Variations of blood flow at optic nerve head induced by sinusoidal flicker stimulation in cats

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1. The present investigation explored, in thirty-four anaesthetized cats, the blood flow changes at the optic nerve head elicited by sinusoidally modulated photic stimuli.

2. The stimuli were achromatic, diffuse and had 30 deg diameter field size; the stimulus frequency was varied from 0 to 100 Hz, modulation depth from 0 to 100% and mean retinal illuminance up to 50000 trolands (td); the blood flow was measured with a near-infrared (810 nm) laser Doppler flowmeter.

3. At various frequencies, modulation depths and mean retinal illuminance, sinusoidal flicker stimulation always caused an increase in blood flow at the optic nerve head relative to steady stimulation.

4. The frequency response and temporal contrast sensitivity function of the blood flow changes had a bandpass shape; the high-frequency slope of the frequency response was 3 decades (dec) per decade and that of the temporal contrast sensitivity function was 1·7 dec per dec, close to the slope for cat 'on' ganglion cells (2·6 dec per dec).

5. In most cats, the magnitude of the increase in blood flow was a sigmoidal function of modulation depth; in the remainder, the relationship was close to linear.

6. The threshold of blood flow changes varied with respect to mean retinal illuminance similar to Ferry-Porter's law and the photopic linear slope was 50 Hz dec^{-1}.

7. In comparison with reported psychophysical and electrophysiological responses elicited by similar stimulations, the results of the present study resemble more those obtained from ganglion cells than those from electroretinograms, visual-evoked potentials and psychophysics. It is suggested that the blood flow changes at the optic nerve head are induced by the activity of ganglion cells.

Since first hypothesized by Roy & Sherrington (1890) more than 100 years ago, the coupling between blood perfusion and functional activity has been amply confirmed for the brain and other regions of the nervous system (e.g. Branston & Symon, 1980; Lou, Edvinsson & MacKenzie, 1987). Studies in the retina and optic nerve, both tissues representing extensions of the brain, are more recent, however. Using predominantly diffuse luminance flicker with multiple short flashes to generate functional activity, and laser Doppler flowmetry to monitor the blood flow changes, these studies have convincingly demonstrated that increases in neural activity induce large increases in blood flow in the optic nerve and retinal tissue in cats and human subjects (e.g. Riva, Harino, Shonat & Petrig, 1991; Mendel, Riva, Petrig & Cranstoun, 1994).

In order to obtain deeper insight into the coupling between flicker-induced neural activity and blood flow in the eye fundus, and also to establish links between the psychophysical and electrophysiological responses resulting from the increased activity and blood flow responses, photic stimulation with sinusoidally modulated waveform is more appropriate than stimulation with multiple flashes. With the former it is possible to stimulate the retina with different contrasts of light–dark variations while maintaining a constant mean retinal illuminance. For this reason, among others, it has been widely applied in the study of the visual system. In psychophysics, it has been used to determine the critical flicker frequencies (CFFs) and temporal contrast sensitivity functions, and in electrophysiology to elicit electroretinograms (ERGs) and visual-evoked potentials

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(VEPs) as well as electrical responses of different cells such as photoreceptors, horizontal and ganglion cells (De Lange, 1958; Cleland & Enroth-Cugell, 1966; Spekreijse, Khoe & Van der Tweel, 1972; Sternheim & Cavonius, 1972; an der Grind & Grüsser, 1981). These results reveal neural activities of different visual cells and at different stages of the visual system.

In the study presented here we investigated the effect of the stimulus frequency, modulation depth and mean retinal illuminance on the blood flow changes at the optic nerve head in anaesthetized cats and compared these results with psychophysical and electrophysiological results obtained under similar stimulations.

METHODS

Preparation

Thirty-four adult cats, weighing between 2 and 4 kg, were used in this study. All experimental procedures complied with the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research and the Presbyterian Medical Center of Philadelphia Guidelines on Animal Research.

Each cat was injected with atropine (0·04 mg kg^{-1} s.c.) and anaesthetized with intramuscular ketamine hydrochloride (22 mg kg^{-1}) and acepromazine maleate (2 mg kg^{-1}). Catheters were placed in a femoral artery and vein, and a tracheostomy was performed. Postsurgically, after an equilibration period, the level of anaesthesia was checked by testing corneal and toe-pinch reflexes. Confirming the absence of these reflexes, we proceeded with the paralysis of the animal with a loading dose of pancuronium bromide (0·2 mg kg^{-1}, I.V.) and the animal was ventilated with 21% O_2 and 79% N_2. Arterial blood pressure, end-tidal CO_2 and heart rate were continuously monitored. Arterial pH, P_{CO_2} and P_{O_2} were monitored intermittently using a blood-gas analyser and adjustments of the inspired gas mixture, end-tidal volume and respiration rate were made to keep pH $\approx$ 7·4, $P_{CO_2} \approx$ 31 mmHg, $P_{O_2} \approx$ 90 mmHg and mean arterial blood pressure between 85 and 119 mmHg. Rectal temperature was maintained at ~38 °C. Enflurane (1·7–2·5%) was administered and pancuronium bromide (0·1 mg kg^{-1} h^{-1}) infused continuously. The adequacy of anaesthesia was ensured by observing the stability of heart rate, arterial blood pressure and the absence of lacrimation, salivation and facial muscle movements. The regime used for anaesthetizing the paralysed animals was the same as would have been used in non-paralysed animals, anaesthetized for the same period of time.

The cats were placed prone on a table with the head secured to a six-degrees-of-freedom structure attached to the table. Only one eye was tested at a time; the other eye was occluded. The pupil of the tested eye was dilated with 1% tropicamide and 10% phenylephrine. A ring was sutured to the eye with three stitches at the limbus and secured to a frame to prevent eye motion. A zero dioptre contact lens was placed on the cornea, which was protected with Healon. At the conclusion of the experiments the animal was killed by administration of 5 ml pentobarbitone (360 mg ml^{-1}) while still under general anaesthesia.

Blood flow recording

Recording of blood flow at the optic nerve was performed by a laser Doppler fundus-camera-based flowmeter, placed approximately 5 cm in front of the eye, using a near-infrared 810 nm diode laser as a probing source and a silicon avalanche photodiode as a detector (Riva, Harino, Petrig & Shonat, 1992). The laser beam had a diameter of approximately 200 μm at the optic disc. The beam power measured at the cornea was approximately 120 μW. The photodetector current generated by the laser light scattered by the red blood cells was processed by the Periflux PF3 (Perimed Inc., Stockholm, Sweden) or the BPM 403A LaserFlo (Vasamedics, St Paul, MN, USA). Both instruments calculated the relative flux of red blood cells in the

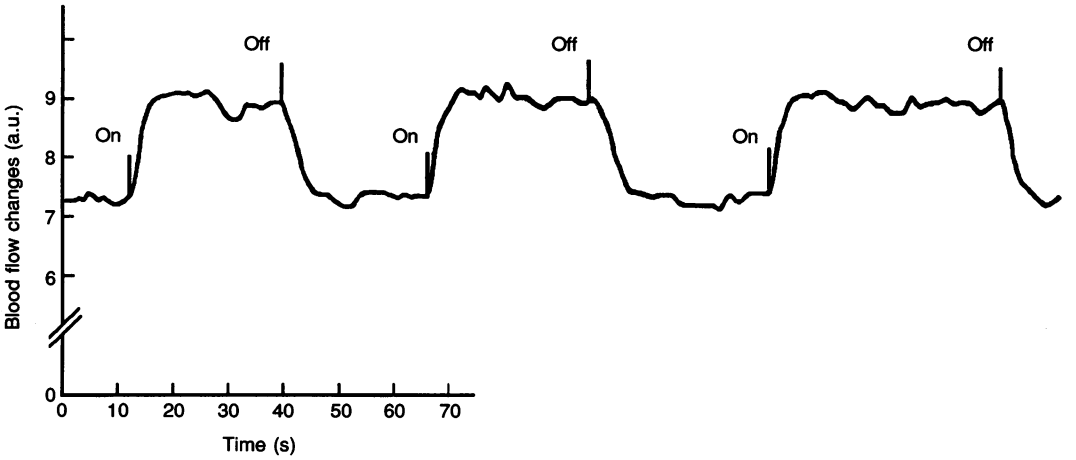

Figure 1. Blood flow changes elicited by sinusoidal stimulation and recorded by laser Doppler flowmetry

The tick marks indicate when the flicker stimulation was on or off. The scales are linear: horizontal, 10 s per division; vertical, arbitrary units (a.u.). Flicker frequency was 10 Hz, modulation depth 50%, field size 30 deg and mean retinal illuminance 3000 td. Stimulation was repeated 3 times and the stimulation duration was arbitrary.

sampled volume (Borgos, 1990; Nilsson, 1990) and the result was plotted on a chart recorder. The time constants of these instruments were 3 and 5 s, respectively.

Visual stimulation

The sinusoidal photic stimulation was generated by a visual stimulator (Vo Van Toi & Grounauer, 1978). In the experiments reported here the original Maxwellian eyepiece of the stimulator was replaced by an extension optical fibre light guide which conducted the stimulus from the original Y-shaped optical fibre to the light-source plane of the fundus camera. Therefore the stimulation light which impinged upon the eye followed the fundus illumination pathway of the camera. The colour temperature of the stimulus was 2200 K. The luminance of the stimulus was measured at the retinal plane of a model eye with a J16 photometer and a J6503 probe (Tektronix, Pittsfield, MA, USA). In the investigation presented here only achromatic stimuli were employed. The stimulation field was diffuse, had a diameter of 30 deg and a dark surround. The stimulus waveform was described by the equation: $L = L_0 M \cos(2\pi f t)$, where L was the instantaneous luminance, L_0 the mean luminance, M the modulation depth, f the frequency and t the time. The three parameters L_0, M and f were varied independently. The mean retinal illuminance, expressed in trolands (td), was computed by multiplying the mean luminance (in cd m^{-2}) by the pupil area (in mm^2). The stimulus modulation depth M was adjusted from 0 to 100% and the stimulus frequency f from 0 to 100 Hz. During the experiments, two of these parameters were kept constant while the third was changed and the elicited blood flow change was recorded.

Experimental protocol

Unless otherwise specified, the stimulation field contained the optic disc in the centre and the laser beam was targeted at a zone of the disc tissue where no vessel could be seen. When the baseline blood flow reached a stable value, a testing stimulus, typically a 10 Hz sine wave, 100% of modulation and 30000 td, was turned on and the blood flow response recorded. This stimulation was repeated many times to check the sensitivity and reproducibility of the blood flow response. Various sites were usually tested in an attempt to optimize these two requirements. The cats were subsequently dark-adapted for approximately 1 h and then adapted for a few minutes at the mean illuminance to be used for the experiment, before flicker was initiated.

Disturbances encountered in the blood flow recordings were caused by spontaneous fluctuation of the blood flow and instrument noise. To avoid mistaking these disturbances for blood flow changes elicited by stimulation, the experiment was started only when the recorded signal was steady and reproducible, and the blood flow increases were at least 2%. Usually, three measurements were performed from which the mean value of the blood flow changes was computed. Figure 1 shows the blood flow changes evoked by three successive sinusoidal stimulations.

Two types of experiment were performed. The first one consisted of recording the blood flow changes elicited by varying either the mean retinal illuminance, modulation depth or flicker frequency. The blood flow changes were then computed using the baseline (which was the blood flow when the cat was adapted to the corresponding mean illuminance and no stimulation was applied) as the reference. They were expressed as percentages and designated, thereafter, as F. The second type of experiment was to determine the thresholds of stimulus parameters which evoked minimum change of blood flow. Hence, either the stimulus frequency or modulation depth was varied by steps until the blood flow increased approximately 2% above the baseline.

In the first type of experiment, two protocols were implemented. (1) The modulation depth of the stimulus was kept constant and, for different levels of mean retinal

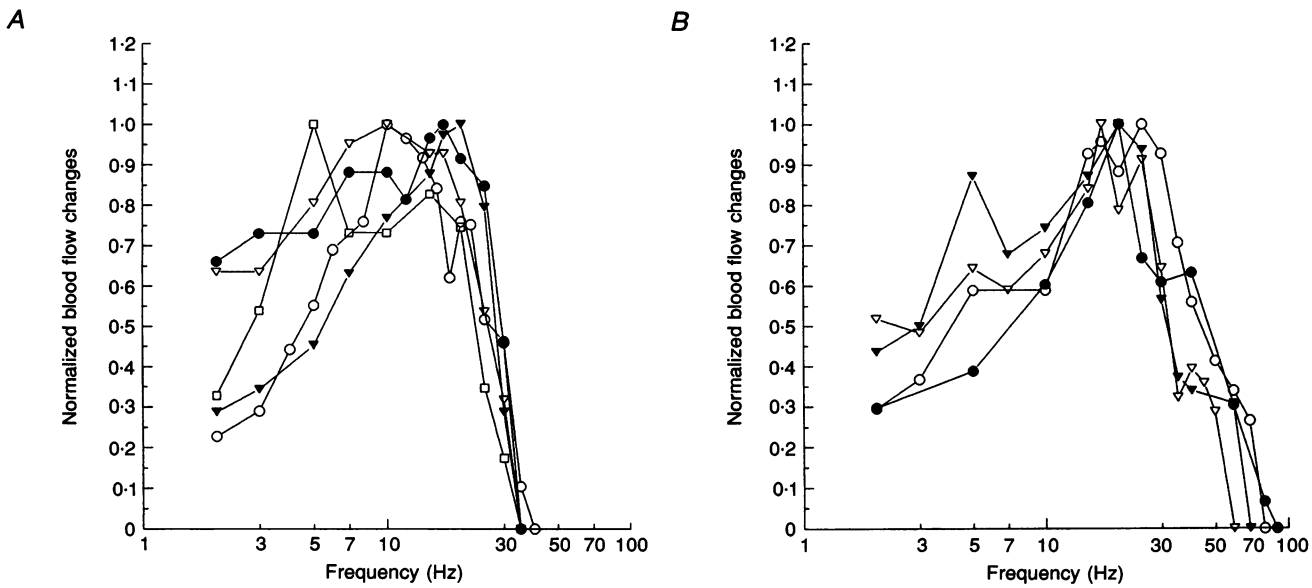

Figure 2. Blood flow changes *versus* flicker frequency

These blood flow frequency responses were recorded in 2 groups of cats at 2 different mean retinal illuminance levels: 3000 td (*A*) and 30000 td (*B*). For the sake of comparison the curves were normalized using the F_{max} of each curve as 100%. Modulation depth was 100% and field size 30 deg.

illuminance, F was established as a function of flicker frequency; this function constituted the F-frequency response. (2) For each level of mean retinal illuminance and frequency, F was determined as a function of modulation depth; this function constituted the F-modulation response.

In the second type of experiment we performed two other protocols. (1) For each mean level of retinal illuminance, the modulation threshold (which produced a minimum change of F) was established as a function of flicker frequency; this function constituted the F-temporal contrast sensitivity (De Lange curve). (2) With the modulation depth of the stimulus kept constant at 100%, the CFF was determined as a function of mean retinal illuminance; this function constituted the F-critical flicker frequency response.

RESULTS

Blood flow changes *versus* flicker frequency

In this experiment the modulation depth was kept constant at 100% and flicker frequency was varied from 2 to 100 Hz. Seven cats were tested; three at 3000 td, two at 30000 td and two at both mean retinal illuminance levels. These levels were arbitrarily selected in the mesopic and photopic ranges.

In all cats, the curves of F *versus* flicker frequency had a bandpass shape. The curves obtained at 3000 td had peaks between 5 and 20 Hz and those recorded at 30000 td had peaks at around 20 Hz. Below 10 Hz, F varied greatly from cat to cat especially at the lower mean retinal illuminance. Figure 2*A* and *B* shows the curves obtained from these cats; for the sake of comparison their heights were normalized using the maximum values as 100%.

The slopes of these curves were evaluated from a log–log scale plot. In order to determine the low-frequency slopes, all the data below 15 Hz were taken into consideration. The mean value and S.D. of the low-frequency slopes obtained at low and high retinal illuminance were $0{\cdot}5 \pm 0{\cdot}4$ and $0{\cdot}3 \pm 0{\cdot}1$ decade (dec) per decade, i.e. not statistically different ($P < 0{\cdot}05$). (The dec per dec unit can be converted into decibels per octave by multiplying by 6 for amplitude, or by 3 for power.) Similarly, the values of the high-frequency slopes of the curves were computed based on data above 15 Hz. The mean values and S.D. of the high-frequency slopes at low and high retinal illuminance levels were $3{\cdot}0 \pm 0{\cdot}5$ and $2{\cdot}9 \pm 0{\cdot}6$ dec per dec, also not statistically different. Figure 3, which shows the curves of one cat at these two illuminance levels, illustrates this fact.

Blood flow changes *versus* flicker modulation

Experiments were performed on ten cats. In these experiments the stimulation frequency was kept constant at 10 Hz while the modulation depth was varied in steps from 0 to 100%. This frequency was selected because it evoked high blood flow changes (see previous section). In four cats, F increased linearly with respect to the modulation depth. Figure 4*A* shows a typical curve for one of these cats.

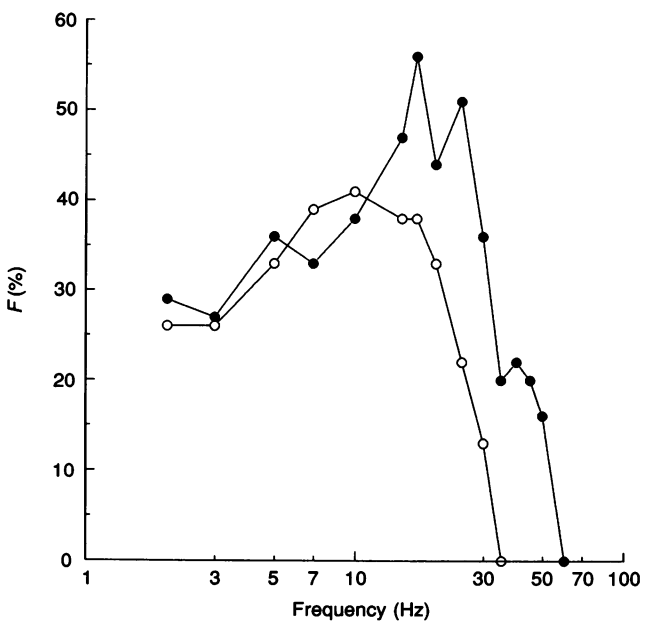

Figure 3. Blood flow changes *versus* frequency recorded on one cat at two different levels of mean retinal illuminance

○, illuminance at 3000 td; ●, illuminance at 30000 td. The modulation depth was 100% and the field size was 30 deg. Here and in the following figures, F is the percentage change in blood flow over baseline (see text).

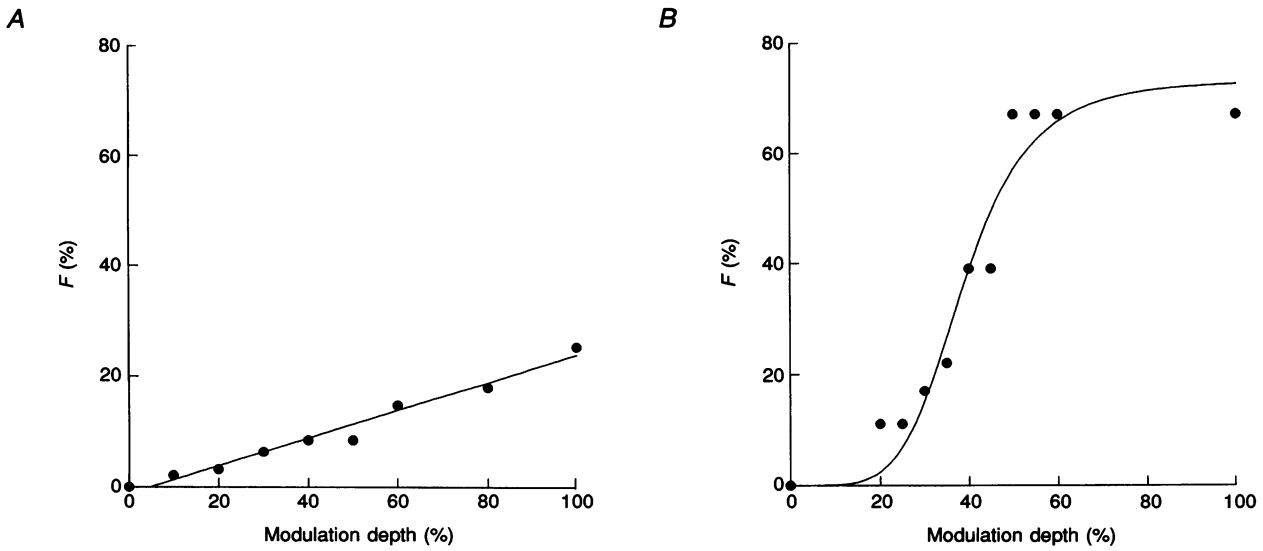

Figure 4. Blood flow changes *versus* modulation depth recorded on two cats

A, modulation response of 1 cat. In this cat no saturation of blood flow was observed. The straight line is a linear regression fit and the dots are experimental values. *B*, modulation response of another cat. The continuous line represents the fit according to a sigmoidal equation (see text) where the maximum blood flow at the saturation level, F_{max}, is 73 and the slope at half-saturation level, Q, is 0·03. The saturation knee is at 50% of modulation depth. In both *A* and *B*, flicker frequency was 10 Hz, field size 30 deg and mean retinal illuminance 30000 td.

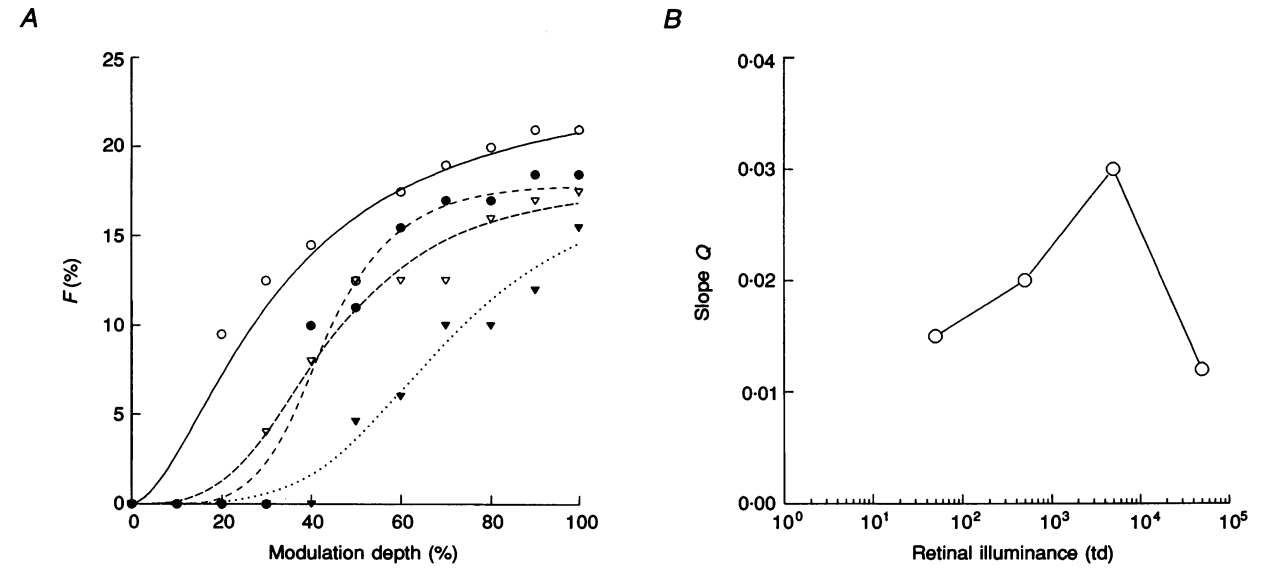

Figure 5. Blood flow *versus* modulation depth recorded on one cat at different levels of mean retinal illuminance

A, the symbols indicate experimental values of mean retinal illuminance: ○, 50000 td; ●, 5000 td; ▽, 500 td; ▼, 50 td. The lines represent the fit according to a sigmoidal equation (see text). *B*, the variation of slope of the curve with respect to the mean retinal illuminance. Slope Q is calculated at the half-level of F_{max} (see text).

In the other six cats, by contrast, F increased with respect to the modulation depth as a sigmoidal function, and rapidly saturated. In four out of these six cats, F started to increase steeply from 20% modulation depth and saturated at 50% modulation depth (illustrated for one cat in Fig. 4*B*). In two other cats, F started to increase at a lower modulation depth (10%) and saturated sooner (at 40%).

Since these curves behaved as a sigmoidal function their data were fitted by the following equation:

$$F = F_{max} M^n / (M^n + k^n),$$

where F is the percentage of blood flow changes at modulation depth M, F_{max} is the value of F at saturation, n is a free parameter and k is the modulation depth at which $F = \frac{1}{2}F_{max}$.

The slope at half-maximum F, determined from the derivative of F/F_{max} and evaluated at k, is:

$$Q = n/(4k).$$

The values computed from these formulae in six cats showed that F_{max} varied from 73 to 360 and Q from 0·002 to 0·03. To explore whether the mean retinal illuminance and stimulus frequency influenced these factors, two new experiments were conducted.

First, the influence of mean retinal illuminance was determined from experiments on seven new cats. All results showed a sigmoidal function (illustrated for one cat in Fig. 5*A*). For all cats, when the mean retinal illuminance increased, Q first increased then decreased while F_{max} varied randomly. Figure 5*B* represents the variation of Q for the same cat as in Fig. 5*A*. This result may be an artifact of the experimental procedure in which higher levels of light were sequentially employed; however, repeated testing at 3000 td did not show a change of curve shape and so it is likely that the change seen in Fig. 5*A* does represent a real effect of light intensity. On the other hand, the feature depicted in Fig. 5*B* may depend on the fitting procedure.

Second, on five other cats, the flicker frequency was varied while the mean retinal illuminance was kept constant. The

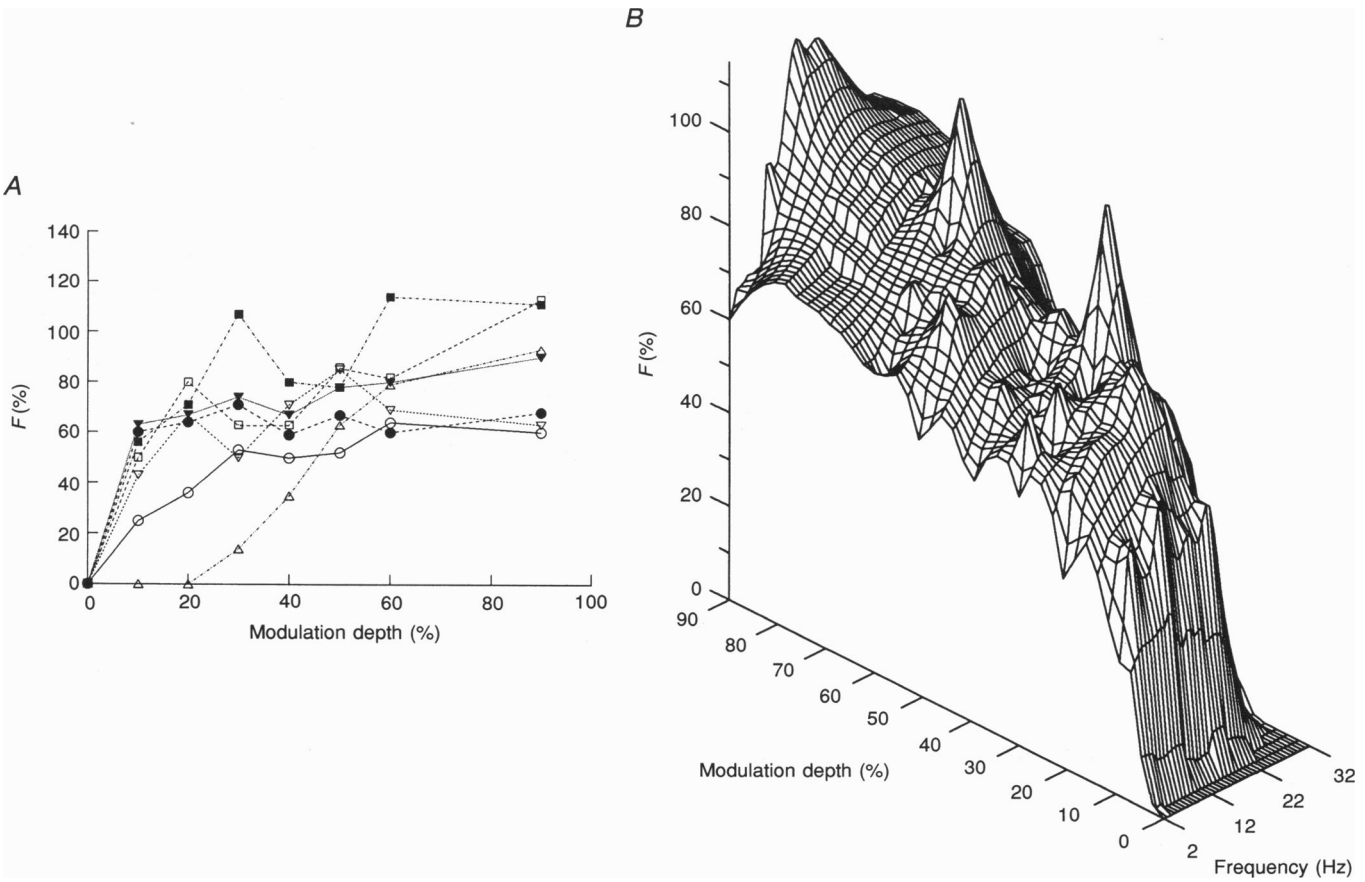

Figure 6. Blood flow changes for different flicker frequencies and modulation depths recorded on one cat

A, blood flow changes (F) at different modulation depths: ○, 2 Hz; ●, 4 Hz; ▽, 8 Hz; ▼, 10 Hz; □, 16 Hz; ■, 20 Hz; △, 32 Hz. Field size was 30 deg and mean retinal illuminance 30000 td. *B*, 3-D curve plotted from the same data.

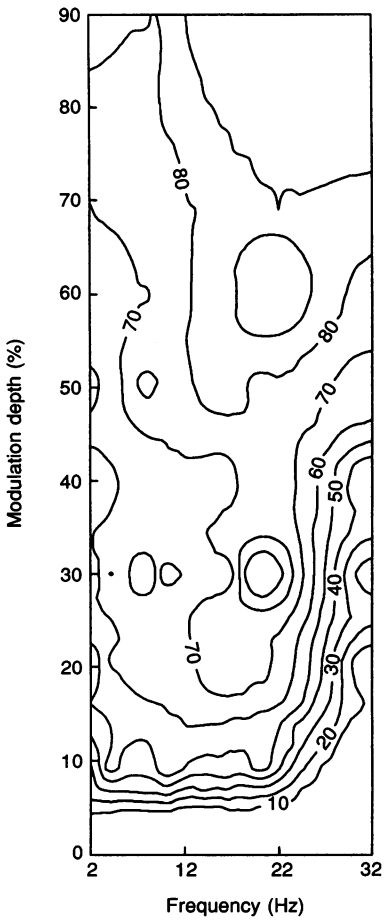

Figure 7. Contour map of modulation depth *versus* flicker frequency for different values of F

Each line corresponds to a constant F value as indicated on the line.

results indicated that the saturation occurred only at frequencies below 32 Hz (illustrated for one cat in Fig. 6*A*) and the variation of Q was not significant within the range of frequencies from 5 to 20 Hz. Figure 6*B* illustrates in 3-D, for the same cat, the variation of F with respect to different values of both flicker frequency and modulation depth.

Modulation thresholds *versus* flicker frequency

In previous studies, different response levels were chosen as criteria for establishing the temporal contrast sensitivity function (Cleland & Enroth, 1966; Sternheim & Cavonius, 1972; Riva *et al.* 1991). In order to determine how the selected criterion may affect this function, based on the data presented in Fig. 6*B*, a contour map which showed the

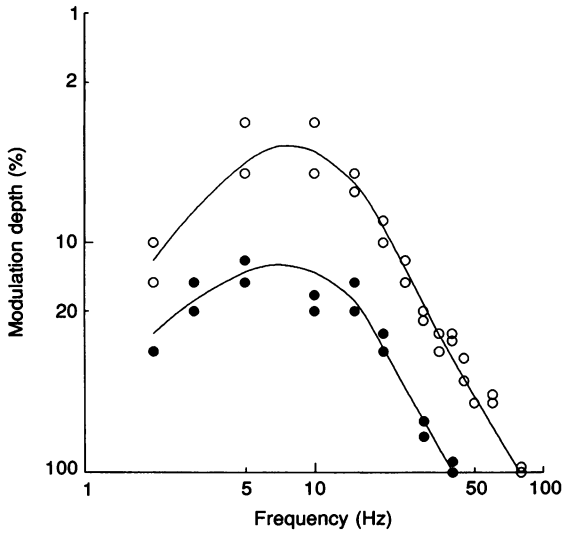

Figure 8. Temporal contrast sensitivity function of the blood flow changes

These results were recorded on one cat at two levels of mean retinal illuminance; ○, 30000 td; ●, 30 td.

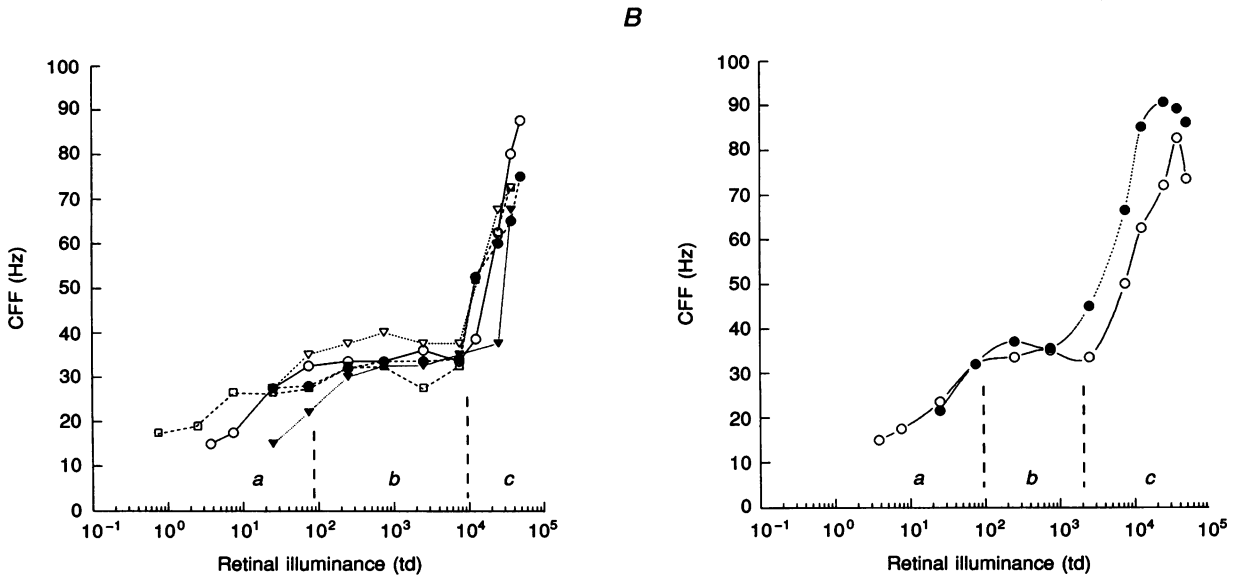

Figure 9. Blood flow critical flicker frequency (CFF) *versus* mean retinal illuminance recorded on two groups of cats

A, the stimulation site was around the optic nerve head. *B*, the stimulation site was at the area centralis. For both *A* and *B*, region *a* is the low-illuminance rising segment, region *b* is the medium-illuminance plateau segment and region *c* is the high-illuminance rising segment.

modulation depth *versus* flicker frequency for different F values was plotted (Fig. 7). Each line corresponds to a constant F value indicated on the line. In other words, these lines represent the temporal contrast sensitivity curves of different F values.

In the range of frequencies tested, for F up to 30% these curves are parallel to each other. Therefore, if, for instance, 30% of F instead of 10% is chosen as a criterion, their modulation depths differ by the same factor throughout the entire frequency range. But this is not the case for higher F values because the related curves are no longer parallel to each other. On another cat, however, these curves were parallel only in the range of F up to 20%. These facts suggest that the curve behaviour may depend not only on the value of the selected criterion but also on the animal. According to De Lange (1958), the visual system shows linear properties when it is stimulated by sinusoidal stimulation near threshold (the 'small signal' criterion). For these reasons we chose a minimum noticeable increase of blood flow (2%) as a criterion to determine the threshold values of parameters such as frequency and modulation depth.

Experiments to establish the temporal contrast sensitivity plots of F were carried out in five cats. The curves had a bandpass shape with a peak located between 10 and 20 Hz. The mean and S.D. of the high-frequency slopes were $1{\cdot}7 \pm 0{\cdot}3$ dec per dec. In one cat tested with different levels of illuminance, we found that, for a mean retinal illuminance of 30 td, the peak was located at about 10 Hz, the CFF was 40 Hz and the high-frequency slope was 1·65 dec per dec. For a mean retinal illuminance of 30 000 td the curve shifted along the vertical axis and the CFF increased to 80 Hz while the high-frequency slope remained unchanged. This behaviour was similar in all cats investigated. Typical curves obtained from one cat are shown in Fig. 8.

Blood flow critical flicker frequency *versus* mean retinal illuminance

The cats were dark adapted for 2–3 h before the experiments started. The stimulus-modulation depth was kept constant at 100% and the frequency was varied by steps. The mean retinal illuminance was varied over 3 dec and neutral density filters were used, starting with the highest density.

The results obtained in four cats showed that, when the mean retinal illuminance increased, the CFF first increased then levelled off before it increased again. The second increase was steeper than the first one. Based on the classification of Conner & MacLeod (1977), we subdivided the curve into three segments: low-illuminance rising (*a*); medium-illuminance plateau (*b*); and high-illuminance rising (*c*) (Fig. 9*A*). The slopes of the low-illuminance and high-illuminance segments were approximately 15 and 85 Hz dec^{-1}, respectively. The height of the plateau was about 35 Hz and the maximum CFF was as high as 90 Hz.

It has been reported that in the area centralis of the cat retina there is an important concentration of cones, while the retinal periphery is dominated by rods (Steinberg, Reid & Lacy, 1973). Thus, according to this finding, Fig. 9*A* shows the variation of the CFF value established in the

rod-dominated zone. In order to inspect whether this variation behaved differently in the area centralis (rod–cone zone), pilot experiments were carried out in two cats. In these cats, F was measured at the optic nerve head while the area centralis was stimulated. The experimental results are shown in Fig. 9*B*.

Comparing Fig. 9*A* with *B*, we found that: (i) the 'rod-dominated curve' had a longer medium-illuminance segment than that of the 'rod–cone curve' (over 2 dec of illuminance instead of over 1 dec); (ii) the high-illuminance segment slope in the 'rod-dominated curve' was 1·7 times as steep as the slope of the 'rod–cone curve'; and (iii) in the high-illuminance segment, the CFFs of the 'rod-dominated curve' increased continuously. In contrast, the CFFs of the 'rod–cone curve' first increased, then saturated and finally decreased.

DISCUSSION

The goal of this investigation was twofold; first, to explore the effects of sinusoidal stimulation on the blood flow at the optic nerve head and, second, to compare the results obtained with those reported in psychophysics and electrophysiology acquired under similar stimulation. As the visual system and visual signal processing mechanism consist of different stages and complex interactions, this same input triggers different outputs at various stages. Numerous investigative methods have been designed to study these outputs along the 'visual pathway'. Some methods have studied isolated cells or groups of the same type of cells (e.g. horizontal and ganglion cells), others have explored global responses of different types of cells. The ERG probes cells at the retinal level, and the VEP explores cells at the visual cortex level. The psychophysical methods investigate visual perception and report information which is completely processed and analysed. It is interesting to compare our data with those obtained by these methods in order to shed some light on how the blood flow at the optic nerve head couples with neural activity.

In the previous section, the influences on the blood flow of sinusoidal waveform stimulation of different frequencies, modulation depths and intensities were reported. In the following, these results will be compared with relevant findings in both cats and humans obtained in psychophysical and electrophysiological studies. For high modulation depth, it has been shown that flicker sensitivity does not depend on the stimulus waveform (Kelly, 1972); therefore our discussion will also include results elicited by other waveform stimulations.

Blood flow frequency response

The F-frequency response had a bandpass shape with a peak in the range from 5 to 20 Hz and its high-frequency slope was about 3 dec per dec. This slope was independent from the mean retinal illuminance. In psychophysics, to the best of our knowledge, no such investigation has been done. In electrophysiology, in the study of the human ERG evoked by sinusoidal waveform stimuli, Spekreijse *et al.* (1972) reported that the frequency response of the amplitude of the ERG's fundamental component behaved predominantly as a low-pass filter. The response started to decrease from 2 Hz but then slightly increased and reached a local maximum around 15 Hz before decreasing continuously with further increase in the flicker frequency. The high-frequency slope was about 2·5 dec per dec and the CFF about 100 Hz. These authors used a sinusoidal waveform stimulus of 30% modulation depth, 20 deg field size and 1590 cd m^{-2} luminance. Although the shapes of the frequency response and the CFF in this study and in the blood flow study (obtained with 30000 td mean retinal illuminance) were different, the high-frequency slope of both curves was similar. On the other hand, Baker & Hess (1984) found that with a pupil of a constant size and a uniform stimulation field, the human ERG-frequency response had a bandpass shape with a maximum range between 6 and 12 Hz, and the high-frequency slope was about 5 dec per dec. In comparison with our results (for a mean retinal illuminance of 3000 td), although the high-frequency slopes in the two studies were different, the curve shape, peak frequency and CFF value were similar. Table 1 reports the data of these studies along with others discussed below.

Van der Grind & Grüsser (1981) used a sinusoidal waveform stimulus of 200 cd m^{-2} luminance and 72% modulation depth to record the signals from three different types of horizontal cells of cats: Hn-, Hm- and Hw-units. They showed that the amplitude of the signals behaved as a low-pass filter. The high-frequency slope of the Hn-unit cell was about 2·5 dec per dec. The CFF of the Hn-unit was between 25 and 40 Hz, of the Hm-units between 55 and 70 Hz and of the Hw-units between 95 and 110 Hz. Hence, although the curve shapes of F and horizontal cell frequency responses were different, the high-frequency slope and CFF value of Hn-unit horizontal cells resembled the slope and the CFF value of F obtained with 3000 td (Table 1).

In the study of the human VEP, using an unpatterned red monochromatic stimulus of 60 deg, modulation depth 33% and 9300 td illuminance, Regan (1968) reported that the frequency response of the second harmonic component had a bandpass shape with a peak around 20 Hz, the high-frequency slope was about 2·9 dec per dec and the CFF about 30 Hz. These results are very similar to our results obtained with a mean retinal illuminance of 3000 td.

In the cat ganglion cell studies, Cleland & Enroth-Cugell (1966) used a sinusoidal stimulation of 50000 td and 50% modulation depth and found that the frequency responses of both on-centre and off-centre ganglion cells had a bandpass shape. However, the high-frequency slope of the on-centre cells is steeper than that of the off-centre cells (4 and 1·2 dec per dec, respectively). Furthermore, the maximum frequency of the on-centre cells was about 20 Hz and of the

Table 1. Characteristics of the frequency responses and corresponding adaptation light levels extracted from different investigations

Reference	Experiment	Adaptation (cd m^{-2})	Peak (Hz)	HF slope (dec per dec)	CFF (Hz)
Spekreijse *et al.* (1972)	Human ERG	1590	—	2·5	100
Baker *et al.* (1984)	Human ERG	100	6–12	5·0	30
Van der Grind *et al.* (1981)	Cat horizontal cells	200	—	2·5	30
Regan (1968)	Human VEP	9300*	20	2·9	30
Cleland *et al.* (1966)	Cat on-centre ganglion cells	50000*	10	4·0	20
	Cat off-centre ganglion cells	50000*	10	1·2	40
Fukada *et al.* (1971)	Cat on-centre Y ganglion cells	1080	10–30	2·3	70
	Cat off-centre Y ganglion cells	1080	10–30	1·7	50
Frishman *et al.* (1987)	Cat on-centre X ganglion cells	340	1, 50	7·7	95
	Cat on-centre X ganglion cells	2·8	10	5·1	53
	Cat on-centre X ganglion cells	0·021	4	3·7	30
	Cat on-centre Y ganglion cells	440	10, 60	8·1	120
Present study	Cat blood flow	600 (30000*)	20	3·0	75
	Cat blood flow	60 (3000*)	10–20	2·9	35

CFF, critical flicker frequency; HF, high-frequency. * Value in trolands.

off-centre cells about 40 Hz. Similarly, Fukada & Saito (1971), in the study of the frequency response of cat optic nerve fibres to flicker stimulation, reported that the frequency response in both on-centre and off-centre Y ganglion cells (phasic type) had a bandpass shape with a peak in the range of 10–30 Hz while the responses of the X ganglion cells (tonic type) were independent of the flicker frequency.

In an extensive study of cat retinal ganglion cells, Frishman, Freeman, Troy, Schweitzer-Tong & Enroth-Cugell (1987) used a stimulation uniform field of 31 × 22 deg size, the luminance of which was modulated sinusoidally. They reported that for the on-centre X ganglion cells or on-centre Y ganglion cells the frequency responses had a bandpass shape. The peak, high-frequency slope and CFF depended on the mean retinal illuminance and the type of cell.

Hence, based on the curve shape, we noticed that the F-frequency response behaved in a similar way to those obtained in VEP and ganglion cell studies. However, the high-frequency slope of the F-frequency response resembled those obtained in human ERG and VEP as well as cat horizontal cells and on-centre Y ganglion cells. Further, the CFF value was closer to those obtained in human ERG, VEP and cat Hn-unit horizontal cell studies. Therefore, in considering the curve shape, high-frequency slope and CFF value all together, the F-frequency response resembles that of human VEP reported by Regan (1968) and cat on-centre Y ganglion cells reported by Fukada & Saito (1971).

Blood flow *versus* modulation response

The magnitude of the increase in blood flow was a sigmoidal function of modulation depth. F started to increase at about 10% modulation depth and reached a saturation level at about 50% modulation depth. In some cats, for reasons yet unknown, saturation was not reached, even at 100% modulation depth. The experimental results also showed that the saturation level and the slope of the curve calculated at half-F_{max} depended on the mean retinal illuminance. However, while the variation of the saturation was random, the variation of the slope was consistent. More experiments need to be done to establish the precise relationship between these parameters and the mean retinal illuminance.

Regan & Beverley (1973), using sinusoidal waveform stimuli in man, showed that the amplitude of the fundamental of the VEP *versus* modulation depth also behaved as a sigmoidal function. This amplitude started to increase at 10% of modulation and reached saturation level at about 40%. By contrast, the second harmonic of the responses and the evoked potential recorded at sites on the scalp other than the visual cortex behaved differently. Furthermore, in comparing these results with those obtained by a psychophysical method which consisted of subjective estimates of the magnitude of flicker sensation at different modulation depths, these authors concluded that psychophysical results correlated poorly with the VEP results.

Spekreijse (1966) also found this sigmoidal behaviour in human VEP studies by stimulating either one eye, or both eyes simultaneously, with sinusoidal stimulation. Further, this author indicated that the stimulus intensity and frequency influenced the saturation level. He suggested that the saturation mechanism may be located in different cortical subsystems.

Our results also resemble ganglion cell activities. According to Cleland & Enroth-Cugell (1966), using sinusoidal stimulation of 8 Hz in cats, the on-centre cell responses rapidly reached saturation level at modulation depths as

Table 2. Peak locations and high-frequency (HF) slopes of the temporal contrast sensitivity plots extracted from different investigations

Reference	Experiment	Peak (Hz)	HF slope (dec per dec)
Vo Van Toi *et al.* (1991)	Human psychophysics	10	3·4–6·3
Sternheim *et al.* (1972)	Human ERG	10	0·7
	Human VEP	10	3·3
	Human psychophysics	10	3·8
Cleland *et al.* (1966)	Cat on-centre ganglion cells	4–6	2·6
Riva *et al.* (1991)	Cat blood flow	10	1·7
Present study	Cat blood flow	10	1·7

low as 20%. By contrast, the off-centre cell responses varied linearly with respect to the modulation depths.

Hence, in comparison with previous investigations, we can conclude that the cat blood flow modulation response resembles that obtained in human VEP reported by Spekreijse (1966) as well as Regan & Beverley (1973), and that obtained with cat on-centre ganglion cells as reported by Cleland & Enroth-Cugell (1966). According to Regan & Beverley (1973), these modulation responses differed from human psychophysical data. The present results suggest that the mechanisms involved in the blood flow and psychophysical studies may not share the same process. It is unclear, however, if there is any relationship between the localizations of the blood flow saturation mechanism and of the VEP-saturation mechanism proposed by Spekreijse (1966).

Blood flow temporal contrast sensitivity function

Using the 'small signal' criterion, we established the blood flow temporal contrast sensitivity plot, which was characterized by its bandpass shape. The maximum CFF value was about 95 Hz and the high-frequency slope was about 1·7 dec per dec. The high-frequency slope in the present study was identical to that reported by Riva *et al.* (1991) although these authors used 30% change in blood flow as a criterion to determine the modulation thresholds. This result is not surprising because, as indicated in Fig. 7, for up to 30% change, the slope was not affected.

In human psychophysics, the same curve shape has been reported. However, the CFF values were never higher than 70 Hz (Vo Van Toi, Burckhardt & Grounauer, 1991) and the high-frequency slope ranged from 3·4 to 6·3 dec per dec, which is 2–3·7 times as much as the results presented here (Table 2).

Sternheim & Cavonius (1972) used a stimulus which consisted of a grating whose luminance was modulated sinusoidally to establish the psychophysical temporal contrast sensitivity plots and record the human ERG and VEP responses. From these responses, and based on a threshold criterion of 0 μV amplitude, these authors also established the ERG and VEP temporal contrast sensitivity plots. They reported that all the curves had a bandpass shape with a peak at 10 Hz and the high-frequency slope of the ERG temporal contrast sensitivity plot was not as steep as those of the VEP and psychophysical temporal contrast sensitivity plots (0·7, 3·3 and 3·8 dec per dec, respectively). They suggested that the mechanism which governed the psychophysical high-frequency perception was more central. In comparison with the present study, although the shapes of the temporal contrast sensitivity curves obtained in all the studies were comparable, the high-frequency slope obtained in the present study (1·7 dec per dec) is 2·5 times that in the ERG study and less than half of that in the psychophysical and VEP studies (Table 2). This suggests that the mechanism which governs the high-frequency sensitivity of the blood flow is located between those which govern the high-frequency sensitivity of the ERG and VEP.

Cleland & Enroth-Cugell (1966) found the same curve shape as in the present study by stimulating the on-centre ganglion cell. However, the peak of the on-centre ganglion cell curve was around 5 Hz which was different from the peak in our study, and the high-frequency slope was about 2·6 dec per dec in comparison with 1·7 dec per dec in our study.

Hence, in all psychophysical, electrophysiological and blood flow studies the temporal contrast sensitivity curves have similar shape but different high-frequency slopes. The high-frequency slope in the blood flow study is close to that in the ganglion cell study but less than half of that in the psychophysical or VEP study and 2·5 times higher than that in the ERG study. These results suggest that the blood flow changes may be closely related to the activities of ganglion cells.

Blood flow critical flicker frequency *versus* mean retinal illuminance function

In the present study the CFF *versus* mean retinal illuminance function was a two-branched curve which could be divided into three segments; low, medium and high illuminance. In human psychophysics, similar results have been reported and the CFF varies with respect to the mean retinal illuminance according to the Ferry-Porter law. Hecht & Verrijp (1933), using flash stimulation,

suggested that the psychophysical two-branched curve resulted from the contributions of both rods and cones in the process of flicker perception. In their results, the CFF at the medium-illuminance segment was about 15 Hz and the maximum CFF was about 55 Hz. On the other hand, Conner & MacLeod (1977), using sinusoidal stimulation in human subjects, reported that the two-branched curve may represent the activity of rods only.

In our study, no attempt was made to isolate rod activities from cone activities. As the same curve shape was obtained when either the area centralis or the zone near the optic nerve was stimulated, we can conclude that, in cats, the two-branched curve indicates rod-dominated activities or rod–cone activities (Fig. 9*A* and *B*). However, in comparison with the rod–cone curve, the rod-dominated curve had a longer medium-illuminance segment and a high-illuminance segment slope twice as steep (85 *versus* 50 Hz dec^{-1}). Furthermore, at a very high mean retinal illuminance, the CFFs of the rod–cone curve started to drop while those in the rod-dominated curve continued to increase.

Hecht & Shlaer (1936) determined the effect of the mean retinal illuminance on the CFF using various monochromatic stimuli. Inspection of the lower branch of their curves, in particular those obtained with 450 and 670 nm, revealed that the 450 nm curve had a longer medium-illuminance segment than the 670 nm curve. Because the 450 nm stimulus elicits mostly rods and the 670 nm elicits mostly cones, these results suggest that a longer medium-illuminance segment is associated with a more important contribution of rods. Our findings support such an interpretation.

In electrophysiology, Dodt & Enroth (1954) recorded the ERG on cats and determined the function CFF *versus* mean retinal illuminance. Figure 10*A* and *B* reproduces the curves from the present study, the cat ERG study (Dodt & Enroth, 1954) and human psychophysical studies (Hecht & Verrijp, 1933; Conner & MacLeod, 1977). These results show that the maximum CFF in the blood flow study is much higher than those in the ERG and psychophysical studies. To the best of our knowledge no similar investigation has been done on ganglion cells.

Table 3 indicates the slopes of the high- and low-illuminance segments derived from these investigations. In comparison with psychophysical results reported by Hecht & Verrijp (1933), the curve shapes in both studies are similar but the high-illuminance segment slopes in our study are 4 times greater (50 and 12 Hz dec^{-1}, respectively). The same factor is found when comparing the low-illuminance segment slopes (15 and 4 Hz dec^{-1}, respectively). Concerning the rod-dominated data, in comparison with the results reported by Conner & MacLeod (1977), the slope of the low-illuminance segment in our study is 2·5 times that in psychophysical study (15 and 6 Hz dec^{-1}, respectively) and the slope of the high-illuminance segment in our study is 12 times greater (85 and 7 Hz dec^{-1}, respectively).

The ERG curve shown by Dodt & Enroth (1954) had a short medium-illuminance segment and the slopes of the low-

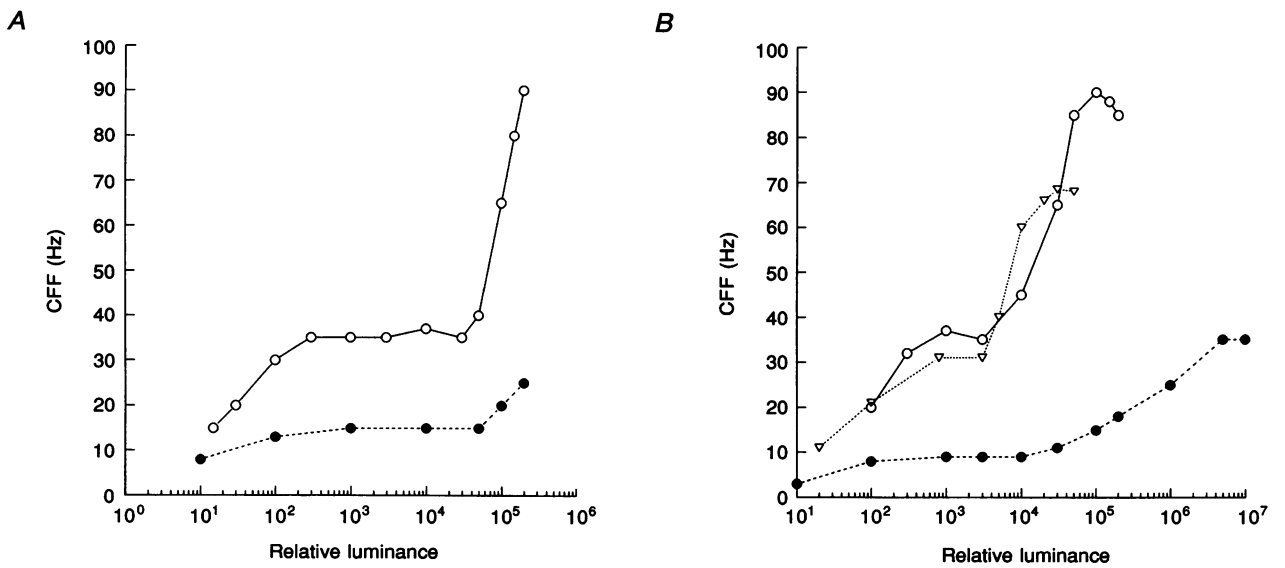

Figure 10. Comparison of data obtained from various investigations of CFF

A, data from present blood flow study (○) and from a human psychophysical study (●) (Conner & MacLeod, 1977, Fig. 1*b*). *B*, data from the present blood flow study (○), from a human psychophysical study (●) (Hecht & Verrijp, 1933, Fig. 2, 5°), and from a cat ERG study (▽) (Dodt & Enroth, 1954, Fig. 3).

Table 3. Comparison of high- and low-illuminance rising slopes of different studies

Reference	Experiment	HIR slope (Hz dec^{-1})	LIR slope (Hz dec^{-1})
Hecht *et al.* (1933)	Human psychophysics	12	4
Conner *et al.* (1977)*	Human psychophysics	7	6
Dodt *et al.* (1954)	Cat ERG	50	10
Present study	Cat blood flow	50	15
Present study*	Cat blood flow	85	15

HIR, high-illuminance rising; LIR, low-illuminance rising. * Data possibly dominated by rod activities.

illuminance and high-illuminance segments were 10 and 50 Hz dec^{-1}, respectively. These characteristics are very similar to the results which we obtained when only the area centralis was stimulated.

Hence, although the curve shapes in our study and in human psychophysical study were similar, the slopes of the high-illuminance and low-illuminance segments were quite different. In contrast, there is a better match between our blood flow data and the cat ERG data.

Overall, the investigation presented here revealed that the blood flow changes observed at the optic nerve head depended on different characteristics of sinusoidal stimulation. The various functions expressing this dependence were similar to those found in psychophysics and electrophysiology. This similarity strongly suggests that the blood flow is tightly coupled to neural activity and this coupling can be seen in the optic nerve head in the same way as in other neural tissues.

As the retinal illuminance influences these functions, it is important to use a stimulation which maintains the retinal adaptation constant. Further, the frequency response and the temporal contrast sensitivity function of the blood flow depend on the flicker frequency. Their bandpass shape implies that, for flicker frequencies lower than 20 Hz, it is better to use stimulation of sinusoidal waveform, rather than complex waveforms, because the latter have harmonics which may make the data analysis more difficult.

The results of four investigative methods showed a general relationship between blood flow change and ganglion cell activities. One of these, the temporal contrast sensitivity function, was particularly appropriate (as indicated in Table 2) to point out the possible location, in the neural pathway, where blood flow is regulated. This may be because 'small signal' criteria were used to investigate the blood flow instead of strongly modulated stimuli.

The variability among individual cats, usually depicted as the variation of the magnitude of the blood flow change from one cat to the others, generally occurred without marked changes in the characteristics of the functions. One exception, however, concerns the modulation responses. In some cats these responses were linear while in others they were sigmoidal, but it is noteworthy that the linear responses occurred when F_{max} was relatively small. The reasons for this discrepancy are still unclear and more investigations would be needed to understand it.

In the investigation of the variation of the critical frequency which elicited minimum change in blood flow with respect to the retinal illuminance, the Ferry-Porter law was demonstrated. Pilot experiments showed that the high-illuminance segment of the function behaved differently depending on whether the stimulated zone was the area centralis or optic nerve head. As a 30 deg field was used, it is unclear if this effect is due to the different contributions of rod or cone.

In summary, in the investigation presented here we explored the blood flow changes evoked by the frequency, modulation depth and luminance of a sinusoidal stimulation. This study showed the following.

(1) The sinusoidal stimulation always caused an increase in blood flow. The amount of this increase depended on several factors – stimulus frequency, modulation depth and luminance.

(2) In the study of the influences of flicker frequency, F had a bandpass shape with a maximum located between 5 and 20 Hz. Overall, the results obtained were different from those of horizontal cells and ERG but similar to those from the VEP and ganglion cells.

(3) In the study of the influences of modulation depth, the results showed that when the modulation depth increased, F increased and, most of the time, rapidly reached a saturation level as a sigmoidal function, similar to that reported in the VEP and ganglion cell studies.

(4) In the study of the influences of mean retinal illuminance, the CFF *versus* mean retinal illuminance curve consisted of two branches which may be attributed to the contributions of both rods and cones; this same curve shape was also reported in the ERG and psychophysical studies.

(5) The temporal contrast sensitivity function established using a minimum blood flow change as a criterion had a

bandpass shape and its high-frequency slope was comparable to the slope obtained in the ganglion cell studies but greater than that obtained in the ERG studies and smaller than that obtained in the psychophysical and VEP studies.

This experimental study therefore demonstrates that sinusoidal waveform stimulation is a powerful tool to investigate the behaviour of blood flow at the optic nerve head in a complete and non-invasive way. The results obtained suggest that, as in other neural tissues, the blood flow in the optic nerve head is tightly coupled to neural activity. Further, the characteristics of the blood flow changes correlate well with those of the ganglion cells, supporting and extending previous findings (Riva *et al.* 1991).

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Acknowledgements

This work has been supported by a grant from the 'Fondation pour la création de l'Institut de Recherche en Ophtalmologie à Sion' (to V.V.T.) and NIH grant no. EYO-3242 (to R.C.). We would like to thank Steve Cranstoun, Benno Petrig and Arthur Uhlir for their assistance.

Received 24 March 1994; accepted 6 June 1994.