*VARIABILITY OF INTERSPIKE INTERVALS IN OPTIC NERVE FIBERS OF LIMULUS: EFFECT OF LIGHT AND DARK ADAPTATION**

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The intervals between the impulses discharged by afferent neurons under steady conditions usually vary considerably. This variability is of interest for several reasons: (1) It must depend in some way on the underlying receptor and neural mechanisms that generate and propagate the impulses. An analysis of the factors that influence variability can therefore be expected to yield some indication of the nature of those mechanisms. (2) The information transmitted to the central nervous system by afferent neurons is coded in terms of the intervals between impulses, that is, in terms of the temporal pattern of the discharge rather than in terms of the shapes or amplitudes of the individual impulses. Any intrinsic variability in the intervals between impulses discharged by the neuron must therefore limit its capacity to carry information about extrinsic events. (3) Although the intrinsic variability may be "noise" as far as external events are concerned, there is nevertheless the possibility that it may actually carry useful information to the central nervous system about the state of the receptor or neuron and the influences that contribute to the variability of the discharge.

The variation of the intervals between impulses has been investigated and described for many different types of neurons,[1-3] but the causes of the variability are largely unknown. One supposed cause is "biological noise"—minute haphazard fluctuations in membrane potential such as those first observed by Fatt and Katz[4] at motor nerve endings in muscle. Recently, for example, it was shown that such random fluctuations, observed in spinal motoneurons of the cat, are adequate to account for the variability of the intervals between impulses discharged by these neurons.[5]

It is usually difficult to control the random fluctuations or "noise" in the membrane potential of a discharging neuron. But such fluctuations are unlikely to be altogether haphazard, and with adequate knowledge of factors that influence their frequency, amplitude, and other characteristics, they may sometimes be brought under control. This is the case with the irregular fluctuations in membrane potential observed by Yeandle[6] in eccentric cell bodies of ommatidia in the compound lateral eye of *Limulus*. At low intensities of illumination, the fluctuations are maximal and occur infrequently. The higher the intensity of illumination, the greater the frequency of occurrence of the fluctuations and, in the steady state, the smaller their amplitudes. Furthermore, the amplitudes of the fluctuations vary markedly with the state of light and dark adaptation of the ommatidium.[7, 8] After some time in the dark, the fluctuations elicited by low-level illumination of an ommatidium are large and distinct, but following a long exposure of the ommatidium to strong light, the amplitudes of the fluctuations become so small that they are barely discernible (Fig. 1).

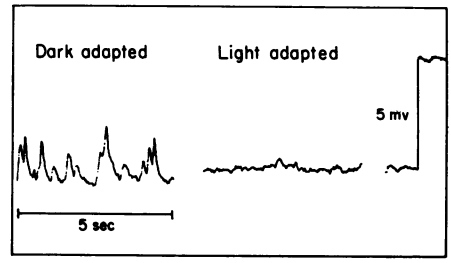

FIG. 1.—Intracellular records of generator potential in an eccentric cell in response to a steady low level of illumination, below the threshold of impulse generation. The record on the left was obtained after the ommatidium had been in the dark for about half an hour. The record on the right was obtained after the ommatidium had been exposed to strong illumination for about 60 sec.

It was suggested by Rushton[9] that these fluctuations in membrane potential may sum to yield, or at least contribute to, the larger so-called generator potential upon which excitation of the eccentric cell and generation of impulses in its axon depends. A recent experimental analysis and theoretical treatment of the relation between the fluctuations and the generator potential by Dodge, Knight, and Toyoda[10] supports this view. Since the amplitudes of these fluctuations in membrane potential and the resulting irregularities in the generator potential vary with intensity and with the state of light and dark adaptation, the variations in interspike interval should be similarly affected. The following experiments were undertaken to make some preliminary tests of this idea.

A lateral eye of *Limulus* was excised and mounted in a moist chamber. Impulses generated by the eccentric cell of an ommatidium were recorded either (first set of experiments) by dissecting the optic nerve and placing a bundle containing a single active fiber on cotton wick silver/silver chloride electrodes or (second set of experiments) by inserting a micropipette electrode directly into the eccentric cell body. In the first set of experiments (Figs. 2 and 3), the ommatidium was stimulated by a small spot of steady light confined to its facet. In the second set of experiments (Fig. 4), the ommatidium was stimulated either by steady light on its facet or by steady electric current passed through the micropipette electrode.

For the "light-adapted" condition in the various experiments, the ommatidium was exposed repeatedly to 20-second periods of fixed high-intensity "adapting" illumination spaced four minutes apart. The intensity of this illumination was such that after a few repetitions a steady state was reached in which a discharge

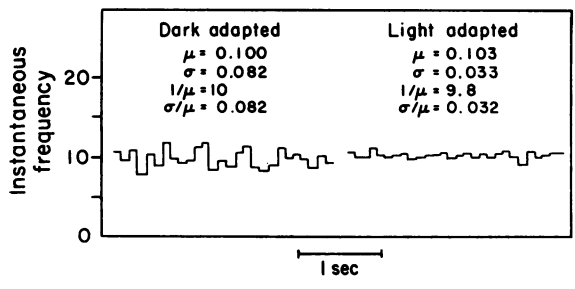

FIG. 2.—Instantaneous frequency (reciprocals of intervals) of impulses discharged by an eccentric cell in response to steady illumination when the ommatidium was dark-adapted (*left*) and light-adapted (*right*). The sample records extend from the 15th to the 20th sec of 20-sec periods of illumination. The numerical data (*above*) are based on a set of records that included these samples but extended from the 10th to the 20th sec of illumination. Intensities of illumination were chosen (low on the dark-adapted ommatidium, high on the light-adapted) that yielded nearly equal frequencies of discharge.

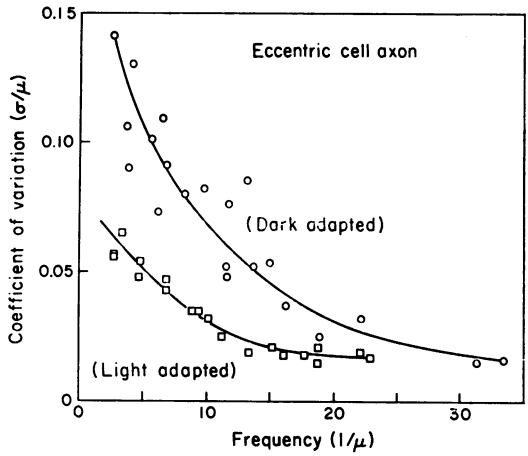

FIG. 3.—Variability of intervals between impulses recorded from the axon of an eccentric cell of an ommatidium when dark-adapted (*upper curve*) and when light-adapted (*lower curve*). The two curves were fitted to the points by eye. (The sample records and numerical data illustrated in Fig. 2 are from this set of observations.)

of about 35 impulses per second was elicited. Midway between these repeated "adapting" exposures the ommatidium was tested by exposure for 20 seconds to steady illumination at various intensities or, in parts of the second set of experiments, by stimulation with steady current. For the "dark-adapted" condition, the procedure was the same as above, except that the repeated exposures to the fixed high-intensity "adapting" illumination were omitted. The differences in these two schedules of illumination were sufficient to produce large differences in the state of adaptation of the eye. Complete dark adaptation was not achieved, however, since the testing exposures themselves, particularly at the higher intensities, inevitably produced some light adaptation.

The times of occurrence of the impulses were recorded on-line by a small digital computer,[11] and the mean length (μ) of the interspike intervals, the mean rate or frequency ($1/\mu$), the standard deviation (σ), and the coefficient of variation (σ/μ) were computed for the final ten seconds of each period. (The first ten seconds were omitted from the computation to avoid the transient changes in frequency that accompany the onset of illumination. Since a slight downward "drift" in the frequency of the discharge always remained, even in these last ten seconds of the 20-second exposure, a smoothed ramp was fitted to the data, and the deviations about it were used to compute σ.) Samples of typical data are illustrated in Figure 2.

In the steady state, the frequency of discharge of impulses increases with increasing intensity of illumination; that is, the mean interval (μ) decreases. But the standard deviation (σ) of the distribution of the intervals about the mean interval decreases more rapidly with increasing frequency than does the mean interval itself. Therefore, the coefficient of variation (σ/μ) decreases with increasing frequency of discharge of impulses. The exact form of the function is markedly affected, however, by the state of light and dark adaptation: the coefficient of variation is greater, for any given frequency, when the ommatidium is dark-adapted (Fig. 3).

These effects cannot be attributed to any long-term changes in the state of the excised eye as a whole. Alternating between light- and dark-adapted conditions

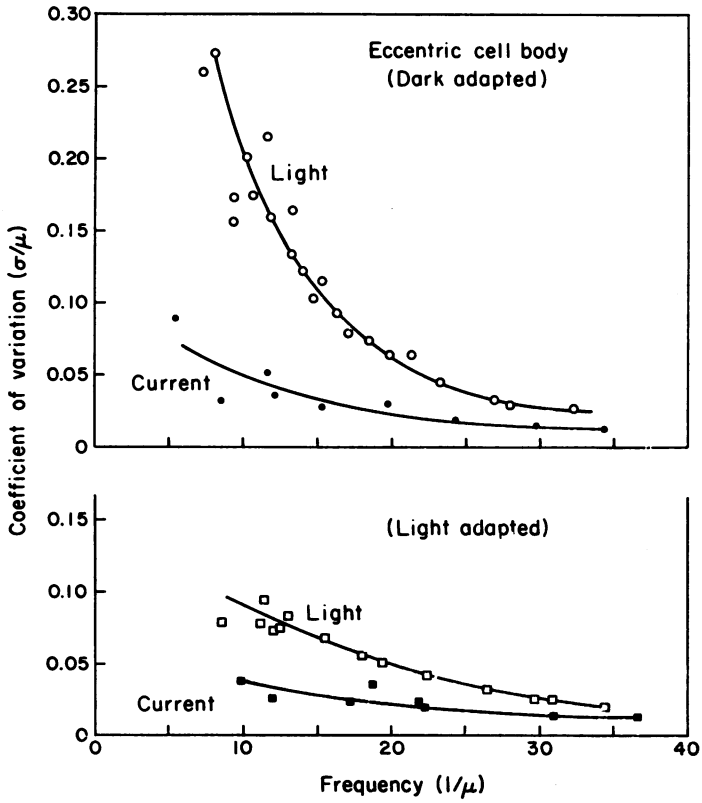

FIG. 4.—Variability of intervals between impulses recorded with a micropipette electrode from the eccentric cell body of an ommatidium when dark-adapted (*upper graph*) and when light-adapted (*lower graph*). The upper curve in each graph represents the normal discharge of impulses in response to steady lights of various intensities. The lower curve in each graph represents the discharge of impulses in response to depolarizing current of various strengths passed through the recording electrode. The curves were fitted to the points by eye.

during the experiment ruled this out. Furthermore, we have observed the same differences in variability while recording simultaneously from two ommatidia in the same eye—one light-adapted and the other dark-adapted. The dependence of the variability of interspike intervals on light and dark adaptation is the principal finding of the research reported here. This result is to be expected if the fluctuations in membrane potential, which are associated with the photoexcitatory process and which are markedly affected by light and dark adaptation, are indeed the underlying cause of the variability.

Further evidence that these fluctuations in membrane potential underlie the variability of interspike intervals was obtained in the second set of experiments. In these experiments, a micropipette electrode was inserted directly into the eccentric cell body and used both to stimulate the cell and to record its activity. This technique is based on the finding of some years ago that the ultimate action of light on an ommatidium is to produce a depolarization of the eccentric cell

(the so-called generator potential), which in turn results in the discharge of impulses in the eccentric cell axon.[12] The depolarization results from a flow of current released by a photically induced increase in the conductance of the cell membrane.[13] The discharge may therefore be generated artificially by the passage of a steady current from an external source through the micropipette electrode in the proper direction to depolarize the eccentric cell.[14] Since the impulses originate in the axon near the cell body, they may be recorded by this same electrode.

The results obtained when the ommatidium was stimulated in the dark by a current passed through the recording electrode were very striking. In all cases and under all conditions, the variation of the interspike intervals was much reduced (lower curve in each of the two graphs in Fig. 4). Indeed, only very slight changes in the coefficient accompanied very large changes in the frequency of discharge. The results obtained lend strong support to the view that the fluctuations in membrane potential resulting from the photoexcitatory process are the principal cause of the variability of the interspike intervals since the variability is much reduced, as expected, when the photoexcitatory mechanism is bypassed in this way. Furthermore, as one would also expect, there is very little difference between the variability in the light-adapted and in the dark-adapted conditions. What little difference there is may be attributed to either the "spontaneous" appearance of some of the minute fluctuations or to their elicitation by very low-intensity light leakage into the box containing the preparation. In either event, whatever fluctuations may occur would be expected to be larger in the dark-adapted than in the light-adapted eye.

The reduced variability is not a consequence of the penetration of the eccentric cell by the micropipette. Indeed, when the ommatidium is stimulated normally by light, rather than by a current passed through the electrode, the results (upper curve in each of the two graphs shown in Fig. 4) are essentially the same as those obtained when the discharge is recorded from a point on the axon several millimeters distant from the cell body (Fig. 3). That is, for any given frequency, the coefficient of variation is greater when the ommatidium is dark-adapted than when it is light-adapted. If there is any difference at all between the variability observed with the recording electrode inserted into the eccentric cell body and that observed with extracellular electrodes in an axon some distance from the cell body, it is usually an increase rather than a decrease, presumably because of injuries caused by the penetration of the ommatidium.

The results obtained when the ommatidium is stimulated with current rather than light must be interpreted with a degree of caution, however. The paths followed by the flow of current passed through the recording electrode and cell membrane were undoubtedly quite different from the paths followed when the eye was stimulated normally by light.

The marked influence of light and dark adaptation on the variability of interspike intervals, which we have demonstrated in the experiments above, is perhaps related to similar changes, with light and dark adaptation, in the "sharpness" of visual thresholds. For example, Hartline, Milne, and Wagman[15] observed that the range of intensities required to elicit a fixed number of impulses, at threshold,

in an optic nerve fiber of *Limulus* is quite large in the dark-adapted eye, but is greatly reduced by light adaptation. Similar effects in human vision were subsequently reported by Mueller and Wilcox.[16]

In summary, the results of these preliminary investigations are in accord with the view that the generator potential in the *Limulus* receptor is the sum of discrete photically induced fluctuations in membrane potential and that these fluctuations are the principal source of the variations in interspike interval. Whether these variations are mere "noise" or are actually carriers of useful information to the central nervous system remains to be determined.

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