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Correlation of Dynamic Responses in the ON Bipolar Neuron and the *b*-wave of the Electroretinogram

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2-Amino-4-phosphonobutyrate (APB) is known to selectively suppress the light response of ON bipolar cells in the vertebrate retina, and reduce the *b*-wave of the electroretinogram (ERG) as a consequence. Using slow drug application, the progressive effect of APB was used to compare the relative response amplitudes of the *b*-wave and the ON bipolar cell. Simultaneous ERG recordings and ON bipolar intracellular recordings were performed in the amphibian retina. The results indicate that there is a strong positive correlation between these two waveforms. This supports the possibility that the *b*-wave of the ERG is the direct result of ON bipolar cell activity.

Electroretinogram *b*-Wave Bipolar cell Müller cell Retina

INTRODUCTION

Various studies have focused on the relationship between the *b*-wave of the electroretinogram (ERG) and the light response of ON bipolar neurons in the vertebrate retina. A working hypothesis that has evolved is that cationic fluxes across the bipolar cell membrane produce the *b*-wave, probably indirectly through polarization of the radially oriented Müller cells. One piece of evidence in support of this hypothesis is that selective neurotransmitter analogs block the *b*-wave if, and only if, they block ON bipolar light responses (Stockton & Slaughter, 1989). For example, APB blocks the *b*-wave and the ON bipolar light response. Kynurenic acid and *cis*-2,3-piperidine dicarboxylic acid suppress light responses of most retinal neurons, but do not suppress the *b*-wave nor the ON bipolar cells. Further support for this hypothesis comes from current source density analysis (Newman & Odette, 1984) and from measurements of the amplitude and latency of potassium fluxes at various retinal depths (Shimazaki, Karwoski & Proenza, 1984; Karwoski, Newman, Shimazaki & Proenza, 1985; Dick & Miller, 1985).

In this paper, we quantitatively compared the amplitudes of the *b*-wave and the ON bipolar light response. APB was used to selectively eliminate the ON bipolar response from the retinal network (Slaughter & Miller, 1981; Shiells, Falk & Naghshineh, 1981). The purpose was to determine how well *b*-wave amplitude correlates with ON bipolar cell voltage responses throughout the dynamic range of these two waveforms. The reasons for performing these experiments were two-fold. First, the *b*-wave has recently been used as a measure of ON bipolar cell responsivity in the formulation of drug dose–response curves, but a quantitative justification for this practice has not been established (Peterson, Thoreson, Johnson, Koerner & Miller, 1991; Thoreson & Miller, 1993; Tian & Slaughter, 1994). The second aim was to extend the comparisons between ON bipolar cells and the *b*-wave. We have reported previously that the waveform of the "isolated" *b*-wave is very similar to the light response of the ON bipolar neuron (Gurevich & Slaughter, 1993). The results presented here demonstrate that there is a strong positive correlation between the amplitudes of these two waveforms. The relationship is similar to that established between the *b*-wave and the Müller cell voltage response (Miller & Dowling, 1970). This provides another link between the ON bipolar cells and one component of the ERG, and raises the possibility that the *b*-wave may be a direct result of ON bipolar activity, as suggested by Xu and Karwoski (1994b).

METHODS

Concurrent recordings of light responses of ON bipolar cells and the ERG were obtained from the superfused retina-eyecup of the amphibian, *Ambystoma tigrinum*. The animal was decapitated, pithed, and the eye enucleated. The cornea, iris, lens, and vitreous were removed, exposing the retina in the remaining eyecup. A perfusion pipette was placed at the rim of the eyecup, allowing Ringers solution to bath the retina. The

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perfusion pipette was connected through a manifold and set of valves to a series of perfusion bottles. One bottle contained control Ringers solution, while another contained 100 μM 2-amino-4-phosphonobutyrate (APB) added to Ringers solution. The bottles were bubbled with oxygen. The solution applied was changed by opening and closing valves connecting the bottles to the perfusion lines. The flow rate over the retina was maintained at approx. 1 ml/min. Due to dead space in the lines, maximal drug effects were generally observed 1 min after switching solutions. The light stimuli consisted of full-field illumination from red or green light emitting diodes (Nygaard & Frumkes, 1982). The intensity of the light stimulus was about 1 μW/cm^2 at the eyecup level. The green light stimulated rods and cones, the red only cones, but the lights were not cone matched. The entire preparation was contained inside a light tight Faraday cage. A detailed description of the methodology has been published (Tian & Slaughter, 1994).

Amphibian Ringer's solution consists of 111 mM sodium chloride, 2.5 mM potassium chloride, 1.8 mM calcium chloride, 1.0 mM magnesium chloride, 10 mM dextrose, and 5 mM HEPES adjusted to pH 7.8 with NaOH. APB was obtained from Calbiochem, all other chemicals were purchased from Sigma Chemical.

The ERG was recorded using low resistance ($\sim$ 1 MΩ) electrodes. The location of the recording site was determined based on the micrometer setting of the microelectrode advancer and the polarity of the ERG components using conventions established previously (Dick & Miller, 1985; Karwoski *et al.*, 1985). Signals were amplified with a Grass P16 amplifier, viewed on an oscilloscope and stored on a Gould penwriter and Vetter videocassette recorder. Intracellular recordings from ON bipolar cells were obtained using high

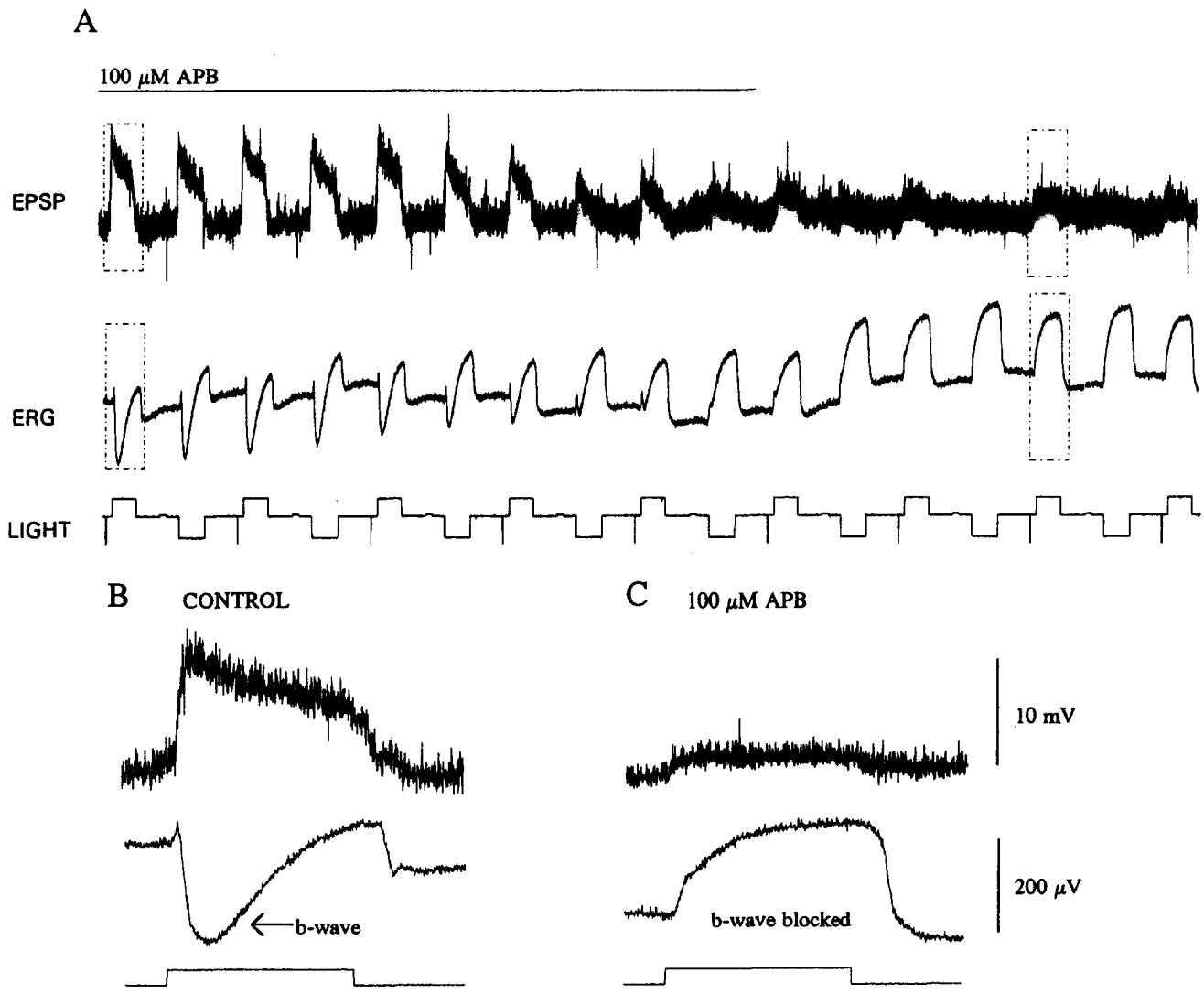

FIGURE 1. Progressive effect of APB on the ERG and the ON bipolar cell light response. (A) Slow application of APB reduced the light evoked voltage response of the ON bipolar cell (EPSP) and the *b*-wave of the ERG. The bar above the traces indicates the timing of APB application. There is a delayed and slow onset of the drug effect due to dead space in the perfusion system and fluid exchange in the eyecup. The square pulse light traces indicate the timing of red (up) and green (down) full field stimuli. (B) An expanded view of the first ON bipolar and ERG traces[boxed responses in (A)] before APB has reached the retina. (C) Expanded view of APB's effect on these two waveforms, taken from the next to last red stimulus in (A) (boxed responses).

resistance (150–300 MΩ) electrodes filled with 2 M potassium acetate and 5 mM HEPES buffered to pH 7.4. The signals were recorded with a Dagan 8100 amplifier and saved on chart paper and videocassette recorder. Offline, signals were digitized at 5 kHz using Axotape software, and then analyzed and graphed using Sigmaplot and Excel software.

The "isolated" b-wave of the ERG was calculated by recording the ERG at a time after APB application when the ON bipolar was fully suppressed and any evidence of a b-wave was eliminated. This ERG was considered to represent all components of the normal ERG except the b-wave. This waveform was digitally subtracted from ERGs obtained under control conditions or after a partial APB suppression had occurred. The result was a waveform that represented the isolated b-wave, as illustrated in Fig. 2. Under control conditions this isolated b-wave was large and resembled the waveform of the ON bipolar cell light response. After APB application, this waveform got progressively smaller, as described in the Results. The percent suppression at any time (pulse) was then calculated using the following formula:

$$\text{percent suppression of } b\text{-wave} = \frac{b_{\text{control}} - b_{\text{pulse}}}{b_{\text{control}} - b_{\text{APB}}} \times 100$$

where b_{control} is the b-wave peak amplitude under control conditions; b_{pulse} is the b-wave peak amplitude due to a partial APB effect; and b_{APB} is the b-wave peak amplitude due to a full APB effect.

RESULTS

Application of APB to the vertebrate retina is known to block the light response of ON bipolar cells and to suppress the b-wave of the ERG. In the superfused amphibian eyecup preparation drug exchange is gradual, so a progressive and simultaneous decline in these two waveforms can be observed. This is illustrated in Fig. 1(A). The upper trace shows the voltage response of an ON bipolar cell (EPSP) while the second trace is the concurrently recorded intra-retinal ERG. As APB slowly takes effect, the b-wave declines gradually and in tandem with the light response of the ON bipolar neuron. The negative going b-wave disappears, and is replaced with a monotonic, positive potential. Both red (upward square pulses) and green light stimuli (downward pulses) are used. The red stimulus is more effective, as demonstrated by the larger ON bipolar response to the red light. During the progressive effect of APB, the green response is suppressed to a slightly greater extent, and

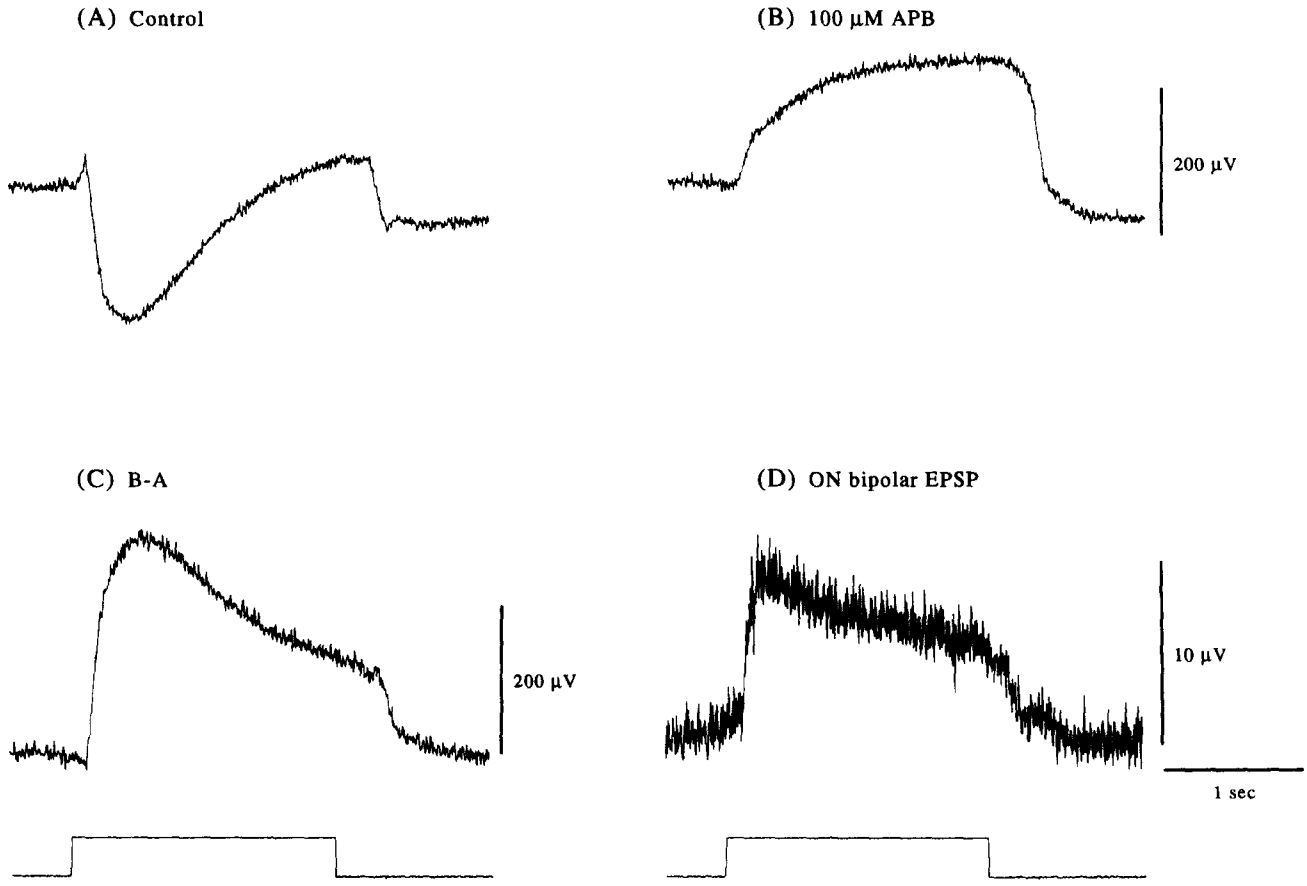

FIGURE 2. The "isolated" b-wave. (A) Under control conditions, the intra-retinally recorded ERG shows a small positive a-wave and a prominent, negative b-wave at light onset and a negative d-wave at light offset. (B) APB application eliminated the b-wave so that the ERG response was a monotonic positive potential. (C) Subtraction of the control ERG from the APB treated ERG results in an "isolated" b-wave that shows the full response of this component. (D) The light response, under control conditions, of a simultaneously recorded ON bipolar cell is shown for comparison.

consequently the positive field potential is larger for the green response. Figure 1(B, C) shows expanded views of the ON bipolar and ERG waveforms taken under control conditions, when APB was just applied and had not yet reached the retina, and during the APB effect [boxed waveforms in Fig. 1(A)]. There is a time lag between drug application and effect because of dead space in the perfusion system. The slow time–course of APB's action permits a correlation of the amplitudes of the ON bipolar and *b*-wave.

In order to quantitatively correlate the relationship between the *b*-wave and the ON bipolar response, it was necessary to have a measure of the full *b*-wave amplitude. The *b*-wave is superimposed on other components of the ERG field potential, so the amplitude of the negative deflection is not necessarily the magnitude of the *b*-wave component. Since the effect of APB on the ERG was not only to nullify the negative deflection of the *b*-wave, but to produce a positive going potential, the difference between these two opposite polarity responses was considered to be the full amplitude, "isolated" *b*-wave. We have shown previously that this difference results in a waveform that is very similar to the ON bipolar cell light response (Gurevich & Slaughter, 1993). This is illustrated in Fig. 2. Figure 2(A) shows an intraretinal ERG recording under control conditions. There is a brief, positive *a*-wave followed by a negative *b*-wave. Figure 2(B) shows the elimination of the *b*-wave after APB treatment. The difference between these two curves is shown in Fig. 2(C), which represents the isolated *b*-wave. An ON bipolar cell light response, recorded simultaneously, is shown in Fig. 2(D) for comparison. Throughout the remainder of this paper, the isolated *b*-wave, calculated as in Fig. 2(C), will be utilized.

A comparison between this isolated *b*-wave and a simultaneously recorded ON bipolar cell light response is illustrated in Fig. 3. Figure 3(A) shows ON bipolar cell EPSPs and the corresponding, calculated, isolated *b*-waves during the course of APB treatment. Each light pulse elicits a progressively smaller response due to the incrementally increasing APB effect. By the seventh light pulse (total elapsed time of approx. 70 sec), the calculated *b*-wave was totally suppressed, while a small

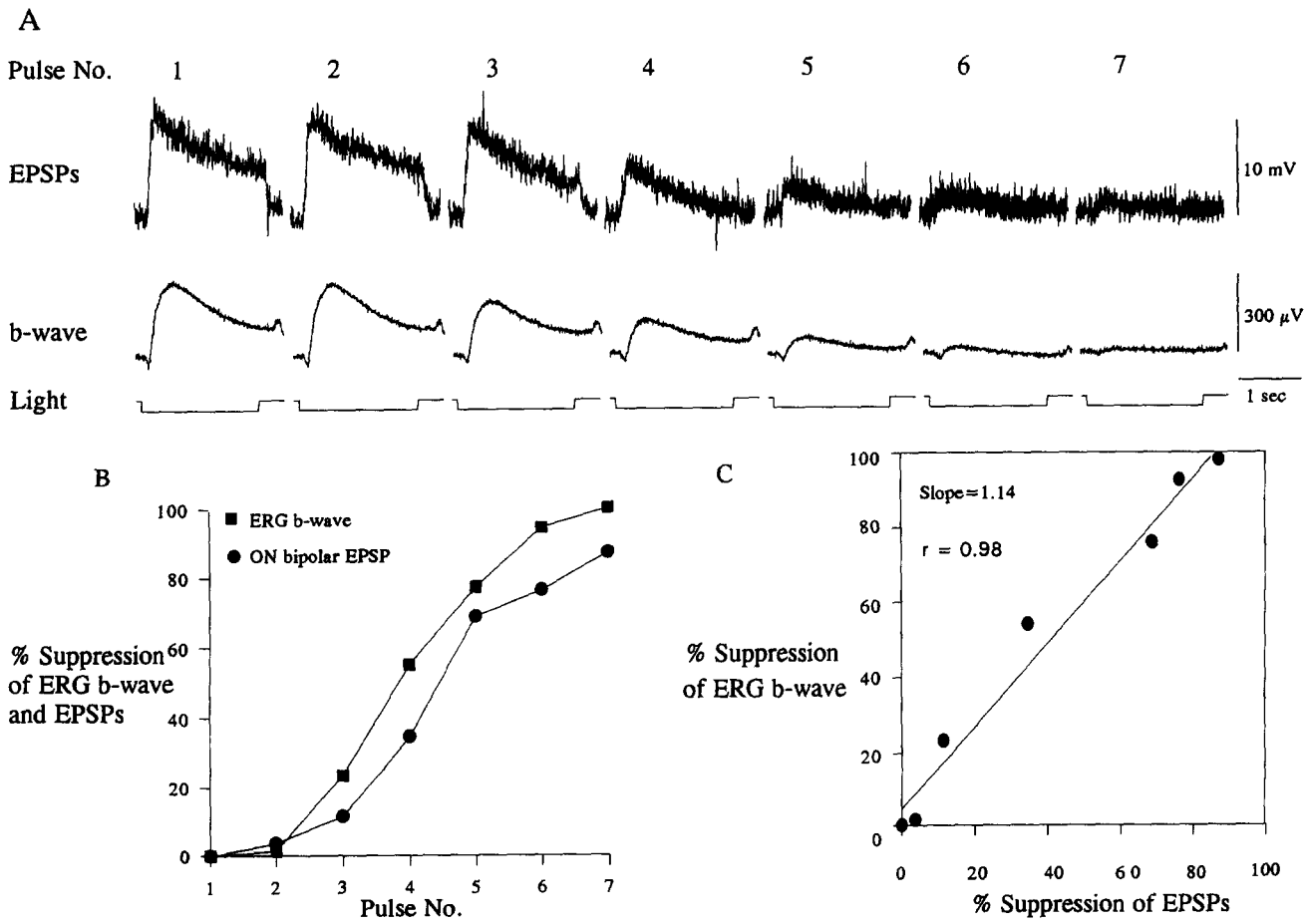

FIGURE 3. Correlation of response amplitudes of an ON bipolar cell and the isolated *b*-wave. (A) This shows the progressive effect of APB on the ON bipolar cell light response and the isolated *b*-wave (calculated as in Fig. 2). Seven light stimuli were evenly spaced while the effect of APB was recorded. (B) The percent suppression of the isolated *b*-wave and the ON bipolar cell response are plotted with respect to the series of light pulses. As the blocking action of APB increased, both the *b*-wave and the ON bipolar cell response decreased. (C) Using the data in (B), the percent suppression of the *b*-wave and ON bipolar cell were correlated by linear regression. The data were fitted by a line with a slope of 1.14 and had a correlation coefficient of 0.98.

ON bipolar light response (approx. 1 mV) remained. The normalized decrease in response of these two waveforms, expressed as percent suppression, is graphed in Fig. 3(B). In this particular cell the *b*-wave suppression was greater than that of the ON bipolar neuron. The cross-correlation between the isolated *b*-wave and the ON bipolar light response is shown in Fig. 3(C). A linear regression line, fitted to the data, has a slope of 1.14, reflecting the slightly greater suppression of the *b*-wave. The goodness of fit, expressed by a Person product moment correlation coefficient, was equal to 0.98 ($P < 0.01$).

Not all bipolar cells have the same dynamic response. Since a field potential is a weighted average of the responses of a group of neurons, we compared the *b*-wave to the responses of a number of ON bipolar cells. A comparison of 14 simultaneous recordings of ON bipolar cell light responses and isolated *b*-waves is shown in the scatter plot of Fig. 4. The protocol described in Fig. 3 was used for each set of simultaneous recordings. In every pair of recordings, the ON bipolar synaptic response and the calculated, isolated *b*-wave declined in tandem. Overall, the linear regression line, fitted to all the data, had a slope of 0.92 and a correlation coefficient of 0.94 ($P < 0.01$).

DISCUSSION

The results indicate that there is a strong correlation between the amplitude of the ON bipolar cell light response and the amplitude of the isolated *b*-wave of the ERG throughout the dynamic response range of the bipolar cell. This provides an additional link between one component of the ERG and one cell type in the retina. Previous reports have shown that the waveforms are similar, that the pharmacology of the *b*-wave matches that of the ON bipolar synaptic response, and that current source density analysis and potassium flux measurements match the anatomical profile of the ON bipolar cell. This compilation of circumstantial evidence suggests that the ON bipolar cell is largely, if not exclusively, responsible for the *b*-wave.

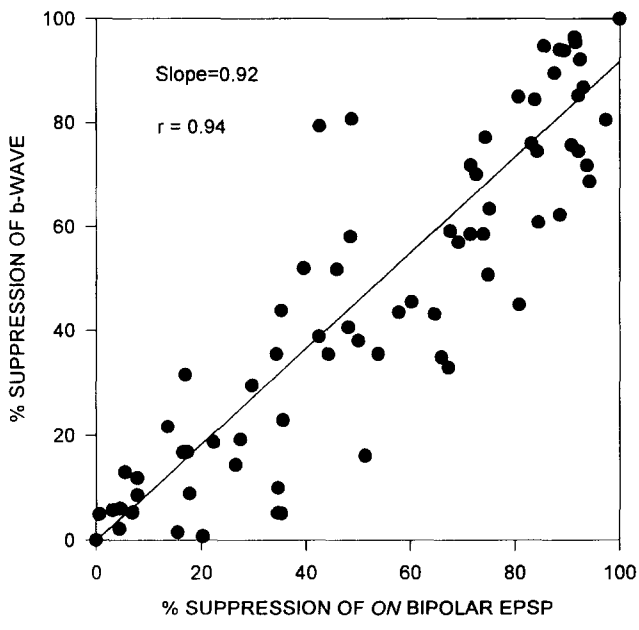

FIGURE 4. Scatter plot correlation of data from 14 paired *b*-wave and ON bipolar cell recordings. Simultaneous recordings of ERG and ON bipolar cell responses were obtained in 14 experiments, similar to that illustrated in Fig. 3. The isolated *b*-waves were calculated and plotted against ON bipolar cell responses during progressive suppression by APB. The data for all recording pairs were expressed as percent suppression and fitted by linear regression to a line which had a slope of 0.92 and a correlation coefficient of 0.94.

The current hypothesis is that the ON bipolar cell produces the *b*-wave indirectly, through depolarization of the Müller cell. This hypothesis is based on studies by Miller and Dowling (1970) showing that the *b*-wave and Müller cell ON response were similar in latency, waveform, and relative response amplitude throughout the intensity–response dynamic range. In contrast, it was observed that none of the retinal neurons correlated so well with the *b*-wave. The selective elimination of the ON bipolar light response by APB has permitted a re-evaluation of this comparison by analyzing the digitally subtracted, isolated *b*-wave. The results indicate that the waveform of the *b*-wave is very similar to the ON bipolar cell light response (Gurevich & Slaughter, 1993) and that there is a strong positive correlation between the *b*-wave amplitude and the ON bipolar cell response. This does not negate the Müller cell hypothesis, but it does not allow exclusion of the hypothesis that the ON bipolar cells themselves are the direct generators of the *b*-wave.

In addition to waveform and pharmacological comparisons, current source density analysis has generally supported the Müller cell hypothesis. Beginning with studies by Faber (1969), several workers have found that current sources and sinks associated with the *b*-wave extended from inner to outer limiting membranes. This implicated the Müller cell because no other cell traverses the full extent between these two membranes. However, as Xu and Karwoski (1994a) point out, there is very little consensus on the sources and sinks associated with the *b*-wave, except for the current sink in the outer plexiform layer. Analytical difficulties arise in separating the sources and sinks associated with the *b*-wave from those associated with other field potentials, such as the *M*-wave (Sieving & Steinberg, 1987) and the proximal negative response (Xu & Karwoski, 1994a). Xu and Karwoski (1994b) have combined current source density analysis with pharmacological studies and found that the *b*-wave sources and sinks are more closely correlated with ON bipolar cell activity than with Müller cell activity. For example, in the presence of 100 μM barium, Müller cell currents are blocked but the *b*-wave persists. In the presence of barium there is a sink in the outer plexiform layer and a source in the inner plexiform layer, which corresponds to the anatomy of the ON bipolar cell.

Sieving, Murayama and Naarendorp (1994) have found that *cis*-2,3,-piperidine dicarboxylic acid and kynurenic acid produce a sustained, positive corneal

ERG in *Macaca* monkeys. Since these agents block OFF bipolar and horizontal cells, as well as third-order neurons, their results indicate that the *b*-wave may result from a push–pull interaction between ON and OFF pathways. In amphibian retina, we have previously observed that these agents prolong the time-course of the *b*-wave (Stockton & Slaughter, 1989) although not as dramatically as demonstrated in monkey. The modest changes we observed could be explained by the loss of antagonistic surround to ON bipolar cells, thereby enhancing and prolonging their response (Slaughter & Miller, 1983). The effect of *cis*-2,3,-piperidine dicarboxylic acid in monkey was additive with the effect of APB, which also suggests input from the OFF pathway.

The results of our experiments also provide a justification for using the isolated *b*-wave as a measure of ON bipolar cell activity in pharmacological studies. It could be argued that the *b*-wave actually is a more representative measure because it represents an average of bipolar cell activity. It also has the technical advantage of greater stability compared to intracellular recordings.

In conclusion, this study shows that there is a strong correlation between the amplitudes of the ON bipolar neuron and the *b*-wave throughout the response range. Coupled with other evidence, this suggests that the *b*-wave results directly from the light response of ON bipolar neurons. This distinction is significant because the ERG, and particularly *b*-wave analysis, is an important diagnostic procedure in clinical settings.

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