

# Relationship between the amplitudes of the b wave and the a wave as a useful index for evaluating the electroretinogram

IDO PERLMAN

*From the Department of Physiology and Biophysics, Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel*

**SUMMARY** The present methods for the assessment of the electroretinogram (ERG) make the exchange of data between laboratories difficult owing to differences in photostimulation and recording techniques. In this study it is suggested that in addition to present techniques the b wave to a wave relationship may be a useful index for evaluating the ERG. The ERG responses evoked by relatively bright flashes are recorded in the dark-adapted state. A plot of the b wave amplitude as a function of the a wave amplitude describes the functional integrity of the retina. A normally functioning retina should elicit an ERG response that fits the normal curve regardless of the apparatus used for ERG recording. The only constraint is the use of full-field illumination. This method is based on physiological considerations regarding the origin of the ERG components. Such a method will facilitate data exchange between centres and can also be used for clinical assessment of the retina.

The use of the electroretinogram (ERG) for objective assessment of visual function has become a routine procedure in many medical centres throughout the world. In most laboratories the ERG evaluation includes measurements of the b wave amplitude and its implicit time and of the a wave amplitude.<sup>1,2</sup> The ERG responses are recorded in the light- and dark-adapted states by means of red, blue, and white stimuli of different intensities.<sup>1,2</sup> However, the exchange of electrophysiological data between laboratories is hindered by the different procedures employed in different places.<sup>1</sup> The temporal characteristics and the amplitudes of the ERG responses depend on many technical factors, the most important of which are the number of quanta absorbed by the photoreceptors<sup>3–6</sup> and the state of adaptation.<sup>3–8</sup> Light absorption in a given state of adaptation depends on the light intensity reaching the retina, which in turn depends on the pupil's size, losses due to absorption and scatter in the ocular media, and the intensity setting of the photostimulator. The latter, being different in different laboratories, gives rise to different normal electroretinograms, thus making the exchange of ERG data for clinical cases rather difficult.

Correspondence to Dr I. Perlman.

In this report an additional approach to the quantitative evaluation of the electroretinogram is suggested in order to facilitate data exchange between laboratories. It is further suggested that this approach will contribute to the existing analytical methods used for the physiological interpretation and the clinical use of the electroretinogram. This new method is based on the current understanding of the origin of the different components of the ERG responses.

Granit<sup>9</sup> isolated 3 components of the ERG, which he termed P-I, P-II, and P-III. The cellular origin of those waves was studied by gradual electrode penetration into the retina<sup>10</sup> and by applying toxic agents as well as by blocking of the retinal circulation.<sup>11</sup> Later studies<sup>12</sup> showed additional components of the ERG, and a different terminology was adopted to identify these waves. These and other studies resulted in the current theory that the ganglion cells in the retina do not contribute to the ERG; the P-I arises from pigment epithelium cells, the P-II from retinal elements proximal to the photoreceptors, and the P-III (late receptor potential) from the activity evoked by light stimuli in the photoreceptors.

Two major waves can be identified in the ERG: the a wave, which is the leading edge of the P-III and is

believed to reflect the activity of the photoreceptors, and the b wave, the cellular origin of which is not clearly understood. Intraretinal recordings from the cat retina placed the origin of the b wave in the inner nuclear layer.<sup>10,13</sup> Intracellular recordings raised the theory that Muller (glial) cells might generate the b wave by a K<sup>+</sup>-regulated mechanism.<sup>14,15</sup> But some experimental results were not easily reconciled with the above interpretation of the cellular origin of the b wave.<sup>16,17</sup> Recent studies employing pharmacological<sup>18</sup> and developmental<sup>19</sup> approaches supported the Muller cell hypothesis. Despite the uncertainty about the cellular origin of the b wave, it is generally accepted that the b wave originates proximal to the photoreceptors and that its amplitude can be used in the assessment of retinal function.<sup>20,21</sup> This idea is supported by experiments in the skate retina, where it was found that the b wave and the ganglion cells responded similarly in different states of adaptation.<sup>22</sup>

From the above data it is assumed that the a wave represents activity in the photoreceptors layer, namely, the primary event evoked by the light stimulus. The b wave is generated in the proximal layer and is therefore a secondary phenomenon. Thus the a wave represents the input to the proximal retina and the b wave its output. The amplitude of the a wave depends on the integrity of the photoreceptors and on absorption of quanta in the photoreceptors, while the amplitude of the b wave depends on that of the a wave and on the functional integrity of the interactions between the a wave and b wave generators. These considerations lead to the hypothesis that a unique relationship between the b wave and a wave amplitudes exists in the normal retina at a given state of adaptation. Any significant deviation from the normal relationship can therefore be regarded as pathological and can be interpreted accordingly.

### Material and methods

The ERG responses were recorded differentially between a Henkes type contact lens electrode (Medical Workshop) placed on the cornea and a reference electrode placed on the subject's forehead. The ground electrode was attached to the left ear lobe. The contact lens electrode contained a -100 dioptre lens in order to achieve a uniform illumination of the entire retina similar to Ganzfeld illumination. The electrical responses were amplified by a Grass P511 AC amplifier set at a bandpass of 0.1 Hz to 1000 Hz. The responses were displaced on an alternating-current coupled oscilloscope (Tektronix 5103N) and were photographed for later analysis.

The photostimulator included an electronic camera flash which elicited 1 ms flashes. The intensity of the

test flash was controlled by a set of calibrated 'neutral' density filters (Schott, NG glass type) spanning a range of 7 log units.

The subject's pupils were fully dilated by 0.5% cyclopentolate hydrochloride (Cyclogyl) and 2.5% phenylephrine hydrochloride (Neosynephrine). The ERGs were first recorded in the light-adapted state with background illumination of 11 foot-lambert. Then, the background light was turned off, and the ERG evoked by a stimulus of constant intensity was recorded in the dark every 2 to 3 min. After 25 min in the dark the dependence of the ERG response on the intensity of the white light stimulus was measured over a range of 6 log units.

The analysis of the electroretinogram consisted in measuring the amplitudes of the a and b waves. The amplitude of the a wave was measured from the baseline to the trough of the a wave. The amplitude of the b wave was defined from the trough of the a wave to the peak of the b wave.

Twenty volunteers, aged 20 to 40 years, with normal or normal-corrected vision, served in this study. Each subject underwent a psychophysical measurement of dark adaptation in order to ensure normal night vision. The dark-adaptation curve was measured intermittently with a 2° field of blue-green light (500 nm) and orange-red light (600 nm) flashed on the temporal retina of the right eye 15° from the fovea. The apparatus and procedure for dark adaptometry were previously described in detail.<sup>23</sup>

### Results

The ERG responses of one subject are illustrated in Fig. 1 (upper trace, left eye; lower trace, right eye). The responses were evoked by flashes of different intensities in the dark-adapted state. The stimulus intensity denoted above each pair of responses is given as the density of the 'neutral' filter interposed in the light path. The ERG pattern strongly depended on the stimulus intensity, in agreement with previous reports on human subjects<sup>3,4,7</sup> and in laboratory animals.<sup>5,6</sup> Dim flashes evoked a small b wave with long implicit time. As the stimulus was made brighter, the amplitude of the b wave increased and its implicit time decreased until an a wave started to appear. The threshold for the a wave was about 2.5 log units above that of the b wave. With brighter stimuli the a wave amplitude increased rapidly and so did the b wave amplitude.

The dependence of the amplitude of the a wave (open squares) and of the b wave (solid squares), recorded in the dark-adapted state, on the flash intensity is given in Fig. 2. The data points represent the mean amplitude  $\pm$  2 standard deviations of the responses obtained from both eyes of 20 subjects with

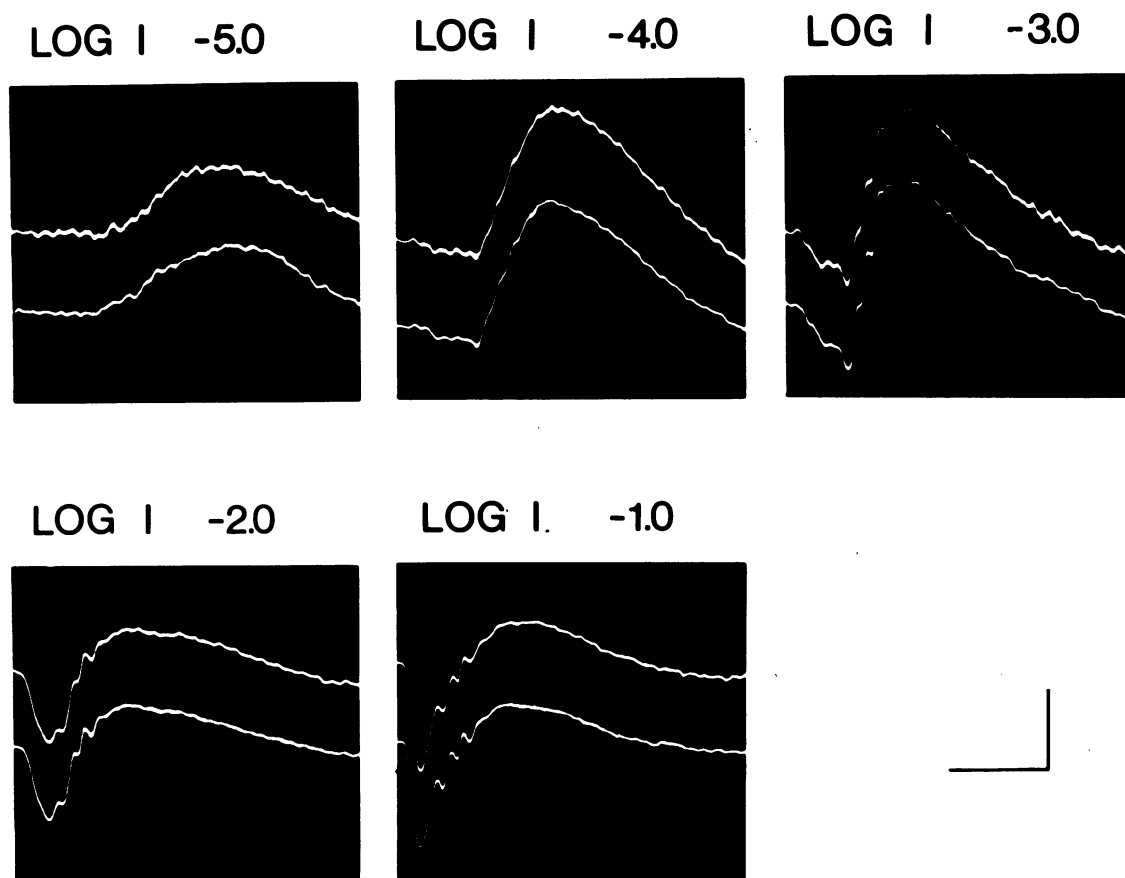


Fig. 1 Normal ERG responses measured in the dark-adapted state. The responses were evoked by 'white' flash, the intensity of which was controlled by 'neutral' density filter, as denoted above each pair of responses. The calibration mark has a width of 50 ms and a height of 200  $\mu$ v for flash intensities  $\log I = -5.0$ ,  $-4.0$ , and  $-3.0$ , and 400  $\mu$ v for flash intensities  $\log I = -2.0$  and  $-1.0$ .

normal vision. Fig. 2 clearly shows the strong dependence of both the a and the b waves on the flash intensity, which determines, with other factors, the intensity of the stimulus reaching the retina. Similar dependence of b wave amplitude on the stimulus intensity was previously reported.<sup>24</sup> Therefore when different light sources with different intensities are used the normal ERG responses differ, and comparison between the different groups is meaningless. It has recently been suggested that this difficulty can be circumvented by recording the largest scotopic response without a preceding a wave and to assess the clinical data in percentages of the normal range.<sup>25</sup> This method has the disadvantage that the a wave threshold is not easily determined, and one has to use for each subject many stimuli of different intensities in order to obtain the correct response. Moreover, different laboratories may use their own criteria for

the definition of the largest response without a preceding a wave, thus making data exchange difficult.

For the past 2 years we have been trying a new approach to the assessment of the ERG responses. The ERG responses, evoked by stimuli of different intensities, are recorded in the dark-adapted state. We measure and plot for each response the amplitude of the b wave as a function of the amplitude of the a wave. In Fig. 3 the normal range (mean  $\pm$  2 SD) of 20 subjects is described. Any ERG response recorded from a normally functioning retina will be represented by a data point within those limits, irrespective of the apparatus used for photostimulation. The only constraint that must be kept is full-field illumination. The general character of the method is demonstrated by the solid and open triangles in Fig. 3. These data points describe the b wave amplitude as a function of a wave amplitude, calculated from normal ERG

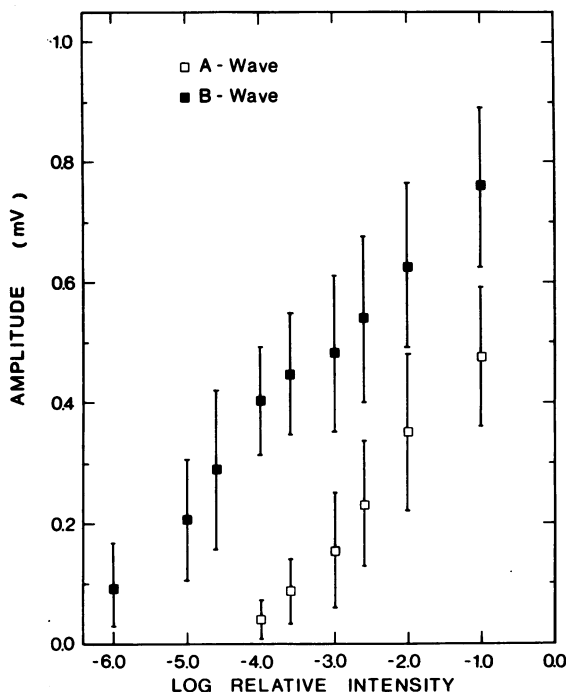


Fig. 2 The dependence of the b wave amplitude (solid squares) and the a wave amplitude (open squares) on the log intensity of the light stimulus. The data points describe the mean  $\pm$  2 SD of the responses obtained in the dark-adapted state from 40 eyes of 20 volunteers with normal vision.

responses given in an article by Berson<sup>2</sup> (open triangles) and by Weleber<sup>26</sup> (solid triangles). The stimulating apparatus used in these works was different from ours, yet the agreement between the 2 sets of normal data and the normal range measured in our laboratory is excellent.

The ERG responses of 2 patients are also illustrated in Fig. 3. One patient, suffering from high myopia, is represented by the open circles. For each stimulus intensity his ERG had a subnormal amplitude. However, the dependence of the b wave on the a wave was normal, indicating that signal transmission in his retina was normal and that the subnormal ERG amplitude was probably due to subnormal rod response. The second patient, suffering from congenital stationary night blindness, had clear ocular media. His ERG responses consisted of a small b wave and a large a wave. Thus his data points (solid circles) fall below the normal curve. This result was interpreted as inferior retinal function, because signal transmission from the photoreceptors to the proximal retina was impaired, resulting in a measured b wave smaller than expected according to the normal relationship. This interpretation is in agreement with

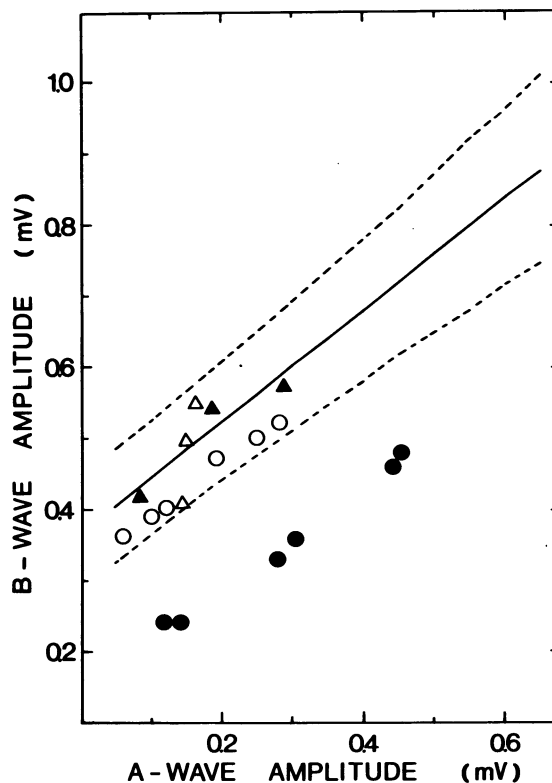


Fig. 3 The relationship between the b wave amplitude and the a wave amplitude obtained from responses evoked in the dark-adapted state. The continuous line describes the mean relationship, while the 2 dashed lines bind the normal range (mean  $\pm$  2SD). Open and solid triangles represent normal ERG data obtained, respectively, from papers by Berson<sup>2</sup> and Weleber.<sup>26</sup> Data of 2 patients are also illustrated; one patient suffers from high myopia (open circles), while the other complained of nyctalopia (solid circles).

previous reports showing that the rod outer segments in such patients were normal and that the defect was placed in the proximal retina.<sup>27 28</sup>

Another way of describing the above method is illustrated in Fig. 4. In this figure the ratio between the measured b wave and the expected b wave is plotted as a function of the a wave amplitude. The expected b wave amplitude was obtained from the mean normal b wave to a wave relationship plotted in Fig. 3. Any normal retina gives a ratio of between 0.8 to 1.2, as is illustrated by the data obtained from Berson<sup>2</sup> (open triangles) and Weleber<sup>26</sup> (solid triangles). The data points obtained from the patient with the high myopia (open circles) fell within the normal range, while those from the patient with the congenital nyctalopia (solid circles) fell consistently below the normal range.

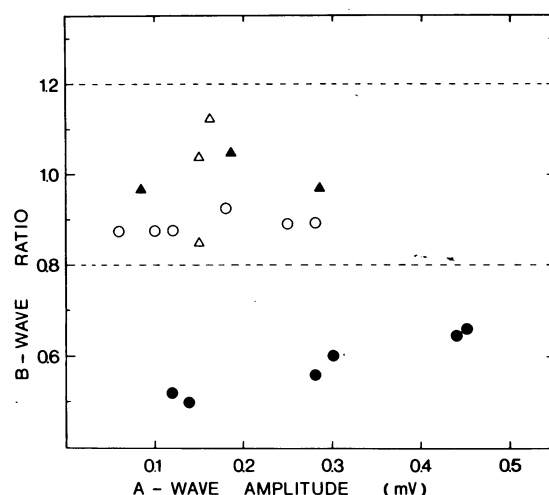


Fig. 4 The b wave ratio as a function of the a wave amplitude. The b wave ratio is the ratio between the measured b wave and the expected one. The expected value was obtained by treating the a wave as the independent variable and using the mean normal b wave versus a wave plot (Fig. 3, continuous line). The dashed lines bind the normal range. Normal ERG data from other studies are described by open triangles (Berson<sup>2</sup>) and by solid triangles (Weleber<sup>26</sup>). The patients with high myopia (open circles) and night blindness (solid circles) are also represented in the figure.

## Discussion

In this report the b wave to a wave relationship is suggested as a useful tool for the assessment of the clinical electroretinogram. According to this method the b wave amplitude is plotted as a function of the a wave amplitude for different flash intensities, thus creating the normal relationship between those 2 variables. The a wave, which describes the activity of the photoreceptors,<sup>9-12</sup> represents the input to the proximal retina, while the b wave represents its output. The a wave amplitude depends on the flash intensities and the integrity of the photoreceptors. The b wave amplitude depends on the a wave and the integrity of signal transmission within the retina. It is therefore concluded that the b wave to a wave relationship depends only on retinal function. Thus a normal relationship should be obtained from a normal retina, irrespective of the stimulating apparatus, provided that full-field illumination is used. The general character of the b wave versus a wave curve is illustrated in Figs. 3 and 4 by the open and solid triangles. These data points were obtained in different laboratories using different techniques for photostimulation. The agreement of these normal data with the normal curve obtained here supports the usefulness of the b wave to a wave relationship as

a general feature of the retina that may facilitate data exchange between centres.

It has recently been suggested that the largest ERG response without preceding a wave should be recorded and the result reported as a percentage from the normal amplitude.<sup>25</sup> Such analysis also circumvents the difficulty of different laboratories using different stimuli to record the ERG. However, the determination of the a wave threshold depends on the experimenter's criteria and on the electrical characteristics of the recording system. Moreover, such a method demands the recording from each subject of many ERG responses evoked by stimuli of different intensities until the specific criterion is met. Furthermore, this method cannot differentiate between different pathological processes. Thus a subnormal b wave can be interpreted as caused by light absorption in the ocular media, by inferior functioning of the photoreceptors, or by abnormal signal transmission from the photoreceptors to the proximal retina.

The use of the b wave to a wave relationship as an additional method for the evaluation of the clinical ERG is done in the following manner. The a wave is treated as the independent variable, describing the activity of the photoreceptors. The b wave is the dependent variable that depends on both the response of the photoreceptors and the integrity of the retina. Thus in a case where ocular media cause light losses but the retina is normal the ERG amplitude will be subnormal, but the b wave versus a wave relationship will be normal. In a patient suffering only from photoreceptor dysfunction the a wave and therefore the b wave will be subnormal in amplitude, but the b wave dependency on the a wave (Figs. 3 and 4, open circles) will be normal. In all other pathological cases where signal transmission from the photoreceptors to the proximal retina is abnormal the measured b wave will be significantly different from the one expected according to the measured a wave (Figs. 3 and 4, solid circles).

The clinical examples presented here were chosen as representative ones that clearly describe the suggested method for assessment of the electroretinogram. In most cases the analysis was not simple, since most patients tested in our laboratory suffered from multiple factors affecting the functional integrity of their retinas.

It should be stressed that the ERG analysis described above is not proposed here to replace currently used methods but as a supplemental one to improve data exchange and analysis. Measurements of the photopic ERG under background illumination and/or with flickering stimuli are most important in the diagnosis of certain diseases, such as retinitis pigmentosa.<sup>28</sup> Dark-adapted ERG responses,



evoked by scotopically matched dim blue and red stimuli, best describe the isolated rod function and separate the rod mechanism from the cone mechanism.<sup>2,8</sup>

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