Influence of diffuse luminance flicker on choroidal and optic nerve head blood flow

G. Garhöfer[1,3], K.H. Huemer[1,3], C. Zawinka[1], L. Schmetterer[1,4] and G.T. Dorner[1,2]

Departments of [1]Clinical Pharmacology, [2]Ophthalmology, and [3]Physiology, University of Vienna, Austria; [4]Institute of Medical Physics, University of Vienna, Austria

Abstract

Purpose. In the retina there is general agreement that blood flow adapts in response to different conditions of light and darkness including diffuse luminance flicker. By contrast, regulation of choroidal blood flow in response to different light conditions is still a matter of controversy. Thus, we investigated the effect of diffuse luminance flicker on choroidal and optic nerve head blood flow.

Methods. In a group of 14 healthy volunteers, choroidal blood flow and ocular fundus pulsation amplitude were assessed with laser Doppler flowmetry and laser interferometry, respectively. Measurements were done before, during and after stimulation with diffuse luminance flicker. Furthermore, the response of optic nerve head blood flow (ONHBF) to flicker stimulation was measured. Flicker stimuli were generated by a Grass® PS2 photostimulator, stimulating at a frequency of 8 Hz. Flicker light consisted of light flashes at a wavelength below 550 nm and produced a retinal irradiance of 140 µW/cm². Blood pressure and pulse rate were measured non-invasively. Paired t-test was used for statistical analysis.

Results. ONHBF increased immediately after onset of flicker stimulation. The maximum increase in ONHBF was 30% ± 10% (mean ± SEM, $p < 0.008$). Both choroidal perfusion parameters were only slightly increased during flicker stimulation, by 2 ± 2% (laser Doppler flowmetry, $p < 0.5$) and by 4 ± 1% (laser interferometry, $p < 0.12$). After the end of stimulation all values returned to baseline levels.

Conclusion. Our study clearly demonstrates that diffuse luminance flicker increases optic nerve head blood flow. In contrast, increased neural activity in the retina has no effect on choroidal blood flow. Thus, choroidal blood flow appears to be largely independent of alterations in retinal metabolism.

Keywords: choroidal blood flow; flicker stimulation; human; optic nerve head blood flow

Introduction

Characterized by a high level of flow and a low level of oxygen extraction, choroidal blood flow differs substantially from that in the retina. In the retina there is general agreement, that retinal tissue has the ability to regulate its blood flow in response to different metabolic demands. This has been confirmed in several animal[1–5] and human studies[6,7] which used diffuse luminance flicker to induce an increase in retinal and optic nerve head blood flow (ONHBF). This increase has been mainly attributed to augmented ganglion cell activity,[2] indicating that in the retina, like in the brain, a coupling mechanism exists between neural activity and blood flow.

In the choroid, blood flow regulation in response to light is still a matter of controversy. It has been hypothesized that the choroid adapts its perfusion to the light level with which the fundus is illuminated to control the retinal temperature.[8] Other investigators, however, failed to detect an effect of light exposure on choroidal blood flow.[9,10] Recent findings[11,12] suggest that the transition from room-light to darkness leads to a reversible decrease in choroidal blood flow due to an unknown, probably neural mechanism.[12]

This study aimed to investigate the effect of diffuse luminance flicker on choroidal blood flow. We set out to investi-

gate this response with two different methods for the assessment of choroidal perfusion.

Materials and methods

Subjects

14 healthy subjects with excellent target fixation participated in the study. Informed consent was obtained from all subjects after the aim of the study and the procedures were fully explained. All subjects were non-smokers and underwent an ophthalmic examination, including slit lamp biomicroscopy and indirect funduscopy. All subjects included had no history of ocular disease, ametropia less then 3 diopters and did not take any medication. All procedures followed the tenets of the Declaration of Helsinki.

Laser Doppler flowmetry

Choroidal blood flow (CHBF) and ONHBF were assessed with a fundus camera based laser Doppler flowmeter (Oculix 4000, Oculix Sarl, Arbas, Switzerland) introduced by Riva *et al.*[13,14] The principles of laser Doppler flowmetry have been described in detail elsewhere.[15] Briefly, the vascularized tissue is illuminated by coherent laser light. Scattering on moving blood cells (RBCs) leads to a frequency shift in the scattered light. In contrast, static scattering in tissue does not change light frequency but leads to a randomization of light directions impinging on the RBCs and consequently a broadening of the spectrum of scattered light (Doppler shift power spectrum, DSPS). From the DSPS the mean RBC velocity, the blood volume and the blood flow can be calculated in relative units. In the present study, a fundus camera based system was used. The laser beam was directed to the fovea to assess blood flow in the submacular fovea. Blood flow in the optic nerve head was measured at temporal sites of the neuroretinal rim. Care was taken to ensure that the measurement location on the optic nerve head did not include visible vessels.

Fundus pulsation technique

Ocular fundus pulsation was assessed by laser interferometry as described in detail by Schmetterer *et al.*[16] This fundus camera based system illuminates the eye by a single beam laser diode (= 783 nm) along the optical axis. The light is reflected at both the front surface of the cornea and the retina. The two re-emitted waves produce interference fringes from which the distance changes between the cornea and the retina during cardiac cycle can be calculated. These distance changes are caused by the pulsatile inflow of blood in the arteries and by the nonpulsatile outflow through the veins. The maximum change in corneo-retinal distance is called fundus pulsation amplitude (FPA). The method has been shown to estimate the pulsatile blood flow in the choroidal vasculature.[17]

Flicker stimuli

For flicker stimulation a Grass® PS-2 Photo stimulator was used, stimulating with light flashes at a frequency of 8 Hz. Flicker light consisted of short light flashes with a duration of approximately 30 µsec and a modulation depth of 100%. Using a 550 nm low-pass cut-off filter, flicker light consisted of wavelengths below 550 nm and produced a retinal irradiance of approximately 140 µW/cm². Flicker stimuli were delivered to the eye through the illumination pathways of the fundus cameras of the laser interferometer and the laser Doppler flowmeter, respectively. The flicker was centered in the macula with an angle of approximately 30 degrees.

Experimental paradigm

The study was performed on two different study days. On one occasion laser-Doppler measurements were performed in the submacular choroid and the optic nerve head, on the second occasion ocular fundus pulsation amplitude in the macula was measured with laser interferometry. All measurements were done in the right eye according to the following time schedule:

Day 1: After a short resting period to obtain stable haemodynamic conditions, 60 seconds of baseline measurements were made on the subfoveal macular region. This was followed by 60 seconds of measurement with flicker light stimulation and again 60 seconds without flicker. Thereafter, the same procedure was carried out for the optic nerve head blood flow measurement.

Day 2: On the second study day, stable haemodynamic conditions were again ensured by an appropriate resting period. Then, measurements with the laser interferometer were started and two readings, each lasting for approximately 5 seconds were done. Thereafter flicker stimulation was applied for 60 seconds. During the last 30 seconds of flicker stimulation, 2 measurements with the laser interferometer were performed, each lasting approximately 5 seconds.

Blood pressure measurement

Systolic, diastolic and mean blood pressures were measured on the upper arm by an automated oscillometric device (HP-CMS patient monitor, Hewlett Packard, Palo Alto, Calif., USA). Pulse rate was automatically recorded from a finger pulse-oxymetric device. Measurements were performed before, during and after flicker stimulation.

Statistics

Changes in the ocular haemodynamic parameters were expressed as percent change over baseline values. Data are presented as means ± SEM. For laser Doppler flowmetry, baseline values were calculated as an average of the last 30 seconds of baseline measurement. The blood flow during flicker was calculated as an average of the last 30 seconds of

light stimulation. For laser interferometry, measurements before flicker and during the last 30 seconds of the flicker period were compared. Paired t-tests were used to calculate the p-values of flicker-induced haemodynamic changes. A $p < 0.05$ was considered as the level of significance.

Results

7 male and 7 female subjects were included in the study. Baseline mean arterial blood pressure and baseline pulse rate values were all in normal range on both study days. Values of blood pressure and pulse rate on both study days are shown in Table 1. No statistical difference in blood pressure and pulse rate was found between the two study days ($p < 0.1$).

ONHBF increased immediately after onset of flicker stimulation. A typical recording of an individual measurement is shown in Figure 1. As shown in Figure 2, the mean increase was $30\% \pm 10\%$ (mean $\pm$ SEM, $p < 0.008$) compared to baseline level. This increase in optic nerve head blood flow was mainly attributed to an increase in optic nerve head blood volume, which increased significantly ($+20\% \pm 7\%$, $p < 0.02$). The change in optic nerve head blood velocity ($+9\% \pm 5\%$, $p < 0.4$) was not significant. After flicker stimulation all blood flow parameters returned to baseline levels. Blood flow in the submacular fovea showed a very small and non-

significant increase of $2 \pm 2\%$ ($p < 0.5$) during stimulation with diffuse luminance flicker. Neither choroidal blood velocity ($+1\% \pm 1\%$) nor choroidal blood volume ($+1\% \pm 2\%$) changed significantly during stimulation with diffuse luminance flicker. Laser interferometric measurements revealed a small and non-significant increase of FPA of $4 \pm 1\%$ during flicker stimulation ($p < 0.12$).

No statistically significant difference was found between male and female subjects regarding flicker responses in ONH or choroid.

Discussion

Using two different methods, the present study indicates that diffuse luminance flicker has no significant effect on choroidal blood flow. Furthermore, we showed that flicker induces an increase in optic nerve head blood flow as evidenced from measurements using laser Doppler flowmetry. The latter result is in keeping with several human[13,18] and animal[2,4] experiments.

In the retina, the coupling between neural activity and retinal blood flow has been attributed to augmented activity in the retinal ganglion cells and associated axons.[2] Although the exact mechanism is not completely clear, the predominant view is now that active neurons liberate vasodilator products which diffuse to the blood vessels, producing a

Table 1. Mean arterial blood pressure (MAP) and pulse rate (beats/min) during the experiments.

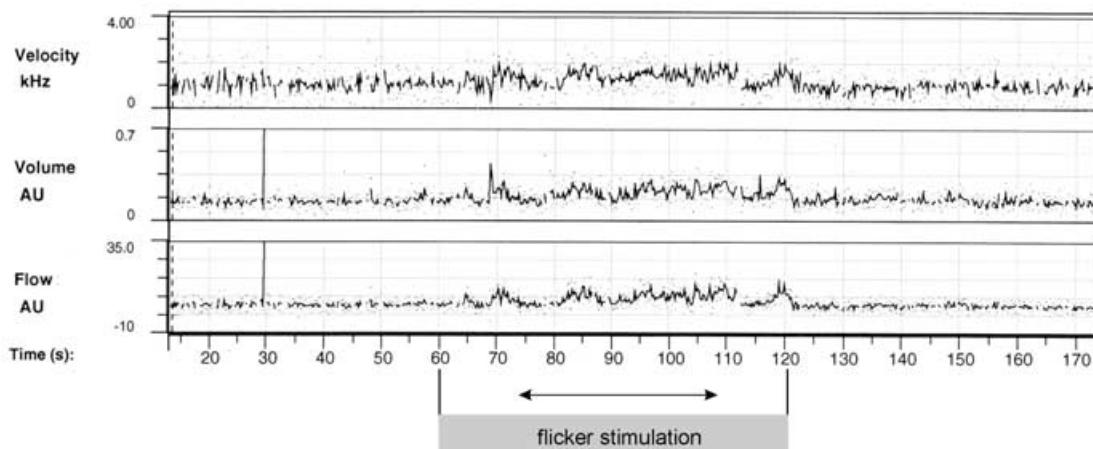|	Day 1			Day 2		
	Before flicker	During flicker	After flicker	Before flicker	During flicker	After flicker
MAP (mmHg)	86 ± 8	83 ± 10	84 ± 6	76 ± 7	79 ± 8	78 ± 10
Pulse rate	72 ± 8	74 ± 10	70 ± 8	65 ± 15	64 ± 9	67 ± 11

Figure 1. Time course of velocity, volume and flow of a representative individual measured at the optic nerve head during flicker stimulation assessed with laser Doppler flowmetry.
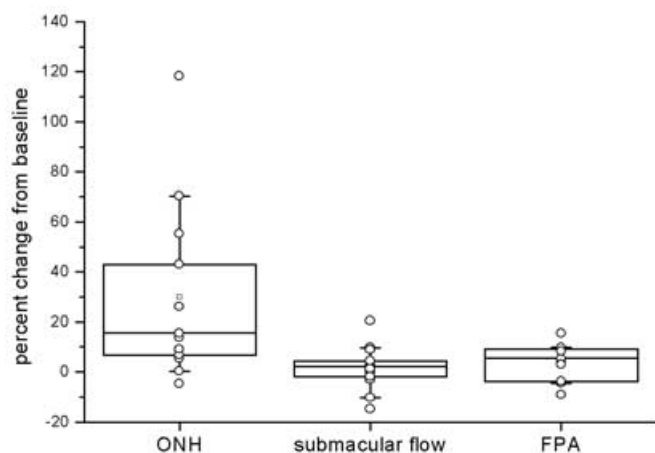
Figure 2. Percent change of blood flow during flicker stimulation. Data are presented for the optic nerve head (ONH) and the submacular choroid as assessed with laser Doppler flowmetry and for fundus pulsation amplitude (FPA) assessed with laser interferometry. The horizontal lines in the box denote the 25th percentile, the mean and 75th percentile values. The error bars indicate the 5th and 95th percentile values. The square symbol in the box denotes the mean. White circles show values of individual measurements ($n = 14$).

relaxation of the vessel wall with a subsequent increase in blood flow.[2] It has been hypothesized that this augmented blood flow is necessary to remove excess potassium and metabolites that accumulate in extracellular space as a result of axonal firing.[3] The exact mechanism of the coupling between increased neural activity and increased blood flow remains, however, to be clarified. Alternatively, based on the findings that glucose consumption of the inner retina is increased during stimulation with flickering light,[19] it has been hypothesized that increased local energy metabolism leads to an increase in blood flow.

The data of our present study indicate that choroidal blood flow is not altered during flicker stimulation. Furthermore, the very high blood flow rate in the choroid may be sufficient to satisfy a potentially increased metabolic demand of the outer retina. However, recent work based on a mathematical model suggests that choroidal blood flow is not superfluous but necessary to ensure proper oxygenation of the distal retina and the photoreceptors.[20] In view of the fact that during flicker stimulation more light reaches the photoreceptors, one could expect a lower oxygen consumption and consequently a change in choroidal perfusion rate. This would of course require choroidal blood flow to be sensitive to changes in pO_2 in the outer retina. Measurements in humans, however, could not confirm this hypothesis.[21]

A limitation of the current study is, however, that laser Doppler flowmetry in the macula as well as FPA only provide information about the submacular region of the choroid. Furthermore, one has to consider that the high concentration of cones in the macula region may differentiate the metabolic needs of the macular from the rest of the retina. Thus, we cannot exclude the possibility that extrafoveolar regions of the choroid may react differently to flicker light stimulation. This issue remains to be clarified.

Recent findings of Longo *et al.*[11] suggest that a transition from room-light to darkness leads to a decrease in choroidal blood flow. This has also been confirmed by a study from our group providing evidence that this effect is due to an unknown neural mechanism.[12] Whereas the latter studies describe an effect that occurs after a single transition from room light to darkness, flicker stimulus consists of repeated transitions from light to darkness. Furthermore, the mean luminance during flicker stimulation stays constant. This indicates that a different mechanism may be responsible for the observed decrease in choroidal blood flow after a transition from light to dark. Our data, however, indicate that in contrast to a transition from light to darkness diffuse luminance flicker does not change choroidal blood flow. This again supports the hypothesis that the effect of light/dark transition is related to a neural rather than a metabolic process.

According to our data, changes in optic nerve head blood flow evoked by flicker light stimulation can be attributed mainly to increased blood volume rather than blood velocity. This indicates that increased blood flow is mainly due to local vasodilatation at the optic nerve head microvasculature. Interestingly, there was a wide variety of responses in the optic nerve head. Whether this is related to inter-individual differences in the flicker response or to local variations remains to be established. The lack of choroidal responses to diffuse luminance flicker was observed with two independent methods assessing choroidal blood flow. This is an important issue, because both methods have considerable limitations: With laser interferometric measurement of fundus pulsation, one has to consider that only the pulsatile component of blood flow can be measured. For laser Doppler flowmetry, the depth from which the signal arises is still a matter of controversy.[22,23] This is especially true for measurements in optic nerve head. A recent study in monkeys[22] demonstrates that after posterior ciliary artery occlusion, blood flow measured in the optic nerve head does not change. This has been attributed by the authors to the limited sampling depth of the laser Doppler system. Consequently, they concluded that the standard laser Doppler flowmetry technique is predominantly sensitive to blood flow changes of the superficial layer in the optic nerve head. Whether this interpretation is correct or not has yet to be confirmed.

In conclusion, this study indicates that choroidal blood flow is not influenced by increased neural activity in the retina. Thus, choroidal blood flow appears to be largely independent of alterations in retinal metabolism.

Acknowledgement

References

1. Bill A, Sperber G. Aspects of oxygen and glucose consumption in the retina: Effects of high intraocular pressure and light. *Graefes Arch Clin Exp Opththalmol.* 1990;228:124–127.

2. Riva CE, Harino S, Shonat RD, Petrig BL. Flicker evoked increase in optic nerve head blood flow in anesthetized cats. *Neurosci Lett.* 1991;128:291–296.

3. Buerk DG, Riva CE, Cranstoun SD. Frequency and luminance-dependent blood flow and K+ ion changes during flicker stimuli in cat optic nerve head. *Invest Ophthalmol Vis Sci.* 1995;36:2216–2227.

4. Buerk DG, Riva CE, Cranstoun SD. Nitric oxide has a vasodilatory role in cat optic nerve head during flicker stimuli. *Microvasc Res.* 1996;52:13–26.

5. Kondo M, Wang L, Bill A. The role of nitric oxide in hyperaemic response to flicker in the retina and optic nerve in cats. *Acta Ophthalmol Scand.* 1997;75:232–235.

6. Formaz F, Riva CE, Geiser M. Diffuse luminance flicker increases retinal vessel diameter in humans. *Curr Eye Res.* 1997;16:1252–1257.

7. Scheiner AJ, Riva C, Kazahaya K, Petrig BL. Effect of flicker on macular blood flow assessed by the blue field simulation technique. *Invest Ophthalmol Vis Sci.* 1994;35:3436–3441.

8. Parver LM, Auker C, Carpenter DO. Choroidal blood flow as a heat dispatching mechanism in the choroid. *American Journal of Ophthalmology.* 1980;89:641–646.

9. Bill A, Sperber GO. Control of retinal blood flow. *Eye.* 1990;4:319–325.

10. Morimoto N. Study on choroidal blood flow at dark and light adaption: II. Choroidal blood flow at light adaptation. *Nippon Ganka Gakkai Zasshi.* 1991;95:235–240.

11. Longo A, Geiser M, Riva CE. Subfoveal choroidal blood flow in response to light-dark exposure. *Invest Ophthalmol Vis Sci.* 2000;41:2678–2683.

12. Fuchsjäger-Mayrl G, Polska E, Malec M, Schmetterer L. Unilateral light-dark transitions affect choroidal blood flow in both eyes. *Vision Res.* 2001;41:2919–2924.

13. Riva CE, Harino S, Petrig BL, Shonat RD. Laser Doppler flowmetry in the optic nerve. *Exp Eye Res.* 1992;55:499–506.

14. Riva CE, Cranstoun SD, Grunwald JE, Petrig BL. Choroidal blood flow in the foveal region of the human ocular fundus. *Invest Ophthalmol Vis Sci.* 1994;35:4273–4281.

15. Bonner R, Nossal R. Principles of laser-Doppler flowmetry. In: Shepard AP, Öberg PA, eds. *Developments in Cardiovascular Medicine: Laser Doppler Blood Flowmetry.* Boston: Kluwer Academic Publishers; 1990:17–45.

16. Schmetterer L, Lexer F, Unfried C, Sattmann H, Fercher AF. Topical measurement of fundus pulsations. *Opt Eng.* 1995;34:711–716.

17. Schmetterer L, Dallinger S, Findl O, Eichler HG, Wolzt M. A comparison between laser interferomteric measurement of fundus pulsation and pneumotonometric measurement of pulsatile ocular blood flow: I. Baseline considerations. *Eye.* 2000;14:39–45.

18. Riva CE, Falsini B, Logean E. Flicker-evoked response of human optic nerve head blood flow: Luminance versus chromatic modulation. *Invest Ophthalmol Vis Sci.* 2001;42:756–762.

19. Wang L, Bill A. Effects of constant and flickering light on retinal metabolism in rabbits. *Acta Ophthalmol Scand.* 1997;75:227–231.

20. Linsenmeier RA, Padnick-Silver L. Metabolic dependence of photoreceptors on the choroid in the normal and detached retina. *Invest Ophthalmol Vis Sci.* 2000;41:2678–2683.

21. Geiser MH, Riva CE, Dorner GT, Diermann U, Luksch A, Schmetterer L. Response of choroidal blood flow in the foveal region to hyperoxia and hyperoxia-hypercapnia. *Curr Eye Res.* 2000;21:669–676.

22. Petrig BL, Riva CE, Hayreh SS. Laser Doppler flowmetry and optic nerve head blood flow. *Am J Ophthalmol.* 1999;127:413–425.

23. Luksch A, Polak K, Beier C, Polska E, Wolzt M, Dorner GT, Eichler HG, Schmetterer L. Effect of systemic NO synthase inhibition on choroidal and optic nerve head blood flow in healthy subjects. *Invest Opththalmol Vis Sci.* 2000;41:3080–3084.