ANALYSIS OF THE RETINA VIA SUPRAFUSION ELECTRORETINOGRAPHY

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ABSTRACT Electroretinographic (ERG) transient responses elicited in monkeys by abrupt changes in the periodicity of a rapidly intermittent (suprafusion) luminance stimulus were studied experimentally, and analyzed and interpreted through a theory of dynamic retinal responses. The suprafusion ERG transients are confirmed to behave in accord with theoretical expectation, as elemental responses (retinal Green's functions). By aid of the theory the ERG wave-forms can be reduced to two significant elements. One element, accounting for approximately two-thirds of the total ERG variance, is strictly linear, and correlates well with simultaneously evoked cortical (VEP) transients which were previously related to suprafusion perception in humans. The other element, comprising approximately one-third the ERG transient, is a rectification, with properties indicating that it may arise from a specific layer of retinal neurons (amacrine cells); on this assumption the theory demonstrates that high-frequency nonlinear ERG flicker can isolate activities proximal and distal to the rectifying (amacrine) layer. Thus, the hypothesis of an amacrine origin for the rectifying element entails the possibility that suprafusion ERG studies could accomplish in vivo "dissection" of the human retina.

INTRODUCTION

The main purpose of this paper is to present an analysis of electroretinographic (ERG)[1] measurements above the flicker fusion frequency, which we shall interpret as showing evidence of substantial amacrine cell involvement. These ERG were elicited in the course of studies of suprafusion visual phenomena (Bird and Mowbray, 1973), using an unorthodox stimulus whose essential feature is the use of suprafusion frequencies. A simple way to induce a visible response with our apparatus is to shift the frequency (Fig. 1). This suprafusion period-jump has been shown (Bird and Mowbray, 1973) in linear theory to elicit an elemental Green's function of the system, its impulse response, so that our theoretical inclination is to suppose the period-jump equivalent to a flash; but our more empirical tendencies warn that linear theory is only approximate, and that impulses and flashes and period-jumps are after all physically different (Bird and Mowbray, 1973; p. 683). In any event, under the essential condition of a variable, rapid intermittency and with apparatus designed accordingly, the period-jump is a simply effected and readily manipulated stimulus, and it has been shown to yield exceptionally sensitive psychophysical responses (Bird and Mowbray, 1973). The ERG results herein give further a posteriori justification for suprafusion stimulation. For some a

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[1] *Abbreviations used in this paper:* EGR, electroretinographic; PC, principal components; PNR, proximal negative response; VEP, visually evoked potentials.

priori rationalization recall the homology between modern flicker studies and their antecedents in radio.

Radio broadcasting utilizes two major modes of modulation, the familiar low-frequency AM and high-frequency FM transmissions. Both modes lead to the same end, an aural response, and they are mathematically related, as two aspects of a Fourier transform of the electromagnetic radiation. But *physically* the two modes are distinctly different, as their usages attest.

In vision, Fourier modulation is now also commonplace, but only in the stimulus mode analogous to low-frequency AM radio. At first blush, high-frequency modulation can seem singularly uninteresting, because it is a classic law of vision that any frequency above flicker fusion yields the same visual sensation as does constant illumination at the same mean level. But this overlooks two points. First, this (Talbot-Plateau) law requires that "the retina is stimulated *always in the same way* by regular periodic impulses of light" (Helmholtz, 1962, emphasis added). If, as in FM radio, the stimulation frequency itself is changed, the law does not preclude a sensation change. Indeed, suprafusion oscillations within the visual system can act like an FM "carrier wave" to transmit sensory signals.

The second and salient point is that suprafusion oscillations within the visual system, although themselves not directly perceptible, exist and carry information about the neural mechanisms. When an FM change is transmitted via these oscillations to yield a visible effect, the latter will be like any visual sensation—a change in brightness (or color)—just as FM and AM radios give similar aural sensations. Likewise, the FM suprafusion sensations can be mathematically related to the more usual AM-type flicker and to flash sensations. However, the history of AM-FM radio warns us against dismissing the suprafusion mode as merely a complex equivalent of low frequency or pulse modes. They are basically different. (And departures from the linear FM-AM analogy are apt to magnify the distinction.) Suprafusion changes take place about a dynamic steady state vs. about a static equilibrium for ordinary flicker or flashes. One may conjecture that the suprafusion oscillations will tend to excite preferentially the more dynamic elements of the visual system—in the retina, primarily the "on-off" amacrine cells (Burkhardt, 1975). But let us see what suprafusion experiments will tell.

Consider as a basic stimulus a step-function form of frequency modulation, i.e. the abrupt period-jump ($t_1 \rightarrow t_2$) in high-frequency luminance modulation depicted in Fig. 1. This stimulus has constant mean luminance and its oscillation periods t_1, t_2 are kept ≤ 10 ms, thus precluding ordinary flash and flicker responses. The period-jump is perceived by human subjects as a transient brightness change, not surprisingly as suggested above.

This suprafusion period-jump stimulus, although not much studied, is not new. In his classic book, Fechner (1860) noted the tactual sensation produced by a toothed-wheel analogue to the stimulus of Fig. 1. In vision, apparatus developed by one of the present authors (Dr. Mowbray) was used at first to study *repetitive* frequency shifts (hence as ordinary flicker sensation, see Forsyth and Brown, 1959). Sen (1964) called attention to the transient response to the isolated period-jump itself, and investigation of its properties was undertaken by Mowbray and Sen (unpublished), Mowbray and Bird (1969). Levinson (1968) also discussed a limiting case of this suprafusion transient, which he dubbed "pseudo-flash", and attempted to determine its perceived polarity (but cf. Bird and Mowbray, 1969). Intensive psychophysi-

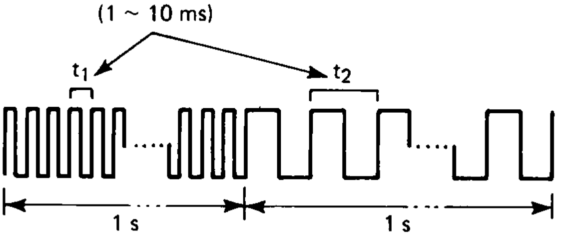

FIGURE 1

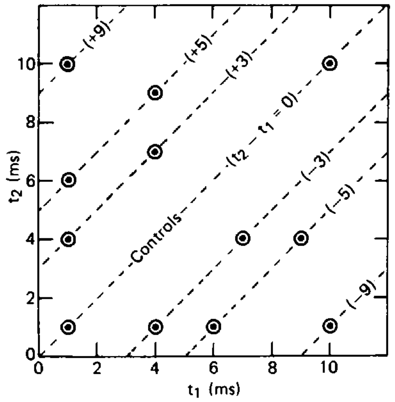

FIGURE 2

FIGURE 1 Schematic of the luminance stimulus containing a period-jump ($t_1 \rightarrow t_2$). The waveform shown was repeated continuously in each experimental run resulting in a series of alternating period-jumps upward and downward, presented in Maxwellian view at retinal illuminance 20,000 td on negligible background.

FIGURE 2 Test matrix indicating the pairs of periods t_1 and t_2 employed in various runs (●). Dashed lines connect the tests theoretically predicted to be experimentally equivalent.

cal investigation and theoretical analysis have since shown the suprafusion period-jump transient to be a sensitive and elemental response of vision (Bird and Mowbray, 1973). The mathematical form of its associated electrophysiological responses was theoretically deduced in that work. Subsequently, the predicted cortical transient was detected and measured in the monkey via visually evoked potentials (VEP) recorded from implanted electrodes (Mowbray et al., 1975). Simultaneously with the VEP, the predicted ERG transient was recorded from electrodes placed on the monkey cornea, as briefly reported by Flower et al. (1975).

The present paper reports detailed measurements, analysis, and interpretation of the suprafusion ERG transient in light of the theory of dynamic visual responses (Bird and Mowbray, 1973). To check the theoretical prediction that the ERG transient would vary with the oscillation periods before and after the jump (t_1 and t_2 in Fig. 1) simply as $\propto(t_2 - t_1)$, the monkey experiments were performed for a matrix of values of t_1 and t_2 (Fig. 2). This is a necessary and sufficient test of the one basic assumption of the theory: linearizability (Bird and Mowbray, 1973; Sec. III). After collating summed ERG wave-forms for each t_1, t_2 pair, we analyze all the data into Principal Components (PC; cf. Mowbray et al., 1975), which reduction has the virtue of being purely empirical, i.e., unprejudiced by theoretical preconceptions.

The results quantitatively confirm the basic theoretical assumption, with the important amendment of a differing linearity for positive and negative period-jumps ($t_2 - t_1 \lessgtr 0$). Thus, the PC analysis evidences a linear rectification by the retina.

But, the neutrality of the PC analysis has as its obverse ambiguity: the PC are not a unique decomposition of the ERG transient (cf. John et al., 1973). To remove the ambiguity requires information or concepts external to the data.

Therefore, we turn to the theory of suprafusion responses, with the added postulate of retinal rectification indicated by the PC analysis. As developed in the Appendix, the theory streamlines the reduction of the ERG data, supplanting the onerous PC computations with a simple calculational algorithm. The outcome is a representation of all the ERG wave-forms in terms of two elemental response functions. One elemental function, accounting for approximately two-thirds of the ERG variance, is strictly linear and closely related to the simultaneous VEP transients which we have correlated previously with other neuronal and behavioral data. The other element, comprising approximately one-third of the ERG variance, is a rectification that we shall argue may represent amacrine cell excitations, and may also permit separate measures of proximal and distal activity. If so, analysis of suprafusion ERG experiments could accomplish a virtual dissection of the retina.

METHODS

The animal preparation, stimulus presentation, experimental procedure, and response processing were as described for the simultaneous VEP experiments (Mowbray et al., 1975). Whereas successful VEP recording required fortunate electrode placement, accomplished in only one case, the ERG transient was reliably obtained in all experimental runs. Thus, the ERG data could be collected over the full matrix of tests planned (Fig. 2), though the VEP could not.

Animal subjects were two adult female rhesus monkeys (*Macaca mulatta*) which had earlier undergone surgical implantation of electrodes on the visual striate cortex for the VEP studies (Flower et al., 1975). For each experimental session, commencing at least 1 wk after surgery, a monkey was anesthetized with phencyclidine HCl and placed under mechanical respiration. Mydriasis was induced in the left eye by 1% tropicamide and the cornea and lids anesthetized by 4% cocaine HCl. A Karpe-type contact lens containing an Ag-AgCl corneal electrode was lubricated with 1% methylcellulose and placed on the treated cornea. A 26-gauge stainless steel needle inserted subcutaneously at the medial canthus served as indifferent electrode. During the data runs the animal had to be kept under general akinesia to obtain the simultaneous VEP records. This involved intravenous administration of gallamine triethiodide (1 mg/kg) and precautions to minimize akinesic stresses as described by Mowbray et al. (1975).

The stimulus light was a Sylvania R1131-C glow modulator tube (GTE Sylvania Lighting Products Div., Danvers, Mass.) driven by electronic generator and logic units to produce the wave-form shown in Fig. 1. This wave-form was verified during experiments by a photocell-oscilloscope monitor. The stimulus was presented in Maxwellian view (Westheimer, 1966) through an optical system adjusted to focus the source image at and completely filling the monkey's mydriatic pupil. The resulting mean retinal illuminance was 20,000 td over a uniform circular field 24° in diameter, for all experiments reported.

In each experimental session, a series of runs was made using various values of the periods before and after the period-jump as indicated in Fig. 2. The dashed lines there connect the different tests which are expected to give equivalent results on the basis of the theoretical deduction that the suprafusion transient is proportional to period-jump $t_2 - t_1$. With each pair t_1, t_2, the wave-form in Fig. 1 was repeated successively every 2 s for a total run duration of 7.5 min, resulting in 225 period-jumps ($t_1 \rightarrow t_2$) interspersed with 225 opposite jumps ($t_2 \rightarrow t_1$). Each session consisted of seven such runs: three with differing periods t_1 and t_2, one control with $t_1 = t_2$, followed by three repeats with t_1 and t_2 values interchanged. Three separate sessions on different days, one with one monkey (I) and two with another (IIA, B), were held. In the last session (IIB) the monkey displayed restlessness during the control run and so we shortened the session by omitting four repeat runs. Consequently, the entire experiment comprised a total of $3 \times 2 \times 7 - 4 = 38$ data runs.

ERG responses recorded in each run were ensemble-averaged and digitalized on a signal processor. The digital averaged wave-forms were then analyzed into PC on an IBM 360/91 computer (IBM Corp., White Plains, N.Y.) as summarized mathematically in Mowbray et al. (1975). The theoretical analysis developed subsequently, however, obviates the need for so powerful a computer.

EXPERIMENTAL RESULTS

The suprafusion ERG transients detected by ensemble-averaging the period-jump responses are displayed in Fig. 3, where the columns correspond to the three experimental sessions: I with one monkey and IIA and IIB with another monkey. In each column, the paired wave-forms from repeated runs are labeled by the period-jump ($t_1 \longrightarrow t_2$ in milliseconds) corresponding to the different tests indicated in Fig. 2. The order of the different runs was such that the repeated pairs are from recordings separated by up to ~1 h in each session. The runs at the middle of each column are the control runs ($t_1 = t_2$).

The signal averaging process showed that as few as 50 individual responses were sufficient to delineate the ERG wave-form indistinguishably from the average of all 225 responses in each run. Likewise, the latency plus duration of the transients is short enough to be comprised within the first 50 ms of the response shown in Fig. 3, although experimental recording extended to as much as five times this long. Samples of the averaged ERG wave-form over 0–125 ms may be seen in Flower et al. (1975).

Oscillatory responses at ~ 100 Hz are obvious in certain of the averaged ERG of Fig. 3, notably in the top and bottom pairs of columns I and IIB and in the controls of IIA, IIB. In all these cases, one of the periods employed (10 ms) corresponds to 100 Hz, so that it is

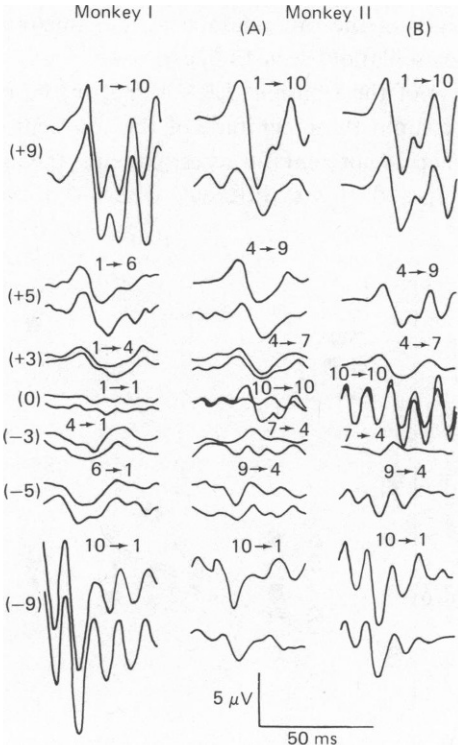

FIGURE 3 Ensemble-averaged ERG waveforms from three sessions (columns I, IIA, IIB) for runs with different period-jumps ($t_1 \longrightarrow t_2$ labels). All traces begin at time period-jump occurs. Each row corresponds to predicted equivalent tests as indicated by period-jump value on left ($t_2 - t_1$, cf. dashed lines of Fig. 2).

reasonable to suppose the oscillations to be simple flicker ERG responses—the frequency not being high enough for effective fusion in the summed wave-forms. Alternatively, for such frequencies the oscillations could be intrinsic "wavelets" evoked concomitantly with the ERG transients (Doty and Kimura, 1957; Ogden, 1973*a*), though the presence of oscillations in the control runs under IIA, B in Fig. 3 militate against this possibility.

(Fourier analysis of the ERG of Fig. 3 shows a strong, sharp line in the spectrum at the putative driving frequency [100 Hz] in all oscillatory cases, supporting the flicker explanation, except for the bottom curves of column I, Fig. 3 which gives a line at 80–90 Hz. We have no satisfactory explanation for the discrepancy and hence cannot discard the possibility that some of the ERG oscillations are intrinsic wavelets. Sporadic oscillations are reported for example in skate proximal negative response (PNR) [Dowling and Ripps, 1977] and wavelet generation has been localized to amacrine cells in rabbit [Korol et al., 1975]. Hence, under the amacrine-PNR hypothesis below [Discussion], wavelets in the suprafusion ERG would not be suprising.)

The primary interest here is the transient ERG; therefore the wave-forms in Fig. 3 will be processed to delete the oscillatory components for some of the later analysis. In column I the highly oscillatory top and bottom pairs will be discarded. For column IIB, the digitalized ERG data will be smoothed by numerically filtering out frequencies $\geq$ 80 Hz. For column IIA, neither selection nor filtering of the data is necessary, because oscillations in those runs are minimal. Subsequent analysis of the three data sets finds no significant differences among them, so the deletion of the oscillations seems satisfactory.

Two main characteristics of the transient ERG signal in the curves of Fig. 3 should be noted. First, within each column the magnitude of the transient decreases as t_1 and t_2 are brought closer together, whereas horizontally across columns the magnitude is approximately the same although both t_1 and t_2 are different. Thus, the magnitude appears to vary

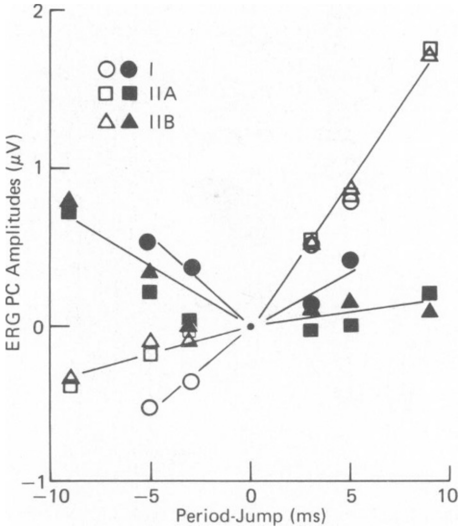

FIGURE 4 PC amplitudes as functions of period-jump size ($t_2 - t_1$) for the two leading PC (first ○ □ △, second ● ■ ▲: cf. Table I) in each of three experimental sessions (I, IIA, IIB).

approximately as the period-jump size, $(t_2 - t_1)$. Second, comparison of upper and lower halves of each column shows that a change of wave-form shape takes place when t_1 and t_2 are interchanged, i.e., when the sign of $(t_2 - t_1)$ changes. These qualitative features are quantitated by the analysis into PC. (The PC analysis comprises computation of the eigenvalues and eigenvectors of the dispersion matrix constructed from the time averages of all binary products of the ERG wave-forms of Fig. 3 and, for each eigensolution ($j = 1, 2, \ldots$), evaluation of the PC waveform ($PC_j[t]$) and PC amplitudes (B_j) from known formulae [cf. Mowbray et al., 1975].) Results for each experimental session are presented in Table I and Fig. 4, as PC eigenvalues and PC amplitudes, respectively.

Table I gives the eigenvalues in units such that each measures the percentage of the total data variance that the corresponding PC represents. It is seen that the ERG transient is dominated by just the first two PC, one accounting for the bulk of the ERG variance and the two together representing from 87 to 97% of the total variance. Notice that the removal of oscillations does not greatly affect the leading eigenvalues in session I (selected data) and only redistributes their relative contributions in session IIB (filtered data) with a slight increase in their partial sum (95.3 → 96.8). (The reason is that the oscillatory component contributed about equally to the two PC in the former case, but predominantly to the second PC in the latter.) The character of the dominant PC with oscillations deleted, indicated by symbols in Table I, is clarified by the behavior of their amplitudes vs. experimental variable, as plotted in Fig. 4 with corresponding symbols.

Fig. 4 displays the amplitudes for the two leading PC as functions of the period-jump $(t_2 - t_1)$. The first PC is seen to be "polar", i.e., its amplitude changes sign on passing through the origin. The second PC amplitude however remains always positive, indicating that the ERG transient has a substantial component that is rectifying with respect to the sign of the period-jump. Further, as indicated by the set of straight lines radiating from the origin in Fig. 4, both PC amplitudes are approximately proportional to $(t_2 - t_1)$ for given sign.

The PC wave-forms themselves are not shown here, because they are essentially ambiguous

TABLE I
PRINCIPAL COMPONENT EIGENVALUES*

Session I		Session IIA	Session IIB	
All data	Selected data	All data	All data	Filtered data
62.8	62.3 (○)‡	75.0 (□)	68.3	80.5 (△)
27.2	28.3 (●)	11.6 (■)	27.0	16.3 (▲)
4.0	4.0	6.2	2.0	1.5
2.9	3.2	2.7	1.3	1.0
1.8	0.9	1.5	0.8	0.5
0.5	0.6	1.1	0.4	0.2
0.4	0.5	0.7	0.2	—
0.3	0.3	0.5	0.1	—
0.1	—	0.4	—	—
0.1	—	0.2	—	—
0.05	—	0.1	—	—

*Percentages of variance
‡Symbols indicate corresponding PC amplitudes in Fig. 4.

to rotations (cf. John et al., 1973), and will be superseded by a better-determined representation below. However, we note that if one rotates the PC to optimize the linearity of the points in Fig. 4, one finds that the filled points transform into a V-shaped locus, representing *symmetric* linear rectification. This adds empirical support for our consideration of such rectification in the theory to follow. The two leading PC wave-forms upon the optimal empirical rotation are not very different from the two elemental functions derived later (Fig. 5).

The behavior of the PC amplitudes in Fig. 4 of course reflects a similar behavior of the ERG amplitude. For example, peak amplitudes of the transients in Fig. 3 also plot vs. period-jump as approximately linear, partially rectified functions. However, PC amplitudes are a better measure of overall size. By definition of PC, the $ERG(t) \equiv \Sigma_j B_j PC_j(t)$. Thus, if $\langle\ \rangle$ denotes time average and because the $PC_j(t)$ are orthonormal ($\langle PC_i(t) PC_j(t)\rangle = \delta_{ij}$), then the total ERG variance is $\langle ERG(t)^2\rangle = \Sigma_j B_j^2$, or $\approx B_1^2 + B_2^2$ by Table I. Therefore, from the straight-line rectified behavior noted above, one has

$$\text{rms ERG} \equiv \langle \text{ERG}^2\rangle^{1/2} \approx (t_2 - t_1) \times \begin{cases} C_+ \text{ for } t_2 - t_1 > 0, \\ C_- \text{ for } t_2 - t_1 < 0, \end{cases} \tag{1}$$

where $C_\pm$ are constants (the rms line slopes in Fig. 4 for positive and negative $t_2 - t_1$, respectively.)

Eq. 1 formalizes the essential conclusion of the data analysis, from which we can develop a theoretical analysis which ultimately leads to an interpretation for suprafusion ERG experiments.

THEORETICAL ANALYSIS

Suprafusion ERG Transients

The theory of suprafusion transients (Bird and Mowbray, 1973) showed that the response of any linearizable part of the visual system to the stimulus $t_1 \longrightarrow t_2$ of Fig. 1 takes the approximate form

$$R_{1\to 2}(t) \approx \frac{1}{4} I_s \cdot (t_2 - t_1) \cdot G(t), \tag{2}$$

with error $\sim(t_{1,2}/\theta)^2 \ll 1$ when $t_{1,2}$ are short compared to the system persistence time θ (i.e., suprafusion). Here I_s is mean retinal illuminance and $G(t)$ the Green's function of the responding part, i.e., its elemental response at time t to an impulse at time 0 (in the present experiments, conditions remained stationary so that the time origin is immaterial). Recall that we deleted fast oscillations, with periods $\sim t_{1,2}$, from the ERG data of Fig. 3. Thus, Eq. 2 here represents smoothed responses in terms of correspondingly averaged G functions, with time constants $\theta \gg t_{1,2}$ (as seen in Fig. 5 below). The validity of the one basic assumption of the theory, linearizability, can be checked a posteriori by comparison of experimental behavior with the predicted Eq. 2.

The experiment showed that the ERG transient varies approximately linearly with period-jumps of given sign. Thus, comparison of Eqs. 1 and 2 indicates that the linearizability assumption is valid for $t_2 - t_1 > 0$ and < 0, separately. In other words, the generation of ERG transient is (at least) the two-part action of a linear rectifier. From the asymmetry in Fig. 4 between $t_2 - t_1 \gtrless 0$, the rectification is asymmetric.

In the Appendix, a dynamical theory of the retina is formulated that involves asymmetric linear rectification. The ERG transient is derived therefrom in terms of strictly linear operations (L) and symmetric linear rectification (R). Such decomposition is not unique of course (cf. van der Tweel and Spekreijse, 1969). However, the L-R formulation has the property that it maximizes the linear portion of retinal operation and places a lower bound on the amount of retinal rectification. The maximal linear operation (L) will be seen below to be responsible for all linear ERG flicker responses (Eqs. 5). The irreducible minimum of symmetric rectification (R) is significant at least as a base-point-index of rectifying action in the retina. Further, we shall argue in the Discussion that the rectification most likely originates from the amacrine-cell layer, which cells show symmetric rectifying action. Also, as remarked above, empirically optimizing the linear behavior regarding Fig. 4 implies symmetric rectification. For these reasons, our specific L-R decomposition seems a preferable working assumption.

The suprafusion ERG transient is expressed by the generalization of Eq. 2 indicated in the Appendix (Eq. A.6 with A.5),

$$ERG_{1\rightarrow 2}(t) \approx \frac{1}{4} I_s \cdot \{(t_2 - t_1) \cdot G_L(t) + |t_2 - t_1| \cdot G_R(t)\}. \tag{3}$$

Here, $G_L(t)$ and $G_R(t)$ are the elemental impulse-response function for the linear and rectifying retinal operations, respectively. These functions could be determined from the PC analysis results, namely as double-summations over all the $PC_j(t)$, with coefficients the B_j weighted for each run (insert the PC expansion $ERG(t) \equiv \Sigma B_j PC_j(t)$ into Eqs. 4 below). However, they are more simply calculated *ab initio* from the original experimental data.

Thus, the elemental responses $G_L(t)$ and $G_R(t)$ are determined by fitting Eq. 3 to all the measured $ERG_{1\rightarrow 2}(t)$ wave-forms of Fig. 3. The least mean squares (lms) fit at the end of the Appendix yields

$$G_L(t) = (4/I_s) \cdot \sum_i w_i \cdot ERG_i(t), \tag{4a}$$

$$G_R(t) = (4/I_s) \cdot \sum_i |w_i| \cdot ERG_i(t), \tag{4b}$$

$$w_i \equiv (t_2 - t_1)_i \Big/ \sum_i (t_2 - t_1)_i^2, \tag{4c}$$

where subscript i denotes the set of experimental runs, i.e., $(t_2 - t_1)_i = 9, 5, 3, 0, -3, -5, -9$ ms from Figs. 2 and 3. In addition, the lms calculation will give an rms measure for the non-L and non-R residue, i.e., any operations besides linear rectification.

Fig. 5 displays the elemental $G_L(t)$ and $G_R(t)$ functions calculated via Eqs. 4 for the three sessions in Fig. 3, all at I_s = 20,000 td (cf. Fig. 1 legend). We used the data of sessions I and IIB that were processed to delete oscillations (cf. Table I), but Fig. 5 shows some 100 Hz oscillation still remaining, which can account for slight differences within the two sets of curves G_L and G_R. There are also latency differences between one monkey (I) and the other

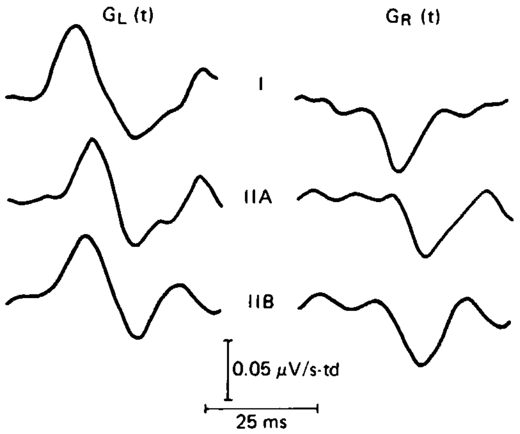

FIGURE 5

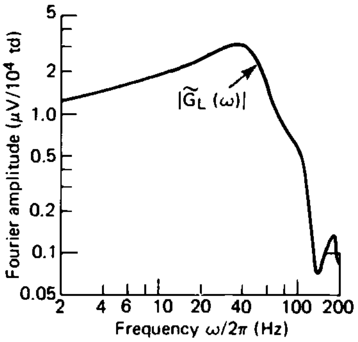

FIGURE 6

FIGURE 5 Elemental responses (Green's function G_L, G_R) calculated via Eqs. 4 from the suprafusion ERG data of Fig. 3 for each session (I, IIA, IIB). $G_L(t)$ is the strictly linear element; $G_R(t)$ the symmetric linear rectifying element. Note the consistency of each between different animals (I, II) and separate runs (IIA, B).

FIGURE 6 Magnitude of Fourier transform of the elemental response function G_L of Fig. 5 as function of circular frequency ($\omega/2\pi$), averaged for three sessions I, IIA, IIB; $|\tilde{G}_L(\omega)|$ is the fundamental "modulation transfer function" predicted from Eq. 5 for high-frequency stimulation.

(II). Nevertheless, the overall consistency among the three separate determinations of both G_L and G_R is to be emphasized. In general Fig. 5 makes clear that $G_L(t)$ has a more-or-less biphasic form and that $G_R(t)$ is essentially monophasic negative.

Table II shows the lms percentage contributions of *L* and *R* components to the total ERG variance, as well as nonlinear contributions beyond linear rectification. As indicated above, the *L* entries represent an upper bound on the strictly linear operation and the *R* entries a lower bound on the linear rectification, both being lms bounds. The residual 5–15% indicates the degree to which the theory, postulating linear rectification, may be considered confirmed in each experimental session. One sees from Table II that at most approximately two-thirds of the ERG variance arises strictly linearly. Rectification is responsible for *at least* roughly one-third of the variance of the suprafusion ERG transient, with implications educed below (cf. Discussion).

TABLE II
ELEMENTAL COMPONENTS VARIANCE (PERCENT)

Component	Session		
	I (selected)	IIA (all)	IIB (filtered)
L(inear)	63	55	60
R(ectified)	31	29	36
Other	6	16	4

Suprafusion ERG Flicker

The relevance of G_L to high-frequency ERG flicker is demonstrated in the Appendix. Intermittent stimulation at a steady frequency ω is transformed by the retinal asymmetric linear rectification into ERG flicker at the fundamental frequency ω plus a dc term and higher even-harmonic terms (Eqs. A.7). The ERG fundamental is determined completely through G_L alone via Eq. A.7c, i.e.,

$$\text{ERG amplitude at } \omega = |\tilde{G}_L(\omega)| \qquad \mu\text{V}/td, \tag{5a}$$

$$\text{ERG phase at } \omega = \arg \tilde{G}_L(\omega), \tag{5b}$$

in terms of the Fourier transform of G_L in Eq. A.3, with magnitude $|\tilde{G}_L|$ and phase $\arg \tilde{G}_L$. The "modulation transfer function" Eq. 5a averaged over the three sessions, I, IIA, and IIB, was calculated from the $G_L(t)$ of Fig. 5 via Eq. A.3 and is exhibited for the subsequent Discussion in Fig. 6. The peak at 35–40 Hz is a reflection of the biphasic form of the G_L in Fig. 5, where one can note a fair match to one cycle of a sinusoid of corresponding period 25–30 ms. At such high frequencies or beyond, the ERG flicker response should be essentially determined via Eqs. 5 by the elemental G_L.

The rectifying contribution of dc and overtone components to high-frequency ERG flicker offers the possibility of determining the frequency sensitivity for separate portions of the retinal transduction channels, proximal (p) and distal (d) to the site of rectification (cf. Appendix). (A technique of sine-wave plus auxiliary-signal stimulation has determined rectifying and proximal-distal linear contributions to both goldfish retinal ganglion responses [Spekreijse, 1969] and human VEP [Spekreijse and van der Tweel, 1972].) The dc shift varies with ω and may therefore be observable. The second-harmonic is likely to be small, but perhaps can be isolated and measured by frequency-selection techniques. If these two contributions are determined experimentally as functions of driving-frequency, as $\text{dc}(\omega)$ and $|2^{\text{nd}}(\omega)|$ respectively, the data will yield via Eqs. A.7*b* and A.7*d* the relative sensitivities

$$|\tilde{G}_d(\omega)| \propto \text{dc}(\omega), \tag{6a}$$

$$|\tilde{G}_p(\omega)| \propto |2^{\text{nd}}(\omega/2)|/\text{dc}(\omega/2), \tag{6b}$$

for the distal and proximal retinal operations, respectively. Of course, nonlinearities other than rectification can also produce a dc shift and overtones. However, if higher harmonics are measurable, one may use as criterion for linear retification that the 3rd harmonic be zero and the 4th have amplitude $|4^{\text{th}}(\omega)| = \tfrac{1}{5}|2^{\text{nd}}(\omega)| \cdot |\tilde{G}_p(4\omega)/\tilde{G}_p(2\omega)|$, using $\tilde{G}_p$ from Eq. 6b (cf. Appendix).

The predictions Eqs. 5 and 6 of the theory remain to be tested by experiment.

DISCUSSION

We conclude that the ERG experiments confirm the theory of suprafusion transients (Bird and Mowbray, 1973) as applied to the retina in the Appendix. In reaching this conclusion we have interwoven experiment and theory, so let us retrace the logical thread for clarity. The data demonstrated, on the neutral ground of PC analysis, a quantitative relation to the predicted form for suprafusion transients (Eq. 2), from which we deduced a linear-rectifying generation for the ERG transient. Then the theory elaborated for such retinal action (Appendix) led to the basic formula, Eq. 3, which finally was shown to fit all the original data within 10% ($\sigma = \sqrt{308/3}\%$ from Table II).

A useful outcome of the theoretical analysis was to streamline the ERG data reduction into two elemental functions (G_L, G_R) determinable with a minim of computation, as compared with PC analysis, by the simple algorithm of Eqs. 4. The simplicity is merely a reflection via Eq. 3 of the elemental nature of suprafusion transients.

The elemental G_L and G_R functions are remarkably consistent between sessions, including the different animals (Fig. 5). They succinctly represent via Eq. 3 the suprafusion ERG transients (as well as other responses, cf. Appendix). Therefore, discussion of the ERG transients devolves into discussion of G_L and G_R. The G_L is of secondary interest here and has been discussed previously, so that we only briefly consider it. Thereafter the discussion will focus on the G_R, as the prime interest of this paper.

The linear element G_L represents two-thirds of the suprafusion ERG transient (Table II) and exhibits a biphasic form (Fig. 5). The two phases could result from competition between excitation and recovery in photoreceptors and later neurons within individual retinal channels, or from the excitatory and inhibitory connections among different channels that are responsible for Mach band phenomena. In the present, wide-field experiments, both effects likely are important. Be that as it may, the biphasic G_L was shown in an earlier, preliminary analysis (Bird et al, 1975) to bear a close relation to cortical suprafusion VEP transients recorded in our first runs (Session I). Further, both the retinal G_L and the corresponding cortical linear response function were shown to Fourier transform (cf. Eq. 5) into close correlates of neuronal and psychophysical flicker responses from other experiments. The ERG "polar PC" and its transform depicted in Bird et al (1975) are essentially the same as the $G_L(t)$ and $\tilde{G}_L(\omega)$ seen in Figs. 5 and 6, respectively, except that the earlier transform was somewhat too low at low frequencies and our calibration was in error by a factor ½ (i.e., the label "ERG × 2" in our earlier figure should read "ERG").

The rectifying element G_R composing one-third of the ERG transient (Table II) is unexpectedly large for the high frequencies here. Low-frequency ERG contain substantial nonlinearities: Gouras and Gunkel (1964) reported a large second-harmonic component in the human cone ERG for stimulus frequencies below 8–17 Hz, and analogous behavior is detected at still lower frequencies in ERG flicker responses from lower species, e.g., below 3–5 Hz in frog (Levett, 1970), 2 Hz in rabbit (Levett, 1973). But with the intermittencies ≥ 100 Hz here, very little of such nonlinear behavior would be expected to persist in our monkey ERG. The time-smoothing (low-pass filter) action of the visual system upon our high-frequency stimuli effects a linearization of the system, up to the limit of its linearizability (Bird and Mowbray, 1973). Therefore, it emerges that *the suprafusion transient technique lays bare the remanent "essential nonlinearity" of retinal rectification.*

How may one interpret the retinal rectification in physiological terms? Since the element G_R turned out to have a monophasic, single-peaked form (Fig. 5), indicating no noticeable delay between the + and − responses, the rectification may well arise from a single operation of individual neurons of a certain class, as opposed to the more diffuse origins for the biphasic G_L indicated above. Considering the unique, rectifying character of the "on-off" class of amacrine cell (cf. review by Burkhardt, 1975), we have conjectured that a likely site of rectification is the amacrine layer (Flower et al., 1975; Bird et al., 1975). (Chan and Naka [1976] point out that the structure of on-off amacrines is not literally "amacrine", but in the absence of a structurally correct term we use current terminology.) That on-off amacrines might contribute significantly to a suprafusion transient is of course consonant with the current idea that these cells are the primary agent for dynamic vision. More specifically the survey of intracellular responses in mudpuppy retina by Werblin and Dowling (1969) clearly singles out the amacrine as the origin of a rectifying action. Details of amacrine operation have been further elucidated in goldfish retina (Kaneo, 1973) and carp retina (Toyoda et al., 1973). The latter studies show that the "on-off" type amacrine (which predominated in the work on mudpuppy and goldfish, though not carp) responds to onset or offset of stimulation about equally in amplitude, latency and form, as well as with the same polarity. In other words, on-off amacrines create an approximately symmetric rectification.

The possibility that amacrine rectification, initially appearing intracellularly as graded depolarization with superimposed spikes, eventually can show up extraretinally in the massed ERG is indicated by studies on the "proximal negative response" (PNR). Burkhardt (1970) identified the intraretinal PNR in frog as extracellular amacrine potentials. Subsequent studies isolated analogous PNR in a variety of vertebrates (Fatechand, 1971; Ogden and Wylie, 1971; Holden 1972; Ogden, 1973b; Proenza and Burkhardt, 1973; Dowling and Ripps, 1977). Species differences are large (e.g., in the PNR time scales), but in general the extracellular PNR exhibit rectification like the intracellular amacrine responses. For the rhesus of our work in particular, a possibly amacrinal PNR that is rectifying and as fast as the suprafusion ERG transient has been reported by De Monasterio and Gouras, (1975, Fig. 13c).

The extraretinal PNR is small, in accord with the lateral orientation of amacrines, but observable in vitreal records (Burkhardt, 1970; Fatechand, 1971; Proenza and Burkhardt, 1973). Most clearly, a symmetric rectifying PNR analogue in frog ERG was revealed by means of transretinal dc current (Knighton, 1975, Fig. 2). Diffuse, photopic illuminance (like ours) gave extraretinal potential ~50 μV, but considerably smaller might be inferred for primates from Ogden (1973*b*). In any event, the suprafusion ERG transient is only a few microvolts (Fig. 3), so its size is compatible with an amacrine-PNR origin.

The G_R wave-forms of Fig. 5 have several features, aside from the question of amplitude, which are consistent with the PNR-amacrine potentials: (*a*) G_R is essentially negative. The PNR associated with the amacrine depolarization also have a negative polarity, which does not change during retinal penetrations (*contra* the sign-reversals of intraretinal a- and b-waves). (*b*) G_R has latency ~ duration ~20 ms here. The PNR time scales in lower species are several times longer, but the putative PNR in the rhesus shows a latency ~30 ms (De Monasterio and Gouras, 1975). The main peak of the PNR is all that persists extraretinally in most studies, with a duration ~ its latency.

Amacrine-rectified signals no doubt influence more proximal cells, e.g., exciting (Werblin and Dowling, 1969) or inhibiting (Miller and Dacheux, 1976) the "on-off" ganglions. However, ganglions at least are not usually considered to contribute to the ERG, because it appears unaffected by ganglion degeneration or by antidromic stimulation of the optic nerve; nor do ganglion discharges appear consistent with PNR properties (Holden, 1977). The

Müller cells thought responsible for the normal ERG b-wave show some possible rectification response after long illumination at moderate intensities (Miller and Dowling, 1970). However, in contrast to the amacrine potentials, the Müller cell responses are incompatible in sign (depolarizing, but reversed extra-retinally) and in time scale with our G_R.

At present one is left with on-off amacrines as the origin, and possibly sole agent, of the suprafusion ERG rectification. Note that a singular site for rectification as indicated for the suprafusion transient here is to be contrasted with the apparently plural origin for b-(on) and d-(off) waves in the normal ERG. Thus, in work on frog ERG flicker to 36 Hz, Troelstra (1971) has considered harmonic distortions as arising from asymmetric rectification with a delay ~36 ms between on and off responses, on the idea of the former as a b-wave effect from Müller cells and the latter a d-wave partly from photoreceptors. However, in our quite different work on the monkey suprafusion transients, there is no such delay noticeable, so that we have postulated a single site of rectification, the amacrine layer.

We therefore propose for further investigation the ***hypothesis*** that the symmetric rectification element (G_R) of the suprafusion transient is an index of on-off amacrine cell activity. A firm test of this hypothesis—and a precise index—requires the intraretinal recording of single-cell suprafusion responses. However, further ERG studies could also go beyond the circumstantial evidence adduced here (G_R size, polarity, latency, duration). As a reviewer has commented, variations in stimulus parameters such as adaptation level and spatial extent or pattern could affect the suprafusion ERG in ways more or less predictable on the amacrine hypothesis. Other possibilities include suprafusion ERG studies with amacrine-rich (e.g., avian) species or with amacrine-specific drugs such as ethanol (Bäckström, 1974). The latter is particularly interesting for human studies, since moderate alcohol ingestion notably reduces the amacrine-mediated ERG flicker response (Ikeda, 1963).

The retinal rectification of steady high-frequency stimulation as noted above produces dc, 2^{nd} harmonic, etc. nonlinear responses, distinct from low-frequency nonlinearities, as well as a linear response at the driving frequency. Measurements on the latter can be cross-checked against suprafusion transient data via Eqs. 4*a* and 5. The high-frequency nonlinearities offer a measure via Eqs. 6 ff. of the retinal operations distal and proximal to the rectification, postulated here as at the amacrine cells. If proven feasible, this would sharpen the ERG as a physiological and/or clinical tool. That is, between suprafusion transient data analyzed via Eqs. 4 and high-frequency tuned-measurements analyzed via Eqs. 6 ff., one might effect a noninvasive "analytical dissection" of the retina.

We emphasize that the foregoing results and possibilities arise from the elemental nature of suprafusion responses, and unique character of amacrine cells, in the visual system.

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APPENDIX

Retinal Dynamics

The theory of suprafusion visual responses (Bird and Mowbray, 1973) when applied at any stage s along the visual pathway expresses the time-varying response of that stage $R_s(t)$ as a result of a rapidly varying illuminance $I(t)$ as $R_s(t) = \Lambda_s I(t)$, where Λ_s is a linear operator, provided the visual system can in principle be linearized up to the stage s. If the retina were linearizable through all stages involved in ERG generation, then the massed ERG response would be a weighted sum of various $R_s(t)$ of the above form, $ERG(t) = \Lambda_{\mathrm{ERG}} I(t)$, where Λ_{ERG} is a linear operator combination of the Λ_s. The initial retinal operations, certainly photon absorption and probably all photoreceptor states, are linearizable. However at some later stages in some retinal channels that generate the ERG, there must intervene the essential nonlinearity of the rectification uncovered by the present experiments. The rectifying action is approximately linear (Fig. 4), and most likely occurs at the on-off amacrines (Discussion) in which case it is also nearly symmetric. Symmetric linear rectification may in general be represented by an operator $\Lambda_p \mathrm{A} \Lambda_d$, where A is the absolute value operator ($\mathrm{A}x \equiv |x|$), while Λ_d and Λ_p denote linear operation distal (d) and proximal (p) to the rectifying stage. Let all other, fully linearizable chanels relevant to the ERG be represented by a linear operator Λ_L. Then, instead of the $\Lambda_{\mathrm{ERG}} I$ above, we have for the dynamic ERG response to rapidly varying illumination,

$$ERG(t) = \{\Lambda_L + \Lambda_p \mathrm{A} \Lambda_d\} I(t). \tag{A.1}$$

Here each Λ combines a multitude of linear retinal operations, both of linear channels (L) and of proximal, distal (p, d) portions of rectifying channels. In the last term $\mathrm{A}\Lambda_d I \equiv |\Lambda_d I|$. Note that Λ_p could conceivably be simply unity; but certainly (as one would expect for photoreceptor operation at least) $\Lambda_d \neq 1$, because otherwise Eq. A.1 would give no varying rectified response for the square-wave of Fig. 1 ($\mathrm{A}\Lambda_d I$ would equal $|I|$ = constant), in contradiction to the present experiments.

(More generally just from the present experiments alone, without the amacrine hypothesis to suggest symmetry (or with significant nonsymmetry in amacrine responses), one is left with an asymmetric linear rectification. However, the ERG can still be expressed in the form Eq. A.1, only with Λ_L and Λ_p redefined as new combinations Λ_L' and Λ_p'. [The $\Lambda_p \mathrm{A}$ above generalizes to $\pm\Lambda_\pm$ for input $\Lambda_d I \gtrless 0$, respectively, or $\Lambda_p \mathrm{A} \rightarrow \frac{1}{2}(\Lambda_+ - \Lambda_-) + \frac{1}{2}(\Lambda_+ + \Lambda_-)\mathrm{A}$; then in Eq. A.1, $\Lambda_L \rightarrow \Lambda_L + \frac{1}{2}(\Lambda_+ - \Lambda_-)\Lambda_d \equiv \Lambda_L'$ and $\Lambda_p \rightarrow \frac{1}{2}(\Lambda_+ + \Lambda_-) \equiv \Lambda_p'$.] The Λ_L' now represent linearizable channels and linear contributions [for $\Lambda_+ \neq \Lambda_-$] from rectifying channels, and Λ_p' gives the irreducible remanent symmetric rectification. The formulation (Eq. A.1) is no longer unique, but remains mathematically convenient and experimentally meaningful [cf. ¶ preceding Eq. 3] even without the amacrine rationale.)

Each Λ operator in Eq. A.1 will be expressed in the usual way, by considering any input as a sequence of impulses whose effects integrate to give the output (Lanczos, 1961):

$$\Lambda_i I(t) = \int_{-\infty}^{\infty} G_i(t, t') I(t')\, \mathrm{d}t', \tag{A.2}$$

where $i = L$ or p or d and $G_i(t, t')$ is the elemental Green's function (response at time t to an impulse at the time t') which completely characterizes the operator Λ_i. We assume the G_i are stationary and define their Fourier transforms, i.e.

$$G_i(t, t') = G_i(t - t') \quad \text{and} \quad \tilde{G}_i(\omega) \equiv \int_{-\infty}^{\infty} \mathrm{d}t e^{-i\omega t} G_i(t). \tag{A.3}$$

In these terms, the basic impulse, oscillatory, and suprafusion transient responses of Eq. A.2 become

$$\Lambda_i E\delta(t) = EG_i(t), \tag{A.4a}$$

$$\Lambda_i e^{i\omega t} = |\tilde{G}_i(\omega)| e^{i[\omega t + \phi_i(\omega)]}, \tag{A.4b}$$

$$\Lambda_i I_{1\rightarrow 2}(t) \approx \frac{1}{4} I_s \cdot (t_2 - t_1) \cdot G_i(t), \tag{A.4c}$$

respectively, with impulse energy $\equiv E$, arg $\tilde{G}_i(\omega) \equiv \phi_i(\omega)$, and $I_{1\rightarrow 2}(t)$ the square-wave period-jump $t_1 \rightarrow t_2$ of Fig. 1. The last Eq. A.4*c* gives the transient with accuracy as indicated below Eq. 2 and assumes any suprafusion frequency "ripple" is negligibly small (Bird and Mowbray, 1973).

One may contrast the above dynamic formulation, deduced from our photopic ERG, with the nonlinear model for the scotopic b-wave proposed by Troelstra (1964), Troelstra and Schweitzer (1968). Their nonlinearity resides in an illumination dependence of the sensitivity function that they postulate as multiplying the input before subsequent linear operations. The model was recently applied (Troelstra and Garcia, 1975), with photopic additions chosen empirically, to human ERG flicker up to 24 Hz. The essential difference between our analytical approach and theirs is that we assume high-frequency stimuli, which isolates the essential nonlinearity of rectification (Discussion), while they treat lower frequencies where other nonlinearities are commingled with it.

ERG Responses

The impulse response of the ERG from Eq. A.1 with A.4*a* is $EG_L(t) + \Lambda_p |EG_d(t)|$, which by Eqs. A.2, A.3 with $i = p, d$ becomes

$$ERG_{E\delta}(t) = E \cdot G_L(t) + |E| \cdot G_R(t), \tag{A.5a}$$

$$G_R(t) \equiv \int_{-\infty}^{\infty} G_p(t - t') \, |G_d(t')| \, \mathrm{d}t'. \tag{A.5b}$$

G_L and G_R are thus the elemental responses of linear and rectifying channels, respectively.

The suprafusion transient generated by the period-jump $t_1 \rightarrow t_2$ of Fig. 1 is seen by comparison of Eqs. A.*4a* and A.*4c* to be approximately equivalent to an impulse response,

$$ERG_{1 \rightarrow 2}\,(t) \approx ERG_{E\delta}\,(t) \text{ with } E = \frac{1}{4} I_s \cdot (t_2 - t_1). \tag{A.6}$$

Eq. A.6 with A.5 gives the basic Eq. 3 of the text. We emphasize that this is a high-frequency (short $t_{1,2}$) approximation which neglects both small transient terms and oscillatory ripples (cf. ff. Eq. A.d4*c*). One can show the respective errors are of relative size $\sim(t_{1,2}/\theta_{L,R})^2$ and $\sim(t_{1,2}/\theta_{L,R})^{n-1}$, where $\theta_{L,R}$ are time scales for *L,R* channels and n the exponent (− logarithmic derivative) of high-frequency channel sensitivities.

The oscillatory response to a sinusoidal stimulus is given by Eq. A.1 and the imaginary part of A.4b as $|\tilde{G}_L\,(\omega)|\sin[\omega t + \phi_L(\omega)] + |G_d(\omega)| \cdot \Lambda_p \cdot |\sin\,[\omega t + \phi_d(\omega)]|$. In the last term we use the Fourier expansion $|\sin x| = (4/\pi)\,\Sigma_{j=0}^{\infty} \cos\,(2jx)/(1 + \delta_{j,0})(1 - 4j^2)$ and apply the real part of A.4b. Thus, the sine-wave ERG response takes the form of a dc shift, the fundamental, and higher even-harmonics,

$$ERG_{\text{sine}}(t) = \mathrm{dc}(\omega) + 1^{\text{st}}(\omega) + 2^{\text{nd}}(\omega) + 4^{\text{th}}(\omega) + \cdots, \tag{A.7a}$$

$$\mathrm{dc}(\omega) \equiv (2/\pi)\tilde{G}_p(0)\,|\tilde{G}_d(\omega)|, \tag{A.7b}$$

$$1^{\text{st}}(\omega) \equiv |\tilde{G}_L(\omega)|\sin[\omega t + \phi_L(\omega)], \tag{A.7c}$$

$$2^{\text{nd}}(\omega) \equiv -(4/3\pi)\,|\tilde{G}_p\,(2\omega)\tilde{G}_d(\omega)|\cos(2\omega t + 2\phi_d + \phi_p)\,, \tag{A.7d}$$

$$4^{\text{th}}(\omega) \equiv -(4/15\pi)\,|\tilde{G}_p(4\omega)\tilde{G}_d(\omega)|\cos\,(4\omega t + 4\phi_d + \phi_p), \tag{A.7e}$$

and so on. (Note that $\tilde{G}_p(0) = \int_{-\infty}^{\infty} \mathrm{d}t\; G_p(t)$ by A.3.) Eq. A.7*c* gives Eqs. 5 of the text. The other expressions combine to yield Eqs. 6 ff.

Notice that if $G_d(t)$ is monophasic then by Eqs. A.5*b* and A.3 we have the relation $\tilde{G}_R(\omega) = (\pm)\tilde{G}_p(\omega) \cdot \tilde{G}_d(\omega)$, where the sign corresponds to that of $G_d(t)$. In that case, the Fourier transform of the G_R determined from Eq. 4*b* with A.3 may be compared with flicker results via Eqs. 6.

Suprafusion ERG Green's Functions

We determine the two functions $G_L(t)$ and $G_L(t)$ above such that the theoretical Eq. 3 best-fits the experimental *ERG*(t) of Fig. 3. For each session (I, IIA, IIB in Fig. 3) separately, let subscript *i* denote the different runs and $ERG_i(t)$ the measured response to period-jump $(t_2 - t_1)_i$. Adopting an lms criterion we then require that

$$\sum_i \left[ERG_i(t) - \frac{1}{4} I_s\{(t_2 - t_1)_i G_L(t) + |t_2 - t_1|_i G_R(t)\}\right]^2 = \text{minimum} \qquad (\text{A.8})$$

under variations of the $G_L(t)$, $G_R(t)$ at all values of the time t. (The overall time-averaged approximation is thus a fortiori optimized also.) Thus, consider small variations $G_{L,R}(t) \longrightarrow G_{L,R}(t) + \delta G_{L,R}(t)$ and require the associated variation of the sum in A.8 to vanish for arbitrary $\delta G_{L,R}(t)$. Two simultaneous equations result, whose solution for the complete march of t is

$$G_L(t) = (4/I_s) \sum_i ERG_i(t)\{w_i - K|w_i|\}/(1 - K^2) \qquad (\text{A.9a})$$

$$G_R(t) = (4/I_s) \sum_i ERG_i(t)\{|w_i| - Kw_i\}/(1 - K^2), \qquad (\text{A.9b})$$

where w_i is as defined in Eq. 4*c* of the text and $K \equiv \Sigma_i w_i |t_2 - T_1|_i$.

In the present experiments, the set of test values $(t_2 - t_1)_i$ was in all cases completely symmetrical between positive and negative signs of $(t_2 - t_1)$ (cf. Figs. 2 and 3). In that case, we have $K \equiv 0$ and Eqs. A.9 reduce to the Eqs. 4 of the text.

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