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Detection of Bimodal Stimuli in the Frog Retina

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The present report addresses the electrical activity of the frog retina assessed using electroretinogram (ERG) recordings of responses to instantaneous changes from a reference line stimulus with fixed luminance and orientation to a test stimulus consisting of a line of different luminance and orientation. The analysis revealed two types of retinal responses. Responses to onset and offset of the line stimulus were analogous to responses obtained by homogeneous illumination of the retina and were characterized by a high-amplitude *b* wave (hundreds of microvolts) and significant asymmetry between the *b* and *d* waves. At the same time, interaction of these two stimuli yielded more symmetrical *b* and *d* waves, with amplitudes an order of magnitude lower, such that this frog retinal activity approached the ERG pattern recorded in response to formed stimuli in higher vertebrates. Analysis of the interaction of the mechanisms detecting the luminance and configurative characteristics of the stimuli, based on construction of V-shaped discrimination functions, showed that when the luminance of the stimulus line was only slightly different from the luminance of the reference line (i.e., discrimination occurred in the retinal stimulus adaptation zone), the activities of the luminance and orientation channels were summed in the ERG. This indicates that these mechanisms function independently and in parallel. From the moment at which the test stimulus luminance became twice that of the reference stimulus, the increase in ERG amplitude grew in a non-linear manner. This two-stage change in amplitude is explained by the existence of two luminance encoding mechanisms in the frog retina, acting simultaneously with the orientation encoding mechanism. One luminance mechanism encodes the action of the stimulus as the discharge power, generating information encoding the absolute level of environmental illumination. The activity of this mechanism is determined mainly by receptors and cells in the outer plexiform layer of the retina. The other mechanism is based on vector encoding of stimulus actions, generating information encoding the spatial and temporal differentiation of light in the visual field and is mainly associated with cells in the inner plexiform layer of the retina.

KEY WORDS: frog pattern electroretinograms (PERG), instantaneous stimulus substitution, discrimination potential, detection of line orientation, achromatic vision.

Retinal electrical activity in higher vertebrates, recorded as electroretinograms (ERG), differs depending on the two types of light stimulation: homogeneous light or a pattern in the form of one- or two-dimensional grids with different spatial frequencies [22–24]. In the latter case, the abbreviation used is for pattern electroretinogram (PERG), designating the response of the retina to a formed stimulus (a pattern), i.e., to the "configurative" characteristics of the stimulus [13]. The term "configurative" in this sense is used to designate the actions on the retina of any light flow with significant inhomogeneity in space or time, with appropriately defined boundaries.

Retrograde degeneration of retinal ganglion cells induced by transection of the optic nerve leaves a normal ERG, while the PERG decreases to the level of noise. These data lead to the conclusion that retinal ganglion cells play a significant role in generating PERG. An additional argument for this conclusion is provided by the effect whereby PERG amplitude increases at low spatial frequencies [24], which links PERG with neurons whose receptive fields show the opponent structure, such as retinal ganglion cells. Intracellular recording of monkey retinal cell activity in response to homogeneous (luminance) and formed (config-

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urative) stimuli [18, 25] has also supported the notion that PERG is linked more with neurons in the intermediate and inner plexiform layers, while the ERG is related to the activity of receptors and cells in the outer plexiform layer. Comparison of the ERG and PERG in nocturnal (cats) and diurnal (monkeys, humans) animals shows that the PERG in diurnal species represents the photopic visual system, while generation of the PERG in cats involves both visual systems – the photopic and the scotopic [18, 24].

Studies demonstrating similarities between the activity of retinal mechanisms in different animal species are very important for identifying the fundamental principles of functioning of the visual system. There is therefore interest in understanding how widely identical functional characteristics of vision are distributed in morphologically and ecologically differentiated visual systems. For example, the universality of the mechanisms encoding homogeneous light stimuli in the retina in both higher vertebrates and lower vertebrates and even invertebrates has been demonstrated by the identical spectral sensitivity functions of photoreceptors seen in numerous investigations and encapsulated in Dartnall's nomograms[14]. Another generalized characteristic is identified by Adrian's observations that stimulus intensity is encoded by the intensity of the discharges of first-order afferents [12]. This mechanism underlies the logarithmic function of the intensity of a light source which is characteristic of the vision of both vertebrates and invertebrates.

A number of studies [29] have shown that a psychometric function – the probability of detecting threshold stimuli (chromatic and achromatic), obtained for humans in psychophysical experiments, corresponds precisely to the analogous function, the probability of detecting threshold stimuli by individual neurons in the cat and monkey visual systems (termed the "neurometric" function). Measurements of this function for the frog ERG have demonstrated that this "retinometric" function is identical to the psychometric and neurometric functions [5]. These are convincing arguments supporting the notion that although the visual systems of higher and lower vertebrates show significant morphological differences, all the mechanisms encoding light intensity in higher vertebrates are the same as those in lower vertebrates.

The present work addresses the extent to which this conclusion is applicable to formed light stimuli, which play a significantly more important role in the lives of animals and humans than homogeneous environmental light. It is important to note that there is a fundamental difference between a homogeneous light field and a temporospatial pattern of light falling onto the retina. In the former, a single photoreceptor apparatus provides a necessary and sufficient mechanism for encoding light intensity. In the latter case, comparison of the intensities of light falling on different parts of the retina have to be compared. In other words, the visual system transfers from reacting to the action of energy to reacting to the ratio of actions, i.e., from encoding the energy of the physical stimulus to encoding the ratio (abstraction, quality).

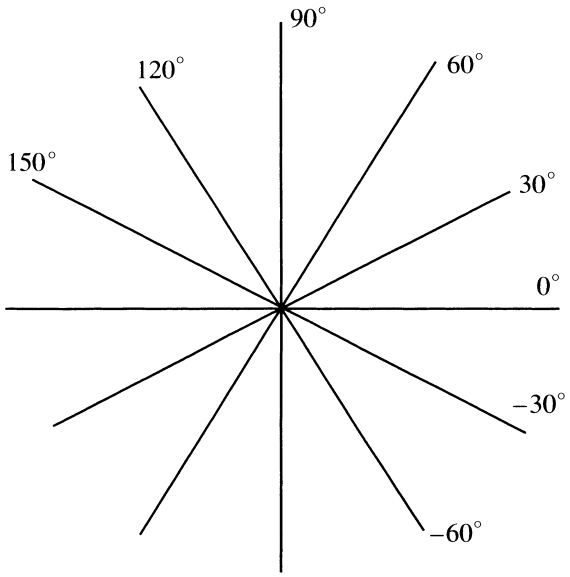

Fig. 1. Diagram of stimuli used in the present experiments. Orientation angles relative to the horizontal are measured in terms of anticlockwise rotation. For lines for which this angle is greater than 90°, the other end is labeled with the smallest angular difference between these stimuli and the horizontal.

Thin lines with different orientations in the frontal plane of the visual field represent one of the simplest types of formed stimuli. The response of the visual system of higher vertebrates to such a stimulus has been studied using a variety of approaches as reported in [19, 30]; see also review [10]. The aim of the present work was to investigate the responses of the frog retina to presentation of lines of different luminances and orientations, with the aim of comparing the mechanisms encoding the intensities of light and formed stimuli in the visual systems of lower and higher vertebrates.

The contribution of the configurative characteristics of the stimulus, such as line orientation, was assessed independently of luminance by using a method based on instantaneous stimulus substitution [1], known as the *stimulus exchange method* or *silent substitution* [16, 17, 27]. The procedure for discriminating different stimulus components during recording of the ERG in frogs has been described in detail in [4]. The main concept of the method is that there is a transfer from measuring visual system responses to a stimulus to measuring responses to differences between stimuli [15, 16]. This method is particularly effective in investigations of the visual systems of animals when different characteristics of the stimuli vary simultaneously. Unlike humans, able to focus attention on a stimulus characteristic specified by the experimenter, ignoring other distractions, animals respond to the most diverse stimulus

characteristics, including contextual features. The result is that the contribution associated with the stimulus character of interest to such integral visual system responses as retinal potentials (ERG) or cortical visual evoked potentials (VEP) is affected not only by random changes, but also by systematic changes in the activity of neural networks. The instantaneous stimulus substitution method allows these factors to be identified by introducing a null substitution into the stimulus set, i.e., substitution of a stimulus by an identical stimulus. In this situation, the null response is taken as a measure of baseline activity providing the basis for assessment of visual system responses to the discrimination of non-null stimuli. Two stimuli may not be discriminated by the part of the visual system in which activity is recorded (although the stimuli may be physically different). The response of the visual system in this case will not differ from baseline. If the visual system discriminates the stimuli, then a change in activity will be detected at the moment of stimulus substitution and, as demonstrated in [7, 21, 26], the greater the difference between the stimuli, the greater the amplitude of the ERG and VEP components of interest. Changes in a given characteristic of a single stimulus in a pair (test pair) relative to another pair (reference pair) arise from the signal associated with physical identity, with both increases in the test stimulus and increases in the reference stimulus. In both cases, the differences between the stimuli increase, the responses of the discrimination system showing corresponding increases at both sides of the null point, generating the so-called V-shaped discrimination function. Changes in the test stimulus in two characteristics simultaneously lead to a parallel shift in the V-shaped function reflecting an increase in the contribution of the second characteristic to the overall response of the discrimination system [27].

On this basis, the present study involved pairwise presentation of stimuli varying in luminance and orientation to construct V-shaped discrimination functions and to determine the contributions of each characteristic to the ERG of the frog retina.

METHODS

Animals. Experiments were performed in conditions of dark adaptation at room temperature using four adult common frogs (*Rana temporaria*), immobilized by injections of diplocin (0.1 mg/kg). Animals were placed in a bath on a waxed platform and covered periodically with wetted gauze. When experiments were complete (3–5 h), animals were returned to the aquarium. Each animal was used in one series of experiments.

Stimuli. Stimuli consisted of thin lines of light on a dark background (Fig. 1), with six orientations (from 0° to 150° with a step of 30°) and six levels of luminance (1–32 cd/m^2). Stimulus luminance was measured using a luminance colorimeter to ±5%. All 36 stimuli passed through the center of the visual field and were of identical length on the monitor screen.

ERG recording. Time plots of stimulus presentation and ERG traces are shown in Fig. 2. Stimuli were presented as sequences of three flashes with instantaneous substitution: background (dark), first stimulus (duration 2 sec), second stimulus (2 sec), first stimulus again (2 sec), and background again (dark). This triad mode of stimulus presentation allowed recording of ERG responses to both direct (reference stimulus substituted by test stimulus) and reverse substitution of the two stimuli in the pair. The interval between triads was at least 2 min, to allow the frog's eyes to return to the initial level of dark adaptation. Retinograms were recorded using a monopolar method from the surface of the cornea using a platinum ring electrode. The reference electrode was established on the skin of the head behind the eyes. The electrical potential from the electrode was passed to a preamplifier and displayed on a CI-103 oscilloscope and then to the analog-to-digital converter of a computer. Digitization was at a frequency of 250 Hz. Stimulus presentation and ERG recording were synchronized using the Conan system [5, 8].

Experiments consisted of two series. Both series yielded V-shaped functions, which allowed the contributions of the luminance and configurative components to the retinogram amplitude to be discriminated. One of the 36 stimuli (the reference stimulus) was of fixed luminance and orientation; test stimuli (all 36 stimuli) were presented sequentially in random order. In both series, the reference stimulus was the line with the horizontal orientation (0°); line luminance was 1 and 4 cd/m^2 in the first and second series respectively.

Data processing. Primary data analysis (elimination of artifacts and filtration and averaging of traces) was performed using the Conan system.

RESULTS

Determination of ERG amplitude. Presentation of stimuli in triads yielded ERG responses both on substitution of the first stimulus for the second and, vice versa, of the second for the first. Trace time was plotted on the abscissa, and ERG amplitude on the ordinate. In Fig. 2, the left-hand column shows three types of retinogram, evoked by different combinations of the two stimulus characteristics: *a*) responses to substitution of stimuli differing both in terms of luminance and orientation, i.e., the total contribution of the retina to the ERG amplitude of the various neural networks, *b*) identically orientated stimuli differing only in luminance, *c*) stimuli of identical luminance differing only in orientation. Diagrams showing stimulus presentation along with the luminances and slope angles of both stimuli are shown above the plots. Each plot was

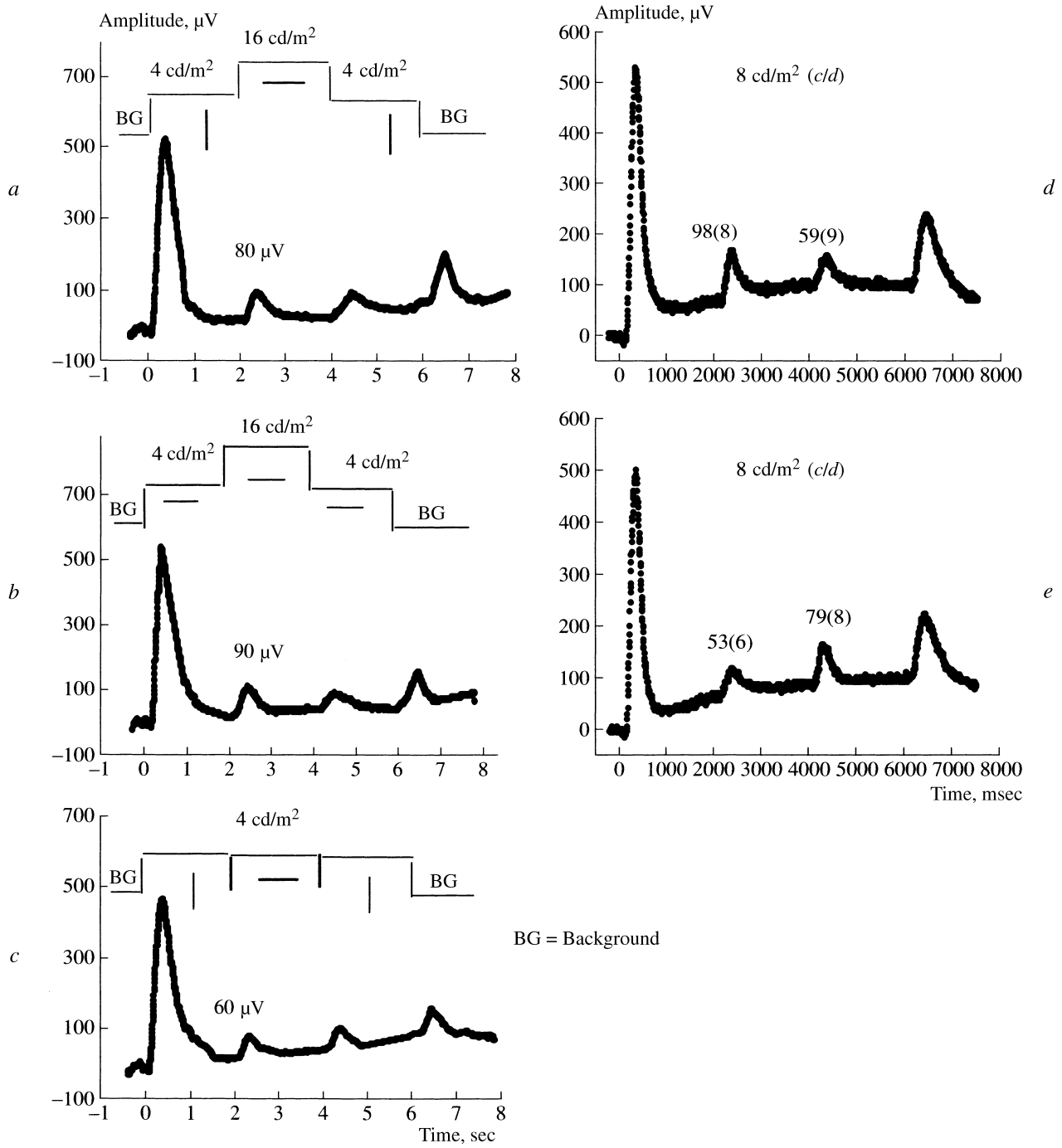

Fig. 2. Digitized frog electroretinogram recorded in response to onset of a reference stimulus on a dark background, substitution of the reference stimulus with a test stimulus, reverse substitution of the test stimulus with the reference stimulus, and offset of the reference signal. The abscissa shows trace time and the ordinate shows ERG amplitude. Stimulus presentation protocols are shown above the plots, along with luminances and slope angles for both stimuli. Mean amplitudes and (in parentheses) standard deviations are shown above the stimulus intersubstitution peaks. See text for explanation.

obtained by averaging five presentations of the corresponding pair of stimuli to a given animal. The right-hand column shows retinograms analogous to the plots shown in Fig. 2, *c*, when stimuli of identical luminance changed only in terms of orientation. These data were obtained at a different luminance level (8 cd/m^2) and with a larger statistical set (averaging of 30 stimulus presentations). Figure 2, *d* shows a stimulus in the vertical orientation substituted with

TABLE 1. Amplitudes of Pattern Electroretinogram *b* Waves (μV) Obtained by Substitution of the Reference Stimulus of Luminance 1 cd/m^2 and Orientation 0° by a Series of Test Stimuli Changing in Terms of Luminance and Orientation

Test stimulus luminance, cd/m^2	Test stimulus orientation (°)					
	–60	–30	0	30	60	90
1	14	9	0	18	23	25
2	25	24	18	23	31	43
4	44	44	74	46	50	60
8	101	98	165	102	120	119
16	118	113	174	117	123	130
32	134	11	175	142	144	148

Notes. The sign of test stimulus orientation values shows the direction of rotation of the line with respect to the horizontal: anticlockwise (+) and clockwise (–) (Fig. 1).

TABLE 2. Amplitudes of Pattern Electroretinogram *b* Waves Analogous to the Data Presented in Table 1, but with a Reference Stimulus of Luminance 4 cd/m^2 and Orientation 0°

Test stimulus luminance, cd/m^2	Test stimulus orientation (°)					
	–60	–30	0	30	60	90
1	48	40	29	40	46	45
2	39	34	22	26	36	37
4	20	16	3	17	22	24
8	22	18	42	26	32	34
16	40	24	54	29	43	43
32	54	50	57	32	53	55

a stimulus in the horizontal orientation. Figure 2, *e* shows the reverse order of stimulus substitution. Mean amplitudes and (in parentheses) standard deviations are shown above the peaks corresponding to the stimulus substitutions. Response amplitudes were calculated from the averaged data as the differences between peaks and the background immediately preceding the moment of stimulus substitution. Peak and background values were also calculated as the means of five sequential measurements of the digitized ERG. Numerical data from both series of experiments are presented in Tables 1 and 2.

The data show that there were significant differences between the responses of the frog retina to formed stimuli (PERG) and to light intensity in conditions of homogeneous illumination of the retina (ERG) [4, 8], as is also the case in higher vertebrates [15, 23].

Comparison of Retinal Responses to Onset and Offset of a Light Stimulus and to Substitution of Stimuli for Each Other

The first discrimination is associated with the nature of the retinal response to changes in the order of the stimuli being discriminated. The ERG recorded in response to a homogeneous light flash (when stimulus luminance was greater than background luminance) always showed a response (the *b* wave) to stimulus onset which was several-fold greater in amplitude than the response to offset of the same stimulus (the *d* wave) [2, 4, 8, 22]. This difference in amplitudes corresponds to the pattern obtained in our studies on onset and offset of line images on a dark background. Figure 2 shows the greater difference in the amplitudes of the *b* and *d* waves in response to onset (first peak) and offset (fourth peak) of lines as compared with the intersubstitution of stimulus lines (second and third peaks). The difference in the amplitudes on substitution of stimulus lines differing in terms of luminance (second and third peaks in Fig. 2, *a*, *b*) is also apparent, though it is less marked. The plot in Fig. 2, *c*, for stimuli of identical luminance, shows that stimulus onset and offset yielded results completely analogous to those obtained in the preceding conditions, though the retinal responses associated with the substitution of stimuli differing only in terms of orientation were of virtually identical amplitude regardless of the order in which the stimuli were presented. The amplitude difference here was no more than a few microvolts, which is comparable to the baseline variations in the retinogram.

The significance of this difference was assessed by performing a separate series of experiments on a new frog for the vertical-horizontal orientation pair at an intermediate luminance level of 8 cd/m^2, increasing the number of presentations from five to 30. Figure 2, *d*, *e* shows the plots yielded by these experiments; these show that the transition from the vertical line to the horizontal yielded a slightly greater PERG amplitude than the reverse substitution. Mean amplitudes, with standard deviations in parentheses, are given above the peaks in each plot. This shows that, as compared with the difference in amplitudes seen on stimulus onset and offset, the amplitudes for the interaction of these stimuli were very similar, though the difference between them was statistically insignificant.

These data show that the response of the retina to onset and offset of a formed stimulus (a pattern) had characteristics coinciding with those of the retinal response to homogeneous illumination of the retina (ERG), but differed from the responses to differences between formed stimuli in terms of both luminance and orientation. This suggests the hypothesis that only the latter situation is related to the PERG – the retinal response intrinsic to the pattern, i.e., a temporally or spatially inhomogeneous distribution of illumination on the retina. The hypothesis that the electroretinogram for the difference between the stimuli represents the retinal response to the pattern suggests that this response to the difference has some origin other than the ERG, as has been demonstrated in higher vertebrates [25]. (It follows from this that calculation of the difference response by calculating the individual responses to identically presented stimuli may lead to significant distortion of the data.)

Another argument supporting this hypothesis arises from the close approximation (symmetry) of the amplitudes of retinograms obtained by mutual substitution of the stimuli as compared with the amplitudes of the ERG *b* and *d* waves. We obtained analogous results for cortical evoked discrimination potentials (EDP) recorded in humans in response to instantaneous substitution of homogeneous light stimuli of different luminances and colors [7, 20]. V-shaped functions constructed using EDP amplitudes were divided into two types. In response to instantaneous mutual substitution of homogeneous light with a luminance change, the amplitude of one component (*N87-P120*) gave a symmetrical V-shaped function, while the amplitude of the other component (*P120*) gave an asymmetrical response with a predominance of on responses over off responses [5]. This result is interpreted in [5] as indicating the presence of two mechanisms encoding luminance in the human visual system. One is an energy mechanism, which is asymmetrical, involves only the on cells of the visual system, and functions as the physical carrier of the visual image; the other involves symmetrical on and off cells, addresses the task of quantitative encoding, and in particular the formulation of the chromatic and achromatic components of light. Recording of EDP to formed stimuli (lines of different orientation in the visual field or schematic diagrams of faces) showed that the amplitudes of the configurative components of the EDP (*P120-N180* and *N180-P230*) also had symmetrical shapes [8, 21].

Thus, this difference in the responses of the frog retina to onset of stimulus lines (ERG) and to changes in the orientation and luminance of the lines (PERG) corresponds not only to the difference between homogeneous illumination of the retina and the formed stimulus (a spatial pattern), but also to differences in the stimulus presentation conditions, i.e., the instantaneous intersubstitution of the stimuli (a temporal pattern). The situation of switching on any light stimulus (both homogeneous light and light formed into some pattern) involves both receptors and many on cells in the outer plexiform layer (OPL) of the retina, which constitute the input level of very diverse networks, explaining the large amplitude of the retinal response to onset. It is very likely that Muller cells, also in the OPL, play a significant role in this process. After this initial stage, activation persists only in those networks specified by the stimulus, activation ceasing in all other networks. Stimulus offset affects fewer off cells, involved in a specific network, so the retinogram *d* wave is significantly smaller than the *b* wave.

Another redistribution of cell activity occurs in retinal responses to instantaneous stimulus substitution. It can be suggested that encoding of stimulus changes (interstimulus differences) is mediated by a more local network, in which the major and identical roles are played by on and off ganglion cells. In particular, our data indicate that local changes in luminance (luminance differences) are determined in the frog retina by a two-channel network of neurons, whose structure and resulting type of electrical code are more complex than a simple multitude of receptors and bipolar cells reacting directly to the actions of the energy of the light stimulus [6]. In other words, the luminance encoding process in the frog retina may function via two mechanisms, while the retinal responses to flashes of light and to changes in light in the visual field are mediated by different networks. One mechanism encodes the action as discharge power [12] and forms information regarding the absolute level of external illumination. The other mechanism is based on vector encoding of the stimulus action [6, 11, 20] and forms information on the spatial and temporal differentiation of the light in the visual field. The first mechanism is characterized by a powerful response to onset of the light stimulus (whether the whole retina or only a part of it is stimulated is less important than the fact of onset of the light) and significant asymmetry in responses to stimulus onset and offset, while the second mechanism is finer, its code being separated from the energy characteristics of the stimulus, such that the responses of this mechanism are of significantly lower magnitude and greater symmetry. This two-channel mechanism in the frog retina can form a response to a configurative difference, which is indirectly

supported by the complete similarity between the retinograms in Fig. 2, *a* and Fig. 2, *b*.

Thus, the first difference between PERG as the response of the frog retina to local stimulus inhomogeneity (i.e., different luminances in areas of the visual field in time or space) and ERG as the response simply to onset of light consists of a significantly smaller amplitude and a tendency to symmetry of both retinogram measures (the *b* and *d* waves). Local inhomogeneity is the luminance difference between areas of the visual field in time or space.

Comparison of Retinal Responses to Simultaneous Changes in Different Characteristics of a Formed Light Stimulus

Another significant feature of PERG in frogs is evident on comparison of the magnitudes of responses to stimuli differing in terms of one characteristic (luminance or orientation) and combinations of characteristics (luminance and orientation together). By analogy with results obtained by recording ERG in response to changes in stimuli in terms of spectral composition and intensity [4, 7, 26], it has been suggested that the response to the combination of independent characteristics will be of greater amplitude than the responses to each individual characteristic [27]. Comparison of Fig. 2, *c* and Fig. 2, *a* shows that this suggestion holds. Wave amplitudes on substitution of stimuli of different luminance and differing only in orientation (~60 μV) were smaller than wave amplitudes on substitution of stimuli differing simultaneously in luminance and orientation (~80 μV). However, Fig. 2, *b*, where the stimuli also differed in terms of one characteristic, i.e., luminance, shows a different picture: the response amplitude was paradoxically increased (~90 μV). With the aim of confirming that this was not an artifact, we return to the construction of the V-shaped functions, which (in accord with the instantaneous substitution concept) should allow the integral response of the visual system to be split into its individual components on exposure to a complex stimulus [1, 4, 27].

Figure 3, *a* shows plots constructed using retinal responses to stimuli with simultaneous changes in luminance and orientation. These plots show only one branch of the V-shaped functions, when the test stimulus changed in the same direction (increases in luminance and rotation angle) relative to the reference stimulus. The points represent experimental data and the lines show predicted functions. The abscissa shows the differences in line orientations between the reference (0°, 4 cd/m^2) and six test stimuli with orientations from 0° to 150° (Fig. 1) and luminance at three levels, 1, 2, and 4 cd/m^2. The ordinate shows the amplitude of the PERG *b* wave in response to stimulus substitution by increments in luminance. Each plot shows a monotonic increase in response amplitude with the difference in the orientation of the test and reference stimuli increasing from 0° to 90°. (As the orientation of the reference stimulus was 0°, this difference is numerically equal to the orientation of the test stimulus.) It should be noted that further increases in the stimulus rotation angle in the same direction (to 120° and 150°) led to decreases in response amplitude. As shown in Fig. 3, *a*, amplitudes for these stimuli (angles are indicated above the abscissa) correspond precisely to values obtained in responses to substitution of orientations to 60° and 30°, respectively. This result – the response to the smallest angle relative to the horizontal – applied in all cases when the difference in the orientations within the pair was greater than 90°. This suggests that the retinal response depends only on the magnitude and not on the rotation direction of the stimulus (clockwise or anticlockwise relative to the horizontal). In Fig. 3, *b*, these same data are presented as classical V-shaped functions. The abscissa covers the range from 0° to ±90° and the response amplitudes for stimuli with orientations of 120° and 150° shown in Fig. 3, *a* are presented on these plots as values of –60° and –30°, respectively. Each V-shaped function identifies the contribution of the orientation-detecting network to the retinal response. The plot also shows that increases in luminance lead to parallel displacements of the plot on the ordinate, i.e., the response amplitude increases by a constant amount. This corresponds to the contribution of the luminance system to the integral retinal response. Thus, it is evident that each of the two stimulus characteristics makes its contribution to the amplitude of the retinal response. For the low luminance amplitudes presented here (1–4 cd/m^2), the minimum point of the V-shaped functions are in the expected locations, where stimuli have equal orientations, i.e., the effect seen in Fig. 2, *a*, where the *b* wave amplitudes for stimuli differing in terms of two characteristics were lower than those for stimuli differing only in terms of luminance (Fig. 2, *b*), is not seen here. We will now consider data for a wider range of luminances.

Figure 3, *c* shows a series of V-shaped functions obtained from another frog and another reference stimulus – a horizontal line of luminance 1 cd/m^2, the test stimulus line changing over six luminance levels, from 1 to 32 cd/m^2, in six orientations. As in Fig. 3, *b*, the data represent the luminance increment situation, though for this reference stimulus this was the amplitude of the first stimulus substitution (second peak in Fig. 2, *a*). The V-shaped difference functions accurately identify the different components of the response reflecting the total electrical activity of the visual system. The plot in Fig. 3, *c* shows data for several test stimuli with luminance identical to that of the reference stimulus (the lower V-shaped function). The minimum of this function corresponds to identity between the test and reference stimuli, the retinogram in this case being simply the background activity of the retina. To the left and right of the minimum, retinal response amplitude increases monotonically as the deviation of the stimulus from the horizontal (reference stimulus) increases in the clockwise and anticlockwise directions. Increases in the luminance of the test stimuli lead to parallel upward displacements of the

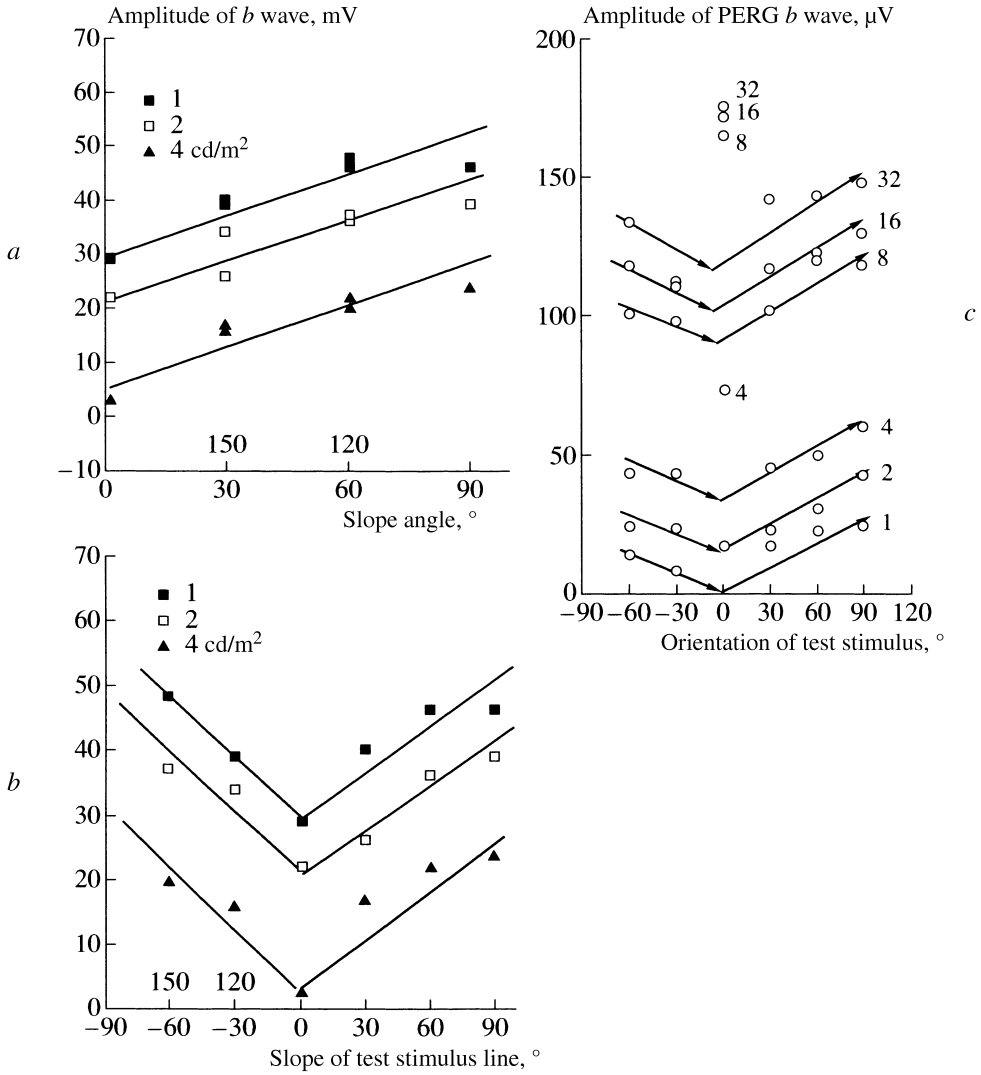

Fig. 3. Plots of V-shaped functions showing changes in the retinal response amplitude (ordinate) in relation to increases in the difference between the reference stimulus (horizontally orientated line of fixed luminance) and a series of test stimuli changing in orientation and luminance. See text for explanation.

V-shaped function on the ordinate (as shown in Fig. 3, *b*). As already noted, this occurs because the luminance difference is added to the stimulus orientation difference. At the point of equality between the orientations of the test and reference stimuli (at 0°, on the abscissa), the retinogram amplitude reflects only differences in luminance, so the minimum pint of the V-shaped function must be displaced on the ordinate by the magnitude of the luminance contribution. However, in this case this condition was fulfilled only for a luminance of 2 cd/m^2, which was close to the luminance of the reference stimulus. For all other test stimulus luminance levels, as in Fig. 2, *b*, the amplitude of the *b* wave on substitution of stimuli of identical orientation was significantly greater even than the amplitude of the wave representing the response to the total difference in luminance and orientation. Thus, the point representing this amplitude on the plot is located significantly higher on the ordinate than the expected minimum of the V-shaped function. These amplitudes in Fig. 3, *c* are, for clarity, shown individually. Verification of this result showed that it was obtained not only for substitution of a darker reference signal by brighter test signal (the *b* wave), but also for the reverse substitution (the *d* wave). However, the *d* wave was characterized by a much smaller amplitude and lack of monotonicity as luminance increased.

This paradoxical effect can be seen particularly clearly on plots showing changes in retinal response amplitude (PERG) in relation to luminance presented as the traditional psychophysical function (Fig. 4). The abscissa shows test stimulus luminance on a natural log scale and the ordinate

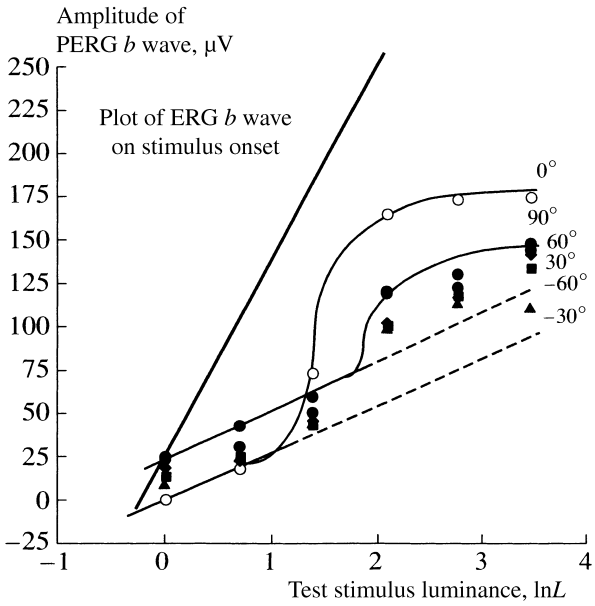

Fig. 4. Changes in the amplitude of the pattern electroretinogram *b* wave as a function of changes in test stimulus luminance. The orientation and luminance of the reference stimulus were kept constant at 0° and 1 cd/m^2. The orientation of the test stimulus (the six levels of orientation are shown at right for each type of symbol) is the parameter for a given value of the function. The thin lines show plots linking the amplitude values for these functions and the dotted lines show the expected luminance discrimination functions. For convenience, only two of the six functions are shown: the lower for the null difference between the orientations of the test and reference stimuli and the upper for the maximum difference. The thick continuous line shows the luminance onset function (the ERG *b* wave from the data presented in Figs. 2 and 3). See text for explanation.

shows amplitudes for substitutions by luminance increment. Each type of symbol characterizes conditions in which the test stimuli had identical orientations (shown in degrees to the right of the plots). The thin continuous lines identify plots linking the amplitudes of these functions, while the dotted lines show the expected standard luminance functions. For clarity, only two of the six functions are shown: the upper is for the null difference in orientation between the test and reference stimuli, i.e., only the luminance function of the PERG; the lower is for the luminance function of the PERG for stimuli with the greatest difference in orientation. All other PERG luminance functions for intermediate test stimulus orientations had the same form and, as shown by the corresponding values, were located in parallel in positions directly related to the test stimulus orientation angle. The thick continuous line shows the luminance function for stimulus onset (the ERG *b* wave for the first retinogram peaks presented in Fig. 2). This shows that initially, over the range of small differences in luminance between the reference and test stimuli, the expected and obtained data are in good agreement with each other. However, as the luminance difference increases, all functions combining luminance and orientation (dark symbols) continued to run parallel, while the pure luminance function of the PERG (light symbols) was sharply displaced towards the luminance function measured from ERG amplitudes (thick line). It should be noted that an analogous displacement of PERG functions for the combination of luminance and orientation (shown in Fig. 4 by the thin continuous line) is also seen, though it is significantly smaller than the displacement of the pure luminance function of the PERG. Considering noise, these latter data may even approximate the standard logarithmic luminance function (shown by the dotted straight line in Fig. 4).

Thus, changes in stimuli over a small range of luminance differences lead to the expected PERG amplitudes (corresponding to the logarithmic luminance function and the minimum of the V-shaped orientation function), while stimuli with large luminance differences gave PERG amplitudes which were significantly greater than expected and close to the ERG amplitudes. This phenomenon – the paradoxical positions of V-shaped function minima – evident on the plots shown in Fig. 3, *c*, is consistent in nature and is expressed as the non-linear function shown in Fig. 4 by the thin continuous line. It can now be suggested that the plot of luminance functions for lines with simultaneous differences in luminance and orientation (shown by the second such line in Fig. 4) is in fact non-linear. The plots in Figs. 3, *c* and 4 suggest an explanation for this phenomenon.

DISCUSSION

Interaction of Mechanisms Encoding Luminance and Orientation in the Frog Retina

The data considered above allow a number of suggestions to be made in relation to the quite complex interaction between the different mechanisms in the frog retina. In particular, this relates to the two mechanisms encoding illumination intensity and the mechanism encoding line orientation. These mechanisms can immediately be divided into two classes: the energy-based mechanism encoding illumination intensity, whose functioning is reflected in the ERG, and the mechanism encoding the luminance pattern, i.e., the distribution of illumination in the visual field space, whose activity is reflected in the PERG. Figure 5 provides a schematic representation of these mechanisms in the neuronal structure of the frog retina. As noted above, onset of a light stimulus immediately activates a large number of receptors and cells in the outer plexiform layer (OPL), which constitutes the input part of a multitude of different visual system neural networks ready to encode "their own" stimuli. This also induces activation of a large number of Muller cells, which explains the large ERG amplitudes of responses to onset after dark of both homogeneous and formed stimuli. This response is shown in Fig. 5 in the plot at upper right as the first wave of the ERG. In the case of

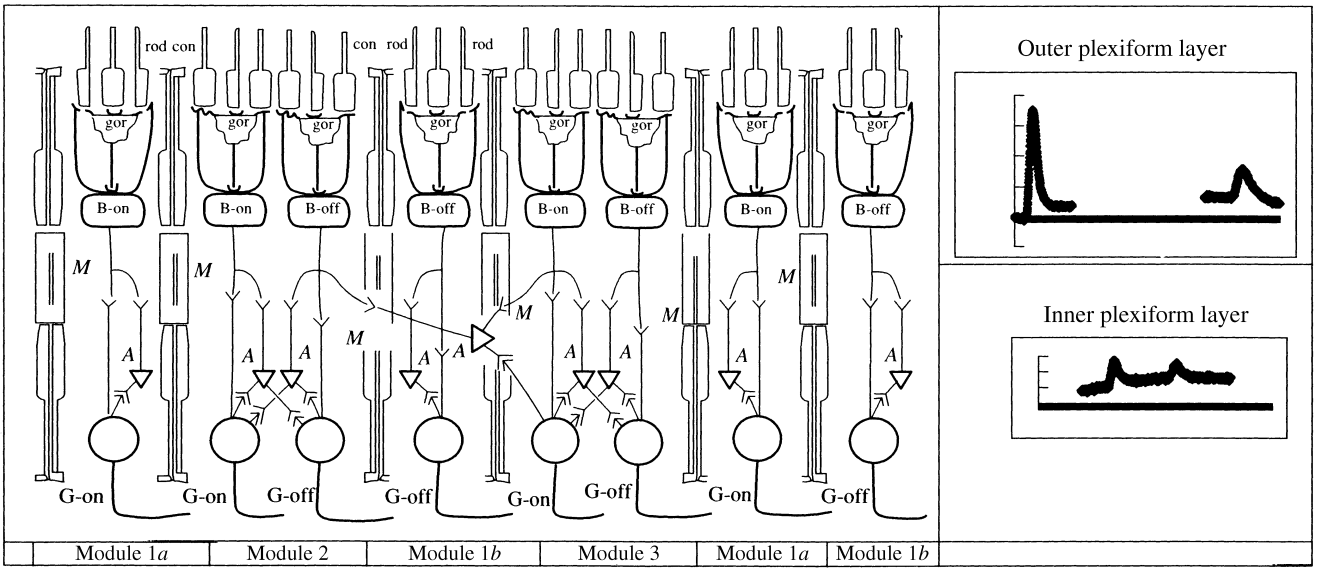

Fig. 5. Diagram of the modular organization of the frog retina. The terms con, rod, and hor identify photoreceptors and horizontal cells constituting the outer plexiform layer (OPL). B, A, and G identify bipolar, amacrine, and ganglion cells, constituting the inner plexiform layer (IPL). *M* = Muller cells. Modules 1*a* and 1*b* form a single-channel network encoding the intensity of light falling on the retina as a monotonic change in the total activity of all elements of the network. Modules 2 and 3 form a two-channel network encoding luminance differences (in the spatial or temporal visual field) forming a pattern. A typical frog ERG recorded in response to stimulus onset and offset (a horizontal light line) in the dark is shown at top right. The response to onset and offset of homogeneous light forms just as in the ERG. The mid part of the retinogram, associated with changes in the configuration of the light pattern (line orientation and luminance) is shown at lower right. This is the so-called PERG, which is characterized by waves with a significantly lower amplitude and greater symmetry in amplitudes as compared with the ERG. See text for explanation.

homogeneous illumination of the retina, massive activation of OPL cells is followed by persistence of activity in the inner plexiform layer (IPL), not only in those bipolar and ganglion on cells (local network of module 1*a* in Fig. 5) encoding the illumination intensity as response intensity. Other networks are not involved in this process, so the stimulus is not "their" stimulus. Offset of homogeneous light lead to activation of off cells in the OPL and IPL in the local network of module 1*b*. As the number of cells in module 1*b*, which process illumination intensity, is significantly smaller than the number of cells in the OPL involved in stimulus onset, the amplitude of the *d* wave is significantly less than the amplitude of the ERG *b* wave. In Fig. 5, this response is shown on the plot at upper right as the second ERG wave. This mechanism is probably one of the most ancient sensory mechanisms, as the basic principle of its construction – that of encoding intensity as a monotonic change in cell activity – appears at the receptor level and is not restricted to light receptors (Adrian's generalization).

In the other case, where onset is not of a homogeneous light but rather a light pattern, powerful activation of OPL cells is also followed by spread of activity to IPL cells, though not only to bipolar and ganglion cells of module 1, but also to cells constituting modules for the various configurative characteristics of the stimulus [9]. Figure 5 shows two such configurative modules, which specify the magnitude of the luminance change (module 2) and the *magnitude of the orientation change* (module 3) between the stimuli used in our experiments. The configurative modules differ from energy module 1 by the fact that their composition includes on and off cells which interact with each other on the principle that the total activity of these cells is constant, which forms a two-channel mechanism for vector encoding of the stimulus, while these cells in module 1 operate independently, each module operating as an independent, single-channel, scalar mechanism. A detailed description of the two-channel mechanism encoding line orientation in the human visual system has been presented in [8], while the mechanism encoding stimulus luminance in the human and frog visual systems has been described in [6, 20]. As on and off cells are tightly linked in the two-channel module, intersubstitution of the pair of stimuli activates this module identically, this being expressed in the symmetry of PERG amplitudes in retinal responses on forward and reverse substitution of the stimuli in the pair. Figure 5 shows that the specific interaction between the channels in the two-channel module may be mediated by amacrine cells, whose dendrites form synapses with both bipolar cells and ganglion cells [2, 28]. The number of configurative modules of each type in the frog retina is even smaller than that in the single-channel module 1, so PERG amplitude in the intersubstitution is even smaller than the amplitude of the *d* wave on

stimulus offset. In Fig. 5, this response is shown on the lower right plot as two PERG waves. Configurative modules 2 and 3 operate in parallel and independently, so their responses are summed in PERG amplitudes, as demonstrated on the V-shaped functions shown in Fig. 3. At the same time, the configurative modules have greater priority and, on activation, they inhibit luminance modules 1*a*. This inhibition can also be mediated by amacrine cells, which form not only the internal structure of the two-channel module, but also the intermodular structure controlling the interaction of different modules.

This priority performs the function of adaptation to the overall illumination level in the visual field. The data presented in Fig. 3 and Tables 1 and 2 show that in situations in which the luminance of the test stimulus is close to the luminance of the reference stimulus, the retinal responses reflect only the activity of the configurative modules. In Fig. 4, this corresponds to a parallel displacement in all PERG luminance functions in the linear range of the plots. When the luminance of the test stimulus is sharply different from that of the reference stimulus, this returns the retina to the stimulus onset situation, and inhibition of the configurative modules is overcome by the sharply increased activity of cells in the OPL and modules 1*a*. The retinal response therefore increases because of addition of the ERG amplitude to the PERG amplitude, which is indeed seen in the non-linear displacement of PERG luminance functions in Fig. 4. It is completely logical that this displacement of the retinal response is greater when the stimulus line changes only in luminance than when there are simultaneous changes in luminance and orientation. In the latter case, module 1*a* experiences the overall inhibition from modules 2 and 3 (Fig. 5), while in the situation of equal orientations, module 3 does not operate and module 1*a* overcomes a twofold lesser inhibition.

CONCLUSIONS

1. The response of the frog retina to onset and offset of a formed stimulus (a thin line with different orientations in the field of vision) coincides in terms of its major components (the electroretinogram *b* and *d* waves) with the retinal response to onset and offset of a homogeneous stimulus and is characterized by high amplitude and significant asymmetry between the electroretinogram *b* and *d* waves, while the response to intersubstitution of stimulus lines (formed stimuli) is characterized by an amplitude an order of magnitude smaller and a tendency to symmetry in the major retinogram components (the *b* and *d* waves).

2. The differences between the electrical response of the frog retina to discrimination of formed stimuli (the pattern electroretinogram) and the response to stimulus onset and offset may be associated with the involvement of different neuronal structures in generating these responses. In particular, the electroretinogram is produced mainly by cells in the outer plexiform layer of the retina, while the pattern electroretinogram is produced by cells in the inner plexiform layer, which is analogous to what is seen in the retinas of higher vertebrates.

3. Simultaneous changes in the luminance and orientation of stimulus lines lead to increases in the amplitude of the pattern-electroretinogram *b* wave as compared with the amplitudes seen in responses to changes in each of these characteristics separately. This means that the mechanisms detecting differences in luminance and orientation, which are based on cells in the inner plexiform layer, operate in parallel and independently. At the same time, a significant increase in the luminance of the stimulus line with a constant difference in orientations leads to a paradoxical jump in the retinogram amplitude. This sharp change is interpreted by the retina as the appearance of a "new" stimulus, and the activity of cells in the inner plexiform layer, which form the pattern electroretinogram, is supplemented by the activity of cells in the outer plexiform layer, which respond to the "onset" of luminance.

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