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Effects of Flicker Adaptation and Temporal Gain Control on the Flicker ERG

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The flicker electroretinogram (ERG) to stimuli varying in temporal frequency and modulation depth was recorded to investigate retinal gain control. With increasing modulation of a sinusoidal flickering stimulus, the flicker ERG shows an amplitude compression and a phase retardation (of the fundamental component) at 16 Hz, an amplitude expansion and a phase advance around 40–48 Hz, and an approximately linear response at 72 Hz. With sum-of-two-sinusoids stimuli, the second stimulus enhances the fundamental response to a 40 or 48 Hz test stimulus at low modulations, and reduces the variation in phase with modulation. This interaction depends primarily on the amplitude of the response to the second stimulus, but not its frequency. With temporally alternating stimuli, a similar but smaller interaction effect is measured. The results suggest that there is an active nonlinear gain control mechanism in the outer retina and this gain control works by adjusting the phase delay of the retinal response. The phase control mechanism is set by the amplitude of the outer retinal response integrated over time.

Flicker electroretinogram Frequency analysis Modulation sensitivity Flicker adaptation Gain control

INTRODUCTION

The ability of the visual system to rapidly adjust its sensitivity to changes in light level (adaptation) is essential for normal visual function. Adaptation seems to occur over a wide range of time scales, ranging from dark adaptation to more rapid types of adaptation. In fact, it has been shown that increment threshold sensitivity can track a 30 Hz flickering background even when the background flicker is not detectable (Boynton, Sturr & Ikeda, 1961). This rapid adaptation to imperceptible flicker may occur in the retina, which has a rapid temporal response as shown by its decreased high frequency attenuation compared to the later stages of the visual system (e.g. see Baron & Boynton, 1975; Kelly, Boynton & Baron, 1976; Burns, Elsner & Kreitz, 1990). In this study we present evidence for the existence of a fast retinal gain control, and test several candidate models for this gain control against measurements of the ERG response to rapidly flickering stimuli. Specifically, we measure the flicker ERG response as a function of stimulus modulation while keeping the mean luminance of the stimulus constant. By maintaining a constant mean luminance, we avoid the effect of slow light adaptation on the flicker ERG, thus concentrating on a temporally-dependent adaptation and/or rapid light adaptation.

Changes in retinal illuminance have been shown to cause changes in retinal responses. For instance, prolonged light adaptation (up to 21 min) increases the flicker ERG response amplitude to a given stimulus by about a factor of 2 compared to the dark-adapted amplitude, and this increase cannot be explained by a recovery of cones from bleaching (Gouras & MacKay, 1989; Peachey, Alexander & Fishman, 1991) or changes in rod–cone interactions (Peachey, Alexander, Derlacki & Fishman, 1992). In addition, even with steady-state stimulation, there still appears to be some form of modulation-related gain control affecting the retinal response. For example, Armington (1964) has reported a change in flicker ERG amplitude following flicker adaptation (2 Hz), and has attributed this amplitude change to a transient effect of light adaptation. In the present study, we do not distinguish between temporal adaptation and rapid light adaptation, since such a distinction may not be meaningful. Psychophysical studies have yielded divergent results concerning how rapid light adaptation can be (e.g. Powers & Robson, 1987), and there are multiple types of adaptive processes with different time constants (Hayhoe, Benimoff & Hood, 1987; Hayhoe, Levin & Koshel, 1992).

In this study we use flicker rates at or above 16 Hz for two reasons. First, glial cells cannot respond rapidly to changes in neural responses (Miller & Dowling, 1970). Thus, the use of frequencies above about 8 Hz restricts the majority of the retinal response to neural elements of the retina. Second, at high flicker rates, the major portion of the flicker ERG arises from the cones or

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pathways directly mediated by cones (Norren, 1973; Neitz & Jacobs, 1984; Abraham, Alpern & Kirk, 1985; Baker, Hess, Olsen & Zrenner, 1988) but not from the rods (Abraham *et al.*, 1985). While it was previously believed that the flicker ERG primarily reflects photoreceptor responses, it has now been shown to also include a considerable contribution from post-receptoral responses (Bush & Sieving, 1993). Thus, the flicker ERG probably represents primarily the summed response of cone photoreceptors and the on- and off-cone bipolar cells, and provides a useful means for studying the response properties of the outer retina.

This study was performed in two steps. In Expt I we examined the relation between ERG amplitude and modulation depth at different temporal frequencies. In Expt II we investigated the interaction between two frequencies, either temporally superimposed or separated in time. Based on our results we will reject the hypothesis that outer retinal response to flicker is governed by a simple static gain control. That is, other than the simple loss of sensitivity at high temporal frequencies, the outer retina should treat all temporal frequencies similarly. We will refer to this as "the uniform temporal gain control" hypothesis. Thus, if the gain control is implemented as a simple multiplicative adjustment of sensitivity with adaptation then for all frequencies the response amplitude should be related to flicker modulation in an identical way. Similarly, the use of complex temporal stimuli should not alter the relation between response amplitude and stimulus modulation for each constituent frequency. After rejecting the null hypothesis, we will then postulate a possible mechanism and delineate its essential features.

GENERAL METHODS

Subjects

Two of the authors served as subjects. Both had normal vision with no evidence of ocular disease. The experimental protocols were reviewed and approved by the local Institutional Review Board. The majority of results are shown for a single subject, although all results were also obtained in the second subject (unless otherwise noted in the text).

Apparatus and stimuli

The apparatus used in this study has been described previously (Chang, Burns & Kreitz, 1993). Briefly, it consists of a two-channel Maxwellian view system using red (633 nm) and green (543 nm) He–Ne lasers as light sources. The red and green lights were both 4.1 log td, at which the amplitude of the retinal response has been shown to be approximately independent of retinal illuminance (Biersdorf & Armington, 1960; Valeton & Norren, 1983; Burns, Elsner & Kreitz, 1992). The mixture of the red and green lights was close to unique yellow, and the two lights were modulated in phase throughout all experiments so that the subjects saw a uniform, flickering yellow field. The modulation depth and the temporal frequency of the yellow field were controlled through a programmable function generator which sends a voltage signal to modulate the laser lights. Under all conditions described in this paper, the time-averaged luminance was constant. The field size was 40 deg in diameter. A white surround produced by a Goldmann–Weekers Ganzfeld bowl was used to suppress stray light response.

Signal acquisition and analysis

ERG responses were recorded using bipolar Burian–Allen contact lenses. Responses were filtered and amplified by two sequential amplifiers (Grass P511K), both were set to pass 3–300 Hz. The gain for the first amplifier was 1000, and for the second, 200. The output of the second amplifier was digitized by a 12-bit analog-to-digital converter (Data Translation) at a sampling rate of 512 Hz.

A total of 2048 samples were recorded for each response (4 sec epoch). Depending on the signal amplitude, either 4, 8, 16, or 32 responses were time-averaged for each trial. Both the sampling and the averaging were synchronized with the stimulus through a single master clock. The averaged responses were analyzed off-line using a discrete Fourier transform (DFT). The magnitude, phase, and the signal-to-noise (S/N) ratio were computed for all stimulus frequencies, their sums and differences, as well as several nonlinear harmonics. The S/N ratio was estimated by computing the ratio of the response at the frequency of interest to the averaged response at nearby frequencies. Data points with a S/N estimate <3 were excluded from further analysis.

Linearity calibration

The linearity of the laser intensity control was calibrated using a photodetector placed at the exit pupil of the optical system. The output of the photodetector to simple sine waves as well as to sum-of-two-sinusoids was recorded at all modulation depths used in the experimental conditions, and was then analyzed using the same Fourier analysis routine used for the ERG. The relation of the photodetector output to modulation depth was linear to the resolution of the recording system, with no nonlinear response components observed under any of the conditions.

EXPERIMENT I

Method

The apparatus, signal acquisition, and recording procedure have been described in the General Methods section. The temporal frequencies tested in this experiment were 16, 28, 40, 48, 56, 64, and 72 Hz. During a typical daily session 2–3 frequencies were tested. At each frequency, there were seven modulation levels, ascending from 0.0156 to 1.0, in equally-spaced logarithmic steps. Depending on the signal amplitude, 8 or 16 responses were averaged at low modulations, and 4 or 8

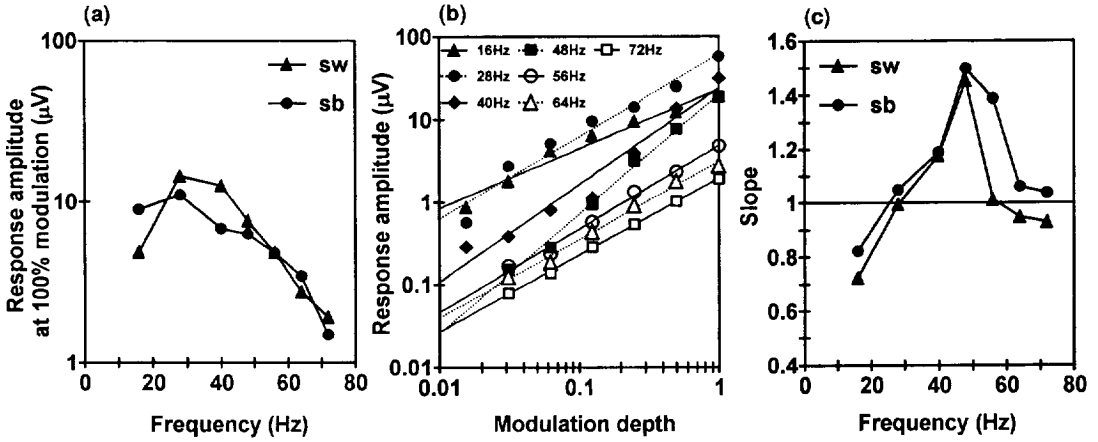

FIGURE 1. ERG fundamental response amplitude for sinusoidally flickering lights. (a) Response amplitude to 100% modulated flickering lights as a function of flicker frequency for two subjects. (b) Amplitude of the fundamental response component of the flicker ERG as a function of stimulus modulation for different temporal frequencies. Data are plotted on log–log scale and are fit with a power law relation (all $r \geq 0.98$). For clarity, responses at 16 and 28 Hz have been displaced upwards by 0.6 log units and at 40 and 48 Hz have been displaced upwards by 0.4 log units. Subject SW. (c) Slope of the fitted lines as a function of temporal frequency for both subjects.

at high modulations. Most of the stimulus conditions have been replicated, and the data presented below (for fundamental response component only) are averages.

Results

The amplitude of the fundamental response varied systematically with temporal modulation at all stimulus frequencies. Figure 1(a) shows the amplitude as a function of stimulus frequency for 100% modulated stimuli. The increase in response amplitude with stimulus modulation was well described by a power law relation between the stimulus modulation and the amplitude (all $r \geq 0.98$), as shown by the straight line relation of the two when plotted on log–log coordinates [Fig. 1(b)]. The exponent required for the best fit power law varied systematically with stimulus frequency for both subjects, as shown in Fig. 1(c). The large deviation from an exponent of 1.0 at 16 and 48 Hz, as well as the large range of modulations (more than 2 log units) used, allow us to reject a linear relation for these conditions ($P < 0.05$). At 16 Hz, a decelerating power relation is observed, where doubling the modulation less than doubles the response. At 40 and 48 Hz this relation is reversed. At high frequencies (≥ 56 Hz), the exponent is not reliably different than 1, indicating an approximately linear relation.

The phase of the fundamental response also varied systematically with stimulus frequency. Figure 2(a) shows the relative phase of the response to 100% modulated stimuli varying in frequency. The phase has been "unwrapped" in increments of 360 deg to produce a continuous function with frequency. The relation

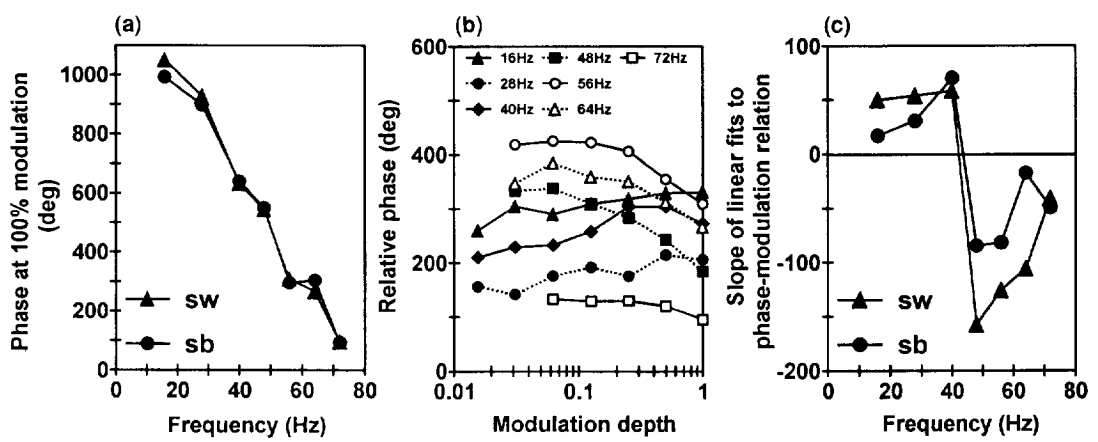

FIGURE 2. ERG fundamental response phase for sinusoidally flickering lights. (a) Response phase to 100% modulated flickering lights as a function of temporal frequency. The phases have been adjusted in 360 deg increments to produce a continuous function with temporal frequency (Burns *et al.*, 1992). (b) Phase of the fundamental response component of the flicker ERG as a function of stimulus modulation. Note that the response phase is plotted on a semilog scale. Response phase at individual frequencies has been shifted in multiples of 360 deg for clarity of presentation. The relation of the phase to modulation changes systematically with frequency. (c) Slope of the linear regression fitted to the response phase as a function of temporal frequency. A positive slope reflects an increase in the phase angle of the fundamental response with increasing modulation.

between the fundamental response phase and stimulus modulation is shown in Fig. 2(b). The response phase increases with increasing stimulus modulation for frequencies up to 40 Hz, and decreases with stimulus modulation for frequencies at or above 48 Hz. A linear relation was fit between the phase of the response and the stimulus modulation to capture the basic trends of the data. The slopes of these linear fits are plotted as a function of stimulus frequency in Fig. 2(c). In general, the linear fits were not as good a description of the data (all $r \geq 0.71$, with one exception, 40 Hz, where $r = 0.56$) as were the power law fits to the amplitude data. For both subjects, the slopes for 16 and 72 Hz approach 0, indicating that response phase is nearly independent of stimulus modulation at these frequencies. However, for 40 and 48 Hz stimuli, large phase shifts with changes in stimulus modulation were measured while the direction of the phase shift reversed (from a positive slope of the phase-modulation relation at 40 Hz to a negative slope at 48 Hz).

EXPERIMENT II

Methods

Simultaneous adaptation

To test for temporal frequency interactions in setting the gain of the retinal response we used stimuli consisting of the sum of two temporal sinusoids. During a typical daily session, we recorded ERG responses to two series of frequency pairs. In one series we kept the stimulus modulation of one frequency (f1) at a constant level, and increased the modulation of the other stimulus frequency (f2) from trial to trial. In another series we either set the modulation of f1 to a different constant level, or switched the two frequencies. The modulation of f2 was varied from 0.0078 to 0.5, in seven equal, logarithmically spaced, steps. For each trial, 8 or 16 responses were averaged, with each response consisting of 2048 samples. Most of the stimulus conditions have been replicated, and the data presented are the averages of all the recordings.

The Fourier analysis program was used to extract response components at the fundamental frequencies of both f1 and f2, as well as at their higher harmonics and at the intermodulation frequencies. We will be concentrating on the fundamental response components only.

Successive adaptation

To test whether temporal frequency interactions occurred over time we recorded responses to a set of stimulus conditions where f1 and f2 were repetitively alternated at 0.5 Hz (1 sec per interval). The test stimulus (f1) was either a 16 or 48 Hz sinusoidally flickering stimulus at a fixed modulation. The adaptation stimulus (f2) was presented in the alternate time intervals. Each response period was sampled over two successive adapt/test cycles (4 sec), giving us two estimates of the test response per trial. For each stimulus condition, 8–32 responses were time averaged. The averaged response was broken into one second intervals and cosine windowed before performing the DFT. Other recording procedures were the same as in Expt I.

Results

Simultaneous adaptation

Amplitude: simultaneous adaptation. The fundamental response amplitude to the test stimulus depended on

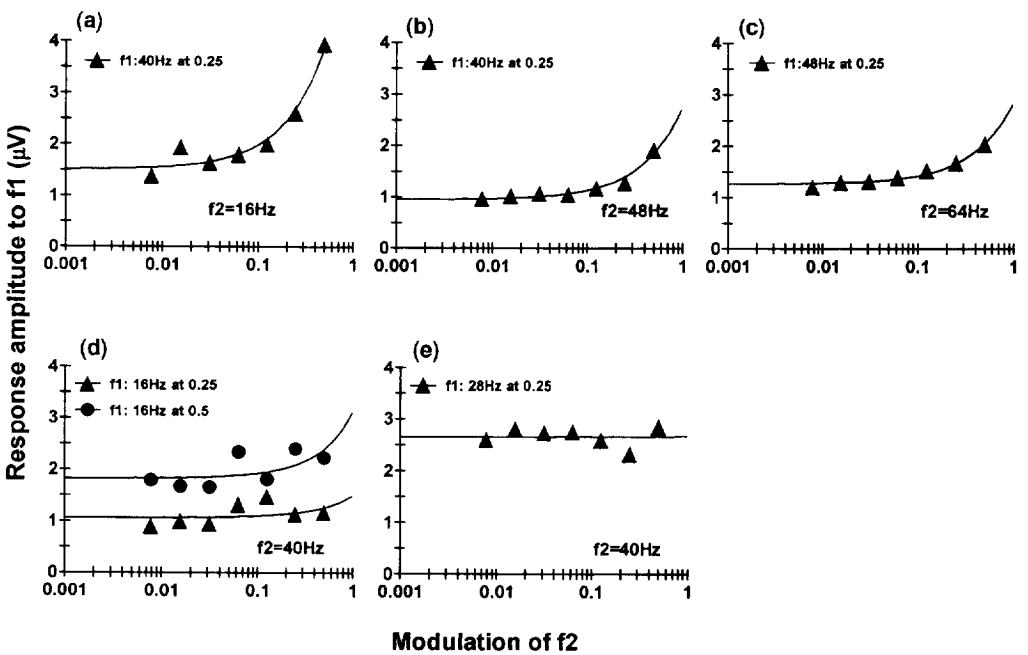

FIGURE 3. Top: response amplitude to a 0.25 modulation 40 and 48 Hz test stimulus (f1) as a function of the modulation of an added stimulus (f2) at frequencies of 16 Hz (a), 48 Hz (b) and 64 Hz (c). Bottom: response amplitude to a 16 Hz (d) and 28 Hz (e) test stimuli (f1) as a function of the modulation of an added 40 Hz adapting stimulus. Subject SW.

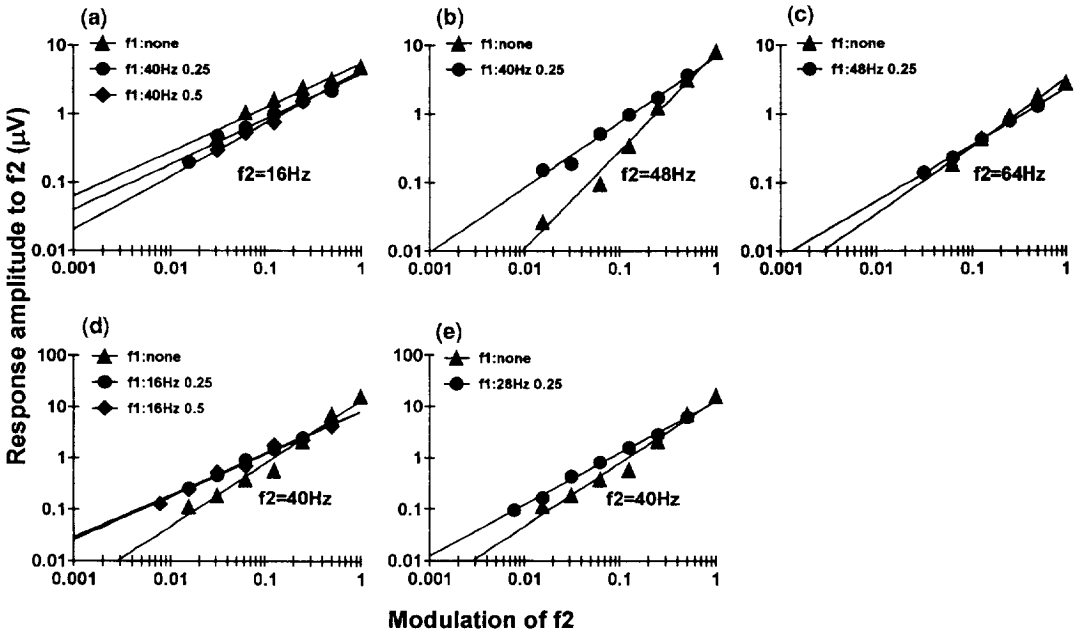

FIGURE 4. Top: response amplitude as a function of modulation to 16 Hz (a), 48 Hz (b) and 64 Hz (c) stimuli varying in modulation in the presence or absence of an added stimulus at a fixed modulation. Note the large change in the slope of the 48 Hz response in the presence of an added stimulus. Bottom: response amplitude as a function of modulation to a 40 Hz stimulus in the presence or absence of a fixed modulation 16 Hz (d) or 28 Hz (e) stimulus.

the modulation and frequency of the added temporal frequency in some cases but not others. Sample results are shown in Fig. 3. Here the response amplitude to the fixed test stimulus (f1) was plotted against the modulation of the added stimulus (f2) on a semi-log scale. If there is no frequency interaction, the response amplitude to f1 should be constant. However, the results show that when the test stimulus was a 0.25 modulation, 40 or 48 Hz stimulus, the response amplitude was increased as the modulation of the added stimulus was increased [Fig. 3(a, b, c)]. The largest increase in response amplitude was measured with the addition of the 16 Hz stimulus (>100% increase in response amplitude). If the test stimulus was 16 or 28 Hz [Fig. 3(d, e)] then there was little effect of adding a higher frequency stimulus on the response amplitude measured at 16 or 28 Hz. Similar results were obtained from a second subject (data not shown).

The relation between stimulus modulation and response amplitude was affected by the presence of another temporal frequency at a constant modulation (Fig. 4). Here we show the response amplitude to the f2 fundamental frequency. The presence of another frequency has no effect on the slope of the response amplitude vs stimulus modulation relation at a stimulus frequency of 16 Hz [Fig. 4(a)], a large effect at 48 Hz [Fig. 4(b)], and a small effect at 64 Hz [Fig. 4(c)]. For the 48 Hz response the exponent of the relation between response amplitude and stimulus modulation decreased from 1.43 to 0.95 with the addition of a 40 Hz, 0.25 modulation stimulus. This resulted in an increased response amplitude to low modulation 48 Hz stimulation, and no change in response amplitude to high modulation 48 Hz stimulation. This change in slope was also apparent for 40 Hz stimuli, with f1 frequencies of 16 or 28 Hz [Fig. 4(d, e)].

Phase: simultaneous adaptation. At 16 and 28 Hz there was not a significant dependence of the response phase on the presence of an additional stimulus frequency [Fig. 5(a, b)]. For the 40 and 48 Hz stimuli however, the conditions in which the amplitude of the response was changed by the presence of an additional frequency, the phase of the fundamental response was also significantly changed [Fig. 5(c, d)]. The effect on the phase of the response occurs at low stimulus modulations, but not at high stimulus modulations, just as the amplitude of the response was most affected at low stimulus modulations. The overall effect of the second stimulus frequency was to decrease the modulation dependence of response phase to 40 and 48 Hz stimulation that was found for the sinusoidal stimulation conditions of Expt I. The only exception is that the response phase at 48 Hz is not affected by the simultaneous addition of a 64 Hz stimulus [Fig. 5(d) ◆].

Successive adaptation

Amplitude: successive adaptation. Figure 6 shows the response waveforms to the 48 Hz test under different adaptation conditions, with the test stimulus at a modulation of 0.25 (a) and 1.0 (b). The response to the 48 Hz test stimulus at a modulation of 0.25 is strongly affected by prior exposure to a high modulation flicker (a), whereas at a modulation of 0.5 (not shown) and 1.0 (b) the response to the 48 Hz test is less affected. At a modulation of 0.25 the response amplitude following high modulation flicker is more than twice the amplitude following a period where the field was maintained at its time averaged luminance.

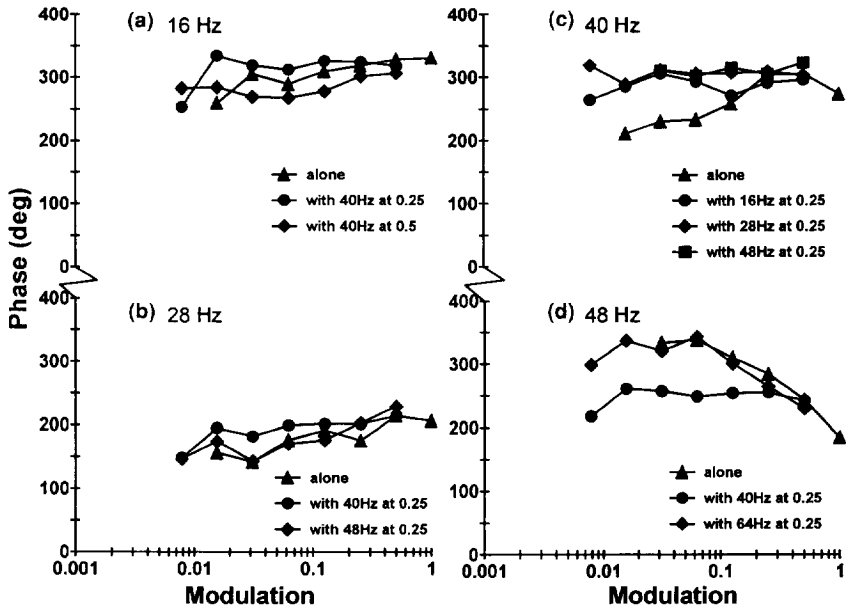

FIGURE 5. Response phase as a function of modulation in the presence or absence of added stimuli for 16 Hz (a), 28 Hz (b), 40 Hz (c) and 48 Hz (d). Subject SW.

The initial portions of the waveforms in Fig. 6 also include a delayed response to the stimulus in the previous interval, which is later removed by the cosine window used in the DFT for amplitude measures. Other than this transient (when it exists), the waveform is essentially constant during the 1 sec test interval. (Figure 6 shows only the first 400 msec, but the later portions of the response behave similarly.) The effect of different adaptation conditions on response phase cannot be readily distinguished in these waveforms.

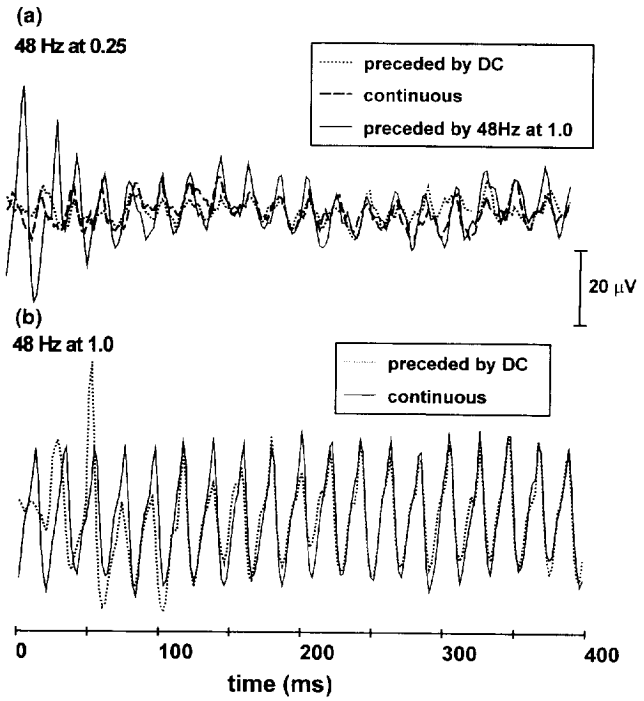

FIGURE 6. Response waveforms to a 48 Hz stimulus at modulations of 0.25 (a) and 1.0 (b) with and without preceding adaptation. The adapting and the test stimuli are alternated at 1 sec per interval. Only the responses in the first 400 msec of the test interval are plotted. The responses in the remaining 600 msec are similar. Subject SW.

We next systematically examined the effects of the modulation and frequency of the adapting stimuli. Adapting stimuli at a fixed frequency (either 16 or 40 Hz) were increased in modulation from 0.0 to 1.0. Then at a fixed modulation of 1.0 the adapting frequency was changed (to either 17, 28, 40, 48, 56 or 64 Hz). Since the waveform comparisons (Fig. 6) did not reveal any significant change of the enhancement effect with time, the subsequent results are expressed in terms of the Fourier amplitudes for the entire 1 sec interval. We show results for the fundamental response component only.

Figure 7(a) shows response amplitudes for a 0.5 modulation, 40 Hz test stimulus preceded (and followed) by a 40 Hz adapting stimulus at various modulations. As the modulation of the 40 Hz adapting stimulus was increased from 0.0 (steady field) to 1.0 (full modulation), the response amplitude to the 40 Hz test increased in an approximately linear fashion. Note that the 0.5 modulation condition (for the 40 Hz adapting stimulus) represents the continuous 40 Hz flicker condition used in Expt I. On the other hand, Fig. 7(b) shows that, for a test stimulus of 16 Hz at 0.25 modulation, the increasing modulation of a 16 Hz adapting stimulus has a very small enhancement effect in one subject (SW) and no effect in the other (SB). This is in agreement with the results from the simultaneous adaptation conditions (Figs 3 and 4), which show no effect of adaptation on the 16 Hz response even when the adapting stimulus was presented simultaneously with the 16 Hz test.

Figure 8 shows the effect of changing the frequency of the adapting stimulus. For a 0.25 modulation, 48 Hz test stimulus (a), all adapting frequencies, when modulated at 1.0, showed enhancement of the test response. However, for a 0.125 modulation, 16 Hz test stimulus

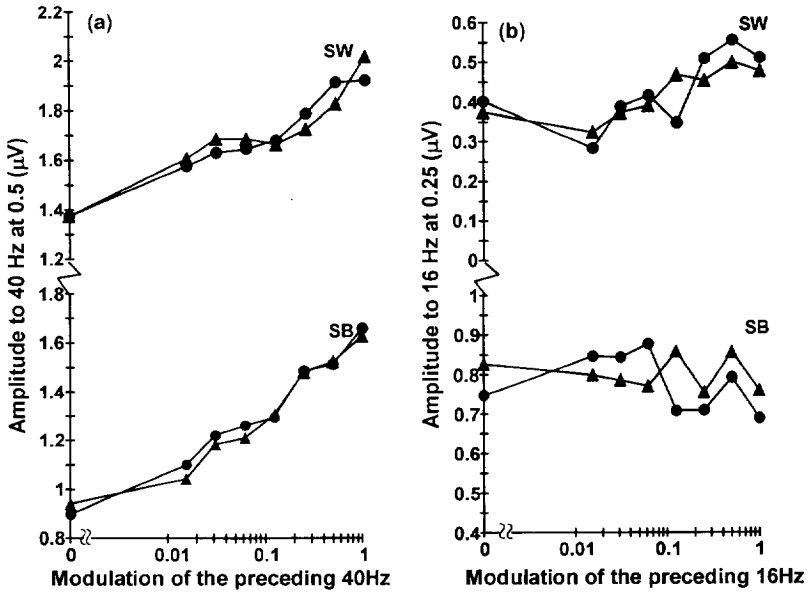

FIGURE 7. Response amplitude to a 40 Hz test stimulus at 0.5 modulation (a) and to a 16 Hz test stimulus at 0.25 modulation (b) as the modulation of the preceding stimulus is varied. In both cases, the preceding stimulus is of the same temporal frequency as the test. The two sets of data in each plot are estimates of the response amplitude to the test from alternate cycles in the same record.

(b), none of the adapting frequencies has a clear and systematic effect on response amplitude.

Phase: successive adaptation. The presence of an adapting stimulus also modifies the response phase to the 40 Hz test stimulus (Fig. 9). As in the case when the test and the adapting stimuli were simultaneous (Fig. 5), the adapting stimulus increases the test response phase at low modulations while having a smaller effect at high modulations. As a result, the phase-modulation relation is flatter when the retina is adapted to flicker.

DISCUSSION

Uniform gain hypothesis

Experiment I documents that the retinal responses do not follow the predictions of the uniform gain hypothesis. The retinal response varies with modulation according to a power law, with a compressive response at 16 Hz, an expansive response around 40–48 Hz, and an approximately linear response at 72 Hz. This behavior rules out a simple, modulation-dependent response

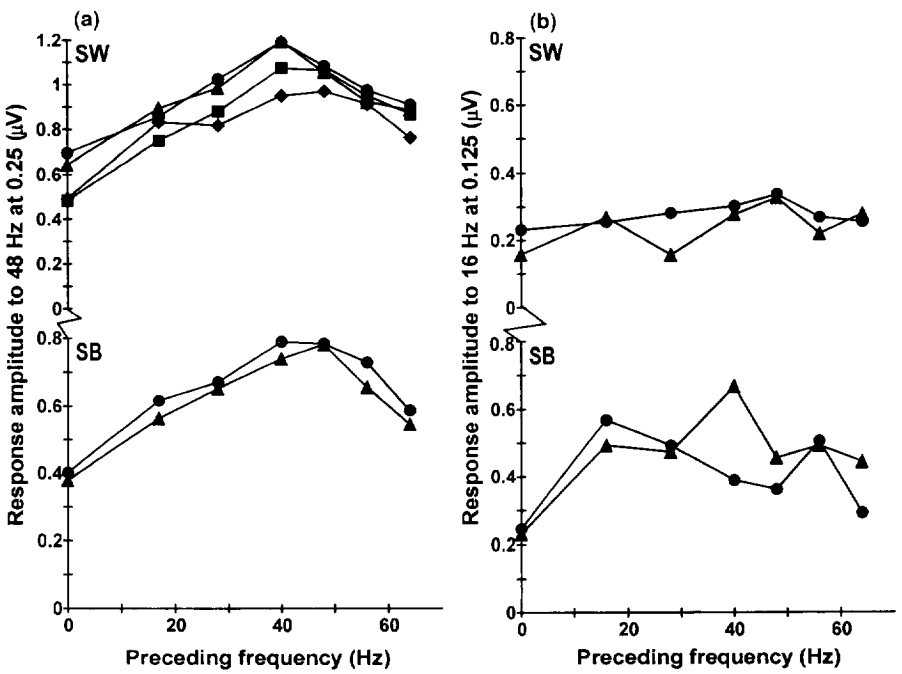

FIGURE 8. (a) Response amplitude to a 48 Hz test stimulus at 0.25 modulation as the temporal frequency of the preceding stimulus is varied. Other than the D.C. (0 Hz) condition, all the preceding stimuli are 100% modulated. Subject SW: two recordings, two estimates each. Subject SB: one recording, two estimates. (b) Results for a 0.125 modulation, 16 Hz stimulus.

nonlinearity as an explanation for our data. However a more plausible nonlinear adaptation mechanism would affect the response properties of the retina according to its sensitivity. Consider a model where the response amplitude is controlled by a simple sigmoidal input output function. In such a model there is an accelerating region of the input–output relation where increasing stimulus amplitude will rapidly increase the response amplitude, then there is a region where the response amplitude is linearly related to the stimulus amplitude, and finally, for large stimulus amplitudes there is a compressive region where the response amplitude increases more slowly than the stimulus amplitude. Even if sensitivity varies with temporal frequency, the response amplitude to different frequencies of stimulation should increase similarly (according to the nonlinear response function) with increasing modulation. In Expt I the response amplitude to a low modulation stimulus was smaller for 48 Hz than for 16 Hz stimulation, whereas at high modulations the 48 Hz response was larger, thus the difference in response properties cannot be attributed to differences in sensitivity causing the retinal response to fall on different portions of a nonlinear operating curve.

A simple nonlinearity can predict frequency interactions, but in general a single static nonlinearity will treat two frequencies which produce the same response amplitude identically. Spekreijse and Oosting (1970) have shown that for certain types of nonlinearities, an auxiliary signal can act as a carrier to shift the response away from the nonlinear portion of the system's operating curve and they have termed this process "linearizing". In Expt II we showed that the response to the 48 Hz test could be linearized by the presence of other frequencies. However, the response to a 16 Hz test was not changed by the presence of other stimulus components. Thus, the results of both Expts I and II rule out a uniform, frequency independent model of the gain control.

Multi-channel model of gain control

Another possibility is that while a single gain control cannot account for our results, there might be multiple temporal channels, each with a simple, linear gain control. A model of multiple *linear* channels predicts specific phase relations that can be compared with our phase data (Fig. 2). First consider the response of a two-channel system under the following assumptions. (1) The two channels produce two fundamental response components, each having a fixed time delay (lag), but one having a longer lag than the other. (2) The output of the two components grow linearly with modulation, but with different gains. From Figs 1 and 2, we assume that 28 Hz is dominated by one component while 72 Hz is dominated by the other. These two frequencies represent conditions for which the increase in amplitude with increasing modulation is approximately linear (Fig. 1) and the phase variation with modulation is small (Fig. 2), two necessary conditions for the model being considered. (3) Due to the different temporal sensitivities

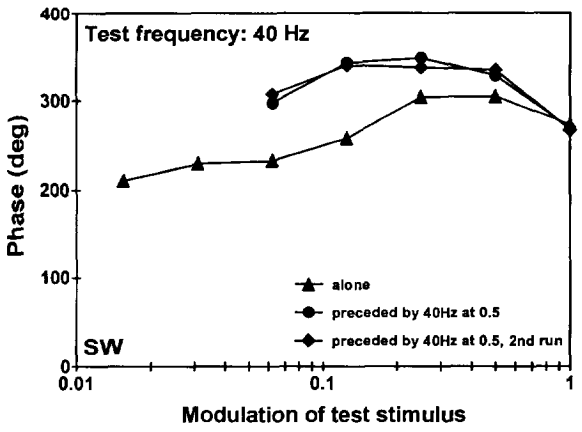

FIGURE 9. Response phase for a 40 Hz test stimulus as a function of modulation with and without adaptation to a preceding 40 Hz at 0.5 modulation. Adapting and test stimuli are alternated at 1 sec per interval. Subject SW.

of the two channels, the variations in stimulus frequency and modulation change the relative contributions of the two components, but not their shapes [from assumption (2)]. Given these assumptions, an increased contribution of the component having a longer phase lag ("the slow component") may account for the retardation in phase with modulation at low frequencies, whereas the response at higher frequencies simply speeds up with modulation due to an increased contribution of the "fast component". That is, the slow component gradually drops out. However, a two-component hypothesis that is consistent with this phase relation between the channels also predicts specific amplitude changes, and these are not seen. For example, between 40 and 48 Hz there is a frequency (e.g. 44 Hz) at which the phase-modulation relation is flat [the zero-crossing point in Fig. 2(c)]. Thus, at this frequency the two putative components must be in a constant proportion at all modulations. If this is the case then the increase in response amplitude with increasing modulation at this frequency (i.e. around 44 Hz) cannot exceed the increase measured at 28 and 72 Hz which are assumed to be dominated by a single component. Indeed, to produce a slope of approx. 1.5 for the 40 and 48 Hz amplitude–modulation relation (log–log scale), the two components must partially cancel at low modulations and add at high modulations. This indicates that the two components cannot have fixed phase lags. We thus conclude that a two-channel system, with each channel having its own linear gain, cannot explain our results.

Nonlinear gain control

One can design a more elaborate multiple-channel system in which each channel has a nonlinear gain, but such a complicated system may not have physiological meaning for the outer retinal responses being measured. A simpler hypothesis is a single, nonlinear, modulation-dependent gain control or adaptation network. The results suggest that the gain is controlled by a feedback (or feedforward) mechanism with at least three components in the control pathway. First, there must be a

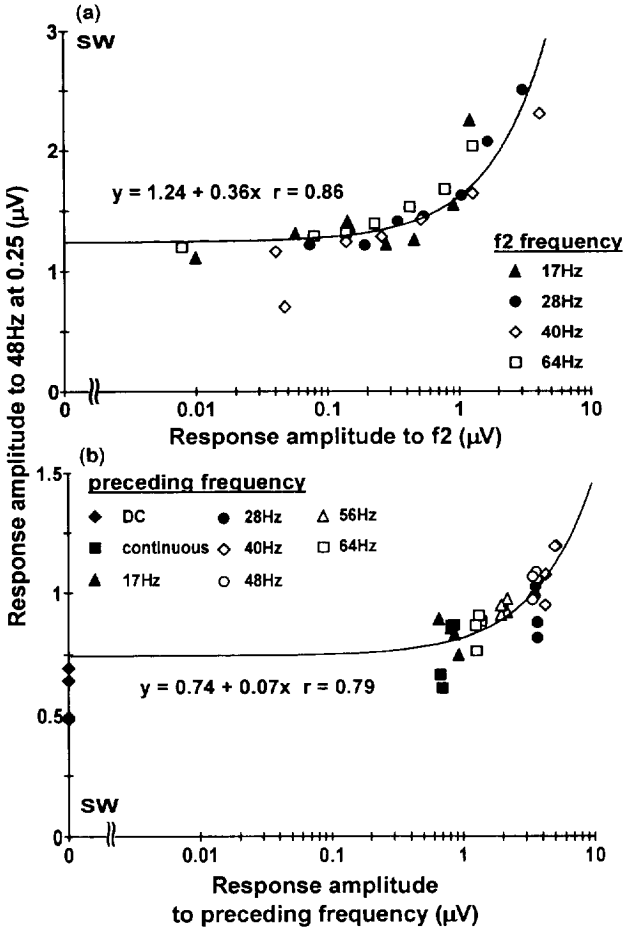

FIGURE 10. Response amplitude to a 0.25 modulation, 48 Hz stimulus as a function of the response amplitude produced by an adapting stimulus. (a) Results for the simultaneous adaptation conditions. The adapting stimulus was varied in modulation and frequency and the response amplitude at 48 Hz is plotted as a function of the corresponding fundamental response amplitude recorded for 17 Hz (▲), 28 Hz (●), 40 Hz (◇), or 64 Hz adaptation (□). The curve is the best fit linear relation between the 48 Hz amplitude and the response amplitude measured at the adaptation frequency. The equation shows the coefficients of the regression and the correlation coefficient. Subject SW. (b) Results from the successive adaptation conditions. In the successive adaptation conditions only the 100% modulated adapting stimuli were used, due to the smaller size of the adaptation effect. The curves are linear fits to the data as in (a). Subject SW.

frequency dependent filtering. This filter will limit the ability of high temporal frequency signals to affect the gain of the system, producing the high frequency linearity that we observed. Second, there must be a nonlinear input–output relation following the linear filter. Thus, at low temporal frequencies, as the stimulus modulation increases, the feedback signal increases at a faster rate, resulting in an overall compressive response for the system. Finally, there must be a time lag or frequency dependent phase shift that inverts the feedback signal at frequencies around 48 Hz. This inversion will cause the output of the system to be expansive, rather than compressive. In addition, the amount of phase lag in the system must be set by the system's response integrated over time, since we found a large change in response in the successive adaptation conditions of Expt II. To test whether the alteration of the response gain recorded in Expt II was dependent on the response amplitude, or whether it had a frequency-specific component we replotted the response amplitude measured at 48 Hz to a 0.25 modulation 48 Hz test stimulus as a function of the response amplitude to the second frequency (Fig. 10). Figure 10(a) shows results for the simultaneous adaptation conditions, and Fig. 10(b) shows results for the successive adaptation conditions. For the simultaneous adaptation conditions response amplitude increases by more than a factor of 2 in the presence of a second stimulus frequency, and the response amplitude at 48 Hz is correlated with the response amplitude at the second frequency. There seems to be no systematic effect of the frequency of the second stimulus on the response amplitude to the constant test stimulus [Fig. 10(a)]. With the successive adaptation [Fig. 10(b)] a similar result was found.* Thus, the gain control mechanism must have a memory of at least 1 sec (the time period tested). However, the slope of the regression line fitted to the response enhancement function is about 5 times steeper in the temporally superimposed case than in the temporally separated case (best fit linear slope of 0.36 vs 0.07). That is, the same adapting stimulus is less effective at enhancing the subsequent response with temporal separation than when it is simultaneously presented. The effect of the adapting stimulus on phase, however, is of the same order of magnitude in both cases (cf. Figs 5 and 9). These data are consistent with the suggestion that the frequency interactions we are measuring occur within a single mechanism, since it can be invoked by a stimulus at any frequency. If the interaction results from multiple channels, then the extent to which two frequencies interact would be determined by the frequency relation between the two stimuli, which is not the case.

An implementation of this type of model using MATLAB was able to qualitatively predict some of the results of this study. However, we were not able to quantitatively fit the results of both the simultaneous and successive adaptation, in part due to the very different time scales over which they were operating. It is possible that there are actually two different mechanisms involved, one with a rapid time–course and one with a much longer time–course. There is ample evidence from other studies that visual sensitivity control occurs on both short and long time scales. For example, psychophysical studies have shown that light adaptation is controlled by mechanisms of different time courses (Hayhoe *et al.*, 1987, 1992). It is not known how these mechanisms are related to the outer retinal mechanisms underlying adaptation-related sensitivity adjustment in the flicker ERG, but in this light, it seems that the long-term light-adaptation induced ERG enhancement observed by Gouras and MacKay (1989) and Peachey *et al.* (1991) must act through an even slower process than

*For the successive adaptation condition all of the adapting frequencies were modulated at 100%. This produced several estimates of the response for each condition and is the reason for the close grouping of the points in Fig. 10(b).

the "adaptation process" discussed here. Thus, the control of sensitivity in the outer retina is much more complex than originally thought.

Other response components

Our data rule out a uniform nonlinear gain control and multiple linear channel models of retinal gain control. These main conclusions were derived primarily to explain the high slopes of the amplitude–modulation curves measured at 40 and 48 Hz and the reduction of these slopes by simultaneous as well as successive adaptation. We now consider two other features of the data, both summarized in Fig. 11. First, there are appreciable nonlinear response components (second harmonics and intermodulation responses) in the data, particularly in responses to low frequency stimuli. Are these nonlinear response components subjected to the same gain control as the fundamental response component? Figure 11 shows that they are not. The third harmonic component to the 16 Hz test stimulus is not affected by any adapting condition. Either the gain control mechanism is located prior to the site generating the nonlinear response components, or the gain of the nonlinear response components are controlled by their own fundamental response component, and thus are not susceptible to adjustment by additional adaptation. Evidence consistent with the latter argument is that the slope of the amplitude–modulation function for the 16 Hz third harmonic is always close to 1 (1.04–1.16), both with and without adaptation.

Second, we have not observed any variation in the responses to 16 and 28 Hz stimuli by simultaneous adaptation in Expt II (Figs 3 and 4). Based on these findings, we have concluded that the gain control is stable at 16 and 28 Hz no matter what the adaptation state is. However, with successive adaptation in Expt II, a small adaptation effect by a preceding 16 Hz stimulus (at various modulations) on the response to the 16 Hz test was measured in one subject but not the other [Fig. 7(b)] and Fig. 11 (the three dashed curves) also shows a consistent, modulation-independent enhancement by a preceding, full modulation, 40 Hz stimulus. This modulation-independent effect is probably not due to the same gain control governing the 40 and 48 Hz response. Since this low frequency adaptation effect is small compared to the other results reported, it has not been incorporated into our framework for the gain control mechanism. However, it also supports the possibility of multiple types of gain control acting on the retinal response.

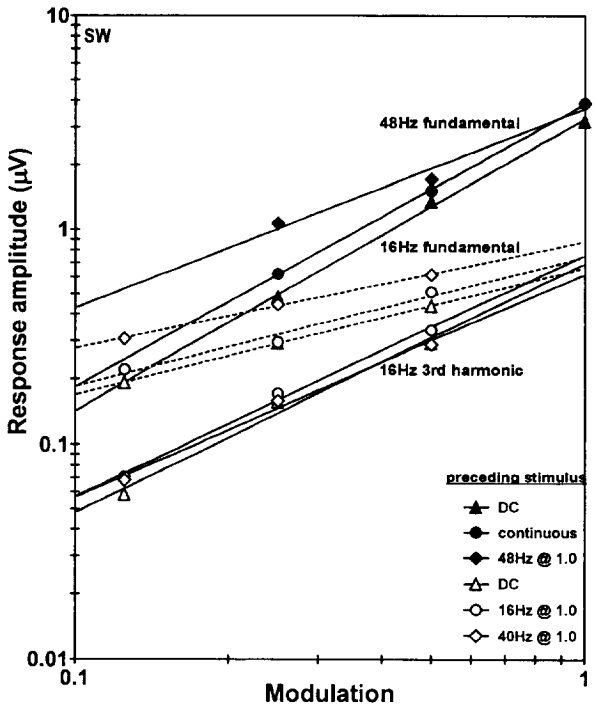

FIGURE 11. Amplitude of the fundamental response component to 16 and 48 Hz stimulation and the third harmonic response component to 16 Hz stimulation as a function of stimulus modulation, measured under three adaptation conditions. The adaptation conditions are D.C., continuous, and 100% modulated 48 Hz for the 48 Hz test stimulus; and D.C., 100% modulated 16 Hz, and 100% modulated 40 Hz for the 16 Hz test stimulus respectively. The slopes at 48 Hz are 1.36, 1.32 and 0.93 respectively, for the three adaptation conditions. For the 16 Hz test stimulus, the slopes under the three adaptation conditions are 0.59, 0.61 and 0.50 for the fundamental response component, and 1.16, 1.13 and 1.04 for the third harmonic. All $r \geq 0.99$. Subject SW.

SUMMARY

(1) The response amplitude of the flicker ERG varies with stimulus modulation with a power law relation. The exponent of the power is 0.7 and 0.9 at 16 and 28 Hz respectively, almost doubles at 40 and 48 Hz (1.2 and 1.5), and is close to 1 at temporal frequencies higher than 48 Hz.

(2) When tested with sum-of-two-sinusoids stimulation, the simultaneous presence of a second stimulus enhances the response to a low modulation, 40 or 48 Hz test stimulus. The magnitude of the enhancement (up to a factor of 2.5) depends primarily on the response amplitude to the second stimulus, but not its frequency.

(3) When the test and the adaptation stimuli are presented successively, the enhancement effect persists for at least 1 sec after the adapting stimulus goes off. However, compared to when the two sinusoids are simultaneously presented, the effect is reduced (to approx. one-fifth).

(4) In both the simultaneous and successive adaptation conditions an adapting stimulus flattens the phase-modulation relation to a 40 or 48 Hz stimulus, which by itself has a phase shift of >100 deg with increasing modulation. On the other hand, the effect of modulation on response phase recorded with 16 and 28 Hz stimuli are not altered by either simultaneous or successive adaptation.

(5) The results of Expts I and II suggest a complex gain control mechanism within which the delay of the control signal must be amplitude-dependent in a nonlinear fashion. We postulate that the general features of this mechanism include a low pass temporal filter,

followed by a nonlinearity in the control pathway for the signal strength and/or its relative delay. The response is then controlled by this delayed signal. However, without additional parameters, this framework cannot quantitatively explain the longer-term changes in gain recorded in the successive adaptation conditions, nor the modulation-independent changes in the low frequency response measured in the successive adaptation conditions.

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