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The effect of background spatial contrast on electroretinographic responses in the human retina

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ABSTRACT

The electroretinogram (ERG) was obtained to contrast modulation (CM). This stimulus is a product of temporal modulation of the contrast of a spatial sinusoid at constant mean luminance. Mean contrast (10–40%), and modulation depth (25–1.0) were modulated at 7.5 Hz to record the pattern electroretinogram (PERG). The spatial pattern was a foveally fixated grating pattern with sinusoidal luminance profile with spatial frequency of 4.6 c/deg. CM resulted in significant first and second harmonic ERG responses. First harmonic amplitude increases then flattens as a function of mean contrast with ΔC = constant, while the second harmonic response remains unaffected by mean contrast. Apparently the first harmonic represents summed signals of local luminance responses arising from on and off neurons. Mean spatial contrast signals modulate preganglionic local luminance responses.

1. Introduction

It is well known that visual sensitivity remains high over a large range of luminous intensity as a result of light adaptation (Crawford, 1947; Baker, 1949; Rushton, 1965). To account for this ability it has been postulated that beyond a compressive nonlinearity of the photoreceptors (Boynton and Witten, 1970; Dunn, Lankheet, & Rieke, 2007) a shift in the dynamic range of neurons at successive stages of retinal processing (Werblin, 1971) serves to maintain responses gain in intensity changes. However, it is also well known that neuronal signals from the distal retina onwards are also determined by differences in the illumination of adjacent retinal areas; i.e., spatial contrast and spatial contrast signals, in addition to overall luminance, may influence the response to incremental stimuli (Shapley & Victor, 1978). In this study we explored in humans how differences in light stimulation of adjacent retinal areas, while maintaining overall luminance levels constant affect the gain of Pattern Electroretinographic (PERG) responses. In humans, Bodis-Wollner (1972) reported that the increment threshold of single slit-like stimuli is dependent on the spatial frequency and spatial contrast of the background grating pattern. Kulikowski (1969) reported (originally published in polish) nearly an identical result. Furthermore, the finding that above 3 c/deg of the background the increment threshold is independent of the background spatial phase led to the conclusion that pooling of spatial contrast signals and not local background luminance alone determines the response. However, a localized incremental stimulus causes a change in the overall luminance and thus itself may affect response gain. A more controlled stimulus is a contrast modulated pattern (Bodis-Wollner, Hendley, & Kulikowski, 1972) since neither background nor increment alter overall stimulus intensity. This stimulus consists of a pattern whose deviation from the mean luminance is a product of a function of space and a function of time (see Fig. 1). The spatial function is a grating pattern with sinusoidal luminance profile and the temporal function is also a sinusoid. The contrast of the spatial function is modulated by the temporal function. Thus, as a result of temporal modulation the spatial contrast changes between a peak and a minimum value, and contains a definite mean (average) contrast half-way between these two spatial contrast values. This stimulus can also be linearly decomposed as the sum of a steady and a temporally modulated counterphase grating of the same spatial frequency where the counterphase component produces the incremental and decremental contrast. A counterphase is sinusoidally temporally modulated grating which changes its spatial phase through 180 deg during one temporal-modulating half-cycle. At peak contrast, the steady and counterphase gratings are in phase while at minimum contrast there are out of phase (see Fig. 1). Regarding a contrast modulated pattern as being composed of two parts is not only formally possible but it is physiologically meaningful. Psychophysical (Bodis-Wollner & Hendley, 1979) and evoked potential (Bobak, Bodis-Wollner, & Marx, 1988) studies provided evidence that responses to modulated contrast are dependent on the contrast of the steady component. Evoked potential measures demonstrated (Bobak et al., 1988) that the function representing response amplitude for a given constant contrast

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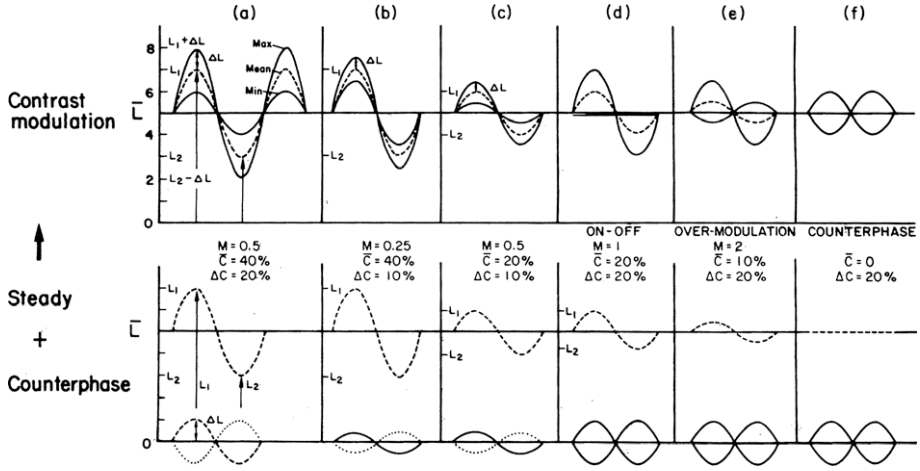

Fig. 1. Luminance profiles of modulated patterns used in this study. Notice that all patterns have the same mean luminance where *L*1 refers to the peak and *L*2 to the trough luminance each unmodulated pattern. Row 1 shows profiles of temporally modulated patterns (contrast modulated), while row 2 shows the counterphase pattern which must be combined with a steady pattern to produce the contrast modulated pattern in row 1. (a) The luminance profile of a contrast modulated grating is shown on the top. Max, the profile of the higher contrast state, alternates with the lower contrast state (Min). The mean contrast profile is labeled mean, and is represented by dotted lines. The highest and lowest luminance levels of the individual bars which establish the mean contrast profile are labeled *L*1 and *L*2, respectively, and have values of 7 and 3 in (a) of this figure. Mean contrast therefore is 0.4. Peak luminance values of the grating during modulation differ from the mean contrast profile by ΔL, which is therefore half of the brightness change at the center of each bar between the contrast levels. By adding ΔL to *L*1, and subtracting ΔL by *L*2, contrast increases by ΔC. The converse decrease occurs when ΔL is subtracted from *L*1 and added to *L*2. Modulation depth is given by $\Delta C/C$, and is 0.5. This means that the maximum contrast is 50% higher and the minimum contrast is 50% lower than mean contrast. Notice that ΔC is 0.2. (b) Row 1 shows a smaller modulation depth at the same mean contrast as (a). Note that the steady component of this contrast modulated grating has the same amplitude as (a). In the (b) column, a mean contrast of 0.4 is modulated with a modulation depth of 0.25, so that ΔC is 0.1. The column (c) has a lower mean contrast (0.2) at the same modulation depth (0.5) as in (a). Notice that $\Delta C = 0/1$ as in (b). In other words, the steady component of (c) has half the amplitude of (a) or (b) while the counterphase component has the same amplitude as (b) and is half that of (a). The column (d) has a mean contrast 0.2, as in section (c), but because the modulated contrast is .20, this results in an on–off pattern. ΔL contrast is 0.4 at its maximum and 0 at its minimum. (e) A mean contrast of 0.1; however the modulated component has the same value as in (a) and (d). This results in "over modulation" as shown in (e). (f) Has no mean contrast, while the counterphase component remains the same as in (a), (d), and (e). This results in a pure counterphase pattern.

increment (ΔC) over various mean contrast levels (C_{mean}) not only fails to be monotonically increasing but shows a distinct peak. That is to say that the slope of the response function first grows and then becomes negative at high mean contrasts when ΔC is constant. Taking advantage of this analytically useful stimulus, in our study we evaluated if spatial contrast signals modify the PERG response to contrast changes, while average luminance remains constant. To anticipate our results, while pure counterphase modulated-grating stimulation produces only second harmonic response, contrast modulation responses consist of both fundamental and second harmonic response components. The amplitude of the fundamental PERG response to contrast modulation is strongly dependent on mean spatial contrast while the second harmonic nonlinear response is similar to the second harmonic response to a "pure" counterphase stimulus.

2. Methods

2.1. Observers

After institutional review board approval was obtained, five healthy male observers (age range: 28–31 years participated in this study. All five subjects took part in Experiments 1 and 2; two (KH and SC) were also tested in additional experiments. Three of the subjects (MB, SC, and FB) were completely naïve to the purpose of the study. Observers had 20/20 visual acuity in the tested eye, two of them using mild myopic correction. The same eye was tested for all experimental conditions in each subject. The other eye was patched with a translucent tissue which blurred spatial detail but allowed light adaptation. Pupil size was between 3 and 5 mm; within experiments, pupil size was not expected to change since the experiments were performed at constant mean luminance. Subjects had no visual complaints. An ophthalmological examination was performed on each subject prior to testing and revealed no abnormalities. The examination included anterior segment and fundus evaluation.

2.2. Stimuli

The contrast of a grating pattern with a sinusoidal luminance profile was sinusoidally modulated at 7.5 Hz for a range of mean contrasts (C) and spatial frequencies. The spatial frequency of a grating pattern is expressed as the number of adjacent pairs of dark and bright bands subtended in one degree of visual angle at the observer's eye (cycles per degree, or c/deg). We report the results for 4.6 c/deg.

A contrast modulated stimulus can be viewed as the sum of a steady grating and a counterphase grating of the same spatial frequency. The contrast of the steady grating components generally defined as the difference of maximum (L_{max}) and minimum (L_{min}) luminance over their sum. The counterphase component alternates its spatial phase between two symmetrical states that are 180 deg out of the phase during each half-cycle of temporal modulation and consequently has no standing mean contrast. The counterphase modulated component induces incremental and then decremental contrast as a function of time. The deviation of the contrast modulated stimulus from mean luminance is determined by the contrast modulation depth of the stimulus ($\Delta C/C_{mean}$), and is therefore a product of both space and time. As a result, the mean contrast changes in time by ΔC is the difference in contrast between C_{max} and C_{mean}, which is equal to the difference between C_{mean} and C_{min}

$$\Delta C = C_{mean} - C_{min} \quad (1)$$

and

$$\Delta C = C_{max} - C_{mean} \quad (2)$$

As a result of the contrast modulation, L_{max} and L_{min} are not constant values. Therefore (as shown in Fig. 1) we substitute $L1$ for L_{max} and $L2$ for L_{min} of the steady grating.

The mean luminance (L_{mean}) is constant and is given by the relation

$$L_{mean} = \frac{L1 + L2}{2} \tag{3}$$

The spatial luminance profile of a sinusoidal grating is

$$L = L_{mean}(1 + C\cos 2\pi Fx) \tag{4}$$

where F is spatial frequency and x is the horizontal distance.

The spatio-temporal luminance profile of a contrast modulated pattern is described by

$$L(x, t) = L_{mean}[1 + C(t)\cos 2\pi Fx] \tag{5}$$

when

$$C(t) = C_{mean} + \Delta C\cos \omega t \tag{6}$$

where C is contrast, L_{mean} is mean luminance, F is spatial frequency, M is depth of modulation, x is distance horizontally, $C(t)$ is instantaneous contrast, and ωt is temporal frequency in Hz (Bodis-Wollner & Hendley, 1979).

Fig. 1 illustrates the luminance profiles of some contrast modulated patterns used in this study. The counterphase component was sinusoidally temporally modulated at a rate of 7.5 Hz (15 reversals per second). Mean contrast was varied from 0% to 40%. Contrast modulation depth, M ($M = \Delta C/C_{mean}$), was varied from 0.25 to 2.0. A mean contrast of 0 represents a counterphase pattern symmetry with 180 deg of shift in spatial phase during each temporal cycle (Fig. 1f). Within this context an "on–off" pattern represents a stimulus with maximum contrast asymmetry without change in spatial phase during each temporal cycle (Fig. 1d). Contrast modulation creates two asymmetric levels of peak contrast during each temporal cycle (Fig. 1a–e). Fig. 1e illustrates an "overmodulated" pattern which has a contrast modulation depth (M) greater than 1. It differs from other contrast asymmetry and a shift in spatial phase during each temporal cycle.

Contrast gain (G) may be defined as the change in response (R) over mean response divided by a given change in contrast (ΔC) over mean contrast,

$$G = (\Delta R/R)/\Delta C/C_{mean}) \tag{7}$$

We were interested if any response that is proportional to ΔC produces different results, depending on C_{mean}. It can be easily shown that any response which is proportional to ΔC can be accepted as being proportional to all local luminance changes since

$$C_{max} = \frac{L1 + \Delta L - (L2 - \Delta L)}{L1 + L2} \tag{8}$$

and

$$C_{min} = \frac{L1 - \Delta L - (L2 + \Delta L)}{L1 + L2} \tag{9}$$

hence

$$(C_{max} - C_{min})/(2) = \Delta C = 4\Delta L(2(L1 + L2) \tag{10}$$

therefore

$$\Delta C = (\Delta L/L_{mean}) \tag{11}$$

We also evaluated whether the PERG response is determined by a flicker difference in each band of the pattern; i.e., local luminance modulation depths were

$$m1 = \Delta L/(L1) \text{ and } m2 = \Delta L/(L2) \tag{12}$$

One can calculate that the local modulation depths ($m1$ and $m2$) have increasingly unequal values as mean spatial contrast increases. As we shall discuss, this asymmetry of the stimulus allows us to separate spatial contrast from local luminance effects.

2.3. Apparatus

Stimuli were presented on an oscilloscope (Joyce Electronics, Model PJ 2, England), which had a display area of 23 × 30 cm. The raster was controlled by a hardwired pattern generator, constructed in our laboratory, modulated by a DEC/LSI-11 (Digital Corp., Florida) microcomputer. The display subtended 18 deg at a viewing distance of 72 cm. Mean screen luminance was 170 cd/m^2. To minimize stray light contamination at the edge of the pattern, the luminance of the screen surround was approximately matched to that of the screen. In addition, each stimulus condition was tested for stray flicker contamination by covering the screen with cardboard. The signals were amplified (gain = 50,000) with a optically isolated pre-amplifier (Neuroscientific, Model 600, USA) with bandpass limits of 1–100 Hz. Sixty seconds of signal were averaged over 20 epochs of 3 s duration each, by a PDP 11/23 microcomputer. Each testing condition was repeated in each subject between two and eight times to ensure reliability. An artifact-rejection algorithm (+90 μV) prevented the addition of large potential changes produced by eye movements or blinks.

2.4. Calibrations

A contrast calibration curve was obtained by measuring the mean luminance of the Joyce oscilloscope using a photometer/radiometer (Spectra, Model 301, USA). For contrast calibration we used a linear photodiode tube (RCE 5583), which was firmly positioned in front of the scope. The output voltage of the photodiode was measured and contrast was calculated from these readings. Measurements were taken over a stationary square wave grating of low spatial frequency by shifting the grating behind the slit maximum and minimum output of the photocell. The amplitude of the carrier was changed in 3 dB (0.15 log unit steps) from 4 to 60 V. Contrast increased linearly up to 80%. The highest peak contrast used in this study was 80%; thus, contrast remained in the linear range.

For each testing condition, the desired level of contrast modulation depth was set by the adjustment of a logarithmic attenuator in steps of 0.05 log units, where 1.0 represents an on–off pattern (or 100% attenuation). The two components of the contrast modulated pattern (the counterphase and the steady) were attenuated independently as required. A peak contrast calibration curve was obtained in order to further insure the accuracy of the various contrast modulation settings used.

2.5. Fourier analysis and statistics

The amplitude and phase of the first (7.5 Hz) and second (15 Hz) components of the pattern ERG response based on 230 s of recorded ERG (Fig. 2) were determined with PDP 11/23 Fourier analysis software.

The bandwidth of each spectral component was 0.5 Hz. Amplitude was expressed as half of the peak-to-trough voltage. When amplitude of a response was close to noise level, a constant phase was considered an indicator of the presence of a response. Constant phase was determined by repeated runs during which the phase was within ±30 deg. There were two "noise" estimates. One was based on the ERG response obtained while viewing an unmodulated blank screen of the same mean luminance. The other was calculated from the amplitude ratio of the harmonic response over the mean adjacent non-harmonic components.

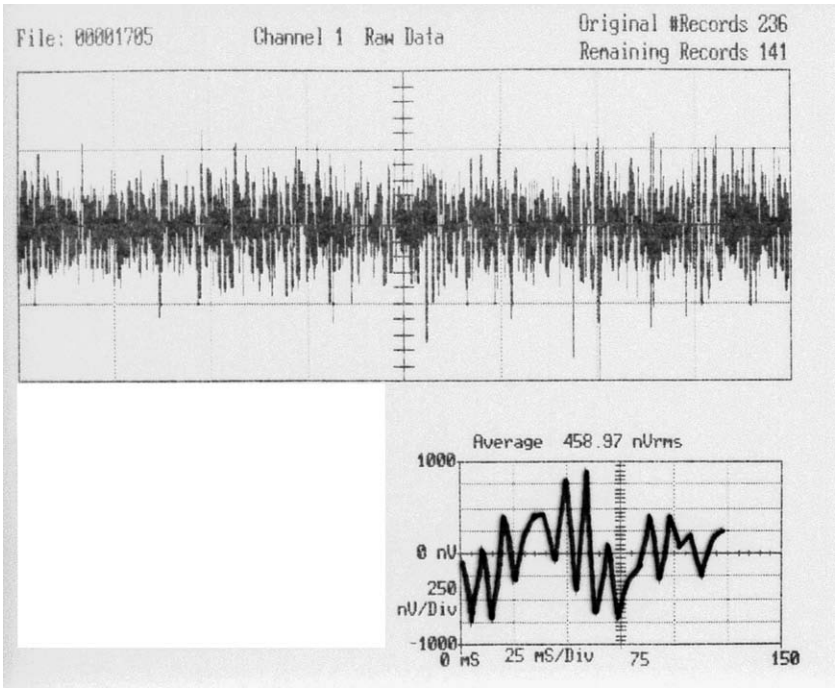

Fig. 2. The top shows 30 s of continuous raw ERG data. Below is the averaged electroretinogram (ERG) for 125 ms. The data have not been filtered and the average ERG shows both low and high frequency components. The first and second harmonic is evident for the trained eye obtained at 50% modulation depth. The ERG was quantified for the first and second harmonic responses using Fourier analysis.

A two-way analysis of variance (ANOVA) was then performed on the Fourier-analyzed amplitude and phase data.

2.6. Testing conditions

Contrast responses in the pattern ERG were explored using two main paradigms. In the first paradigm the mean contrast (C_{mean}) was kept constant at 40% and the modulation depth ($\Delta C/C_{mean}$) was randomly varied from run to run between 0.25 and 1 (“on–off”) by accordingly varying ΔC between 10% and 40%. In the second experimental paradigm, ΔC was kept constant at 20% and C_{mean} was randomly varied from 0% (counterphase) to 40%. Spatial frequency was 4.6 c/deg.

2.7. Method of recording the pattern ERG and control experiments

We recorded the PERG with C-glide electrode referred to a gold-cup electrode placed on the contralateral temple. This montage was decided after we conducted extensive preliminary studies to compare various types of eye electrodes and reference positions under identical testing conditions. The stimulus pattern used (except where noted otherwise) was a sinusoidal grating, counterphase modulated at 7.5 Hz with a peak contrast of 80%.

2.7.1. A comparison of electrode types

We compared the C-glide, a disposable commercially available eye electrode, to three electrodes commonly used for obtaining the electroretinogram (the JET contact lens, Universo, Switzerland; DTL fiber, Tallahassee, Fl, USA; and gold-cup dermal electrode, Grass, USA). The C-glide is placed over the lower lid in the tested eye. It uses a micro-thin saline pad for contact with the eyeball, and a carbon fiber connects this to a miniature socket. The fiber is laminated in polyethylene/polyester for stability. The material is firm and easy to handle. One drop of local anesthetic (Proparacaine Hydrochloride 0.5%) was used. Scalp electrodes were the gold-cup type.

The following electrode comparisons were made: (a) active C-glide with a contralateral reference being either a C-glide, DTL, or palpebral gold; (b) active DTL with a contralateral C-glide or gold-cup reference. For evaluating the placement of references we compared the responses obtained with an active C-glide referenced to the other eye and to the temple and to the forehead and to the midfrontal electrode. The C-glide electrode used monocularly (OD) referenced to either a gold-cup electrode placed on the lower lid of the patched fellow eye (OS) or to a DTL gave a well-isolated retinal signal with good repeatability and a high signal-to-noise ratio. In each combination the C-glide alone or combination with a C-glide reference yielded the best signal-to-noise ratio. Signal-to-noise ratio was determined as the ratio of the response amplitude at 15 Hz over the mean amplitude of the response over adjacent (±0.5 Hz) response frequencies (i.e., the mean over 14.5 and 15.5 Hz).

2.7.2. A comparison of various reference sites

A gold-cup electrode was placed below the skin of the fellow eye, which was patched to serve as a reference. The forehead was grounded. This “interocular recording” montage reduces passively transmitted potentials by canceling them. Volume conducted potentials, even if of different amplitudes, would have identical timing. Recording an interocular PERG has an additional advantage, as first suggested by Fiorentini, Maffei, Pirchio, Spinelli, and Porciatti (1981), in that it also cancels signals related to conjugate eye movements. However, we found little eye movements pickup when the active electrode was referenced to the temple. In the closed eye condition there was little noise.

We found a reference electrode placed on the ipsilateral temple equally effective to a contralateral reference.

2.7.3. Control experiments for evaluating “passive” pickup of the corneal electrode either of occipital signals (VEP) or from the other eye

With the C-glide–C-glide (reference) yielding a good PERG we simultaneously recorded the signal at Z5 (occipital) using conven-

tional VEP (gold-cup) electrodes referenced to the C-glide inserted at the patched eye. The VEP was clearly recordable suggesting that the patched eye was indifferent and did not significantly register either a volume conducted VEP or PERG (from the unpatched eye). As an additional control, we attempted to record a PERG from the patched right eye with the reference electrode being placed on the ipsilateral temple: the ERG response was at noise while the VEP (through stimulation of the open eye) was unaffected. In other words, the control experiments suggested the suitability of the C-glide PERG recordings by showing its good signal-to-noise ratio, secondly revealed that it does not pick up significant passively conducted signals.

2.7.4. The effect of the corneal placement of the electrode on the response

We evaluated the stability of the C-glide obtained PERG by exploring in one subject the influence of electrode position on the amplitude and phase of the signal. We recorded simultaneously from two adjacent active C-glide electrodes placed in the same eye. The stimulus was a contrast modulated pattern with a spatial frequency of 4.6 c/deg, modulated at 7.5 Hz. As illustrated in Fig. 3, after Fourier analysis the two positions result in similar amplitude and phase. Hence the exact position of the C-glide electrode has no significant influence on the amplitude of phase of the PERG signal.

2.7.5. The effect of eye movements on the PERG

The subject was requested to follow the apparent movement of the one of the bars of a sinusoidal grating across the screen. The pattern had 80% contrast. When the reference electrode was placed in the contralateral open eye, the response degraded to noise level. However, when the reference electrode was referred to the ipsilateral temple the response degraded but remained above noise. Hence an interocular montage is more effective in canceling the effect of active eye movements. The temple reference has however a higher signal-to-noise ratio.

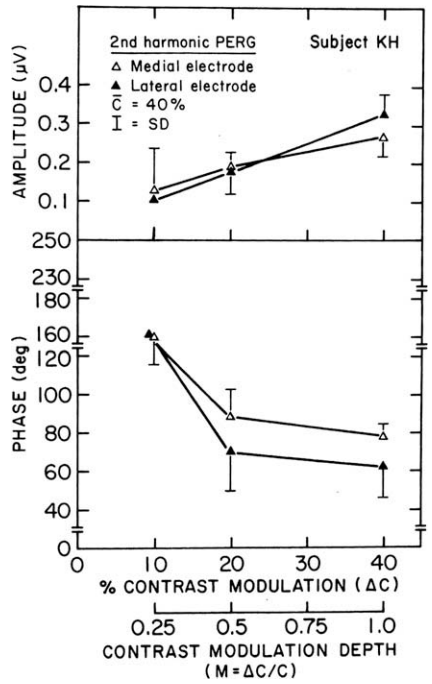

Fig. 3. A comparison of the pattern electroretinogram (PERG) response of two c-glide electrodes placed simultaneously medially and laterally in the same eye of one subject. The top panel shows the amplitude (in microvolts) of the PERG, while the bottom panel shows the phase (in degrees). These results show that the exact position of the electrode does not appreciably affect the PERG phase or amplitude.

3. Results

A two-way analysis of variance (ANOVA) performed on the Fourier-analyzed amplitude and phase data revealed that the monocular PERG to a contrast modulated pattern contains significant first and second harmonic frequency components. Hence we will discuss each harmonic function separately for each experimental condition.

3.1. Experiment 1: PERG response as a function of modulated contrast (ΔC)

Fig. 4 is a plot of the amplitude (top panel) and phase (lower panel) of the first and second harmonic components of the PERG as a function of modulated contrast (ΔC). Mean contrast (C_{mean}) was 40%. Each datum represents the mean of five subjects. As shown in the top panel, the amplitude of the first harmonic component grows monotonically as ΔC increases [$F(2,8) = 16.87$; $p < .01$]. Second harmonic amplitude also increases with ΔC [$F(2,8) = 34.65$; $p < .01$], although the slope of the function is somewhat less steep. The amplitude of subjects' individual first and second harmonic components for this condition are represented if Fig. 5. In all subjects first and second harmonic amplitudes increased with ΔC.

The lower panel of Fig. 5 represents PERG phase as a function of ΔC. Neither the first nor the second harmonic functions were significantly changed by varying modulated contrast. However, although the first harmonic phase function is smooth and monotonic, the second harmonic function is somewhat more compli-

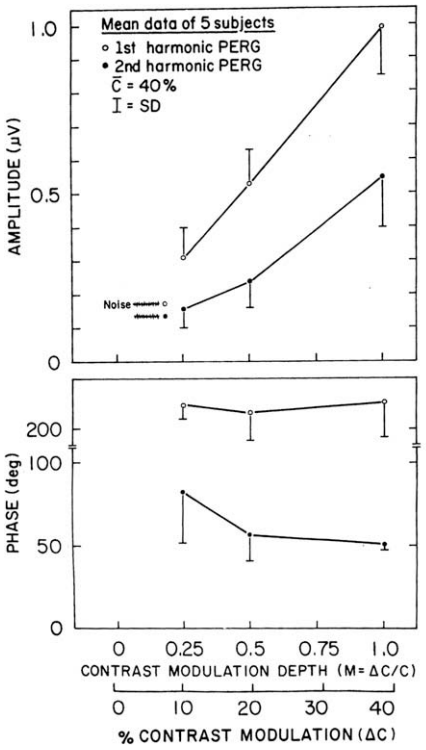

Fig. 4. The effect of contrast modulation depth on the PERG. The mean data of five subjects are represented. Bars represent one standard deviation. The top panel shows the amplitude (in microvolts) of the first and second harmonic components, while the bottom panel shows their phase (in degrees). The amplitude of both components increases monotonically with contrast modulation depth. Notice that there is no saturation evident even at the highest instantaneous contrast ($C_{max} = 0.80$ at $C = 0.4$ and $M = 1$) for either response. Phase however does not change.

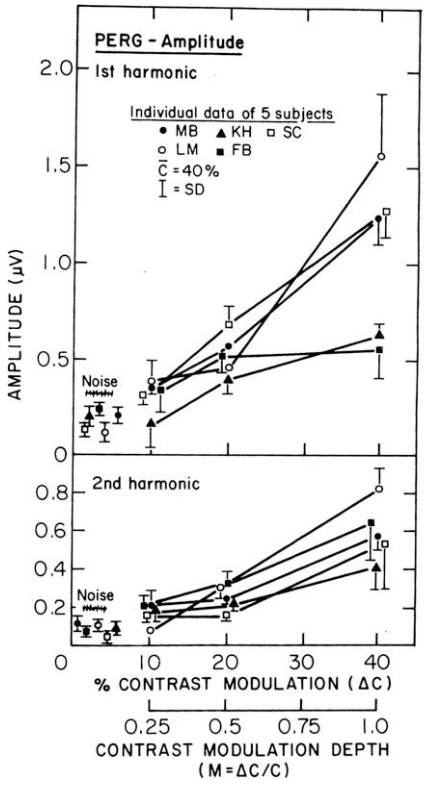

Fig. 5. Individual data showing the effect of increasing contrast modulation depth on the first and second harmonic amplitude of the PERG.

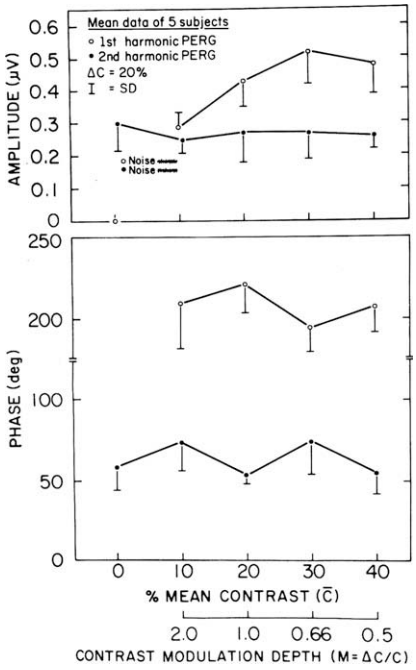

Fig. 6. The effect of mean contrast on the PERG response to constant modulated contrast. The mean data of five subjects are shown. Bars represent one standard deviation. The top panel shows the amplitude (in microvolts) of the first and second harmonic components, while the bottom panel shows their phase (in degrees). The amplitude of the first harmonic amplitude function first grows and then slightly dips in respect to mean contrast, while the second harmonic function remains flat.

cated. However, for both the first and second harmonic functions, results indicate that there is no response saturation as a function of peak instantaneous contrast, which can be simply calculated as $\Delta C + C_{mean}$. This is relevant for interpreting the results illustrated in Fig. 6 which show a flattening of the first harmonic function beyond 30% mean contrast. Based on the results presented here (Fig. 4) it is clear that this flattening of the curve is not caused by saturation to high C_{max}.

The results strongly suggest that the amplitude of the pattern ERG is dependent on modulated contrast. This is true for both odd and even components. This result could be obtained if the response was proportional to either instantaneous (peak) contrast (C_{max}), irrespective of average contrast, or relative contrast change ($\Delta C/C_{mean}$). Our second set of experiments was designed to distinguish between these alternatives.

3.2. Experiment 2: The PERG as a function of mean contrast

The effect of changing mean contrast (C_{mean}) on the PERG is illustrated in Fig. 6. ΔC was kept constant at 20%. The amplitude (top panel) of the first harmonic component grows as C_{mean} increases from 0% to 30% (where it saturates). This trend was significant [$F(3,12) = 6.27$; $p < .1$]. The amplitude of the second harmonic component was not affected by C_{mean}. Individual first and second harmonic amplitude data are shown in Fig. 7, and all subjects show an accelerated trend, i.e., an increase of the first harmonic amplitude with increasing C_{mean} (top panel). For the second harmonic responses (lower panel), there is greater inter-subject variability but most subjects show no effect of C_{mean} although one (represented by filled circles) showed an apparent increase in amplitude at 20% contrast. However, another subject (represented by filled triangles) showed decreased amplitude at this same point. The first harmonic data clearly suggest that mean contrast does control the PERG response. The positive slope of the function represents contrast gain (see Sections 2 and 4).

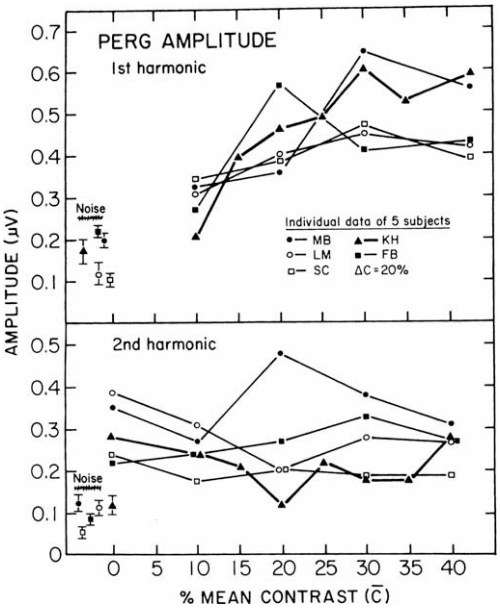

Fig. 7. Individual data showing the effect of mean contrast with constant modulated contrast on the PERG. In four of five subjects the first harmonic amplitude grows with mean contrast while the second harmonic shows little change with mean contrast in any subject.

In the lower panel of Fig. 6, the phase of the PERG is plotted as a function of increasing C_{mean}. It is clear that first harmonic phase is not changed by different levels of mean contrast. Surprisingly, the second harmonic shows a slight phase shift between 10% and 20% contrast [$F(4,16) = 3.24$; $p < .004$].

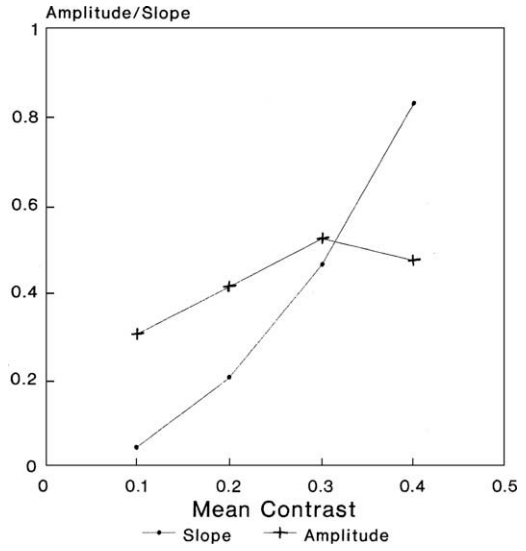

Fig. 8. A comparison of the predicted response function if local flicker determines the response to mean contrast, and determines the observed first harmonic of the PERG as a function of mean contrast. The slope scale represents values derived for each mean contrast condition assuming that at any given mean contrast the response to ΔC is proportional to the difference of light bar and dark bar flicker (see text). The scale for the amplitude of the first harmonic PERG is expressed in microvolts, while the theoretical function is expressed in relative units multiplied by 10 in order to bring in register the two functions. It is clear that the fundamental PERG response to mean contrast cannot be accounted for by a model which assumes that local flicker asymmetries determine the response.

We have evaluated whether the slope of the fundamental response as a function of mean contrast is proportional to a function derived from the asymmetry of local flicker values. Assume that there is a response mechanism with a spatial profile narrower or equal to the half-cycle of a pattern. Given the luminance difference created by the steady spatial contrast pattern, temporal contrast modulation will induce different depths of local flicker (m) in the dark (higher depth) and lighter (lower depth) bands of the pattern. Based on the assumption that response is determined by this local flicker difference ($m1 - m2$), Fig. 8 represents the predicted response function. It is clear that the fundamental response function does not show the monotonic and accelerated slope that would be expected if local flicker difference determined the response. It should also be noted that a response function based not on the difference but the sum of local flicker would predict a similar function to the one in Fig. 8 with the same shape, the only difference being in scale.

4. Discussion

The strength and weakness of PERG response is that it represents the mass activity of the central retina (Bobak et al., 1983; Hollander, Bisti, Maffei, & Hebel, 1984; Maffei, Fiorentini, Bisti, & Hollander, 1985; Maffei & Fiorentini, 1990). Therefore we consider the spatially tuned PERG response curve as an envelope function of all retinal ganglion cells covering the central retina (Bodis-Wollner & Tzelepi, 2002). The spatially bandpass ERG response curve may be considered as the response of the "equivalent" retinal ganglion cell. This model is clearly insufficient to discern fine spatial and temporal structure of the retina which is inhomogeneous. Using a system analytical approach it is nevertheless possible to deduce some logically coherent inferences of sequential (Spekreijse & Reits, 1982) and infer feedback connections of the human retina (see for instance Brannan, Bodis-Wollner, & Storch, 1992).

We evaluated the dependence of the ERG response of the "equivalent retinal ganglion cell", using 4.6 cpd, the pattern spatial frequency near the optimum for ganglion cells of the primate central retina (Purpura, Kaplan, & Shapley, 1988). The responses were analyzed to dynamic contrast ("counterphase component) riding on standing retinal contrast ("pedestal" contrast). Using counterphase modulated patterns, contrast gain control as opposed to contrast response properties cannot be derived from either psychophysical or electrophysiological measures. In a counterphase stimulus the magnitude of local luminance change (the maximum and minimum flicker at each point) and spatial contrast change (the maximum and minimum luminance difference between adjacent bands of the pattern) are equivalent (Bodis-Wollner et al., 1972). Hence using counterphase stimuli it is not possible to separate response to spatial contrast versus local flicker responses. Non-periodic stimuli, in particular classical incremental stimuli locally change the mean luminance of the stimulus; hence conclusions concerning gain in reference to spatial contrast alone would not be rigorous either. If for instance the luminance of unmodulated elements of a pattern is varied to discern the effect of luminance on contrast responses (Riemslag, Ringo, Spekreijse, & Verduyn Lunel, 1985) the average retinal illumination is also affected and spatial contrast is not constant. Our stimulus ensured constant average luminance and constant average spatial contrast. Our results suggest that spatial contrast alone without changing mean luminance adjusts the gain to the sum of local luminance changes in the human ERG. These data therefore suggest that retinal signals derived following center–surround interactions participate in this circuit.

Several functionally relevant nonlinear operations are expressed in the human PERG (Brannan et al., 1992; Hess & Baker, 1984). Our studies show that the ERG response which expresses a retinal nonlinear mechanism is the fundamental ERG component. It is known that a steady contrast stimulus modulated in contrast around its mean introduces a strong fundamental response in addition to the second harmonic visual evoked potential response (Bobak et al., 1988), however, its presence in the PERG was controversial. Hess et al. (1984) reported that no fundamental response occurs in the PERG to an on–off stimulus (which is the extreme of contrast modulation) when an equiluminant surround is provided, while Harnois, Bobak, and Bodis-Wollner (1982) claimed its presence. In our study (Brannan et al., 1992) we confirm that the fundamental PERG is present to contrast modulation without changes in the mean luminance of either the stimulus or the surround. Our present study shows that for constant ΔC and constant mean luminance the fundamental PERG response amplitude first grows with mean contrast and then flattens. Therefore the fundamental response function cannot be predicted by the magnitude of local flicker. Its presence cannot be due to surround luminance mismatch, either. What is the origin of the fundamental response?

It was shown that when a pattern is modulated in the counterphase mode with two temporal frequencies simultaneously, the PERG contains fundamental response components as well as intermodulation terms arising from interaction between the fundamentals (Brannan et al., 1992). It was established that this fundamental PERG response is not noise. Furthermore, it cannot arise in a linear system as equal and opposite responses would cancel at the corneal electrode sampling all spatial phases of the pattern. Additionally, the fundamental component cannot arise from the interaction of the second harmonics beyond their point of generation, since no subharmonics are generated in the PERG to single frequencies of modulation (Regan & Regan, 1988). Hence the fundamental component is likely to be generated prior to or in parallel with symmetrical (second harmonic) responses. Sieving and Steinberg (1985) using in depth and corneal recordings in the cat reported that the waveform of the local intraretinal PERG depended on the spatial phase of the grating pattern. At the center of the band, fundamental components dominated the local ERG, while at the

corneal electrode all fundamentals cancelled. However, their stimulus was counterphase modulated while the stimulus we used is asymmetrical. The asymmetry of a contrast modulated pattern arises from the fact that the temporal average luminance of adjacent (dark or light) levels is unequal. Taken together, these results suggest that a fundamental response is present as a result of a local spatio-temporal asymmetry.

We have evaluated if the fundamental response function is determined by the difference of local luminance modulation (flicker) of the stimulus pattern. If this was the case the fundamental response would be consistent with a preganglionic nonlinear/linear sequential model. The fundamental PERG response curve does not fit this prediction (see Fig. 8). It is also not possible to account for the shape of the fundamental response function as a result of saturation at high instantaneous peak contrast, since the fundamental response does not saturate even at 80% peak instantaneous contrast (see Fig. 4). This suggests a stability of the retina to maintain high sensitivity to small changes even at high contrasts. The data in Fig. 7 do not suggest that the response "runs away" at high contrasts, and this result is consistent with the operation of gain control. We define gain as $(\Delta R/R)/\Delta C/C_{mean})$. From the experiment keeping ΔC constant it is evident that $G = K/\Delta R/R/C_{mean}$. A decrease in gain would be shown if response as a function of mean contrast had a negative slope, while constant gain would occur if ΔR and C_{mean} were inversely proportional, resulting in a flat function. An increase in response, as shown in Fig. 8, suggests that gain increases up to 30% mean contrast and then remains constant or decreases slightly. Possibly therefore our results could be accounted for with an additional nonlinearity (Sutter & Vaegan, 1990) from a simple sandwich model (Spekreijse, 1966) to the model of Shapley and Victor (1978), Shapley and Victor (1981) for explaining the contrast dependence of second order responses in individual retinal ganglion cells in the cat. Their model accounts for contrast gain control based on a basic nonlinearity of preganglionic retinal circuit (Shapley & Enroth-Cugell, 1984; Shapley & Victor, 1981) providing negative feedback. In this model the feedback signal is neither added or subtracted but is used as a controller of the response of distal retinal elements. This model significantly departs from the concept of adaptation of successive neurons but it is consistent with physiological and anatomical data. Our results depart somewhat from a preganglionic model based on single ganglion cell properties in that they do not show perfect agreement between the amplitude and phase functions of the PERG. By perfect agreement we assume that whenever there is a shift in the amplitude function there should be one in the phase function. Although we do not have an explanation, it has been observed in recordings using massed responses, such as the VEP (Milner, Regan, & Heron, 1974; Regan, 1968) that there are no correspondence phase shifts at points where the amplitude function peaks. One possibility could be that the model of Shapley and Victor (1981) is based on responses of single ganglion cells in the cat, while the PERG samples retinal contrast gain mechanisms over an extended retinal area and possibly records an average of the massed ganglion cell responses including preganglionic lateral and feedback connections.

While the retinal circuitry of this contrast gain control mechanism which is expressed in the fundamental ERG response component cannot be determined using corneal recordings alone, the results suggest some candidate structures. Bodis-Wollner and Tzelepi (1998, 2002) considered two proximal to distal feedback paths: one onto horizontal cells and another to cone receptors.

They modeled a push–pull effect of the two feedback pathways on retinal ganglion cell center/surround interaction and retinal ganglion cell output. A feedback pathway arising from the proximal retina following center–surround interactions on distal retinal elements could regulate local luminance responses preceding the ganglion cell.

It was shown in the turtle retina (Reifsneider & Tranchina, 1995) that background contrast modulates kinetics and the lateral spread of local response properties in the outer retina. In the primate some studies of the center/surround organization of preganglionic neurons show a potential mechanism of the influence of spatial contrast on first harmonic PERG responses in humans. Axon bearing primate AI amacrine cells Davenport, Detwiler, and Dacey (2007) are on–off cells hence generate second harmonic responses to dynamic spatial contrast. Their dendritic tree establishes the center/surround organization.

The sum total of "On" and "Off" cone bipolar cells with center–surround organization (Dacey et al., 2000) could be suited to generate massed first harmonic, "local luminance", responses of the PERG. A luminance dependent divergence of the contribution of "On" and "Off" neural channels to the non-monotonic photopic responsive curve flash ERG has been shown by Ueno, Kondo, Niwa, Terasaki, and Miyake (2004). As we have discussed, with increasing C_{mean} local flicker

$$m1 = \frac{\Delta L}{L1} \text{ and } m2 = \frac{\Delta L}{L2}$$

grow more and more apart: $m2$ (the local flicker in the dark bar stripe) becomes larger and larger compared to $m1$, the local flicker in the light stripe. Our study shows (Fig 8) at maintained luminance a non-monotonic fundamental response curve of the PERG with increasing spatial contrast perhaps reflecting divergent weight of "On" and "Off" responses.

Irrespective of the precise explanation, our results may resolve the paradoxical interpretation of Riemslag et al. (1985) compared to the results reported by van den Berg, Boltjes, and Spekreijse (1988). The former study concluded that the PERG is of luminance origin in man and not the result of spatial contrast mechanisms. Our results suggest that the amplitude of the local luminance (fundamental) response component is under the control of retinal spatial contrast and hence indirectly responds to spatial contrast. The advantage of a retinal mechanism which samples contrast signals over a considerable area of the central visual field and adjusts distal luminance response gain is perhaps to optimize responsiveness to relatively small local changes even in the presence of high prevailing contrast.

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