Frequency and Luminance-Dependent Blood Flow and K⁺ Ion Changes During Flicker Stimuli in Cat Optic Nerve Head

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Purpose. The purpose of this study was to investigate whether blood flow in the cat optic nerve head (ONH) is related to increased neuronal activity elicited by diffuse luminance flickering light stimulation.

Methods. ONH blood flow was measured by laser Doppler flowmetry in anesthetized cats during 1 to 3 minutes of flickering light stimulation at controlled luminance and frequency (n = 227 measurements in 18 cats) using either a conventional visual stimulator (repetitive short flashes) or a sinusoidally varying light stimulator. Potassium ion concentration ([K⁺]) changes in the vitreous humor immediately in front of the optic disk were measured with neutral carrier K⁺ ionophore liquid membrane microelectrodes. Effects of varying flicker frequency (2 to 80 Hz) at constant luminance were quantified. Effects of luminance were quantified by varying the modulation depth of the stimulus at constant frequency.

Results. Both ONH blood flow and [K⁺] increased during flicker stimulus with an average slope of 0.305% ± 0.064% (SE)/µM [K⁺] (257 measurements in 18 cats). The peak ONH blood flow increase was 59% ± 11% above baseline at 33.3 ± 3.1 Hz. The peak [K⁺] increase was 188 ± 42 µM above baseline at 38.3 ± 3.3 Hz. Both ONH blood flow and [K⁺] changes had similar bandpass characteristics with frequency, first increasing, then dropping off at higher frequencies (122 measurements in 10 cats). Both frequency responses were described by power law functions (y = αfⁿ). Luminance responses for both ONH blood flow and [K⁺] changes could be fit by a modified Hill model and were 50% of maximum at light modulation depths of 21.2% ± 4.6% and 22.5% ± 3.7%, respectively (53 measurements in 5 cats).

Conclusions. Increases in ONH blood flow were correlated with changes in [K⁺]. Both responses were remarkably similar, with no significant differences in the frequency for peak responses in ONH blood flow or [K⁺], in low- and high-frequency power law exponents of the two responses, or in the 50% response to light modulation. The results are consistent with close coupling of neuronal activity and ONH blood flow. Invest Ophthalmol Vis Sci. 1995; 36:2216-2227.
by tissue, and the resultant photocurrent of the detector is processed electronically. The principle of measurement is based on the Doppler shift in frequency caused by the moving red blood cells within a small volume of illuminated tissue. Although the measurement cannot be used for absolute blood flow measurements because of uncertainty in knowing the volume sampled by the incident laser light and the multiple light-scattering properties of tissue, the LDF technique is capable of making relative blood flow measurements with high sensitivity and with high spatial and temporal resolution. Previous LDF studies in cats have shown that ONH blood flow increases with systemic hypoxia, remains relatively constant when the intraocular pressure is elevated, and can be influenced by pharmacologic agents.\(^6\)\(^7\) Optic nerve head blood flow also has been shown to increase with flickering light stimuli in the cat eye,\(^5\)\(^8\) an effect that demonstrated for the first time that ONH blood flow is coupled to increased neuronal activity.

In the current in vivo study, we measured changes in ONH blood flow in the cat eye during flickering light stimuli. Indirect measurements of neuronal activity were made from changes in \([K^+]\) in the vitreous humor with ion-sensitive microelectrodes placed immediately in front of the optic disk. The variation in both ONH blood flow and \([K^+]\) with frequency of the flicker stimulus were quantified. In addition, the effect of luminance was investigated by varying the depth of light modulation. These measurements correlated with changes in ONH blood flow during stimulation by flickering light and confirmed that there was tight coupling between ONH blood flow and neuronal activity.

**METHODS**

The cat eye preparation has been described in detail.\(^2\)\(^3\)\(^5\)\(^7\) All experimental protocols conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and guidelines of the Presbyterian Medical Center of Philadelphia. Cats (2 to 4 kg) were premedicated with atropine (0.04 mg/kg) and anesthetized with ketamine hydrochloride (22 mg/kg) and acepromazine maleate (2 mg/kg) during initial preparation. Catheters were placed in the femoral artery and vein, and tracheostomy was performed. Volatile anesthetics were delivered during experimental measurements. Each cat was mechanically ventilated with 21% \(O_2\), 79% \(N_2\), and 1.5% to 2.5% enflurane. Pancuronium bromide was delivered continuously (0.15 mg/kg per hour) after an initial dose (0.2 mg/kg). A stainless steel ring was sutured to the eye with stitches at the limbus to stabilize eye position. The pupil was dilated with 1% tropicamide and 10% phenylephrine, and a zero dioptr contact lens was placed on the cornea along with a drop of Healon (Pharmacia Ophthalmics, Monrovia, CA).

Theoretical principles for neutral carrier ionophore liquid membrane \(K^+\) ion-sensitive microelectrodes are described by Ammann\(^8\) and Buerk.\(^9\) The potential (EMF), including possible interferences from other ionic species, can be described by the semi-empirical Nicolsky–Eisenman equation

\[
EMF = E_0 + \frac{RT}{z_iF} \ln \left[ \frac{[K^+]_{\text{external}}}{[K^+]_{\text{internal}}} \right] \tag{1}
\]

where \(E_0\) is a reference potential (which may include constant liquid junction, metal–metal, or boundary potentials), \(R\) is the gas constant, \(F\) is Faraday's constant, \(T\) is absolute temperature, and \(S_i\) are the membrane selectivity factors for each of the interfering ions. The ionic species of interest has an activity \(a_i\) and charge \(z_i\), and each interfering ion has an activity \(a_j\) and charge \(z_j\). With the selectivity of the ionophore membrane, the interference from other ions is negligible. Therefore, the EMF across the membrane depends on the \(K^+\) concentration difference between the external medium and the internal solution filling the \(K^+\) microelectrode, given by

\[
EMF = \frac{RT}{2.303F} \log_{10} \frac{[K^+]_{\text{external}}}{[K^+]_{\text{internal}}} \tag{2}
\]

The potential is negative when \([K^+]_{\text{external}} < [K^+]_{\text{internal}}\). Microelectrodes with neutral carrier ionophore liquid membranes have lower impedances and faster time responses than ion exchange resin \(K^+\) microelectrodes.

Glass micropipettes were pulled out and the tips ground off at an angle of approximately 30°. After cleaning with acetone, the tip was silanized (dimethyl-dichlorosilane; Fluka Chemicals, Buchs, Switzerland) and baked. The tip was filled with 100 mM KCl solution by applying vacuum. Then a small amount (5 to 20 \(\mu\)m long) of a neutral carrier ionophore mixture (cocktail A; Fluka Chemicals) was sucked into the tip. A syringe was used to back-fill the body of the electrode with 100 mM KCl solution, and a chlorided silver wire was inserted to complete the electrical circuit. After construction, \(K^+\) microelectrodes were stored with the tips submerged in 100 mM KCl solution and a light-tight container and were used within 1 to 2 weeks of fabrication. Before each experiment, calibrations were made in a recirculating system by sequentially diluting an initial volume of 100 mM KCl to 3 mM or less. A typical calibration curve for a \(K^+\) microelec-
FIGURE 1. Semilogarithmic calibration curve for a neutral carrier ionophore membrane K⁺ microelectrode obtained at 20°C by sequential dilution, followed by the addition of concentrated K⁺ solutions. Slope is 42 mV/decade [K⁺] (r = 0.997).

trode at room temperature is shown in Figure 1. Data points were obtained by dilution, then by adding K⁺. Semi-logarithmic sensitivities averaged approximately 45 mV/decade [K⁺] at 37°C. Potentiometric measurements were made with a high impedance electrometer (model 610C; Keithley Instruments, Cleveland, OH).

To achieve the best resolution for measurements in the eye, the baseline potential (typically between 100 to 120 mV) was zero-suppressed, and the electrometer gain increased by at least 10-fold (typically from 300 mV to 30 mV full scale).

K⁺ microelectrode measurements were limited to sites either touching the ONH surface or in the vitreous humor very close to the ONH. The fundus was observed through a Zeiss (Thornwood, NY) operating microscope at ×40 with a halogen lamp for illumination during placement of the microelectrodes using a custom three-dimensional microdrive system designed by Pournaras et al. The mechanical linkage rotated about the point of insertion, allowing precise positioning anywhere within the field of view. The microdrive can advance or withdraw in 1-μm steps along the penetration axis. Buerk et al. used this microdrive with PO₂ microelectrodes to measure spatially detailed O₂ gradients around retinal arterioles and venules in the cat eye. Single-barrel K⁺ ion-sensitive microelectrodes with 1-mm shaft diameters were inserted into the vitreous through a hypodermic needle. Measurements were made relative to a grounded, chlorided silver wire placed in the vitreous through a second hypodermic needle puncture.

After positioning the microelectrode, a laser beam was focused through the operating microscope onto a nearby spot (approximately 150 μm in diameter) on the ONH, avoiding visible blood vessels. The microscope was modified for laser Doppler measurements as described by Shonat et al. The laser system uses diode lasers that emit light in the near-infrared region (wavelength at 812 nm), allowing measurements to be made in the dark. An optical fiber in the microscope’s field collected the scattered light within a 5° angle, which was amplified by a photodetector. Electronically separated DC and AC components of the photodetector current were then processed through the electronics of a commercial instrument (Periflux PF3; Perimed, Stockholm, Sweden) to determine red blood cell flux in relative units. After positioning the microelectrode and focusing the laser beam, the halogen lamp and room lights were turned off and the eye was allowed to dark adapt for 20 to 30 minutes before testing flicker responses.

Diffuse luminance flickering light was delivered to the eye through the operating microscope by a fiber-optic cable connected either to a Grass PS-22 visual stimulator (20-μsec flashes) (Grass Instruments, Quincy, MA) or to a custom-built sinusoidal light source. The sinusoidal stimulator varied its light output by rotating polarized filters. The light source had a color temperature of 2200 K, with maximum luminance of 30,000 trolands. The sinusoidal waveform can be described as

\[ L = L_0(1 + M \cos(2\pi ft)) \]  

where \( L_0 \) is the average luminance, \( M \) is the modulation depth, \( f \) is the frequency, and \( t \) is time. The instrument could maintain constant average luminance over a wide range of flicker frequencies (1 to 100 Hz), or it could be used to vary the depth of light modulation from maximum (100%) to nearly zero at any frequency. With the sinusoidal stimulator, it was possible to maintain the same average luminance over a wider range of flicker frequencies or to make continuous variations in the luminance at a fixed frequency. Flickering light for either stimulator was centered at the ONH, covering an area of approximately 30° of the fundus.

Experimental LDF and K⁺ microelectrode responses were measured with flickering light at frequencies between 2 to 80 Hz or with varying depths of light modulation at a fixed frequency near the maximum ONH blood flow response, usually in the 20- to 30-Hz range. Trials were conducted in a randomized order, with the period of flickering light stimulation lasting for 1 to 2 minutes. Flicker tests were spaced several minutes apart to permit sufficient time for the LDF signal to return to prestimulus levels. Laser Doppler flowmetry and microelectrode signals were continuously recorded on a video cassette recorder-based
ONH K+ and Blood Flow During Flicker

FIGURE 2. Experimental K+ microelectrode potential measurement (top) and laser Doppler blood flow measurement (bottom) in the cat optic nerve head during 20-Hz flickering light at full modulation (100%). The flickering light stimulus started at $t = 0$ and lasted for approximately 105 seconds. Initial rates of increase, time trends during the final minute of stimulus, and return rates are indicated by dashed lines.

tape recorder (model 820; A.R. Vetter, Rebersburg, PA) and on a strip chart recorder. Tape recorded signals were later played back and digitized on a data acquisition system (80286-based computer, 12-bit accuracy) at sampling rates between 1 to 5 Hz, with low-pass filtering to eliminate 60-Hz aliasing. Data sets were then separated into smaller segments for subsequent computer analysis and plotting of individual responses (Sigmastat 1.01 and Sigmaplot 5.0; Jandel Scientific, San Rafael, CA). When plotting potential changes, upward changes are shown to correspond with increases in [K+]. Otherwise, concentration changes are shown after converting the K+ microelectrode signal (mV) using the semi-logarithmic calibration curve (equation 2) obtained for each microelectrode. Frequency responses and light modulation responses were curve fit to mathematical models described below, using the nonlinear Marquardt-Levenberg least squares optimization method (Sigmaplot 5.0) to estimate model parameters.

RESULTS

Optic nerve head blood flow increased when the eye was stimulated with flickering light from either the Grass PS-22 or the sinusoidal visual stimulator. The K+ microelectrode potential change with either type of flickering light stimulus always corresponded to an increase in [K+]. A typical experimental measurement is shown in Figure 2.

Simultaneous changes in K+ microelectrode po-tential (top panel) and ONH blood flow by LDF (bottom panel) are shown during 20-Hz flickering light delivered with the sinusoidal visual stimulator at maximum luminance (100% modulation depth). Often, a biphasic LDF response was observed, with a rapid increase within the first 15 to 30 seconds (upward dashed line, bottom panel), followed by a smaller, but sustained, slower increase over the next 1 or 2 minutes (second dashed line). The LDF change was usually faster than the [K+] change, reaching 50% several seconds before the 50% [K+] change. Usually, [K+] rose in a more monotonic fashion than the biphasic ONH blood flow response and was essentially at steady state by 1 to 2 minutes. Average changes during the last 45 seconds to 1 minute of the flicker responses were used for the analyses described below. In some cases, the LDF signal was still increasing by a small amount. It was not unusual to see low-frequency (5 to 10 per minute) ONH blood flow oscillations during the prestimulus control period and during the flicker response. Oscillations in [K+] were usually not observed. After stopping the flicker stimulus, both ONH blood flow and the K+ potential returned rapidly back (downward dashed lines) to prestimulation baseline levels (horizontal dashed lines). In most cases, there was a small undershoot in ONH blood flow also was seen after stopping the flicker stimulus.

Frequency-Dependent Changes

The magnitudes of ONH blood flow and K+ potential responses during flicker were found to vary with the frequency of the stimulus. Three-dimensional plots of seven individual pairs of K+ microelectrode (left panel) and ONH blood flow (right panel) responses are shown in Figure 3. A series of measurements in one cat experiment are shown during 90 seconds of flicker stimuli at frequencies from 5, 10, 20, 30, 40, and 60 to 80 Hz (increasing from right to left). The largest increase in K+ potential occurred at 30 Hz (fourth curve, left panel) whereas the largest ONH blood flow increase occurred at 40 Hz (fifth curve, right panel). The magnitude of both responses increased with frequency up to their respective maximum levels, then declined at higher frequencies.

Mean changes in relative ONH blood flow and [K+] were obtained by averaging data during the final minute of the flicker stimulus for each flicker stimulus trial. Both blood flow and [K+] responses could be fit to a general power law function of frequency. For low frequencies, data were fit to the increasing function.

$$y_{\text{low}}(f) = a_{\text{low}} f^\gamma_{\text{low}} \quad \text{for } f < f_0$$

(4)

and at high frequencies by the decreasing function

$$y_{\text{high}}(f) = a_{\text{high}} f^{-\gamma_{\text{high}}} \quad \text{for } f > f_0$$

(5)
where $f_0$ is the frequency

$$f_0 = \frac{1}{a_{\text{low}} r + a_{\text{high}} r}$$  \hspace{1cm} (6)$$

where the two functions intersect. The peak value

$$f_{\text{peak}} = a_{\text{low}} f_0^{n_{\text{low}} r} = a_{\text{high}} f_0^{n_{\text{high}} r}$$  \hspace{1cm} (7)$$

occurs at $f_0$. The coefficients $a_{\text{low}}$ and $a_{\text{high}}$, and exponents $n_{\text{low}}$ and $n_{\text{high}}$ were determined by nonlinear regression (Marquardt–Levenberg) and illustrated by double logarithm plots of the mean ONH blood flow change or the $[K^+]$ change with frequency. The peak value and $f_0$ were calculated from equations 6 and 7 for each parameter set estimated from the data.

Examples of experimental responses are shown in Figure 4 for frequency-dependent changes with constant average luminance measured in three cat experiments. In Figure 4A, the logarithm of the mean $[K^+]$ changes with the logarithm of flicker frequency are plotted. One cat (top panel) had a more complex four-component, bandpass filter-type response with a slow increase for $f < 15$ Hz, a rapid rise in the 15 to 20 Hz range, a flat response in the range between 20 to 40 Hz, then a drop to $f > 40$ Hz. The majority of responses displayed a simpler bandpass behavior, such as the responses shown in the middle and bottom panels, and could be described by the two power law functions described above. Most of the cats (5 of 9) had peak $[K^+]$ responses at approximately 50 Hz, as illustrated in the middle panel. The highest peak, $f_0$, was at approximately 50 Hz for the cat illustrated in the bottom panel. Measurements at two sites are shown, near the center of the optic disk (solid circles) and closer to the rim (open circles). Both blood flow and $[K^+]$ responses were larger near the center, but the peak $f_0$ was similar for both locations.

In Figure 4B, the double logarithm plots of the mean increase in ONH blood flow with flicker frequency are shown for the same three cats. The cat with the atypical $[K^+]$ response (top panel, Fig. 4A) had a similar four-component, bandpass filter description of blood flow (top panel, Fig. 4B) with a flat ONH blood flow response in the 20- to 40-Hz range. The simpler two-component analysis (equations 4 to 7) was adequate for all the ONH blood flow responses measured in the other cat experiments. The corresponding blood flow responses are shown for the previous two $[K^+]$ examples (middle and bottom panels of Fig. 4B). For the two sites measured in one cat (bottom panel), the ONH blood flow was higher near the center (solid circles) than at the site closer to the rim (open circles). The peak flow near the center was also at a slightly higher frequency (approximately 50 Hz) than near the rim (40 Hz) for this experiment.

In Figure 4C, linear regressions (dashed lines) between the increase in ONH blood flow and the $[K^+]$ change are shown for these three cats using the same symbols as in Figures 4A and 4B. The 95% confidence intervals (dotted curves) also are shown. The corresponding slopes and correlation coefficients from top to bottom panels were (all in % per μM) 0.398 ($r = 0.672$), 1.693 ($r = 0.876$), 0.548 ($r = 0.680$, center), and 0.363 ($r = 0.858$, rim).

One hundred twenty-two pairs of mean ONH blood flow and $[K^+]$ responses to sinusoidal flicker stimulus, with varying frequency and constant average luminance from experiments with 10 cats, were analyzed. Average results and ranges for the power law fit to these data are summarized in Table 1. Although the peak $[K^+]$ change occurred at a higher frequency (38.3 Hz) compared to the peak ONH response (33.3 Hz), this difference was not significant (paired $t$-test). Also, there were no significant differences between power law exponents ($n_{\text{low}}$ and $n_{\text{high}}$) for either ONH blood flow or $[K^+]$ frequency dependence (paired $t$-tests). Correlations between the increase in ONH blood flow and the $[K^+]$ change are described later in this section.

**Luminance-Dependent Changes**

Both ONH blood flow and $[K^+]$ responses to flicker were smaller as the luminance was reduced. The effect of reducing the modulation depth from 100% to 60%, 30%, 20%, and 10% in one trial is shown by three-dimensional plots in Figure 5. There was relatively little change in the $K^+$ microelectrode potential (left panel) until the modulation depth was <20%. Similarly, there was relatively little change in the ONH
FIGURE 4. Examples of frequency-dependent changes measured in three cats. (A) Double logarithmic plot of average [K⁺] increases above baseline from microelectrode potential changes with frequency. One cat (top, triangles) had a distinct range with a wide bandpass characteristic, whereas others (middle, bottom) could be separated into low- and high-frequency components. Measurements at two sites in the same eye are shown in bottom panel (open and closed circles). (B) Double logarithmic plot of average percent increases in optic nerve head blood flow changes with frequency. Frequency characteristics were similar to those for [K⁺], with wide bandpass range for one cat (top, triangles), with separate low- and high-frequency components for others (middle, bottom). (C) Linear correlations (dashed lines) and 95% confidence intervals (dotted curves) between average changes in optic nerve head blood flow and [K⁺] for the previous examples.
TABLE 1. Frequency-Dependent Changes in Potassium and Optic Nerve Head Blood Flow With Sinusoidally Flickering Light Stimulus

<table>
<thead>
<tr>
<th>Peak ± SE</th>
<th>Range</th>
<th>( f_0 ± SE ) (Hz)</th>
<th>( n_{\text{low}} ± SE )</th>
<th>( n_{\text{high}} ± SE )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Delta[K^+] ) (( \mu \text{M} ))</td>
<td>188 ± 42</td>
<td>69–413</td>
<td>38.3 ± 3.3</td>
<td>+0.35 ± 0.07</td>
</tr>
<tr>
<td>( \Delta \text{LDF} ) (%)</td>
<td>58.9 ± 11.2</td>
<td>16–132</td>
<td>33.3 ± 3.1</td>
<td>+0.34 ± 0.08</td>
</tr>
</tbody>
</table>

122 measurements, \( n = 10 \) cats.
NS = not significant.

The effect of varying luminance with different modulation depths could be characterized by a general Hill model of the form

\[
y = y_{\text{max}} \frac{M_{\text{inh}}^{n_{\text{inh}}}}{M_{\text{inh}}^{n_{\text{inh}}} + K_{50}^{n_{\text{inh}}}}
\]

where \( M \) is the modulation depth (in %), \( y_{\text{max}} \) is the maximum value, \( K_{50} \) is the Hill parameter for 50% of the maximum, and \( n_{\text{inh}} \) is the Hill coefficient. This function was fit to the experimental data using the Marquardt–Levenberg least squares optimization (SigmaPlot 5, Jandel) to estimate the model parameters. Because the modulation depth cannot exceed 100%, the Hill model was redefined in terms of the peak value

\[
y_{100\%} = y_{\text{max}} \frac{100^{n_{\text{inh}}}}{100^{n_{\text{inh}}} + K_{50}^{n_{\text{inh}}}}
\]

at 100% modulation depth, and the 50% value

\[
y_{50\%} = y_{\text{max}} \frac{M_{50}^{n_{\text{inh}}}}{M_{50}^{n_{\text{inh}}} + K_{50}^{n_{\text{inh}}}}
\]

at the modulation depth \( M_{50} \).

Representative examples of light modulation trials showing the mean \( [K^+] \) (top panel) and mean ONH blood flow (bottom panel) changes with modulation depth from two cat experiments are shown in Figure 6A. The resultant Hill model fits (solid curves) and estimated 50% \( (M_{50}) \) values are indicated (vertical dashed lines). As illustrated in Figure 6B, changes in ONH blood flow and \([K^+]\) were linearly correlated with slopes of 10.1% (\( r = 0.923 \))/\( \mu \text{M} \) and 17.8% (\( r = 0.898 \))/\( \mu \text{M} \) for these two examples.

Fifty-three measurements \( (n = 5 \text{ cats}) \) were made for modulated light trials at fixed frequencies in the 20- to 30-Hz range. Overall results for the estimated Hill model parameters are summarized in Table 2. There were no significant differences between the \( M_{50} \) or \( n_{\text{inh}} \) parameters that were determined for either ONH blood flow or \([K^+]\) responses (paired \( t \)-tests).

Average Responses

Two hundred fifty-seven \( K^+ \) microelectrode flicker responses were measured at various frequencies and light intensities \( (n = 18 \text{ cats}) \). Slopes for the relationship between ONH blood flow and \([K^+]\) changes were determined by linear regression, which are summarized in Table 3 for the different light stimulation conditions. Ranges for the experimental measurements also are listed. None of the measured \([K^+]\) increases exceeded 500\( \mu \text{M} \). Flicker response data were obtained from eight cats using the Grass model PS-22 visual stimulator (repetitive 20-msec flashes) at lower frequencies (2 to 40 Hz) than the sinusoidal stimulator. Peak responses for ONH blood flow and \([K^+]\) were observed in the 20- to 40-Hz range. The differential effects of frequency and luminance could not be separated easily from responses obtained with the Grass stimulator. Correlations between the increase in ONH blood flow and the \([K^+]\) change with frequency or with modulated light using the sinusoidal waveform are listed separately in Table 3. A linear relationship

\[
y_{50\%} = y_{\text{max}} \frac{M_{50}^{n_{\text{inh}}}}{M_{50}^{n_{\text{inh}}} + K_{50}^{n_{\text{inh}}}}
\]
between ONH blood flow and \([K^+]\) was found for both types of visual stimulators, with no significant differences in the mean slopes. Overall, the mean slope was 0.305\% \pm 0.064\%/\mu M \([K^+]\). Although there was a wide range of slopes for different cats, in all cases ONH blood flow was closely associated with the increase in \([K^+]\) with greater neuronal activity during flicker.

**DISCUSSION**

The current study confirms earlier LDF observations with flickering light stimuli in the cat ONH by Riva et al\(^{15}\) and adds new information with regard to changes in ONH blood flow in relationship to changes in local \([K^+]\). Our studies suggest that the rise in ONH blood flow and \([K^+]\) in the vitreous humor near the ONH are associated with changes in neuronal activity. Furthermore, there is a good correlation between the changes in ONH blood flow and \([K^+]\), which confirms a strong coupling between blood flow and neuronal activity. The increase in \([K^+]\) appears to be similar to the well-known rise in extracellular \([K^+]\) during increased neuronal activity in the central nervous system. Our results are also consistent with the fact that \([K^+]\) ions can be potent dilators.\(^{15}\) On the other hand, we cannot conclusively say that the increase in \([K^+]\) had a direct vasodilatory effect on the ONH microvasculature.

The regulation of ONH blood flow probably is not controlled by a simple functional relationship to neuronal activity and associated \([K^+]\) changes. Besides \(K^+\), other ions and vasoactive chemical species—such as \(H^+, CO_2,\) adenosine, lactate, nitric oxide, and neurotransmitters\(^{11,16,17}\)—are likely to modify ONH blood flow responses during the increase in neuronal activity with flicker stimulation. We have recently reported\(^{16}\) preliminary measurements of increased nitric oxide with flicker stimuli in the cat ONH. Optic nerve head blood flow is also likely to be coupled to oxidative metabolic requirements (glucose, \(O_2\)). Sperber and Bill\(^{19}\) have shown from autoradiograms with a monkey optic nerve that radiolabeled 2-deoxyglucose uptake was enhanced with 4- to 8-Hz flickering light stimulation, compared to paired measurements from the nonstimulated opposite eye. The primate ONH microcirculation appears to be able to regulate its \(O_2\) supply. Shonat et al,\(^{18}\) using a noninvasive phosphorescence imaging technique to map \(O_2\) distributions in the cat ONH, showed that there is little decrease in intravascular \(PO_2\) with reduced perfusion pressure during acute increases in intraocular pressure. Riva et al\(^{15}\) have shown that ONH blood flow remains fairly constant until intraocular pressure exceeds 40 mm Hg. However, as pointed out in a review on cerebral blood flow by Lou et al,\(^{16}\) there can be situations in the brain in which blood flow changes are independent of changes in metabolic demand. There also may be circumstances in the ONH when this may occur. Possibly other factors (myogenic, neurogenic mechanisms) are involved in modulating vascular tone. Of course, there are also anatomic differences in the ONH microvasculature in primates, humans, and cats that could be major factors in determining the effectiveness of blood flow regulation. Clearly, the regulation of blood flow can be a complex control system with the potential for interactions among multiple feedback loops.

Although the tips of the \(K^+\) microelectrodes in our study were small and allowed us to make highly
localized measurements, [K⁺] in the vitreous humor near the ONH must result from a summation of [K⁺] fluxes from a relatively large region. Besides the increased neuronal activity of the ONH itself, activity in nearby retinal tissue also may contribute to the total [K⁺] flux at the measurement site. Steinberg et al.20 reviewed the role of [K⁺] in the generation of components of the electroretinogram and described the [K⁺] buffering role of the vitreous humor. Karwoski et al.21 found the greatest increase in extracellular [K⁺] after light stimulation in the plexiform layers of the retina. This response was enhanced with flickering light. Using longer (4-second) single flash light stimuli, Frishman and Steinberg22 found that the largest increases in extracellular [K⁺] occurred in the proximal retina near the vitreous. Increases of approximately 0.5 mM were measured at higher light intensities, with smaller responses at lower intensities. At deeper penetrations in the retina near the choroidal microcirculation, extracellular [K⁺] also increased with lower light intensities but decreased at higher intensities. These changes were presumed to be caused by K⁺ siphoning from deeper layers by Müller cells, as proposed by Newman et al.23 Increased blood flow through the choroidal microcirculation also might be a factor. Müller cells in the retina have specialized ion channels that allow efficient buffering of excess [K⁺]. The morphology and anatomic location of the Müller cells in the retina may be important, particularly around the optic disk where the retina is thicker and the Müller cells are longer. Frishman and Steinberg21 observed larger increases in extracellular [K⁺] in the proximal retina near the optic disk. Glial cells in the ONH also may have a role in clearing excess [K⁺] because there appears to be a similar function for brain glial cells. Odette and Newman24 proposed that K⁺ siphoning by glial cells could provide a mechanism for the tight coupling between neuronal activity and blood flow in the brain. There may be a similar involvement for astrocytes in the ONH.

The relationship between blood flow and [K⁺] that we observed at the ONH surface might not be the same at all locations within the ONH, especially at anatomic sites at which [K⁺] could be influenced by local microcirculatory exchange. Transport effects were found in the retina by Poitry-Yamate et al.,25 who used double-barrel K⁺ microelectrodes in the preretinal vitreous of miniature pigs to measure [K⁺] and local electroretinographic changes in response to single-flash (4-second) light stimulation. They reported

### Table 2. Hill Model Fits for Changes in Potassium and Optic Nerve Head Blood Flow With Modulated Light

<table>
<thead>
<tr>
<th>Parameter</th>
<th>M50% ± SE (μM)</th>
<th>n100% ± SE (μM)</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>∆[K⁺]100% ± SE (%)</td>
<td>149 ± 44</td>
<td>22.5 ± 3.7</td>
<td>21.2 ± 4.6</td>
</tr>
<tr>
<td>∆LDF100% ± SE (%)</td>
<td>38.4 ± 6.4</td>
<td>21.6 ± 0.36</td>
<td>1.83 ± 0.24</td>
</tr>
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</table>

53 measurements, 20 < f < 30 Hz, n = 5 cats.
NS = not significant.

### Table 3. Summary of Laser Doppler Blood Flow and K⁺ Microelectrode Measurements

<table>
<thead>
<tr>
<th>Stimulator</th>
<th>ΔLDF/Δ[K⁺] ± SE (%)/μM</th>
<th>Range (%/μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grass PS-22 stimulator</td>
<td>Varying frequency and luminance (82 measurements, n = 8 cats)</td>
<td>0.277 ± 0.066</td>
</tr>
<tr>
<td>Sinusoidal stimulator</td>
<td>Constant luminance, varying frequency (2 to 80 Hz; 122 measurements, n = 10 cats, data from Table 1)</td>
<td>0.347 ± 0.124</td>
</tr>
<tr>
<td></td>
<td>Constant frequency (20 to 30 Hz), modulated luminance (53 measurements, n = 5 cats, data from Table 2)</td>
<td>0.233 ± 0.052</td>
</tr>
<tr>
<td>Overall mean (257 measurements, n = 18 cats)</td>
<td></td>
<td>0.305 ± 0.064</td>
</tr>
</tbody>
</table>
a transient fall in [K+] (≈200 μM) after the light flash for measurements at which their K+ microelectrode tip was placed near arterioles, whereas transient [K+] increases (≈500 μM) were seen in retinal tissue zones that were relatively avascular (free of arterioles). In avascular retinal tissue zones, Poitry-Yamate et al25 reported that the [K+] increase usually reached a maximum several seconds after the peak electroretinogram was measured. However, with the more prolonged time period of the flickering light stimulus in our study and with the different microvasculature of the cat ONH compared to the miniature pig retina, it is possible that, if these microvascular effects were present, they may not have been noticeable.

Even though many factors in addition to [K+] can be involved in regulating ONH blood flow, the current study demonstrated that there were remarkably similar relationships between changes in [K+] near the ONH and blood flow in the ONH that depended on the frequency and luminance of the flickering light stimulus. The sinusoidal stimulator allowed us to quantify this dependence. It should be noted that sinusoidally modulated photic stimulation has been widely used in experimental electrophysiology and for human psychophysical measurements. Our LDF and K+ studies have interesting similarities to these other measurements, and they may have some bearing on interpreting results for both types of studies.

The frequency response of cat optic nerve fibers has been studied by Fukada and Saito.26 Electrophysiological measurements of flicker responses for both on and off Y ganglion cells (phasic type) had a bandpass filter-like shape, with peaks in the 10- to 30-Hz range. In contrast, X ganglion cell (tonic type) responses appeared to be independent of frequency. Under in vivo conditions, the neuronal input to the optic nerve is derived from a variety of cell types in the retina that have different sensitivities and frequency responses to the stimulus. Direct measurements of electrophysiological responses to sinusoidal flickering light have been made in photoreceptor, horizontal, and ganglion cells of the retina. Cleland and Enroth-Cugell27 measured bandpass filter characteristics with fast roll-off at high frequencies in cat retinal ganglion cells. On-center ganglion cells had a maximum frequency response at approximately 20 Hz, whereas off-center ganglion cells had a higher peak at approximately 40 Hz. The high frequency slope (n_{high}) of the on-center cells was approximately −4, and the off-center cells were approximately −1.2, within the ranges of the slopes for the [K+] and LDF responses in the current study (Table 1). Cleland and Enroth-Cugell27 also reported that on-center ganglion cells in cat retina reached saturation during 8-Hz sinusoidal stimulation at modulation depths as low as 20%. However, off-center ganglion cell responses varied linearly with modulation. Frishman et al28 extended these studies of cat X and Y ganglion cells to higher frequencies, with complex spatial and temporal results. A wide range of peaks was found, as high as 60 Hz. Under photopic adaptation levels, the high-frequency slopes of both X and Y ganglion cells ranged between −6.7 to −8.3. In our study, none of the high-frequency slopes for [K+] and only one of the LDF responses were in this range (Table 1). There was no significant difference between the average n_{high} for [K+] or LDF (around −3) in our study, showing a slower roll-off at high frequency. The low-frequency slopes (n_{low}) for the different conditions in the study by Frishman et al28 were small, ranging from 0.4 to 0.7. Our averages for n_{low} were just below in this range for both [K+] and LDF responses.

Vo Van Toi and Riva29 also have investigated sinusoidal flicker frequency and luminance effects on blood flow in the cat ONH. They reported a slightly smaller value for the high-frequency roll-off in ONH blood flow (n_{high}), averaging approximately −2.5. The data from cat eye experiments were compared to related measurements in the literature, and the relevance to human psychophysical measurements was discussed. In human psychophysics, the observer’s perception of a flickering light depends on the depth of modulation and frequency of the stimulus (De Lange30). A sinusoidal stimulus can be used to generate a temporal modulation function (De Lange curve) and determine the critical flicker frequency or the highest perceptible frequency. The De Lange curve can serve as a diagnostic tool for detecting visual abnormalities. Sinusoidal waveform stimulation has been used to produce electroretinograms or visual-evoked potentials. The current study demonstrates that changes in ONH blood flow also are induced by sinusoidal stimulation. The ability of the ONH to regulate its blood flow with varying neuronal activity may be an important factor in human electrophysiology and may play a role in human psychophysical measurements.

Multiple peaks have been observed for De Lange curves measured in humans (for example see Vo Van Toi et al31). There were no obvious multiple peaks seen in the current cat eye experiments, although some data sets could be interpreted as having more than one peak. For example, the experiment illustrated in the middle panels of Figure 4 appears to have a second, lower peak in [K+] at 20 Hz, compared to the peak at 30 Hz, where the two power functions intersect (dashed lines, middle panel, Fig. 4A). Similarly, the LDF response appears to have a second, lower peak at 10 Hz (middle panel, Fig. 4B). To confirm whether there are multiple peaks, our experiments would have to be repeated using finer incre-
ments in frequency. The apparent peaks may be caused by random variability.

Human psychophysical testing controlled for changes in retinal illuminance with age by Kim and Mayer,⁵² and results from earlier studies reviewed in their article suggest that there are three phases of temporal contrast sensitivity related to normal, healthy aging. There is an increase in sensitivity up to 16 years of age, followed by a plateau or much slower increase in sensitivity up to 44 years of age. Thereafter, sensitivity declines with age. It is of interest that laser Doppler velocimetry studies in human optic nerve by Rizzo et al³⁵ indicate that with aging there is a decrease in red blood cell speed in the capillaries. Reduced ONH blood flow might be a factor contributing to the reduced temporal contrast sensitivity with age. In our laboratory, Cranston et al²⁴ have reported a decrease in ONH blood flow with age in humans that averaged 11% per decade from LDF measurements at the periphery of the ONH. If there is any impairment in ONH blood flow regulation, this may cause a greater susceptibility to disease with age. The noninvasive LDF technique could be used to generate a De Lange type of ONH blood flow curve with sinusoidally flickering light, similar to those generated in the cat eye in the current study, as another diagnostic tool for the earlier detection of pathologic changes with glaucoma or other conditions.

Key Words
autoregulation, blood flow, flicker sensitivity, laser Doppler velocimetry, neuronal activity, optic nerve, potassium transport

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