Flicker-Evoked Responses of Human Optic Nerve Head Blood Flow: Luminance versus Chromatic Modulation

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Purpose. To determine the response of human optic nerve head blood flow ($\mathbf{RF}_{onh}$) to heterochromatic equiluminant flicker modulation and compare it to the response induced by pure luminance flicker.

Methods. In five normal volunteers, F_{onh} measured at the neuroretinal rim was monitored continuously by laser Doppler flowmetry. Stimuli were generated by green and red light emitting diodes and delivered to the fundus in Maxwellian view (field of 25°). Both green (*G*) and red (*R*) illuminances were square-wave modulated, 180° out of phase, with a maximum value of 10.4 for *G* and 2.64 lux for *R*. Flicker frequency was varied from 2 Hz to 40 Hz. $\mathbf{RF}_{onh}$ was defined as the change in F_{onh} during stimulation relative to the prestimulus F_{onh}.

Results. Defining the color ratio *r* as $R/(R + G)$, the $\mathbf{RF}_{onh}$, measured for a 15-Hz flicker, was largest at pure luminance ($r = 0$ and 1), declined at mixed luminance and chromatic modulations, and reached a secondary maximum at $r = 0.45$, the value of psychophysical equiluminance. $\mathbf{RF}_{onh}$ versus flicker frequency displayed the characteristics of a low-pass function for the equiluminance flicker stimulus and of a band-pass function, with a maximum at intermediate frequencies, for the luminance flicker stimulus.

Conclusions. $\mathbf{RF}_{onh}$ in humans can be evoked by heterochromatic flicker, modulated either in luminance or chromatic equiluminant conditions. $\mathbf{RF}_{onh}$ may be specific for luminance and chromatic modulations, similar to neural responses dominated by the magno- and parvocellular activity, respectively. These findings offer a new approach to study the neurovascular coupling at the optic nerve head in both physiological and diseased conditions involving predominantly or selectively the magno- and parvocellular pathways. (*Invest Ophthalmol Vis Sci.* 2001;42:756–762)

Laser Doppler Flowmetry (LDF) can measure optic nerve head blood flow (F_{onh}) and its change ($\mathbf{RF}_{onh}$) in response to diffuse luminance flicker.[1,2] In cats, $\mathbf{RF}_{onh}$ appears to be tightly coupled with the change in optic nerve head neural activity. Evidence for this neurovascular coupling is the similarity between $\mathbf{RF}_{onh}$ and neural activity change when the parameters of the flicker stimulation, such as the intensity, wavelength, frequency, and modulation, are varied.[3,4] Although few in numbers, various studies also suggest that a neurovascular coupling exists at the retina and ONH in monkeys and humans.[5,6]

The primate visual pathway is characterized by two main cellular systems. The parvocellular system consists of tonic, wavelength-opponent retinal ganglion cells projecting to the parvocellular layers of the lateral geniculate nucleus. The majority of neurons belonging to this system receive antagonistic input from medium-wavelength–sensitive and long-wavelength–sensitive cones.[7] The magnocellular system is made up of phasic, nonopponent ganglion cells that project to the magnocellular layers of the lateral geniculate nucleus. Magnocellular retinal ganglion cells receive combined input from medium- and long-wavelength–sensitive cones at both center and surround. Uniform field stimuli, whose luminance is varied periodically over time (i.e., the luminance flicker modulation) at relatively high temporal frequencies, are known to be optimal, though not specific, for evoking the response of the magnocellular neurons.[7] On the other hand, low-temporal frequency, counter-phase modulation of red and green fields, whose luminance has been matched by heterochromatic flicker photometry (i.e., the equiluminant chromatic flicker modulation), represents a strong stimulus for the parvocellular neurons, eliciting predominantly their activity.[7,8] At both retinal and postretinal levels, sensitivity of the human visual system to luminance modulation is maximal at intermediate temporal frequencies (10 Hz to 20 Hz) and falls off at both low and high frequencies, whereas sensitivity to equiluminant chromatic modulation is maximal at low temporal frequencies (<5 Hz) and falls-off at frequencies greater than 5 Hz to 10 Hz.[7,9–11]

It is presently unknown whether an $\mathbf{RF}_{onh}$ can be evoked by chromatic, whole-field flicker modulation. The occurrence of such a response would imply that vascular changes in the ONH are associated, under specific experimental conditions, with the neural activity dominated by the parvocellular system. The aim of the present study was, therefore, to evaluate whether: an $\mathbf{RF}_{onh}$ can be evoked in normal humans by red–green equiluminance flicker modulation and, if so, whether the temporal frequency dependence of $\mathbf{RF}_{onh}$ evoked by this stimulus differs from that elicited by luminance flicker of either pure *R* or *G* luminance modulation.

Methods

Subjects

Five normal observers (1 female and 4 males, ages 28 to 61 years) participated in the study. They all had excellent target fixation and had previously taken part in several LDF studies. They had normal general and eye examinations, including color vision tested with the Farsnworth 100 Hue (Luneau Ophtalmologie, Paris, France). Informed consent was obtained from all subjects after the aim of the study and the procedures were fully explained. The research followed the tenets of the Declaration of Helsinki.

LDF Measurements

ONH blood flow was measured using the Topcon TRC-based near-infrared laser Doppler system (Topcon, Inc., Tokyo, Japan) described previously.[1,12] Briefly, a probing laser beam (wavelength, 810 nm; power at the cornea, 90 μW; diameter at the fundus, ~150 μm) was directed at the ONH tissue at temporal sites on the neuroretinal rim.

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The laser light scattered by the ONH was collected by an optical fiber at the image plane of the fundus camera and guided to a photograph-detector. The aperture of the light-collecting optical fiber (diameter at the fundus approximately 180 μm) was centered on the site illuminated by the probing laser beam.

An infrared video camera placed in the retinal image plane of the fundus camera allowed the operator to monitor the location of the beam and of the light-collecting aperture at the disc and, when necessary, reposition both at the desired site. The camera was positioned in front of the eye so that an edge of the pupil of the tested eye could also be observed on the video monitor. In this manner, the observer could monitor the position of the camera relative to the subject's eye pupil and perform the necessary corrections to keep it steady. Guided by color photographs of the disc, care was taken to avoid recording from large vessels. The output signal of the detector was analyzed using a software implemented on a NeXT computer.[13] It was monitored continuously in real time throughout the experiment. F_{onh}, in the volume sampled by the light collecting fiber, was obtained from the relationship $F_{onh} = k \times Vel_{onh} \times Vol_{onh}$, where k is an instrumental constant, Vel_{onh} is the first moment frequency of the Doppler shift spectrum, which is proportional to the mean velocity of the red blood cells, and Vol_{onh} is the relative number of red blood cells.[2]

Flicker Stimuli

Stimuli were generated by two independently controlled ultrabright green and red light emitting diodes (Sloan, Precision Optoelectronics, Model SL905GCU-15 and SL905OCU-16), with dominant wavelengths at 524 and 630 nm, respectively. They were delivered to the eye through the fundus illumination's optical system of the Topcon camera and uniformly illuminated in Maxwellian view, a 25°-diameter area of the posterior pole. The currents of the diodes were square-wave modulated, 180° out of phase, between zero and a maximum illuminance of 10.4 lux for green and 2.64 lux for red. Temporal frequency of this modulation ranged from 2 to 40 Hz. The color ratio defined as[14] $r = R/(R + G)$, where R and G represent the illuminance of the red and green stimuli, was varied from 0 to 1. $r = 0$ corresponds to a G–Black and $r = 1$ to a R–Black modulation. Intermediate values of r defined a R–G chromatic modulation. CIE coordinates were $x = 0.7$, $y = 0.3$ for R and $x = 0.17$, $y = 0.7$ for G. The excitation of each cone type (i.e., the medium- and long-wavelength–sensitive cones, the short-wavelength cone excitation being negligible) was estimated by multiplying each LED spectral component with the psychophysically based cone fundamentals[15] and integrating over the wavelength range. Data showed that under the prior experimental conditions, long- and medium-wavelength–sensitive cones do not modulate at $r = 0.34$ and $r = 0.72$, respectively, the r values correspond to silent substitution.[16] For $r = 0.46$, the responses of long- and medium-wavelength–sensitive cones were equal and opposite. At this r value, cone modulation, expressed as Michelson luminance modulation depth (see definition later), was 0.35 for the long- and 0.43 for the medium-wavelength–sensitive cones.

Experimental Protocols

In each subject, trial recordings of the LDF parameters were performed for various locations of the probing laser beam in the temporal region of the optic disc to obtain the largest response possible. These consisted of 30 seconds of constant maximum G or R illuminance, followed by 30 seconds of flicker at 15 Hz. At the location of the disc where this response was 20% or more, the protocols pertinent to the various studies described in this article were started. For each subject, attempts were made to aim the laser beam at the same site of the optic disc in the various experimental sessions. Some of the experiments described next were performed in separate sessions on the same day. Most of them, however, took place on different days.

Changes in blood flow were measured in response to three different modes of stimulation: pure green flicker stimulus of different modulation depths at 10 Hz; flicker stimulus with different color ratios at 15 Hz; and flicker stimuli of pure luminance and equiluminance, changing the temporal frequencies.

Pure Green Flicker Stimulus of Different Modulation Depths at 10 Hz

This measurement was performed to identify possible saturation effects in $\mathbf{R}F_{onh}$ at high luminance modulation, which could represent a source of potential artifacts, distorting the relationship between $\mathbf{R}F_{onh}$ and the parameters of chromatic flicker under investigation.

$\mathbf{R}F_{onh}$ was measured in 2 subjects for a pure G stimulus ($r = 0$, 10 Hz) as a function of modulation depth defined according to the Michelson formula, as $(G_1 - G_2)/(G_1 + G_2)$. G_1 and G_2 represent the green illuminance during the first and second phase of the modulation, respectively. The mean illuminance $(G_1 + G_2)/2$ was kept constant and equal to 3 lux for all modulation depths. Two experimental protocols were followed:

Protocol 1. After a 30-second baseline recording of F_{onh} with constant illuminance (modulation depth = 0), stimuli with stepwise ascending values of the modulation depth from 0.2 units up to 1 were presented each for a duration of 40 seconds The same sequence was then followed in reverse.

Protocol 2. After delivery of each stimulus, F_{onh} was allowed to return to baseline, which occurred in less than one minute, before the next modulation depth was tested. The subjects were also tested twice, in separate days, to determine the reproducibility of $\mathbf{R}F_{onh}$ as a function of modulation depth. In one of them, reproducibility of $\mathbf{R}F_{onh}$ was evaluated from a number of successive measurements with a pure G stimulus ($r = 0$, modulation depth = 0.6, frequency = 10 Hz) and a pure R stimulus (1, 1, 25 Hz).

Flicker Stimulus with Different Color Ratios at 15 Hz

In 5 subjects, $\mathbf{R}F_{onh}$ was measured in response to R–G stimuli with different values of r, from 0 to 1 (10 to 12 steps). The stimuli were modulated at 15 Hz. Reproducibility of $\mathbf{R}F_{onh}$ versus r was obtained from two subjects (stimulation at 15 Hz) each run on two separate days.

Flicker Stimuli of Pure Luminance and Equiluminance, Changing the Temporal Frequencies

For stimuli of pure luminance (i.e., $r = 0$ or $r = 1$) and stimuli with r corresponding to the point of subjective equiluminance, $\mathbf{R}F_{onh,}$ was measured in three subjects for several frequencies between 2 Hz and 40 Hz. The point of subjective equiluminance was estimated psychophysically for every subject by adjusting r to produce a minimum perceivable flicker of the R–G modulation when alternating at 15 Hz. Mean r at equiluminance was found to be 0.45 ± 0.02.

$\mathbf{R}F_{onh}$ was also measured in response to pure luminance (modulation depth = 1) and equiluminance flicker stimuli alternating at 2 and 15 Hz. Four out of the five subjects were tested at both 2 and 15 Hz with the luminance flicker stimulus, while all the five subjects were evaluated with the equiluminance stimulus.

In one subject, $\mathbf{R}F_{onh}$ induced by luminance flicker obtained with pure G stimuli of different modulation depths (1, 0.6, and 0.4), but with the same mean illuminance, were also recorded as a function of frequency.

Data Analysis

$\mathbf{R}F_{onh}$ was defined as $Av F_{onh,st}/Av F_{onh,bl}$, where $Av F_{onh,st}$ represents the average over the last 20 seconds of F_{onh} measured as the tested eye was exposed to a specific flicker stimulus of 1 minute duration (if not stated otherwise). $Av F_{onh,bl}$ represents the average of F_{onh} over the last 20 seconds measured as the eye was exposed to a homogenous, unmodulated field of approximately the same mean luminance as that of the flicker stimulus (baseline condition). $\mathbf{R}F_{onh}$ was plotted as a function of r, modulation depth, and frequency of the stimulus. Com-

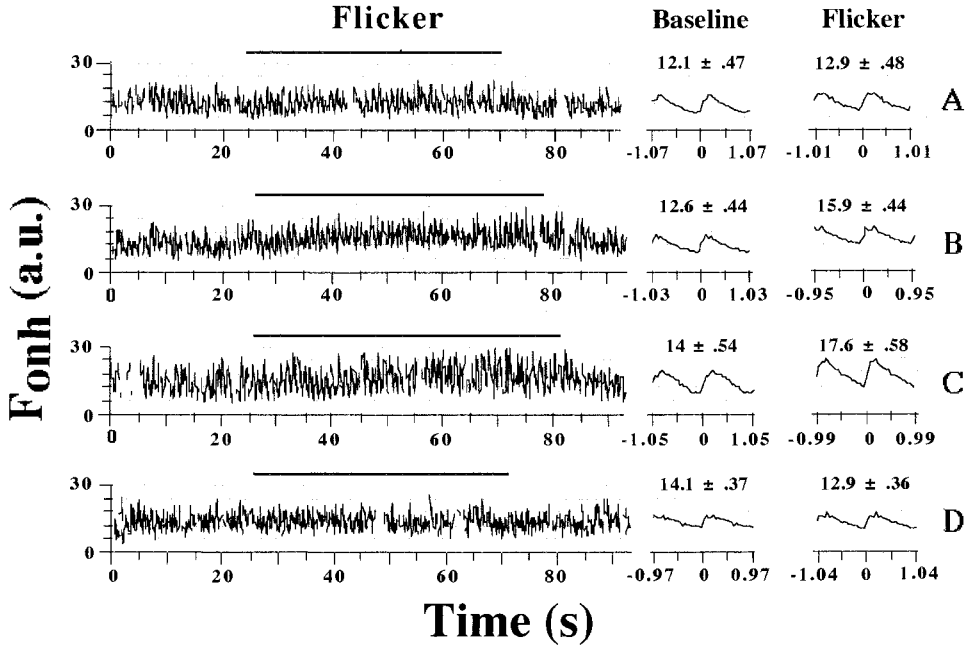

FIGURE 1. Recordings of F_{onh} in one subject during baseline and red (*R*)-green (*G*) flicker stimulation at 2 Hz (**A**, **B**) and 15 Hz (**C**, **D**). Color ratio $r = R/(R + G) = 1$ for the recordings (**A** and **C**) and 0.46 (the equiluminance point for this subject) for (**B** and **D**). The *horizontal line* above each recording indicates the occurrence of the flicker stimulus. At the *right* are plots of F_{onh} during the heart cycle (2 cycles are shown) during baseline and flicker. These variations were obtained by averaging F_{onh} over 20 seconds in phase with the heart cycle, as previously described.[27] Means ± 95% confidence limits of F_{onh} during the heart cycle are shown on *top* of each plot.

parisons across conditions were performed by repeated measures ANOVA (with post hoc adjusted *t*-tests) or paired *t*-tests, assuming normal data distribution.

RESULTS

Figure 1 represents sample recordings of approximately 90 seconds duration obtained in the same session from one subject during baseline and *R-G* flicker stimulation at 2 Hz, with $r = 1$ (A) and $r = 0.46$ (B) and at 15 Hz, with $r = 1$ (C) and $r = 0.46$ (D). The lower value of *r* corresponded to the equiluminance point for this subject. At the right of the recordings, we show the average variation of F_{onh} during the heart cycle for both baseline and flicker conditions. The mean values (± 95% confidence limits) of F_{onh} during the heart cycle are shown on top of each of these plots. For the equiluminance flicker at 2 Hz (B), F_{onh} increased immediately after the beginning of the stimulation to reach an average value of approximately 27% above baseline ($\mathbf{RF}_{onh} = 1.27$). For pure luminance flicker (A), F_{onh} changed by only 7% ($\mathbf{RF}_{onh} = 1.07$). The magnitude of the changes in F_{onh} was reversed when the flicker was modulated at 15 Hz. At this frequency, $\mathbf{RF}_{onh}$ in response to luminance flicker (C) was 1.26, whereas for the equiluminance flicker (D), a slight decrease in F_{onh} was observed ($\mathbf{RF}_{onh} = 0.91$).

The reproducibility of $\mathbf{RF}_{onh}$ was determined based on 5 consecutive trials performed during sessions of less than 19 minutes duration. The laser beam was aimed at 8 o'clock at the rim of the ONH in the right eye. Using a stimulus consisting of pure *R* illuminance flicker, $\mathbf{RF}_{onh}$ values were obtained ranging from 1.46 to 1.95, with a mean of 1.63 ± 0.16 (SD), corresponding to a coefficient of variation of the flicker-induced flow change [i.e., $100 \times (Av\mathrm{F}_{onh,flicker} - Av\mathrm{F}_{onh,baseline})/Av\mathrm{F}_{onh,baseline}$] of 26%. In another sequence of 4 measurements from approximately the same region of the disc and a pure *G* stimulus, a coefficient of variation of 12%, the lowest achieved in all our studies, could be obtained.

$\mathbf{RF}_{onh}$ versus Modulation Depth for Pure Green Luminance Flicker at 10 Hz

Figure 2 shows $\mathbf{RF}_{onh}$ versus modulation depth for a pure *G* luminance flicker ($r = 0$) at 10 Hz, when the modulation depth

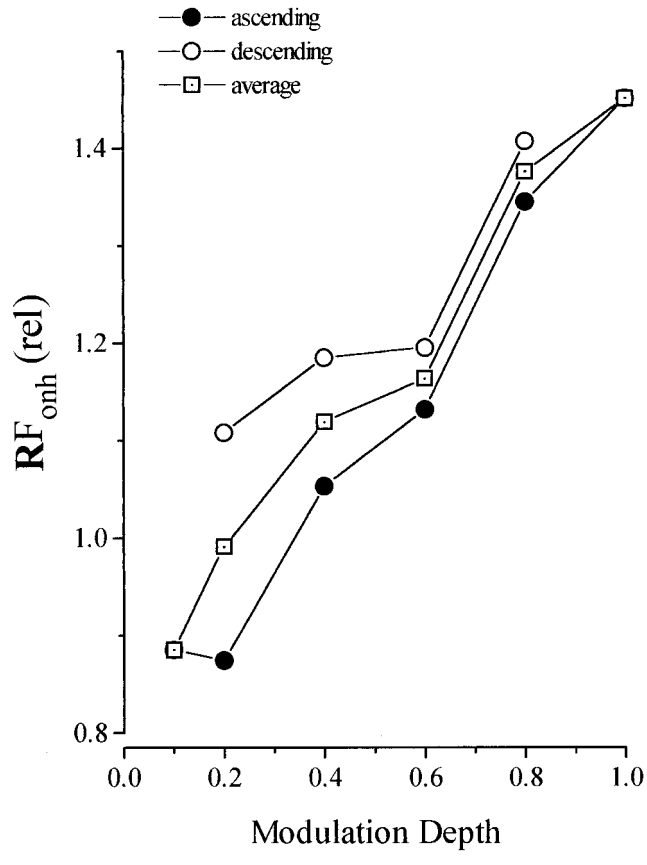

FIGURE 2. $\mathbf{RF}_{onh}$ versus modulation depth varied according to protocol 1. The average $\mathbf{RF}_{onh}$ of both ascending and descending data are also shown. The stimulus was pure *G* luminance flicker ($r = 0$) at 10 Hz. The data were fitted by a linear model ($y = ax + b$). Correlation coefficients are 0.98, 0.91, and 0.98 ($P < 0.01$ for all) for the ascending, descending and average data, respectively. The parameters of the fits are: $a = 0.67$, $b = 0.78$ for the ascending, $a = 0.45$, $b = 1$ for the descending, and $a = 0.61$, $b = 0.8$ for the average data.

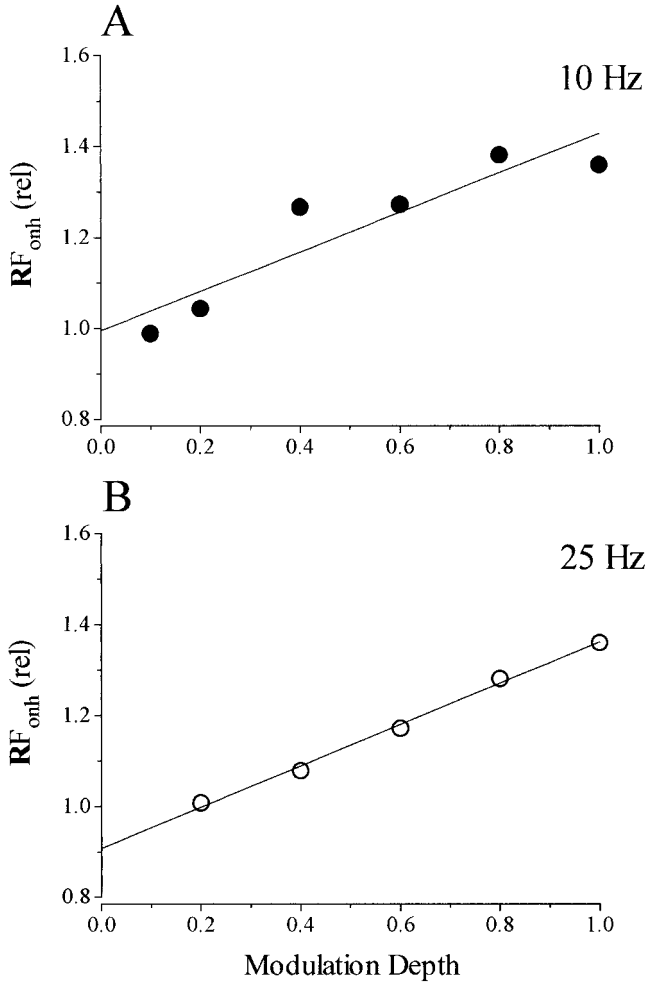

FIGURE 3. $\mathbf{RF}_{onh}$ versus modulation depth according to protocol 2. Stimulation consisted in pure *G* luminance flicker at 10 Hz (**A**) and pure *R* luminance flicker at 25 Hz (**B**) in the same subject as in Figure 2. The data were fitted by a linear model ($y = ax + b$). Correlation coefficients are 0.92 (**A**) and 0.99 (**B**), ($P < 0.01$ for both). The parameters of the linear fits are: $a = 0.43$, $b = 1$ for (**A**) and $a = 0.456$, $b = 0.91$ for (**B**).

was varied in an ascending and descending manner in one subject (protocol 1). Also plotted is the average of both $\mathbf{RF}_{onh}$ data. A linear model is adequate to fit the $\mathbf{RF}_{onh}$ versus modulation depth data ($P < 0.01$), with correlation coefficients equal to 0.98, 091, and 0.98 for the ascending, descending and average data, respectively. $\mathbf{RF}_{onh}$ measured in one subject from the temporal part of the disc, according to protocol 2 and a pure *G* luminance flicker at 10 Hz and pure *R* luminance flicker at 25 Hz are shown in Figure 3A and B. The linear model adequately fits the $\mathbf{RF}_{onh}$ versus modulation depth data ($P < 0.01$), with correlation coefficients of 0.92 for Figure 3A and 0.99 for Figure 3B, respectively. Linearity between $\mathbf{RF}_{onh}$ and modulation depth is improved by using this second protocol, as indicated by the higher values of the correlation coefficients in Figure 3 compared to those associated with the data shown in Figure 2.

Flicker Stimulus with Different Color Ratios at 15 Hz

Figure 4A shows two sets of $\mathbf{RF}_{onh}$ data obtained in two different sessions from the previous subject for a 15-Hz *R–G* flicker modulation when the *r* value was changed from 0 to 1 in 12 steps. In the set represented by black squares, $\mathbf{RF}_{onh}$ showed a local peak corresponding to the equiluminant point measured psychophysically ($r = 0.46$). In the other (white squares), this peak was less clear. In addition, there is an extra dip at $r = 0.42$.

Based on five subjects, average $\mathbf{RF}_{onh}$ ($\pm$ SEM) as a function of r has been plotted in Figure 4B. $\mathbf{RF}_{onh}$ was largest for pure luminance flicker ($r = 0$ and 1) and lowest for mixed luminance chromatic modulation ($r = 0.34$ and 0.64), with a secondary peak near $r = 0.45$, the average point of psychophysical equiluminance. An ANOVA performed on the data of Figure 4B showed that average $\mathbf{RF}_{onh}$ changed significantly as a function of *r* [F-ratio (degrees of freedom: 11,44): 6.25, $P = 0.01$]. The post hoc *t*-tests revealed that the decrease in mean $\mathbf{RF}_{onh}$, when *r* was changed from 0 or 1 to $r = 0.34$ and 0.64, was statistically significant ($P < 0.01$). The secondary maximum in average $\mathbf{RF}_{onh}$ at around equiluminance flicker ($r = 0.45$) was also significantly larger than the values observed at $r = 0.34$ and 0.64 ($P < 0.01$).

Flicker Stimuli of Pure Luminance and Equiluminance, Changing the Temporal Frequencies

Figure 5 shows how the average $\mathbf{RF}_{onh}$ of the data obtained from three subjects, for luminance and chromatic equilumi-

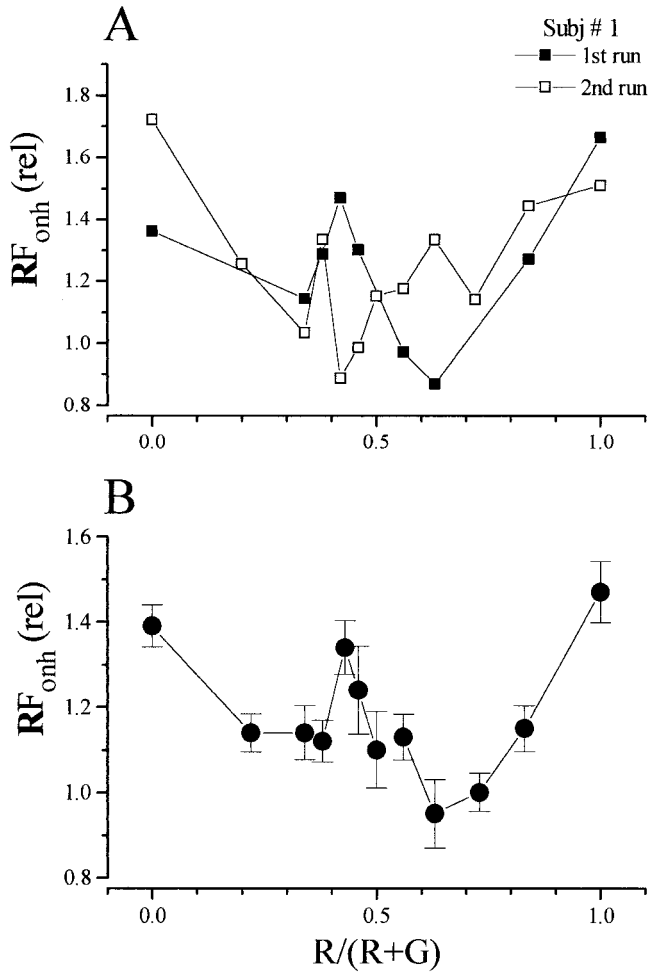

FIGURE 4. $\mathbf{RF}_{onh}$ versus color ratio, $r = R/(R + G)$, when the *r* was changed from 0 to 1 in 10 to 12 steps for a 15 Hz stimulus. (**A**) Data obtained in two different sessions in one subject. (**B**) Average of the $\mathbf{RF}_{onh}$ data obtained from 5 subjects. *Error bars* are $\pm$ 1 SEM.

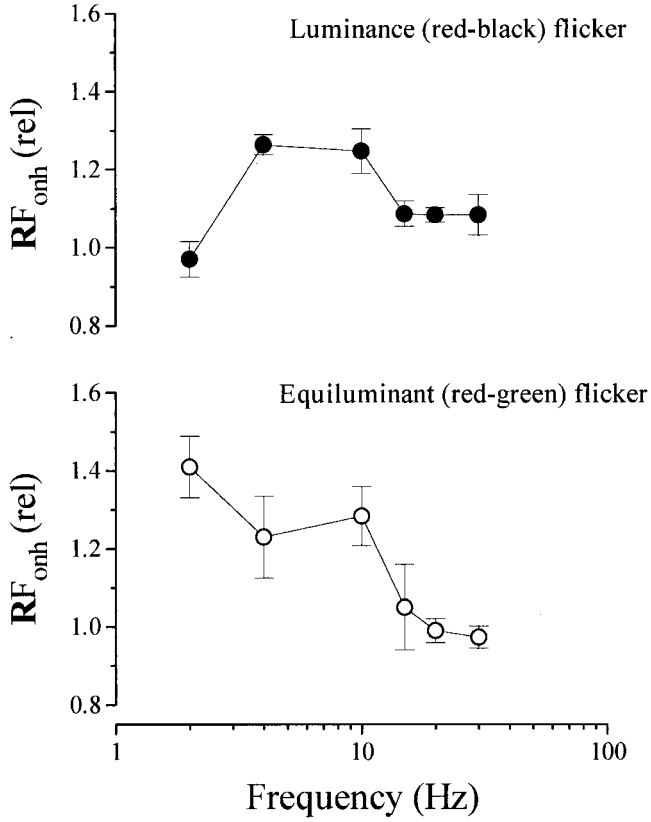

FIGURE 5. Average RF_{onh} versus stimulus temporal frequency obtained from three subjects, for luminance and chromatic equiluminance flicker.

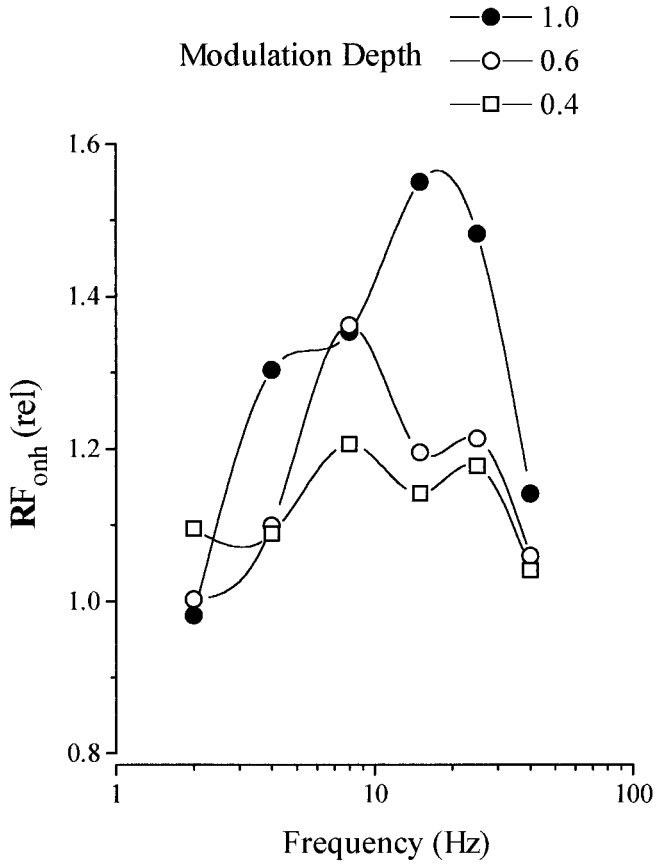

FIGURE 6. RF_{onh} versus stimulus temporal frequency for three values of modulation depth obtained in one subject for a pure *G* luminance flicker ($r = 1$)

nance flicker, varied as a function of frequency. For luminance flicker a band-pass function having a broad maximum at 5 Hz to 10 Hz was found. A response, though small, was still consistently recorded at 30 Hz. For the equiluminance flicker, a low-pass type of function was observed, with a maximum at 2 Hz, an attenuation above 10 Hz, and no significant contribution beyond 15 Hz. Repeated testing in two subjects showed that these characteristics were reproducible.

RF_{onh} for 2 Hz versus 15 Hz

The dependence of RF_{onh} on flicker frequency was further evaluated by comparing the RF_{onh} obtained at 2 Hz and 15 Hz for both stimulations. Individual values of RF_{onh} are reported in Table 1 for every subject. For the luminance flicker condition, RF_{onh} was larger at 15 Hz than at 2 Hz in all four subjects. For the equiluminance condition, the opposite occurred in four out of five subjects. Both comparisons approached but did not reach statistical significance by paired *t*-test ($t = 2.17$, $P = 0.11$ and $t = 2.41$, $P = 0.07$, respectively). The average ratio of RF_{onh} at 2 Hz to that at 15 Hz was 0.86 ± 0.05 for the luminance flicker and 1.26 ± 0.12 for the equiluminance flicker. This difference was statistically significant ($t = 3.3$, $P = 0.01$).

RF_{onh} versus Frequency for Various Modulation Depths

Plots of RF_{onh} versus frequency were obtained in one subject during the same session for pure *G* luminance flicker ($r = 1$) at modulation depths = 1, 0.6, 0.4, and 0.2. Because the RF_{onh} data for modulation depth = 0.2 were within the noise level for most frequencies, only the RF_{onh} for the three other modulations have been plotted in Figure 6. At all modulations, RF_{onh} versus frequency displays a band-pass shape. Coefficients of variation were determined for the case of the modulation depth = 0.4, based on of three measurements of RF_{onh} at 5 frequencies of a *G* luminance flicker. These varied be-

TABLE 1. Individual RF_{onh} Values Obtained from Normal Volunteers in Response to 2 Hz and 15 Hz Flicker Modulated in Luminance and Chromatic Equiluminant Conditions

Subject, Age, and Sex	RF_{onh} 2 Hz Luminance Flicker	RF_{onh} 15 Hz Luminance Flicker	RF_{onh} 2 Hz Equiluminance Flicker	RF_{onh} 15 Hz Equiluminance Flicker	2 Hz RF_{onh}/15 Hz RF_{onh} Ratio (Luminance Flicker)	2 Hz RF_{onh}/15 Hz RF_{onh} Ratio (Equiluminance Flicker)
1/60/M	1.26	1.69	1.30	1.04	0.74	1.25
2/28/M	1.01	1.04	1.56	1.21	0.97	1.29
3/31/M	0.88	1.07	1.06	1.17	0.82	0.90
4/32/M	1.12	1.22	1.51	1.26	0.92	1.20
5/45/F	—	—	1.38	0.84	—	1.64

tween 10% (at 25 Hz) and 48% (at 8 Hz), with an average of 26%.

Discussion

The present findings show that a $\mathbf{RF}_{onh}$ can be recorded in volunteers selected for their excellent target fixation in response to luminance modulation, equiluminant chromatic modulation, or both, of a *R–G* flickering stimulus (Fig. 1). In the subjects tested in this investigation, $\mathbf{RF}_{onh}$ measured under different stimulus conditions demonstrated a satisfactory reproducibility, with a coefficient of variation of less than 26%. The response as a function of modulation depth could be fitted by a linear function with a highly significant correlation coefficient for both stimulus frequencies tested, that is, 10 Hz (Figs. 2 and 3A) and 25 Hz (Fig. 3B). Thus for the characteristics of the flicker stimulus pertaining to and the various functional relationships sought in the present investigation, it appears that the range of our blood flow changes is below that corresponding to maximum vasodilatation, where saturating effects could distort these relationships.

$\mathbf{RF}_{onh}$ decreased significantly when the ratio between the *R* and *G* illuminance of the chromatic flicker stimulus was varied at 15 Hz from a condition of pure luminance flicker modulation to a condition where mixed luminance and chromatic modulations were present (Figs. 4A and 4B). In most experiments, $\mathbf{RF}_{onh}$ also showed a local peak corresponding to the equiluminant point measured psychophysically. This peak is quite clear in Figure 4B.

The $\mathbf{RF}_{onh}$ to luminance flicker displayed a different dependency on frequency compared to the response to equiluminance flicker. With the former stimulus, $\mathbf{RF}_{onh}$ was band-pass tuned and well detectable up to 30 Hz (Fig. 5); with the latter stimulus, $\mathbf{RF}_{onh}$ had a low-pass behavior with a cutoff at 15 Hz. The results of Figure 6 suggest that this difference is not attributable to a difference in cone modulation amplitude between luminance and equiluminance flicker because reducing the modulation depth of a *G–G* stimulus left unaltered the band-pass tuning behavior of $\mathbf{RF}_{onh}$. In accordance with the band-pass versus low-pass behavior of $\mathbf{RF}_{onh}$, the average ratio between the $\mathbf{RF}_{onh}$ obtained at 2 and 15 Hz for luminance flicker was significantly smaller and reversed, compared with that for equiluminance flicker (Table 1).

The dependence of $\mathbf{RF}_{onh}$ on *r* and frequency (Figs. 4B and 5) is similar to that observed for the magno- and parvo-cellular retinal ganglion cells activity[7] and for the pattern electroretinogram (PERG), a response correlated with ganglion cell activity.[9,10,17,18] By recording the responses of magno- and parvocellular ganglion cells to luminance and *R–G* equiluminance flicker modulation in monkeys, it was found that, at high frequency, response sensitivity of magnocellular ganglion cells was substantially weaker for the equiluminance than for the luminance flicker.[7] By contrast, at low frequency, the response sensitivity of parvocellular ganglion cells to chromatic equiluminance was stronger than the corresponding responses to luminance flicker. Recordings of the steady state PERGs at relatively high temporal frequencies ($\geq$6 Hz) as a function of *r* of *R–G* patterns revealed that the response amplitude decreased significantly when *r* was changed from luminance to mixed luminance and chromatic contrast modulation.[9,18,10] Furthermore, in both humans and monkeys the PERG amplitude showed a local maximum at equiluminance.[9,10] By decreasing stimulus temporal frequency, this maximum was relatively more pronounced, compared to the responses to patterns of pure luminance contrast.[9] These similarities suggest a correlation between the observed $\mathbf{RF}_{onh}$ behavior and the physiological responses of retinal ganglion cells to respectively high-frequency luminance flicker modulation and low-frequency equiluminance flicker chromatic modulation. Although the neural responses to luminance flicker may have a contribution of both magno- and parvocellular systems,[9,18] it may be suggested that the responses to equiluminance flicker reflect, selectively or prevalently, the activity of the parvo system.[7,8] It should be noted, however, that the neural parvo-response might change depending on the type of stimulus. It is indeed known, from single cells studies[7] that the temporal response function of the parvo-system is low-pass with chromatic, but band-pass with luminance modulation.

The mechanisms underlying the flicker induced $\mathbf{RF}_{onh}$ are still matter of investigation. In the cat, the extracellular K^+_{onh} concentration at the ONH increased in parallel with $\mathbf{RF}_{onh}$. Lack of evidence of a direct vasodilatory effect of K^+_{onh} on the ONH microvasculature raised doubt regarding the role of K^+_{onh} in the flicker-induced $\mathbf{RF}_{onh}$.[4] The role of nitric oxide (NO) in ONH vaso-activity has been recently demonstrated.[19,20] In the cat, flicker-evoked changes in NO_{onh} concentration paralleled $\mathbf{RF}_{onh}$.[19] In addition, inhibition of NO synthase markedly attenuated both the changes in NO_{onh} concentration and $\mathbf{RF}_{onh}$. These data strongly implicate NO as a putative mediator of the coupling between blood flow and neuronal activity, although the role of other factors (pH, pCO_2, circulating hormones) cannot be excluded. It is presently unknown whether the same or different mediators may underlie the specific vascular responses at the level of the ONH induced by luminance and chromatic equiluminance flicker stimuli observed in this study. Pharmacological approaches in experimental animal models could help clarify this issue.

The study described here was performed in volunteers who could maintain excellent target fixation and minimal head motion, since no bite bar was used. Subjects were instructed to fixate during the whole flicker period. This condition could be relaxed by having subjects fixate the target only during the last 20 seconds or so of the 1-minute flicker period, while the tested eye remains exposed to the flickered field during the whole period. From a clinical standpoint, the measurement of $\mathbf{RF}_{onh}$ in response to luminance and chromatic equiluminance flicker stimulation, even if only applicable to patients selected for their adequate target fixation, could provide a new, powerful tool to investigate ONH and retinal diseases. First, it may bring new insights into the pathophysiology of blood flow, examined through its relationship with neural activity losses. For example, it is still unknown whether ischemic damage to the ONH in glaucoma is a primary determinant or whether it develops secondarily to compression-induced neural damage. In the former situation, it could be conceivable that an abnormal vascular response to flicker precedes any detectable neural loss, due to impaired vasoactivity. Investigation of the neurovascular coupling could also be of interest in early diabetes, where both ischemic and neural damage occur in the inner retina.[21] Second, the present approach allows separate evaluation of neural visual activities, coupled with corresponding hemodynamic changes, carrying luminance and chromatic information. For instance, a considerable body of anatomic evidence indicates that, in early glaucoma, large retinal ganglion cells, subserving primarily the magnocellular pathway, are selectively or predominantly damaged.[22–24] Although this selective vulnerability may not necessarily correlate with visual dysfunction,[25] it may be of interest to investigate whether the measurement of $\mathbf{RF}_{onh}$ reveal specific losses elicited by luminance versus chromatic stimulation. Electrophysiological and psychophysical results[26] have shown that, in multiple sclerosis patients with or without optic neuritis, the function of the parvocellular pathway may show specific losses. The relationship of these losses to the neurovascular activity changes are still unknown.

In conclusion, the present findings indicate that changes in human ONH hemodynamics can be evoked by both luminance and equiluminance *R-G* flicker modulation, and that the temporal frequency response characteristics of these changes differ for the two types of stimulation, similar to the neural responses of retinal ganglion cells. These data suggest that equiluminance-evoked $\mathbf{R}F_{onh}$ is associated with neural parvocellular activity, yet luminance flicker-induced $\mathbf{R}F_{onh}$ may be correlated with the activity of both magno- and parvocellular neurons. The specificity of $\mathbf{R}F_{onh}$ elicited by chromatic flicker modulation may offer new opportunities to study visually evoked vascular activity of the ONH in both physiological and diseased conditions involving predominantly or selectively the magno- and parvosubsystems.

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