

# Influence of Flicker Frequency on Flicker-Induced Changes of Retinal Vessel Diameter

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**PURPOSE.** To investigate the effect of diffuse luminance flicker of different frequencies on retinal vessel diameter in the human eye.

**METHODS.** Nine healthy subjects participated in the study. A retinal vessel analyzer (RVA; Zeiss, Jena, Germany) was modified to allow for continuous measurement of vessel diameter during flicker stimulation. With this technique, the light used for measurement of vessel diameter consists of wavelengths between 567 and 587 nm, whereas the spectrum of the stimulation is low-pass, with a cutoff wavelength at 550 nm. Flicker frequencies ranged from 2 to 64 Hz.

**RESULTS.** In retinal arteries an increase was observed at all flicker frequencies, with a less-pronounced effect at 64 Hz. In retinal veins, all flicker frequencies except 2 and 64 Hz induced vasodilation. Generally, the flicker-induced diameter response was less pronounced in retinal veins than in arteries.

**CONCLUSIONS.** The flicker-induced diameter response of the larger retinal arteries and veins can be continuously measured with a modified RVA system that spectrally separates the flicker stimulation from the fundus illumination light. Vasodilation of retinal arteries was observed in response to short wavelength flicker with frequencies between 2 and 64 Hz, indicating that the parvo- and magnocellular neural pathways are activated with this stimulation. (*Invest Ophthalmol Vis Sci.* 2002;43:2721-2726)

In their classic paper published more than 100 years ago, Roy and Sherrington<sup>1</sup> hypothesized that the brain possesses an intrinsic mechanism by which its vascular supply can be varied locally in correspondence with local variations of functional activity. Using various techniques to measure regional cerebral blood flow, such as laser Doppler flowmetry, transcranial Doppler sonography, and positron emission tomography, researchers have since amply verified this hypothesis.<sup>2</sup> Evidence is accumulating that such a mechanism also operates in the optic nerve head (ONH) and retina in cats,<sup>3-5</sup> primates,<sup>6</sup> and humans.<sup>7,8</sup>

Because retinal blood flow (RBF) in a main retinal vessel is equal to  $\pi D^2 V_{\text{mean}} / 4$ , where  $D$  is the diameter of the vessel and  $V_{\text{mean}}$  the mean velocity of blood, a twofold increase in  $V_{\text{mean}}$  leads to a twofold increase in RBF, whereas a twofold

increase in  $D$  leads to a fourfold increase in RBF. This underlines the importance of measuring precisely the changes in  $D$  when attempting to detect changes in RBF in response to a stimulus. The most favorable sites in a vascular bed to detect a change in  $D$  are the arterioles, which have a  $D$  below approximately 20  $\mu\text{m}$ , because these vessels are the major sites of resistance to flow in the absence of precapillary sphincters. However, quantification of changes in  $D$  in these vessels is difficult because of the limited resolution of present techniques. In contrast, changes of less than 3% in  $D$  of the larger retinal arteries are routinely detectable. Although they do not constitute the main component of vascular resistance, these arteries are known to show vasoreactivity in response to various physiological stimuli.<sup>9,10</sup>

Previous methods to examine the response of  $D$  and RBF to flicker in humans did not allow for assessment of these parameters during flicker,<sup>7,8</sup> but measured their response after cessation of flicker. We set out to develop a method that allows for the on-line registration of the changes in  $D$  during flicker. For this purpose we modified a commercially available system for the measurement of  $D$  of retinal vessels in humans and investigated the flicker-evoked change in  $D$  at various flicker frequencies.

## METHODS

### Subjects

The study was performed according to the guidelines set forth in the Declaration of Helsinki. Seven men and two women participated (mean age,  $34 \pm 11$  years [ $\pm$ SD]). After the nature of the study was explained, written consent was obtained from all participants. All subjects were asked to refrain from alcohol and caffeine for at least 4 hours before the trial. They had no history of ocular disease or epilepsy and a refractive error of less than 3 diopters. Mean arterial blood pressure ranged from 85 to 100 mm Hg.

### Study Protocol

Measurements of  $D$  were performed continuously for 3 minutes during each experiment. Flicker stimulation was scheduled from second 60 to second 120. To investigate the reproducibility of the flicker-evoked response of  $D$ , as obtained with our experimental setup, we performed five experiments within 30 minutes with flicker stimulation at a frequency of 8 Hz. From these experiments, the coefficient of variation of the  $D$  response was calculated.

The protocol was repeated eight times, each at a different frequency, with a time span between two flicker stimulation experiments of at least 3 minutes. Measurements using a flicker frequency of 4, 8, 16, 24, and 32 Hz were performed in both eyes in eight subjects and in one eye in one subject. Measurements with 40 and 64 Hz were performed in both eyes of six subjects and in one eye in two subjects, whereas measurements with 2 Hz were performed in both eyes in four subjects and in one eye in four other subjects.  $D$  was measured at the trunk of the inferior or superior temporal retinal arteries and veins, proximal to the bifurcation of their first major branches.

Additional  $D$  measurements were made off-line by using the video recordings of the experiments with 8 and 16 Hz to investigate regional differences in the  $D$  response. We measured again the  $D$  of the inferior

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Supported by Project 32-53785 from the Swiss National Science Foundation and Project P14262 from the Austrian Foundation for the Promotion of Scientific Research.

Submitted for publication January 15, 2002; revised March 22, 2002; accepted March 29, 2002.

Commercial relationships policy: N.

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or superior temporal retinal arteries and veins. One measurement window was located at the trunk of these vessels within one disc diameter of the optic disc. Another window was placed at the same trunk in a distance between one and two disc diameters from the optic disc. Finally, we measured the  $D$  of arterial and venous branches, which originated distal to the described fundus locations.

### Retinal Vessel Analyzer

The RVA comprises a fundus camera (field of 50°; model FF 450; Zeiss, Jena, Germany), a video camera, a real-time monitor, and a computer with image-analysis software for the accurate determination of retinal arterial and venous diameter.<sup>11</sup> Retinal vessel  $D$  is analyzed in real time with a maximum frequency of 40 Hz, so that every second a maximum of 25 readings of vessel diameter can be obtained. For this purpose the fundus is imaged onto the charge-coupled device (CCD) chip of the video camera. The consecutive fundus images are digitized with a frame grabber. Evaluation of the retinal venous  $D$  was performed on-line, and evaluation of arterial  $D$  was performed off-line, from the recorded videotapes.

Because of the absorbance properties of hemoglobin, each blood vessel has a specific transmittance profile. Measurement of retinal vessel  $D$  is based on adaptive algorithms that use these specific profiles. Whenever a specific vessel profile is recognized, the RVA can follow this vessel as long as it appears within the measurement window. This means that the system can correct automatically for alterations in luminance that are induced, for instance, by slight eye movements. If the requirements for the assessment of  $D$  are not fulfilled temporarily, as occurs during blinks, the system automatically stops the measurement of  $D$ . As soon as an adequate fundus image is achieved again, measurement of  $D$  restarts automatically.

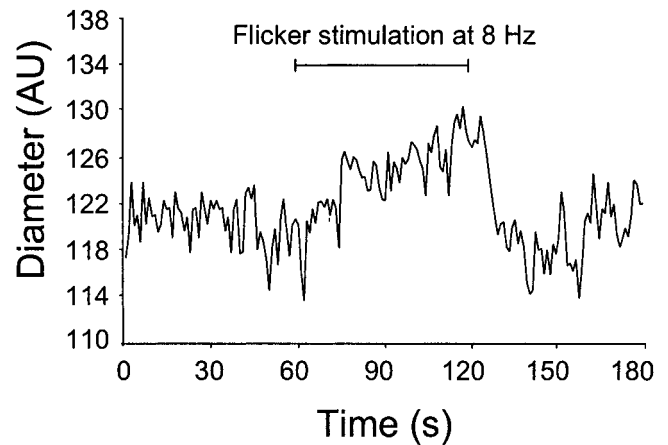
To select a region of interest, a rectangle that includes the particular vessel can be defined on the screen of the real-time monitor.  $D$  is then calculated along the arterial or venous segment that lies within the rectangle. As long as the vessels under study are within the selected rectangle during the eye movements, the system automatically corrects for the eye movements. This is again permitted by the adaptive nature of the  $D$  analysis software. We have shown that this system provides high reproducibility and sensitivity of the  $D$  response to pharmacologic stimulation.<sup>12</sup>

### Flicker Stimulation

Flicker was generated by focusing the light from a 150-W xenon arc lamp system (Oriol, Stratford, CT) onto a sector disc. A low-pass filter with a cutoff at 550 nm (Laser Components, Olching, Germany) was placed in front of the xenon arc lamp. The light passing through the disc was delivered to a fiber optics cable placed behind the disc. The light at the output of the cable was introduced into the illumination pathway of the fundus camera. Mean retinal irradiance of the stimulation was approximately 150 W/cm<sup>2</sup>.

To separate the flicker light spectrally from that used to illuminate the fundus and measure retinal  $D$ , an interference filter with a center wavelength of 577 nm and a bandwidth at half height of 10 nm (Laser Components) was placed in front of the light source of the fundus camera. This spectral window between 567 and 587 nm was chosen to optimize the contrast between blood vessels and the surrounding tissue. Retinal irradiance of this fundus illumination was approximately 220 W/cm<sup>2</sup>.

During stimulation, the subject perceived a blue-green/yellow flicker, because the light at the output of the sector disc alternated blue-green light and no light and the continuous fundus illumination appeared yellowish (567–587 nm). To increase the perception of the flicker, the 50° field of illumination of the fundus camera was reduced to 30° by placing a ring-shaped diaphragm in the retinal plane of the fundus camera that lies behind the ophthalmoscopic lens. This diaphragm was transparent to the flicker light but opaque to the fundus illumination light. As a result, the flicker light, which was delivered over a 50° field, was mostly perceived in the periphery of the field



**FIGURE 1.** Sample measurement of the diameter of an inferior temporal retinal artery made with the modified RVA system (Carl Zeiss, Jena, Germany) during flicker stimulation. The diameter was measured during 60 seconds of constant illumination, followed by 60 seconds of flicker stimulation, and another 60 seconds of constant illumination. AU, arbitrary units.

(30–50°). An interference filter that exactly matched the spectrum of the continuous fundus illumination and therefore was opaque to the flicker light was placed in front of the CCD.

### Data Analysis

The flicker-induced change in  $D$  was expressed as the percentage change from baseline (no flicker)  $D$ . The average over the last 30 seconds of  $D$  recordings during baseline was considered to be the baseline  $D$ . During flicker,  $D$  was averaged over periods of 10 seconds. The average over the last 20 seconds of flicker was used to calculate the percentage change in  $D$  relative to baseline  $D$ .

Correlations between flicker-induced  $D$  responses between the left and right eye and between the  $D$  response and baseline  $D$  were analyzed with the Pearson product-moment correlation. The statistical significance of the  $D$  responses was assessed with repeated-measures ANOVA and paired  $t$ -tests for post hoc analysis. A two-tailed  $P < 0.05$  was considered statistically significant.

### RESULTS

Repeated flicker stimulation at 8 Hz resulted in a mean flicker response in venous  $D$  of 2.1%. The coefficient of variation of the change in  $D$  for the five trials was 25%. A representative  $D$  response to flicker in a retinal artery is shown in Figure 1.

### $D$ Response in the Left and the Right Eye

In arteries, the  $D$  responses in the right and left eyes were not correlated at flicker frequencies between 4 and 64 Hz. In contrast, a significant correlation was found at 2 Hz ( $r = 0.99$ ,  $P = 0.006$ ). In retinal veins, however, the  $D$  responses in the right and left eyes were negatively correlated at 4 Hz ( $r = -0.92$ ,  $P = 0.002$ ) and 40 Hz ( $r = -0.87$ ,  $P = 0.03$ ). No correlation was found at the other frequencies.

### Influence of Flicker Frequencies on Flicker-Induced $D$ Response

Flicker stimulation significantly increased  $D$  in arteries and veins with each applied frequency (Table 1). Flicker at 64 Hz caused a significantly smaller  $D$  response in the retinal arteries than did the other frequencies ( $P = 0.0072$ ). In Figure 2 the frequency dependence of the  $D$  response in arteries is depicted in a double-logarithmic scale. In retinal veins, flicker stimuli at

**TABLE 1.** Retinal Arterial and Venous Diameters at Baseline and during Flicker

| Frequency (Hz)      | Baseline <i>D</i> | <i>D</i> during the Last 20 Seconds of Flicker | <i>P</i> |
|---------------------|-------------------|--|----------|
| <b>Arteries</b>     |                   |  |          |
| 2 ( <i>n</i> = 12)  | 118 ± 16          | 121 ± 16                                       | <0.0002  |
| 4 ( <i>n</i> = 17)  | 119 ± 17          | 124 ± 16                                       | <0.0001  |
| 8 ( <i>n</i> = 17)  | 120 ± 16          | 124 ± 16                                       | <0.00001 |
| 16 ( <i>n</i> = 17) | 119 ± 16          | 121 ± 17                                       | <0.0002  |
| 24 ( <i>n</i> = 17) | 120 ± 16          | 124 ± 17                                       | <0.0005  |
| 32 ( <i>n</i> = 17) | 121 ± 16          | 125 ± 15                                       | <0.0003  |
| 40 ( <i>n</i> = 14) | 117 ± 15          | 119 ± 15                                       | <0.003   |
| 64 ( <i>n</i> = 14) | 117 ± 14          | 118 ± 14                                       | <0.05    |
| <b>Veins</b>        |                   |  |          |
| 2 ( <i>n</i> = 12)  | 153 ± 19          | 154 ± 20                                       | NS       |
| 4 ( <i>n</i> = 17)  | 148 ± 19          | 151 ± 20                                       | <.0001   |
| 8 ( <i>n</i> = 17)  | 150 ± 21          | 153 ± 20                                       | <.00002  |
| 16 ( <i>n</i> = 17) | 149 ± 18          | 151 ± 19                                       | <.0002   |
| 24 ( <i>n</i> = 17) | 150 ± 20          | 151 ± 20                                       | <.03     |
| 32 ( <i>n</i> = 17) | 151 ± 19          | 153 ± 20                                       | <.003    |
| 40 ( <i>n</i> = 14) | 151 ± 20          | 153 ± 20                                       | <.01     |
| 64 ( <i>n</i> = 14) | 151 ± 18          | 151 ± 18                                       | NS       |

Data are expressed as mean AU ± SD.

all frequencies except 2 and 64 Hz significantly increased *D* (Table 1). The flicker-induced *D* increase was comparable between the other frequencies tested ( $P = 0.16$ ). *D* responses in retinal veins as a function of frequency are also shown in Figure 2.

### Time Course of *D* Changes during Flicker

The flicker-induced changes in arterial and venous *D* are presented for all applied frequencies in Figure 3. The change in *D* was always an increase, most of which occurring within the first 20 seconds. Thereafter, *D* remained almost stable during the following 40 seconds. The *D* response in veins was less pronounced than that in arteries ( $P < 0.001$ ). The time course in arterial *D* during flicker, however, was comparable to that in venous *D*.

### *D* Response after Cessation of Flicker

In most cases, a decrease in *D* was observed after cessation of flicker. A fit obtained from an individual *D*-versus-time curve is shown in Figure 4. This fit was based on an exponential decay with a linear component. *D* at baseline, during the last 20 seconds of flicker, and at the local minimum are indicated. In addition, the time span from the cessation of flicker to minimum *D* is shown. In 96 of 119 individual measurements in the arteries and in 69 of 119 individual measurements in the veins, a minimum *D* of the fit curves was identified. In the other cases, *D* returned to baseline, but no clear undershoot was seen. The minimum *D* after cessation of flicker was observed after 10 to 40 seconds.

### *D* Response and Fundus Location

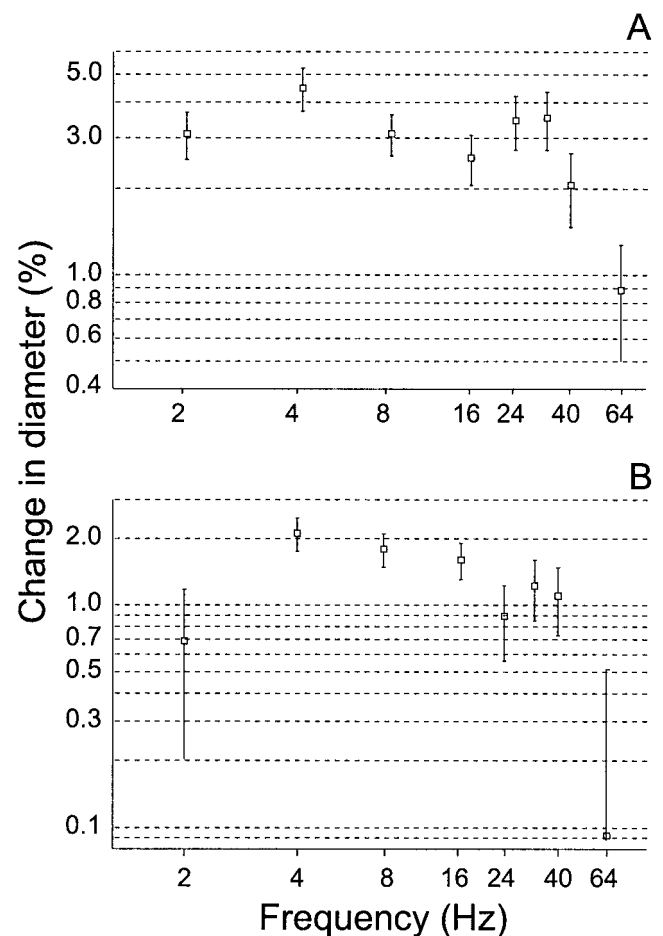
The effect of flicker stimulation at 8 and 16 Hz on *D* at different fundus locations is presented in Table 2. Again, the increase in *D* was more pronounced in retinal arteries than in retinal veins. In retinal arteries, the *D* responses in the trunk and the branch of the vessel were comparable (8 Hz:  $P = 0.96$ ; 16 Hz:  $P = 0.35$ ). In retinal veins, the response in the branches tended to be higher, but this effect was not significant (8 Hz:  $P = 0.15$ ; 16 Hz:  $P = 0.62$ ).

A negative correlation was observed between baseline *D* and the *D* response to 8-Hz flicker stimulation in the veins ( $r = -0.497$ ,  $P < 0.0001$ ) but not in the arteries ( $P = 0.4$ ), as shown

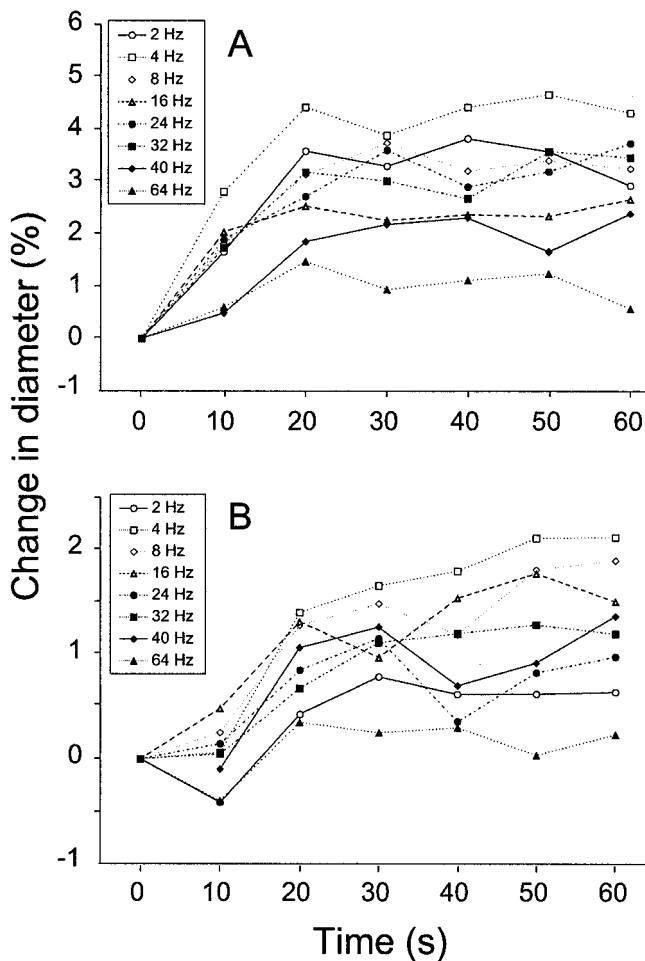
in Fig. 5. However, no correlation was observed between baseline *D* and the *D* response to 16-Hz flicker stimulation in arteries and veins.

## DISCUSSION

In the present study, a novel technique was used for the measurement of retinal vessel diameter during flicker stimulation. Several approaches have been tried to investigate the blood flow change in the retina during neuronal stimulation in humans. In one of these studies, the blue-field entoptic technique was used and an increase in retinal white blood cell flux in response to flicker stimulation was reported in healthy volunteers.<sup>7</sup> However, the flicker light prevents visibility of leukocytes, and therefore retinal macular blood flow cannot be quantified during stimulation. Another study measured *D* on fundus photographs taken immediately after flicker cessation to assess the change in *D* in response to flicker.<sup>8</sup> From these measurements, it was inferred that *D* increased during flicker. In contrast to this previous study, the present work describes measurements of *D* during flicker. The *D* response in arteries was more pronounced than that in veins, which indicates that the arterial bed plays an important role in the decrease in retinal vascular resistance during flicker. However, the *D* response was not dependent on baseline *D*. Although we do not have data on small arterioles (<40 μm) or capillaries, our results indicate that all retinal vessels showed an increase in



**FIGURE 2.** Mean (±SD) of the diameter of retinal vessels versus stimulus frequency. (A) Arteries; (B) veins. Number of vessels measured were: at 2 Hz: *n* = 12; at 4, 8, 16, and 32 Hz: *n* = 17; and at 40 and 64 Hz: *n* = 14.



**FIGURE 3.** Mean retinal arterial (A) and venous (B) diameter during 60 seconds of flicker stimulation. Number of vessels measured were: at 2 Hz:  $n = 12$ ; at 4, 8, 16, and 32 Hz:  $n = 17$ ; and at 40 and 64 Hz:  $n = 14$ .

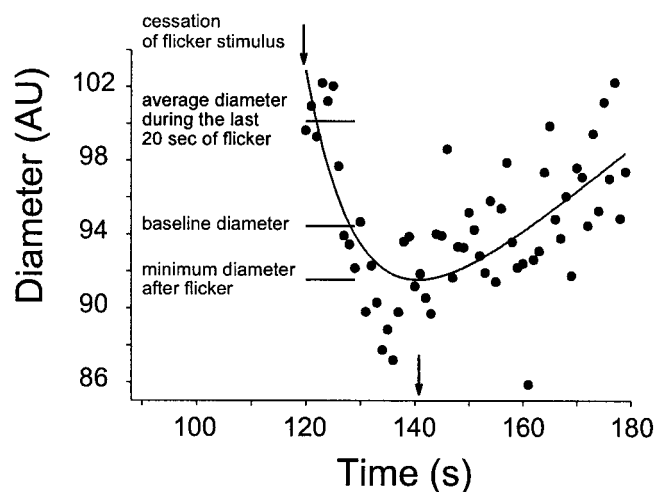
diameter during stimulation. If we assume that much of the retinal response to flicker occurs within the small arterioles, it may be expected that blood velocity in retinal arteries and veins increases considerably. Bidirectional laser Doppler velocimetry (BLDV) or Heidelberg Retinal Flowmeter-based laser Doppler flowmetry<sup>13,14</sup> could in principle show such an increase. However, BLDV requires excellent target fixation, which may be difficult to achieve during flicker stimulation, and the applicability of HRF to measure retinal blood flow changes during flicker stimulation remains to be explored. In contrast, the method based on the RVA is attractive because of its high reproducibility and its applicability in patients with ocular vascular disease. In the optic nerve head, measurements of flicker-induced blood flow changes were obtained with laser Doppler flowmetry,<sup>14,16</sup> but this technique is currently not applicable in the retina.

The general absence of correlation in the flicker-evoked  $D$  response between right and left eyes allowed us to treat the responses in each eye as statistically independent. The local variability of the  $D$  response may be caused by local regulatory mechanisms within retinal vessels, but may also depend on the specific angioarchitecture. However, a clear flicker-induced vasodilation was seen in all selected vessel segments, suggesting that, in clinical studies, the selection of the fundus selection of a segment is not very critical.

Two important characteristics of the RVA-based setup must be mentioned. First, because of the high light level required for the measurement of  $D$ , the contrast of the flicker stimulus was low. Second, because the fundus and the flicker illuminations were spectrally separated, the perceived flicker was a mixture of luminance and chromatic flicker. This last feature is important for the understanding of the frequency response of the flicker-evoked vasodilation because, as suggested by previous findings in cats and humans,<sup>16,17</sup> the  $D$  response is expected to depend, among other variables, on the luminance, wavelength, and modulation depth of the stimulus, as well as on the mean retinal illuminance and fundus location. In the present study, the  $D$  response versus flicker frequency displayed the characteristics of a band-pass function with a wide plateau between 4 and 40 Hz. From electrophysiological studies, it is well known that the sensitivity of the human visual system is at its maximum at frequencies between 10 and 20 Hz for luminance flicker, whereas the sensitivity to equiluminant chromatic modulation reaches its maximum at much lower frequencies ( $<5$  Hz).<sup>18-20</sup>

Thus, the similarity between these electrophysiological data and the behavior of the  $D$  response versus temporal frequency strongly supports a contribution from both luminance and chromaticity, in accordance with the specific illumination characteristics of our RVA-based setup. This suggests that both the parvo- and magnocellular pathways are stimulated. Although our method is not suitable for investigating either pure luminance or pure chromatic flicker-induced  $D$  responses, it nevertheless may provide a new tool in the study of vascular responses in glaucoma. Electrophysiological studies in patients with early glaucoma have demonstrated that this disease affects the functional response to both types of flicker stimulation,<sup>21</sup> suggesting that both the magnocellular and the parvocellular layers of ganglion cells are affected in this disease. Moreover, the characteristics of our stimuli are particularly relevant in the study of glaucoma, because computer perimetry<sup>22,23</sup> has revealed alteration of the blue-yellow system in the early stage of this disease.

An important novel finding of the present study is the time course of the changes in  $D$  during flicker stimulation. Approximately 20 seconds after the start of flicker stimulation, a stable response was normally seen. After cessation of flicker, there



**FIGURE 4.** Sample measurement of the decrease in retinal vessel diameter observed after the cessation of flicker in one subject (flicker frequency, 8 Hz). A fit based on an exponential decay plus a linear component was applied to the diameter data after cessation of flicker, and the minimum of this fit was identified. The preflicker and the minimum diameters are also shown. AU, arbitrary units.

TABLE 2. Response of Arterial and Venous Diameter to 8- and 16-Hz Flicker at Different Vessel Locations

| Location<br>(number vessels)       | % Change from<br>Baseline <i>D</i><br>at 8 Hz | <i>P</i>  | % Change from<br>Baseline <i>D</i><br>at 16 Hz | <i>P</i> |
|------------------------------------|---|-----------|--|----------|
| Arteries                           |   |           |  |          |
| Trunk proximal<br>( <i>n</i> = 17) | 3.5 ± 2.3                                     | <0.00005  | 2.7 ± 2.0                                      | <0.0001  |
| Trunk distal ( <i>n</i> = 17)      | 3.5 ± 2.4                                     | <0.00002  | 1.9 ± 1.5                                      | <0.0001  |
| Branch ( <i>n</i> = 17)            | 3.3 ± 2.4                                     | <0.00006  | 1.7 ± 2.7                                      | <0.02    |
| Veins                              |   |           |  |          |
| Trunk proximal<br>( <i>n</i> = 17) | 1.9 ± 1.2                                     | <0.000019 | 1.8 ± 1.1                                      | <0.00005 |
| Trunk distal ( <i>n</i> = 17)      | 1.8 ± 1.6                                     | <0.0003   | 1.1 ± 0.8                                      | <0.02    |
| Branch ( <i>n</i> = 17)            | 2.9 ± 2.2                                     | <0.0002   | 1.4 ± 2.8                                      | NS       |

was a rapid decrease in *D*, which reached baseline in approximately 6 seconds, in agreement with previous results.<sup>8</sup> However, our measurements also demonstrate that *D* continues to decrease below baseline, reaching a minimum at approximately 10 to 40 seconds after cessation of the stimulus. The reason for this undershoot deserves further investigation.

Based on photographs taken during the first 6 seconds after flicker stimulation, Formaz et al.<sup>8</sup> reported a mean change in *D*

of 4.2%. In view of the rapid decrease in *D* we are reporting in the current study, it is likely that this value underestimates the actual magnitude of the change in *D* during flicker. In the present study, the average *D* response measured during flicker was even smaller than 4.2%. This can be attributed to the relatively low flicker contrast perceived with the RVA system because, in the RVA method, the flicker stimulation is superposed on a bright-illumination light (the light needed to measure *D*), whereas in the method of Formaz et al. the contrast of the stimulus was almost equal to 1. However, although our RVA-based setup produced a smaller *D* response, it allowed the precise recording of the time course of this response, a feature that may be of interest in the investigation of various retinal vascular alterations.

The mechanism underlying the flicker-induced increase in retinal vessels *D* and, presumably, in retinal blood flow remains to be elucidated. A number of studies have identified factors involved in the modulation of vascular tone during increased neural activity. The putative role of various substances in the vasodilation induced by increased retinal activity, such as K<sup>+</sup> ions,<sup>24</sup> nitric oxide,<sup>5,25</sup> PO<sub>2</sub>, pH, Pco<sub>2</sub>, circulating hormones, and others<sup>4</sup> have been investigated, but other contributing factors cannot be ruled out. Further insight into the mechanisms underlying flicker-induced vasodilation may be obtained when the undershoot that was commonly observed after cessation of flicker is analyzed in more detail. For this purpose, however, longer observation periods after the cessation of flicker are necessary.

In the present investigation, a square-wave flicker induced the *D* response. The retinal circulation, however, responds also to sine-wave flicker, sequences of flashes (such as those generated by a Grass photic stimulator; Grass Instruments, Quincy, MA) and red-green chromatic flicker.<sup>17</sup> Further investigations will therefore attempt to optimize the *D* response by varying the parameters (mean luminance, contrast, frequency, and wavelength) of various types of stimuli.

In conclusion, a new method has been presented to record the flicker-induced retinal vessel diameter vasodilation and the dependence of this dilation on the flicker frequency for a mixed luminance-chromatic stimulus. It is suggested that the response of the diameter with frequency of the flicker is due to the activation of both the magno- and parvocellular layers of ganglion cells.

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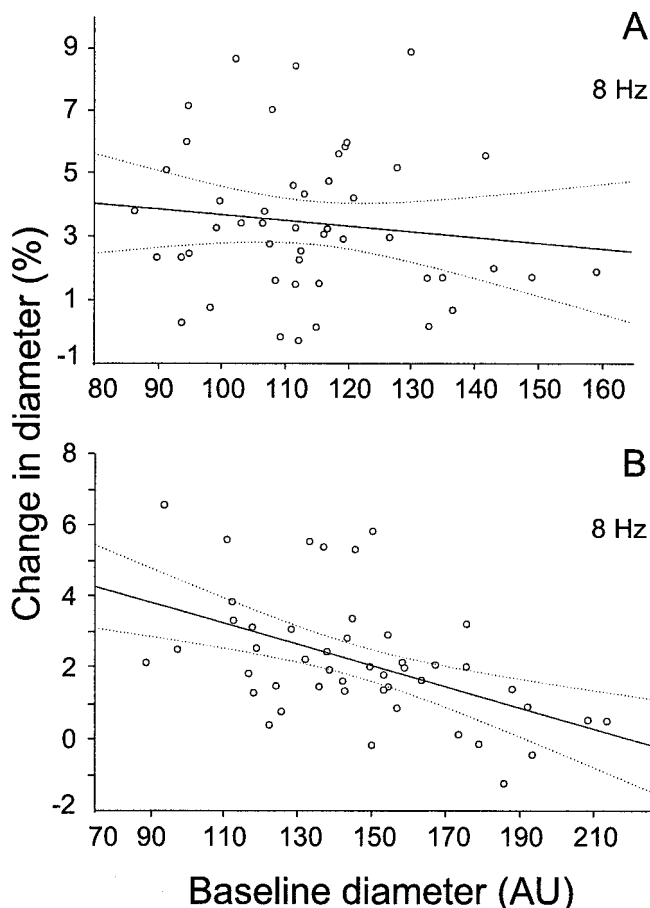


FIGURE 5. Correlation between baseline diameter of retinal arteries (A) and veins (B) and the flicker-induced change in this diameter. The *D* response was calculated based on the last 20 seconds of flicker stimulation. Solid line: correlation; dotted lines: 95% confidence interval. The 51 data points correspond to the responses obtained from three fundus locations in 17 eyes.

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