating a larger participation of rods in the recorded ERG. When the blue flash was at its dimmest level, the ERGs before and after adaptation to room light were identical and almost extinguished. These changes in the ERG profiles differed markedly from those elicited by the same light stimuli but presented in reverse order.

**DISCUSSION**

**ChBF Response to Flicker**

To our knowledge, direct driving of ChBF by light stimulation of the retina had not been reported previously by other groups for the human eye. In fact, the linkage between ChBF and a light-activated increase in retinal metabolism had been specifically rejected (13, 18). These latter reports were not consistent with the notion that the human retina requires all the oxygen provided by the choroid (17). If the choroid could not increase the availability of oxygen to the retina during photic stimulation by increasing ChBF, such stimuli might not be processed normally for lack of metabolites, and other ongoing physiological processes could also be compromised. The first report of an association between light-induced neuroretinal activity and changes in ChBF was provided by Lovasik et al. (20). This group reported that the amplitude of both flash ERGs and the subfoveal ChBF increased during red flicker stimulation of the retina. The present follow-up study is the first to provide a detailed report on the systematic effects of light flashes modulated in luminance and temporal frequency on the subfoveal ChBF in the human eye.

**Anatomic and Physiological Evidence for Differential Blood Flow Requirements**

Early psychophysical studies established that the spectral sensitivity of the retina some 7° from the fovea was best described by overlapping scotopic and photopic luminosity functions that peaked at about 500 nm (14). The observation that the greater quantal sensitivity to light of rods over cones increased with distance from the fovea matched the anatomic increase in rod density toward the retinal periphery (14). The blue-sensitive s-cone population is minimal in the fovea, occurs in highest density in an annulus 2.5–7° wide about the fovea, half this density outwards to 20°, and a further drop off beyond to the far periphery (2). This distribution of rods and s-cones led to our proposal that the 473-nm dim-to-bright flicker series preferentially increased neural retinal activity in the rod-populated periphery and that this heightened neural activity could direct ChBF in favor of the retinal periphery. The progressive decrease in subfoveal ChBF during blue flicker is consistent with this interpretation. The absence of blood flow changes at the fovea for bright-to-dim flicker was consistent with ChBF being driven by the s-cone population that dominated the foveal-macular zone. Such s-cone-dominated activity for the highest blue flicker luminance (375 cd/m²) is supported by psychophysical studies that showed the rod system sensitivity to be reduced by several log units at this light level and, conversely, the cones to be insensitive to very low light levels (14). To our knowledge, there have not been any previous reports of a physiological reallocation of ChBF in the human eye. We believe that these data are the first to reveal this unique capability of the human choroid.

The notion of a topographically specific increase in blood flow to meet the demands of a focal increase in retinal metabolism has been advanced in animal studies. Kiryu et al. (16) reported that 488-nm 8-Hz flicker increased retinal blood flow in baboons by about 30%, more so in the midperipheral region, where ganglion cells and nerve fibers were found in greater density. This was interpreted as a regulatory response to meet the increased metabolic demands at discrete retinal sites, similar to zones of increased retinal activity induced by the graded luminance flicker used in the present study. The possibility of similar regulatory responses in the choroidal vasculature of subhuman primates was raised by Fryczkowski (11), who distinguished two models of choroidal lobuli, anatomic versus functional, where the latter could modify flow according to metabolic need. In a follow-up study, Flower et al. (9) also reported that lobular filling of blood in the choriocapillaris could be altered by acute elevations in the IOP and by oxygen and carbon dioxide breathing. Thus ChBF is not merely a passive network of vessels but can be modulated according to regional metabolic needs. Therefore, the anatomic and physiological bases for our proposal have already been reported in subhuman primates. Consequently, the progressive decrease in the subfoveal CHBF during increased flicker luminance (Fig. 1A) and the observation that the flicker caused the volume of blood to decrease (Fig. 1B) are consistent with this differential choroidal perfusion across different retinal zones.

Such preferential perfusion of metabolically active tissue is well known in functional MRI studies of the brain, which consistently show blood moving preferentially toward metabolically active sites (23, 25, 29). For the human eye, however, this proposal demands experimental validation possibly by simultaneous ChBF measurements in foveal and eccentric regions of the fundus during flicker. The technology to realize dual ChBF measurements at different retinal sites is yet to be developed.

If one rejects the notion that a preferential increase in rod activity draws blood away from the foveal-macular zone and into the retinal periphery, it would have to be assumed that the human eye can withstand a 30% drop in ChBF and still maintain normal physiological function. Such a large decrease in retinal perfusion would not only impair retinal function (15) but would also have effects on overall vascular hydrodynamics, thereby making this argument untenable.

**Photoreceptor Activity and Choroidal Vascular Tone**

The present data show discrete changes in choroidal hemodynamic parameters throughout blue flicker stimulation of the retina. When the percent change in ChBVel and ChBVol parameters was correlated with the percent change in ChBF during blue flicker stimulation, the results indicated that the
change in ChBVol was responsible for the decrease in the subfoveal ChBF (Fig. 4A).

Inasmuch as this trend for the volume was seen in all test subjects, we propose that vasoconstriction was the mechanism by which ChBF was reduced at the fovea. The overall effect of a regional vasoconstriction would likely be a displacement of blood toward the periphery for greater perfusion of a large stimulated rod population. Furthermore, it may be hypothesized that the reduced ChBF at the fovea returns to the prestimulus value by the reverse process involving vasodilation where blood from the periphery is returned to the foveomacular zone. This notion has yet to be verified experimentally.

Correlation Between Retinal Activity and ChBF

This light-induced change in ChBF (Fig. 1) was not found in previous studies, likely because of the broad spectral nature of the test flashes (13, 18). The successful modulation of the subfoveal ChBF in the present study was due to light stimuli that first stimulated the peripheral rods at very low flicker luminance, then the rods and s-cones at higher luminance, and finally the s-cones at maximal flicker luminance. These changes in ChBF were clearly linked to electrical indexes of global retinal function as indexed by diffuse blue flash ERGs. Figure 5 shows that the amplitude and timing of the ERG components were modulated in proportion to the blue flash luminance. Together, these data sets show a clear association between neuroretinal activity and blood flow in the choroid.

In an earlier study using the cat model, Buerk et al. (4) reported flicker-induced changes in blood flow in the optic nerve head as well as changes in the ganglion cell axons that comprise the optic nerve. This relationship between blood flow and neural activity has been coined “neurovascular coupling.” In the present study, direct measurements of rod or cone activity by invasive probes were not possible for obvious reasons. However, in clinical neurophysiology, it is well established that the early negative potential wavelet referred to as the a-wave of the flash ERG originates from the photoreceptors that are specialized sensory neurons containing light-sensitive pigments. In this context, our findings can also be interpreted as a form of neurovascular coupling. However, neurovascular coupling as a general descriptor of the phenomenon reported here is precluded by the observation that flicker of decreasing luminance (Fig. 5B) elicited changes in the ERG but not in the subfoveal ChBF.

The observation that reversing the step changes in flicker luminance had no effect on the subfoveal ChBF across flicker luminance (Fig. 2A) is consistent with the notion of ChBF being directed to retinal zones with highest neuronal activity. The ERG records in Fig. 6 revealed that before each flicker set, the global retinal response was much smaller than that for the same flash luminance but presented in the forward (dim to bright) direction. As such, less ChBF would have been needed to support an attenuated rod-dominated response; consequently, the subfoveal ChBF would be minimally affected (Fig. 6B), as verified empirically during the reversed luminance flicker study (Fig. 2A).

Referring to Fig. 5A, the diffuse blue flash ERG b wave elicited by the dimmest luminance level (0.0375 cd/m²) was relatively flat and delayed. As the flash intensity was increased, the ERG amplitude increased rapidly to its largest and quickest value for a 37.5-cd/m² flash, after which it decreased by about 50% and showed the fastest response time to flashes of 375 cd/m². For all these test conditions but the last, rods were progressively recruited into the response. For the 37.5-cd/m² blue flash, s-cones also contributed to this response, whereas the smallest and fastest ERG was a s-cone-dominated ERG. The progressive recruitment of rods by blue flashes of increasing luminance was presumed to require increased choroidal perfusion in the periphery, where rods occur in greatest density.

The ERG response profiles in Fig. 5, A and B, show that the same flash luminance elicited different retinal responses depending on whether the test luminances were increased (Fig. 5A) or decreased (Fig. 5B). Parallel to these objective indexes of retinal function, ChBF also responded differently when the flicker luminance increased or decreased. Thus the argument that a singular stimulus should elicit the same retinal response is untenable. Clearly, the preceding retinal light history strongly influences the retinal response to a light flash with fixed spectral and luminance properties. The variation in neural and vascular responses to identical stimuli is illustrated most clearly in Fig. 6. These data show that the neural retinal and vascular responses differed considerably when the flicker luminance changed from dim to bright (Fig. 6A) versus bright to dim (Fig. 6B). The vascular counterpart for this neural response profile was a large progressive decrease that totaled ~32% when flicker luminance changed from dim to bright.

The present report, which shows that blood flow in the human choroid is linked to light-induced retinal activity, is fundamental to our understanding of retinal processing of visual stimuli. This newly defined association between photoreceptor activity and blood flow in the choroid may provide new avenues for research into the pathophysiology of sight-threatening diseases of the eye such as age-related macular degeneration. In addition, this vascular response to photic stimuli may be used for diagnosing hemodynamic abnormalities in the choroid and also for evaluating the effectiveness of therapeutic procedures aimed at minimizing vision loss.

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