The Rat Electroretinogram

II. *Bloch's law and the latency mechanism of the b-wave*

RICHARD A. CONE

From the Department of Physics, The University of Chicago

ABSTRACT   Electroretinograms were obtained from the all-rod eye of the rat with uniform illumination of the entire retina and stimulus flashes of less than 3 msec. duration. Bloch's law of temporal summation was verified for the b-wave latency by varying the time between two equal intensity flashes and observing that no change occurred in the latency when measured from the midpoint of the two flashes. The results of this and other experiments are described in terms of a simple but general model of the latency-determining mechanism. It is shown that this latency mechanism acts as if it depends on a linear additive process; and also that a hypothetical excitatory substance which triggers activity in the sources of the b-wave must accumulate rapidly in time after the flash, approximately as t^8. The rate at which this substance accumulates is accurately represented by the diffusion equation for more than 4 to 6 log units in the flash intensity. This suggests that the rate-determining step in the latency mechanism may be diffusion-limited.

INTRODUCTION

In the preceding paper (1) it was shown that the b-wave latency does not depend on the amplitude of the b-wave but depends instead primarily on the absolute stimulus intensity in terms of the number of quanta absorbed per flash. These characteristics suggest that the latency-determining mechanism is closely linked to the initial photochemical events occurring in the rods. If this is the case, one would expect the latency mechanism to depend upon additive processes linear in the stimulus intensity because a wide variety of experiments have shown that the earliest stages of vision obey linear additive laws such as Ricco's law of spatial summation and the Bunsen-Roscoe and Bloch's law of temporal summation (2). It is of some interest, then, to know whether or not the b-wave latency satisfies a linear additive law such as Bloch's law of temporal summation.

In the past, evidence on this point has been weak and inconclusive. For

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example, Creed and Granit (3) and Johnson and Bartlett (4) have reported that stimulus duration does not affect the latent period when the latent period is measured from the onset of the stimulus. This fact, if true for all stimulus durations, would contradict any form of temporal summation, but the evidence is not conclusive because very short durations were not investigated. Apparently, no careful investigation has been made to determine whether linear temporal summation occurs in the mechanism responsible for the latent period even though several studies (3–7) have indicated that temporal summation occurs in the amplitude-determining processes. In the following experiments an attempt was made to determine under what conditions and to what extent temporal summation occurs in the mechanism responsible for the latent period of the b-wave.

METHODS

The ERG recording conditions used here were the same as those described in the preceding paper (1). The data are again given in terms of an intensity, I, which is the number of quanta absorbed by the average rod per stimulus flash. This is abbreviated to read quanta/rod. In the two-flash experiments reported here, the eye was allowed to fully dark adapt after the presentation of each pair of flashes. One flash for each pair was delivered by each beam of the stimulator. In this way, the flashes could be controlled with complete independence. Because the a-wave obscures to some extent the initial appearance of the b-wave the latency of the b-wave was again measured to the leading edge of the second peak (b_2) as described in the preceding paper. The following experiments were designed to depend only on observations of changes in this latency because these changes can be measured with high precision and are not subject to the ambiguities involved in determining the absolute latency.

First Experiment: Bloch's Law

For a response to rigorously satisfy Bloch's law, not only must temporal summation occur, but also the response must depend only on the total stimulus energy arriving within some critical duration (2). In the following two-flash experiment, both flashes were set to deliver the same number of quanta to the rods, and then only the time between these equal intensity flashes was varied. In this way the sum of the effective energy of the two flashes was held strictly constant. Under these conditions, if no change occurs in the latency as the flash separation is varied, the latency must obey Bloch's law. Furthermore, with this stimulus pattern, the boundaries of the critical duration are clearly delineated because all of the stimulus energy arrives only at the beginning and the end of the interval.

In Fig. 1, the latency of the b-wave, L, measured from the midpoint of the two flashes, is shown as a function of the flash separation for four different intensities. To cancel a small error caused by the different durations of the flashes from each beam of the stimulator, the flashes were presented in alternate order and the average latency was determined. The results shown in Fig. 1 are typical of those obtained from four different rats. It can be seen that no change occurs in the latency measured from the mid-

point until the flash separation exceeds about one fourth of the latent period. This is the case for all intensities, including intensities so low that very few rods absorb quanta from both flashes. Therefore, the b-wave latency obeys Bloch's law for all intensities, and the critical duration is about one fourth of the two-flash latent period. When the
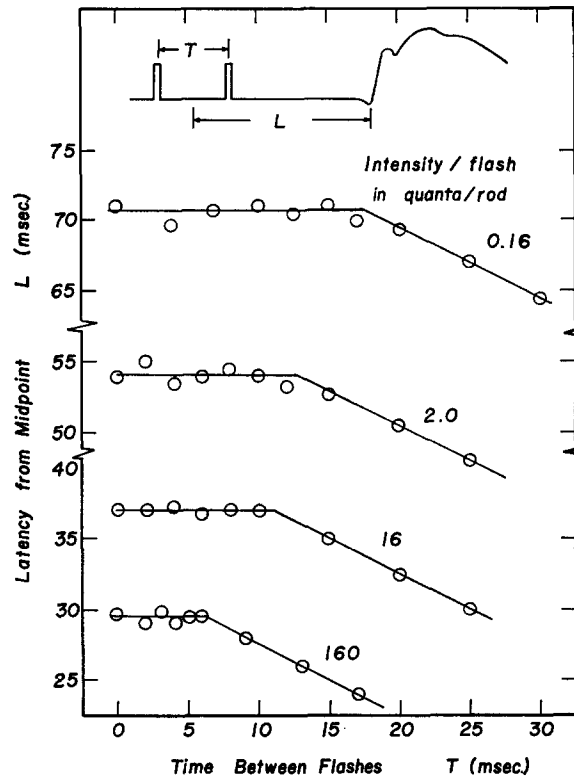

FIGURE 1.   Latency of the b-wave as a function of the time between two equal intensity flashes. The latency from the midpoint, L, and the time between flashes, T, were measured as shown in the diagram. Data for four different flash intensities are shown. The intensity per single flash is given in quanta absorbed per average rod. The straight lines were drawn by eye and represent the asymptotes of the experimental curves. The durations of the flashes were 1.5 and 3 msec. (For flash separations greater than the critical duration, the latency from the midpoint is obtained by subtracting one-half of the flash separation from the latency for the first flash. Thus the slope of each line in this region is $-\frac{1}{2}$.)

flash separation exceeds the well defined critical duration, the latency is not affected by the presence or absence of the second flash. Therefore, in this region the latency is entirely determined by the first flash even though the second flash is delivered long before the b-wave appears.

Discussion of First Experiment

These results indicate that the sum of the excitations produced by the two flashes must always have the same effect on the mechanism which determines

the latency for any flash separation less than the critical duration. This suggests that the excitation to which the latency mechanism responds is linear in intensity because if the flash separation is zero there is effectively only one flash with doubled intensity. These results also imply that the latency mechanism acts as if the excitations from the two flashes add linearly. These implications have been incorporated in the following general model of a latency mechanism.

Light absorbed by the visual pigment almost certainly leads to the production or activation or release of some excitatory substance (ions, enzymes, hormones, etc.). It is reasonable to suppose that the amount or concentration of this substance will increase rapidly in time following a stimulus flash, or possibly that it will suddenly be released and then diffuse away to some excitable surface or membrane where, again, its concentration will increase rapidly in time. Whatever happens, we might suppose that the state or concentration of this substance *at its site of action* can be described by some time-dependent function, f, which in this discussion will be called the excitor function. Furthermore, because the latency of the b-wave is due to a true latent period during which the b-wave sources are not active (1), it seems reasonable to assume that the concentration of this substance must increase beyond some threshold value, f_θ, before the next step in the production of the b-wave is initiated. Since this kind of formal analysis can cover a variety of different processes, it need only be supposed that such an excitor function describes one main rate-determining process which is responsible for a major fraction of the latent period.

If, as suggested in the above discussion, the excitation is linear in the intensity, the excitor function, f, must have the form $f(I, t) = Ig(t)$ where I is the intensity of the flash in quanta/rod and $g(t)$ is the time dependence of the excitor function. Furthermore, if the excitation does in fact add linearly as suggested by the above results, then the total excitor function will be the linear sum of the excitor functions for each flash, $f_{total} = f_1 + f_2$.

In addition to these inferences the results of this experiment suggest certain restrictions on the form of $g(t)$, the time dependence of the excitor function. For example, as soon as the flash separation exceeds the critical duration, the second flash has little or no effect on the latency even though this flash is presented long before the b-wave appears. Thus there is a minimum latent period, L_M, which must elapse before the excitation produced by the second flash can detectably alter the latency of the b-wave produced by the first flash. In Fig. 1, this minimum latency is about four-fifths of the dark-adapted latency for a single flash. Therefore, if this model is appropriate, $g(t)$ must be negligibly small during the first four-fifths of the dark-adapted latent period compared to its value at the end of this period. Moreover, in this model $g(t)$ for one flash should not be altered by presenting another flash because the

excitor functions are assumed to add linearly. Therefore, this model predicts that for a given flash intensity (a) the minimum latency, L_M, should be independent of the intensity or arrival time of another flash, and (b) L_M should always be about four-fifths of the dark-adapted latency.

Second Experiment: Minimum Latency

This experiment is depicted in terms of the model in the upper right hand corner of Fig. 2. If only the flash labeled a is presented, the resulting hypothesized excitor function would be as shown by the solid black line labeled f_a. If another flash, labeled b,
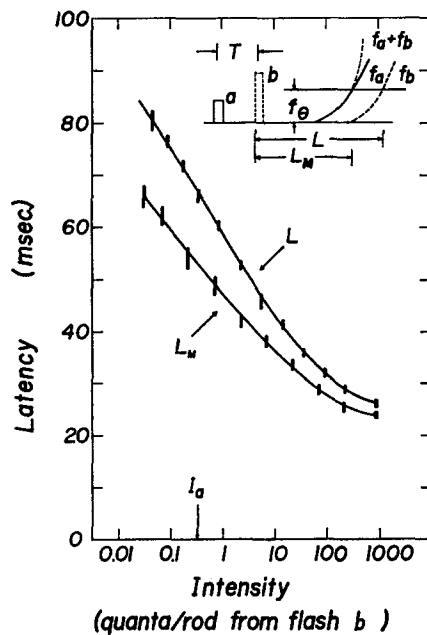

FIGURE 2. Normal and minimum observable latencies of the b-wave for flash b. The time, T, between flashes was adjusted so that adding flash b produced a just detectable decrease in the latency for flash a. The normal latency, L, and the minimum observable latency, L_M, were measured as shown in the diagram, which also depicts the interpretation of this experiment in terms of the excitor functions. The flash durations were 3 msec. for flash a, 1.5 msec. for flash b. The length of each vertical bar represents the estimated measuring uncertainty.

is also presented, it would initiate its own excitor function, f_b, which would add to f_a. (See dashed lines in Fig. 2.) In this case, the presence of f_a should effectively reduce the value which f_b must attain before the excitor function threshold, f_θ, is reached. In particular, if flash a is adjusted in time relative to flash b until the response for both flashes arises just detectably earlier than the response for flash a alone, the excitor functions will be as shown in the diagram. Presumably, this is the condition in which the minimum time must elapse after flash b in order to experimentally observe an effect by flash b on the latency of flash a.

In this experiment, the intensity of flash a was held constant while varying the intensity of flash b over a wide range. The oscilloscope was triggered by flash a, and the oscilloscope trace was expanded so that changes of less than 1 msec. in the latency could be easily detected. For each intensity, flash b was adjusted in time relative to flash a until the latency of the b-wave for both flashes was just detectably shorter than the latency for flash a alone. When this condition was attained, the latency of the b-

wave for both flashes was measured from flash b. This measurement indicated the minimum time which had to elapse for flash b to alter the latency of the response for flash a. This minimum observable latency, L_M, and the normal dark-adapted latency, L, for flash b are plotted in Fig. 2 as functions of the intensity of flash b. It can be seen that across the entire intensity range the minimum latency is about four fifths the normal latency, the value predicted by the above model from the results of the first experiment in which both flashes were of equal intensity.

The intensity of flash a in Fig. 2 was about 0.3 quanta/rod. At this intensity less than 1 out of 3 rods absorbed quanta during flash a. In other experiments the intensity of flash a was increased by as much as $3\frac{1}{2}$ log units and in each case the minimum latency for flash b was found to be about four fifths of the normal dark-adapted latency. Therefore, the minimum observable latency does not depend on the intensity of the adapting flash a.

Discussion of Second Experiment

The results of the second experiment are thus consistent with the linear additive model discussed above. In addition, these results bring out another important characteristic of the latency-determining mechanism. In the first experiment, the critical duration for temporal summation was found to be about one-fourth of the two-flash latency, and in no case was the duration longer than about 15 to 20 msec. Therefore, these first results do not rule out the possibility that the critical duration might be limited by a breakdown in summation, $i.e.$ excitation from the first flash might no longer add to excitation from the second flash if the second flash arrived after some critical duration. However, in Fig. 2, the flash separation, T, was more than 43 msec. when the intensity of flash b was about 900 quanta/rod. Thus, summation must occur over times longer than 43 msec., which is longer than the entire latent period of moderate to high intensity flashes. This fact argues against any model in which the critical duration is limited by a breakdown in summation, but it is consistent with the present model in which the critical duration is determined by the time dependence of the excitor function, $g(t)$.

Besides the above results, other characteristics of the latency-determining mechanism can also be described consistently with the present model. For example, a comparison of Fig. 2 with Fig. 6 in the preceding paper (1) suggests that the reduction which occurs in the latency during both light adaptation and rapid dark adaptation can be simply and adequately described by a reduction in the excitor function threshold, f_θ. This threshold would remain unchanged during photochemical dark adaptation.

Time Dependence of the Excitor Function

If the latent period is in fact determined by the time it takes for the concentration of some substance to reach a threshold value, and if the initial amount of

this substance is proportional to the intensity of the flash as indicated in the first experiment, then the rate at which this substance builds up at its site of action should be revealed by the dependence of the latency on the intensity. That is, if the concentration of this substance is described by an excitor function $f = I g(t)$, then for any given intensity I, and its corresponding latency L, the excitor function must equal the threshold value f_θ; i.e., $f = I g(L) = f_\theta$. If, for simplicity, $g(L)$ is approximated by t^n, a latency *versus* intensity curve is generated which fairly closely resembles the latency curve shown in Fig. 3 of the preceding paper if $n = 7 \pm 2$. Thus the latency curve also indicates that $g(t)$ is a rapidly increasing function of time as implied by the above two experiments. In fact, from the precision with which the minimum detectable latency could be measured, it is estimated that the excitor function must increase by about a factor of 10 during the last one-fifth of the latent period. This provides an independent estimate for n. For example, if $g(t)$ is to increase by a factor of 10 during the required interval, it must increase about as t^8. Thus both these methods indicate that the concentration of the excitor substance must increase approximately as the seventh or eighth power of the time after the flash.

Diffusion Model

Up to this point, the discussion of the latency mechanism has been kept as general as possible so that the terms employed would not bias the description. However, the experiments considered above serve to specify the characteristics of the latency mechanism with sufficient completeness and precision to warrant consideration of specific physicochemical processes which may be involved in this mechanism. One such process has been suggested by Wulff *et al.* (8, 9), in an attempt to explain the characteristics of the latent period of the electrical response in the grasshopper eye. They found that the characteristics could be accounted for by an autocatalytic reaction which was initiated by a dose of enzyme proportional to the intensity of the flash, and which triggered the production of the electrical response as soon as the concentration exceeded some threshold value. This process yields a latent period which decreases linearly with log I. With the rat, the *reciprocal* of the latency is more nearly proportional to log I. This relationship is also appropriate for some of the data reported by Wulff *et al.* (9) and by Fry *et al.* (10) for the grasshopper eye, and it is the relationship found in the eye of the frog as well (11, 12). Such a relationship suggests that the rate-determining step of the latency mechanism is diffusion-limited. This can be seen as follows.

If it is assumed that an amount of excitor substance proportional to the intensity is released relatively suddenly by a light flash (*i.e.*, within a few milliseconds) and that this excitor substance must then diffuse across a fixed dis-

tance to some site of action before initiating the next step in b-wave production, then the excitor function would have the form of the diffusion equation (13):
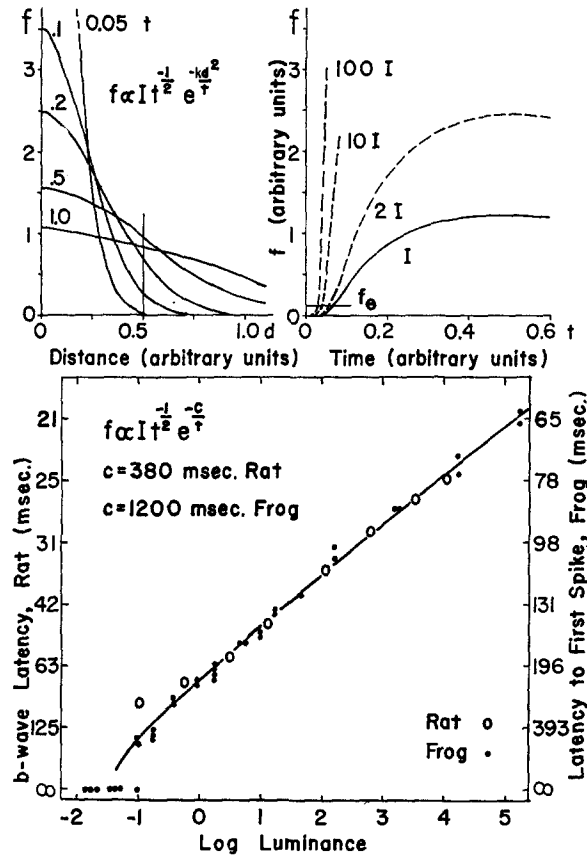
$$f \propto It^{-1/2}e^{-kd^2/t}$$

FIGURE 3. Characteristics of the diffusion equation (top) and comparison of frog and rat latencies with the latency curve predicted by the diffusion equation (bottom). The latencies are plotted on reciprocal scales. Data for the latency to the first spike in frog ganglion cells are replotted from Chapman (*J. Opt. Soc. America*, 1961, **51**, 1102. Fig. 2).

where d is the distance to the site of action and k is a constant involving the rate of diffusion. Certain characteristics of this equation are illustrated in the two diagrams at the top of Fig. 3. On the left, the concentration of a diffusing substance is shown as a function of the distance from the plane at which it was suddenly released. The curves are drawn for various times after the time of release to show the way in which the distribution of the substance changes with time. If the concentration at the distance $d = 0.5$ is considered, it is seen

that the concentration is essentially zero until the time increases beyond $t = 0.05$, at which point the concentration rises rapidly and then reaches a gradual maximum value at about $t = 0.5$. These changes in concentration at this distance are shown by the solid curve in the diagram on the right in Fig. 3. The concentrations for increased flash intensities are shown by the dashed curves. It can be seen that as the intensity is increased, the time, or latency, at which the concentration reaches a constant threshold value, f_θ, decreases. This intensity dependence of the latency is given by the equation:

$$It^{-1/2}e^{-kd^2/t} = f_\theta \quad \text{when} \quad t = L.$$

Since both k and d would probably be constant, kd^2 may be set equal to a constant C, $kd^2 = C$. Also, f_θ is treated as a constant in the above description. Therefore, after conversion to the logarithm to the base 10, and rearrangement, it can be seen that the reciprocal of the latency is linearly related to log I except for a slowly varying term in log L:

$$\frac{C}{2.3L} + \frac{1}{2}\log L = \log\frac{I}{f_\theta}$$

Such a linear relationship was found by Hartline (11) and more recently by Chapman (12) between the logarithm of the stimulus luminance and the reciprocal of the latency to the first spike occurring in the ganglion cells of the frog. For comparison with the above equation, Chapman's data have been replotted in the lower graph of Fig. 3 (solid dots). The solid line in the graph is generated by the diffusion equation when $C = 1200$ msec. and the reciprocal latency scale on the right is used. It can be seen that the curve predicted by the diffusion equation fits these data very well for over 6 log units in intensity. However, these data were obtained with a 0.1 sec. stimulus duration; the intensity scale is therefore shown in terms of the luminance and is not quite equivalent to the scale used here for the rat.

The latency of the b-wave of the rat is also plotted in Fig. 3 (left hand reciprocal scale). The open circles represent the average latency from four dark-adapted rats whose latency curves were quite similar. The data for the rats were obtained with a 1.5 msec. flash, and therefore these data are again given in terms of the absolute intensity of quanta/rod. (Log luminance = 0 corresponds to $I = 1$ quanta/rod for the rat data.) The curve predicted by the diffusion equation when $C = 380$ msec. (using the scale on the left) fits the latency of the rat b-wave for 4 log units in intensity. It can be seen from this figure that the diffusion equation predicts latency curves which fit the data for both rats and frogs well enough over 4 to 6 log units in intensity to suggest that the time dependence of the latency mechanism may well be determined by a diffusion process.

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