Effect of Flicker on Macular Blood Flow Assessed by the Blue Field Simulation Technique

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Purpose. To determine the effect of diffuse luminance flicker on the motion of leukocytes in the retinal macular capillaries of normal subjects.

Methods. Using the blue field simulation technique, subjects were asked to match the motion of simulated leukocytes displayed on a video monitor to that of their own entoptically seen white blood cells (WBCs). The changes in velocity and density of the WBCs were recorded after stimulation with diffuse luminance flicker of various durations (0 to 16 seconds), either immediately or at various delays (2, 4, 8 seconds) after cessation of the stimulus.

Results. White blood cell velocity increased as flicker duration increased from 0 to 16 seconds. After cessation of flicker, leukocyte motion decreased to baseline within 15 seconds.

Conclusions. The authors' findings suggest a coupling between retinal neural activity and blood flow in the macular region of the retina. The rapidity of both the flicker-induced increase in WBC motion and the disappearance of the effect after flicker cessation resembles the time course of blood flow changes previously observed in the microcirculation of the cat optic nerve. Invest Ophthalmol Vis Sci. 1994;35:3436-3441.

When asked to look into a blue field entoptoscope, an instrument that elicits the perception of the observer's own WBCs moving in retinal macular capillaries, nearly all subjects reported that these WBCs move more swiftly or in greater number for a few seconds after the blue light had been flickered for a short period of time. This qualitative observation was thought to be important because it suggests a possible coupling between local retinal function and blood flow in humans. The existence of such a coupling has been established for the brain where the vasomotor response to local activity occurs within seconds and allows for rapid changes in blood flow. Consequently, we have attempted to quantitate the relationship between flicker and blood flow in the macular region of the retina using the blue field simulation technique, a noninvasive technique that does not require injection of a dye to quantitate blood flow in the capillaries of this region. In this paper, we report on the effect of the duration of the diffuse luminance flicker on the motion of the WBCs and the motion of WBCs after cessation of the flicker stimulus.

MATERIALS AND METHODS

Blue Field Simulation Technique

The blue field entoptic phenomenon allows the observation of one's own WBCs passing through the macular capillaries. Under uniform retinal illumination with blue light at a wavelength $\lambda = 430$ nm, ($\Delta \lambda = 20$ nm at half-height), many tiny corpuscles are seen moving in random directions throughout a $25^\circ$ field of view. They repeatedly proceed along the same pathways defined by the inner retinal capillary loops. They feature a bright "head" and dark "tail" and move in a rhythmic fashion, synchronous with the heart cycle.

In the blue field simulation technique, the subject compares and matches the global motion of a $25^\circ$ field of computer-simulated particles (simulated leukocytes [SLs]) displayed on a color graphics video monitor connected to an Amiga 2000 computer (Commodore Business Machines, West Chester, PA) to the global motion of their own WBCs. This field is defined as...
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Subjects

Ten males and two females ranging in age from 17 to 53 years, each of whom had normal eye examination results, participated in the study. Before entering the actual study, subjects were asked to match two computer simulations of the blue field phenomenon to evaluate their ability to perform the matching task. All subjects had an error smaller than 20% in matching either the velocity or the density of the particles. Tenets of the Declaration of Helsinki were followed, informed consent was obtained from all subjects, and institutional human experimentation committee approval was granted.

Effect of Flicker Duration on WBC Motion

After the subjects had been in darkness for 10 minutes to insure identical initial conditions of adaptation, three matching trials, each consisting of the paradigm described schematically in Figure 1a, were performed. Subjects viewed with the right eye (OD) a diffusely illuminated field at a wavelength of 450 nm ($DIF_{450}$) of approximately 25° diameter, centered at the fovea, with a retinal irradiance of $4.9 \times 10^{-6}$ Watt/cm² for 10 seconds. This field was then flickered at 8 Hz for a duration ($T$) using a Uniblitz model 225L shutter and a model SD-10 driver (Uniblitz, Vincent Associates, Rochester, NY). This electromechanical system produces a flicker stimulus that is nearly a square wave (opening time = 3 msec, closing time = 4.2 msec). After the diffuse luminance flicker, subjects observed at the same irradiance a blue field diffusely illuminated at 430 nm ($DIF_{430}$) for less than 4 seconds and then were given 10 seconds to adjust the simulation seen with the left eye (OS). The whole sequence was repeated as many times as needed, without interruption between each sequence, until the subjects thought the motion of the SLs matched that of the WBCs. When this occurred, subjects pressed a button that ended the matching trial, and the V and D values were stored in the computer. From three such trials, average values of V, D, and $V \times D$ were calculated. In the first seven subjects studied, D was kept at the value adjusted by the subject at baseline (no flicker), whereas in the last five subjects measured, the protocol was modified to allow the adjustment of D throughout the experiment. Identical experiments were conducted with values of $T = 0, 1, 2, 4, 8, \text{ and } 16 \text{ seconds}$. The motion of the SLs in this field is defined by three variables: the number or density, D, seen on the monitor; the time-averaged velocity, V; and the pulsatility, P, defined as $1 - \frac{V_{dia}}{V_{sys}}$, where $V_{dia}$ and $V_{sys}$ are the diastolic and systolic particle velocities, respectively. The subject is asked to set these parameters by the method of adjustment so as to obtain the best match between the motion of the SLs in the test field and that of the WBCs. In this work, we kept P constant and equal to 0.5.

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subjects. Subjects followed the paradigm described above were conducted for the following four values of At: with OD a DIF450 for 10 seconds (irradiance 1.75 \times 10^{-6} \text{ Watt/cm}^2). This field was then flickered for 8 seconds approximately 2 seconds. After this observation, the subjects adjusted the motion of the SLs to obtain a match. The first statistically significant change occurred at 2 seconds (paired t-test, \( P < 0.05 \)), and the change in V increased with flicker duration. For the change in D (Table 1), statistical significance (paired t-test, \( P < 0.05 \)) was seen for 1-, 8-, and 16-second durations. For the quantity \( V \times D \) (Table 1), statistically significant changes (paired t-test, \( P < 0.05 \)) were seen at 1-, 4-, 8-, and 16-second durations.

In the control experiment, V, D, and \( V \times D \) of the SLs in the reference field during constant illumination were 0.77 ± 0.02 (SD), 197 ± 13, and 151 ± 10, respectively, and 0.78 ± 0.07, 202 ± 24, and 157 ± 20 after 8 seconds of 8-Hz flicker. These values after flicker were not statistically different (\( P > 0.05 \)) from the ones obtained during constant illumination.

Figures 3a and 3b display the change in V and D, and in \( V \times D \), respectively, obtained during a 2-second observation time at various delays after cessation of 8 Hz flicker. These changes are expressed relative to the value at constant luminance. At zero delay, the mean values of V and \( V \times D \) were significantly higher (paired t-test, \( P < 0.05 \)) than the corresponding values for no flicker. This was not the case, however, at 4 and 8 seconds. The mean value of D was not significantly different from the no flicker value at any time after cessation of flicker. A regression line through the mean values revealed a significant decrease in \( V \times D \) (correlation coefficient 0.995, \( P < 0.01 \)) with increasing delay times, but not in V or D alone. The control experiment did not show any significant change in any of the motion parameters.

Reproducibility of the flicker-induced change in V can be derived from measurements of V after 8 seconds of 8-Hz flicker in all groups of subjects. The average relative changes in V from the no flicker conditions were (mean ± 95% confidence interval of mean): 1.4 ± 0.22 (\( n = 12 \)), 1.33 ± 0.19 (\( n = 8 \)), and 1.5 ± 0.4 (\( n = 5 \)). These values, which correspond to

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\begin{array}{cccc}
\text{Duration} & \text{V (mm/sec)} & \text{D (number)} & \text{V \times D} \\
\text{(seconds)} & & & \text{(number \times mm/sec)} \\
\hline
\text{No flicker} & 0.60 ± 0.07 & 107 ± 22 & 67 ± 16 \\
1 & 0.73 ± 0.10 & 216 ± 32* & 156 ± 29* \\
2 & 0.71 ± 0.04* & 172 ± 39 & 120 ± 25 \\
4 & 0.90 ± 0.14* & 171 ± 40 & 150 ± 39* \\
8 & 0.90 ± 0.09* & 192 ± 39* & 171 ± 35* \\
16 & 1.01 ± 0.11* & 227 ± 43* & 226 ± 42* \\
\end{array}
\]

Values are mean ± SEM.

* Significantly different from the no flicker conditions, \( P < 0.05 \).
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FIGURE 3. (a) Velocity (V) and density (D) of WBCs measured after observing the motion of the WBCs for 2 seconds at various time delays, Δt, after 8-Hz flicker presented for 8 seconds. The values of V and D were expressed relative to the corresponding values at constant illumination (= 1). Error bars represent the standard error of the mean for n = 8. Closed circles represent the mean relative values for V. Open circles represent the mean relative values for D. The correlation coefficient for the linear regression line through the data was not significant. (b) Velocity × density (V × D) of WBCs measured after observing the WBCs for 2 seconds at various time delays, Δt, after 8-Hz flicker presented for 8 seconds. Error bars represent the standard error of the mean for n = 8. The value of 1 represents the mean of V × D for unflickered diffuse illumination. The solid lines are the linear regression and 95% confidence intervals of the regression. Correlation coefficient r = 0.995 (P < 0.01).

the groups of subjects in Figures 2 and 3a and Table 1, are not statistically different.

DISCUSSION

In this study, we used the blue field simulation technique to quantitate blood flow in the retinal macular capillaries. The obvious advantage of this technique is that it is applicable to humans without the need for intravenous injection of a dye. Because the WBCs cannot be observed during flicker of the type used in this study, we adopted the paradigms described above, where flicker was stopped intermittently to perceive the WBCs and to make a rapid judgment on their motion.

Changes in WBC motion were evaluated based on the changes in V and D, and also on V × D, a quantity that can be considered a measure of the flux of WBCs through the field of observation. To simplify the matching procedure, the pulsatility (P) was maintained at a constant value because it has little influence on the matching of V and D. Among the parameters mentioned above, it appears from Table 1 that V can be determined more precisely than D or V × D and is, therefore, a more sensitive indicator of changes in the motion of the leukocytes than the other two parameters.

The effect of flicker duration on the flow parameters, as well as the time course of the flow parameters upon cessation of the stimulus, were determined using a frequency of 8 Hz. The choice of this frequency was based on previous studies of flicker sensitivity determined from flicker fusion experiments, which showed high sensitivity of the human visual system at this frequency.

Determining whether the observed flicker-induced changes in the flow parameters represent real flow changes or are due to a psychophysical effect of motion perception causing a merely apparent change in flow is important. The control experiments designed to answer this question were identical to those related to the motion of the WBCs, except that, instead of measuring the motion of WBCs in a DIF430, a blue field of SLs moving at a known speed was used. The control experiments show that this speed was adjusted correctly, even after flicker. Therefore, assuming that psychophysical effects after flicker stimulation would affect the perception of WBCs and SLs equally, we rule out a psychophysical interpretation of the observed changes in the motion of the WBCs.

For blood flow to the macula to increase during flicker stimulation, a decrease in the resistance of the vascular system feeding this region is required. After the observation of Friedman et al., it has been generally assumed that the retinal capillaries have rigid walls. If this is so, the fact that changes in both V and D occurred during flicker suggests dilatation of the arteriole(s) feeding the macular region and an increase in the number of WBCs perfusing the capillaries. Whether the latter results from recruitment of capillaries or increased number of WBCs per unit time being diverted to the macular region cannot be answered by our technique.
Previous work in cats and miniature pigs has demonstrated that diffuse luminance flicker increases optic nerve head and retinal blood flow. Increases in blood flow of similar magnitudes were also recorded from the macular microcirculation of the primate with a technique based on laser-targeted delivery of fluorescein and, recently, from the optic nerve head microcirculation in a human volunteer by means of laser Doppler flowmetry. This effect has been attributed to increased activity in the retinal ganglion cells and associated axons. Our measurements also suggest a coupling between retinal activity in the human macular area and blood flow in this region of the fundus. Furthermore, the rapidity of both the flicker-induced increase in WBC motion, 90% of which occurred in less than 8 seconds, and the disappearance of the effect after stimulus cessation, also within approximately 15 seconds, closely corresponds to the rapidity of the blood flow changes previously reported for the cat optic nerve. There, the flicker-induced increase in blood flow in the optic nerve and its decay after cessation of flicker occur within similar time durations (see, for instance, Riva et al).

The coupling mechanism between neural function and blood flow in the retina or, in fact, in any other region of the nervous system remains to be elucidated. Numerous studies have been performed since the pioneering work of Roy and Sherrington in 1890 to identify the mediators of the vascular response. However, to this day our knowledge is highly speculative. Because an extensive discussion of possible mechanisms is beyond the scope of this paper, we will only briefly mention some of them.

Increased neuronal activity has been shown to induce the release of intracellular K+ into the extracellular space of the brain, retina, and optic nerve head tissues. This release could lead to an increase in blood flow in the macular microvasculature; K+ is known to be a potent vasodilator in such cases as, for example, the brain vasculature. On the other hand, a flicker-induced increase in metabolism, as reported by Sperber and Bill, could produce a decrease in tissue pO2, which in turn could trigger a flow increase. Such pO2 changes have been previously demonstrated in the optic nerve of the cat. The recent discovery that nitric oxide, a powerful vasodilator, is produced by active neurons has made this agent a strong candidate as the mediator of the coupling between activity and blood flow. Nitric oxide has been shown, for example, to play a role in the coupling of cerebral blood flow to neuronal function induced by stimulation of vibrissae in rats. The possibility that neurotransmitters or neuropeptides participate in the regulation of inner retinal blood flow during neural activity should also be considered because a number of these substances are released in the inner retina during the visual process and have the potential to act as vasodilators. However, studies in the brain suggest that these intrinsic pathways may have only modulatory influence and perhaps do not represent the main mechanism mediating the changes in blood flow associated with neural activity.

This study represents a first attempt to characterize the flicker-induced macular blood flow increase in humans. Further studies aimed at determining the effect of various flicker stimulus parameters, such as frequency and wavelength, on the blood flow response are needed to describe fully the normal response and to establish a basis for investigations in patients with various diseases affecting the retinal macular circulation.

**Key Words**
- flicker, macular blood flow, leukocyte velocity, blue field phenomenon, blue field simulation, retinal activity

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**References**


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