

Thermal Sensitivity of Lateral Inhibition in *Limulus* Eye

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ABSTRACT The effectiveness of lateral inhibition, measured as spike response decrement in a test ommatidium, produced by activity in a group of neighboring ommatidia, decreases as temperature decreases (Q_{10} of 2.6). The corresponding sensory transducer-spike encoding processes have a weaker temperature dependence (Q_{10} of 1.6). Relative synaptic delay, the time difference between the latency of inhibition onset and the latency of test receptor excitation, has a strong temperature dependence (Q_{10} of 5), while receptor potential onset latency (Q_{10} of 1.4) and optic nerve spike conduction velocity (Q_{10} of 1.7), two factors inherent in relative synaptic delay, are less temperature sensitive. Oscillations of optic nerve spike response ("bursting") may be produced by thermal adjustment of temperature-sensitive parameters of the lateral inhibitory network in the retina. Burst interval has a strong temperature dependence (Q_{10} of 2.4) and broad interspike interval distribution compared to the thermal sensitivity (Q_{10} of 1.4) and narrow spike interval spectrum of the response of a single unit within the bursting group.

INTRODUCTION

Many investigators have long-held interests in *Limulus* lateral eye as an extensively studied sensory receptor and a neural network exhibiting lateral inhibition (Wolbarsht and Yeandle, 1967). The lateral inhibitory property of the eye, in which spike activity arising from stimulation of a test ommatidium is reduced or suppressed by stimulation of its neighboring ommatidia, has been well defined functionally (Hartline and Ratliff, 1972). Its physiological and morphological bases have received somewhat less study (Purple and Dodge, 1965; Knight et al., 1970; Gur et al., 1972). Initial studies on the pharmacology and neurochemistry of inhibition in the lateral eye (Adolph and Tuan, 1972), in addition to the results of the earlier investigations mentioned above, suggest that chemical synaptic transmission is the basis of the lateral interaction. Temperature is a physical factor which strongly affects chemical synaptic transmission (Katz and Miledi, 1965) and a study of the thermal sensitivities of putative synaptic neural processes may provide evidence of the role of chemical synaptic transmission in the neural process. This report

describes the effects of temperature on light-evoked lateral inhibition in *Limulus* eye, including the effects on the transducer-spike encoding process and on synaptic delay. Thermal "tuning" of the frequency of spike bursts in the neural response of groups of interacting neurons in the lateral eye is also reported in this paper.

METHODS

The electrophysiological and optical techniques used consist essentially of extracellular recording of optic nerve spikes with suction-electrodes or intracellular recording of ommatidial responses with glass micropipette electrodes (3 M KCl filled) and stimulation by light spots applied directly to the cornea by appropriately sized fiber optic bundles. Antidromic stimulation of the optic nerve is used in the conduction velocity experiments. The eye is mounted in a 15 ml perfusion chamber with Peltier module (Cambridge Thermionic Corp., Cambridge, Mass.) cooling of the artificial seawater bathing the eye. Temperature of the bath can be controlled to within 0.1°C of set temperature by a feedback system incorporating a thermistor sensor and proportional control of module current. Another thermistor records the bath temperature as an indication of eye temperature. The bath may be heated by reversing current direction through the module. The details of most of these techniques are given in an earlier paper (Adolph and Tuan, 1972).

RESULTS

The inhibition produced by the illumination of a group of ommatidia on the spike firing response of a neighboring test ommatidium is shown in Fig. 1. The test response is isolated from other units in the same small nerve fiber bundle through restricted test stimulus area, the result of applying a 70 μm diameter fiber optic bundle directly to the cornea. The test receptor is stimulated for 4 s in isolation, 3 s inhibited by the neighboring group, and a final 3 s in isolation. The responses at three temperatures (22°C, 16°C, and 9°C), all other conditions kept constant, are shown in the figure. There are several features of interest in these responses. The latency to the first spike of the initial transient of the test response increases as temperature decreases. The latency to inhibition onset also increases, and at a greater rate than test response onset latency. The firing rate in response to a constant light stimulus intensity decreases as temperature decreases and so does inhibitory decrement, the difference in test response without, and with, lateral inhibition.

The result of one experiment of the type described above, carried out for a number of test stimuli intensities and eye temperatures, is illustrated in Fig. 2. It shows the means of responses at four intensity levels of test receptor stimuli with the same inhibitory stimulus intensity at all test intensities. The control (uninhibited test receptor) responses and inhibitory decrements are normalized to control response at 20°C. The inhibitory decrements and control responses, at each temperature, may be normalized and averaged since

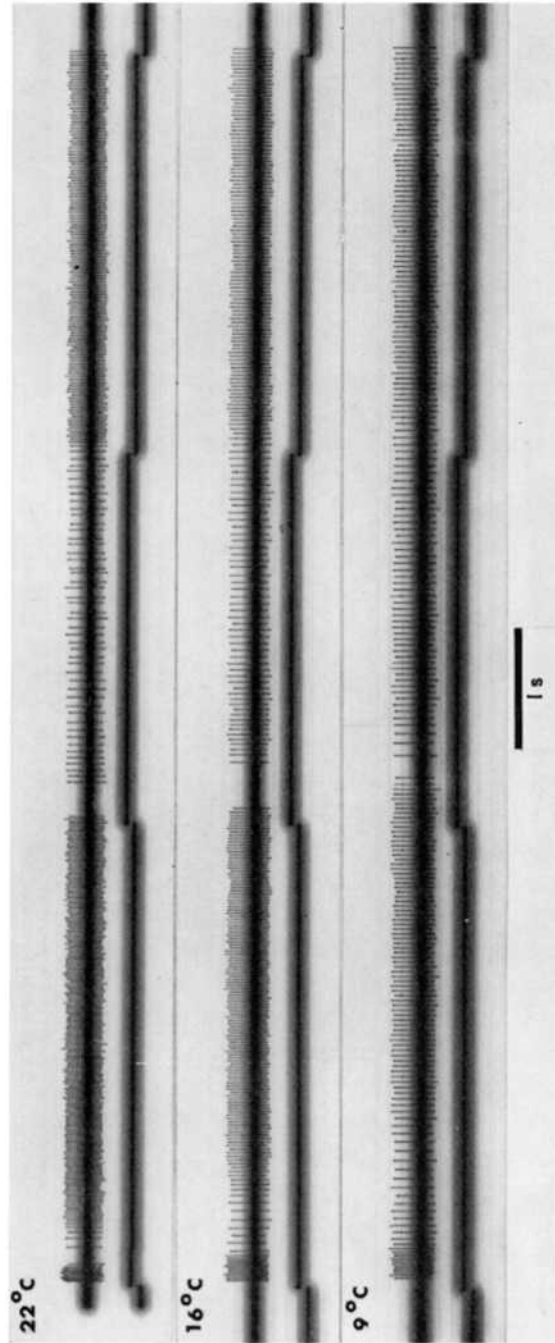


FIGURE 1. Test receptor spike response to lateral inhibition at three eye temperatures. Constant test-light and inhibitory light intensities. Upper tracing of pair at each temperature, optic nerve spikes. Lower tracing of pair, test light and inhibitory light durations.

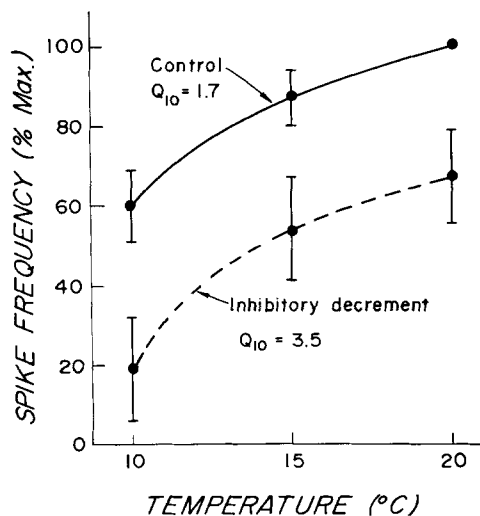


FIGURE 2. Control spike frequency and inhibitory decrement as a function of temperature. Results from four test receptor intensities (neutral density: 0, 0.5, 1.0, 2.0) with constant inhibitory group intensity at each of the three temperatures. Normalized to uninhibited test receptor responses (control frequency) at highest temperature. Inhibitory decrement is the difference in test receptor response between uninhibited control condition and response during inhibition. Each point is the mean of the normalized responses at the four test receptor intensities. Bars indicate 1 SD above and below mean.

the decrements produced by constant inhibitory stimuli are a constant percentage of control responses to different test receptor stimuli intensities (Adolph and Tuan, 1972). The percentage decrement is not a constant function of temperature, however, as indicated by the results of the experiments reported in this paper.

The results of nine experiments, in which the Q_{10} for inhibitory decrement and control response were determined, as illustrated by the results of the experiment given in Fig. 2, are summarized in the scatter-plot in Fig. 3. The appropriate Q_{10} is plotted as a function of the control frequency at 20°C, for each experiment. The Q_{10} fall into two groups which can be graphically separated into nonoverlapping domains. The inhibitory decrement group has a mean Q_{10} of 2.64 ± 0.78 SD, and the control frequency has a mean Q_{10} of 1.58 ± 0.24 SD. The two groups are statistically independent, as assayed by a t test of the means ($t = 3.66$; $P < 0.01$). These results suggest that some processes other than encoder-transducer processes predominate the temperature sensitivity of lateral inhibition. The working hypothesis in these experiments is that the processes predominating thermal sensitivity of lateral inhibition are closely related to chemical synaptic transmission in the lateral plexus.

Some indication of synaptic delay may be obtained by taking the time dif-

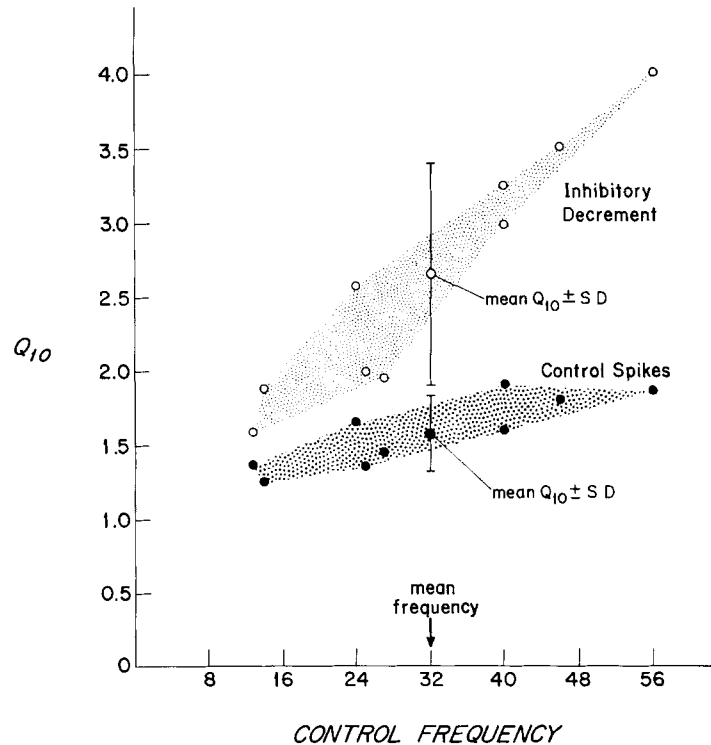


FIGURE 3. Scatter plot of temperature dependence (Q_{10}) as a function of control spike frequency. The Q_{10} of inhibitory decrement and control spike frequency from nine experiments, similar to the one whose results are shown in Fig. 2, are plotted at abscissa values corresponding to control frequency at 20°C, in each experiment.

ference between the latency of inhibitory onset and on-latency of the test response. Fig. 4 illustrates the responses, recorded simultaneously from their optic nerve fibers by a single, extracellular electrode, of a test receptor and one unit in the group inhibiting the test receptor. The transient and steady components of the inhibitory unit's response are reflected by the inhibition of test response, although in a delayed and somewhat modified form. The time difference between the onset latency of the inhibitory unit and the latency to inhibition of the test receptor is a measure of relative synaptic delay. It is a relative measure since it includes transport delay in axon collaterals and a distribution of inhibitory unit onset latencies which cannot be factored out of the latency difference measurement used here to indicate relative synaptic delay. However, if the temperature sensitivity of synaptic delay, rather than its absolute measurement is the feature of primary interest, then it is possible to examine separately the contributions of onset latency and transport delay. This is done in a following section.

The situation in which responses from a unit in the inhibiting group as well

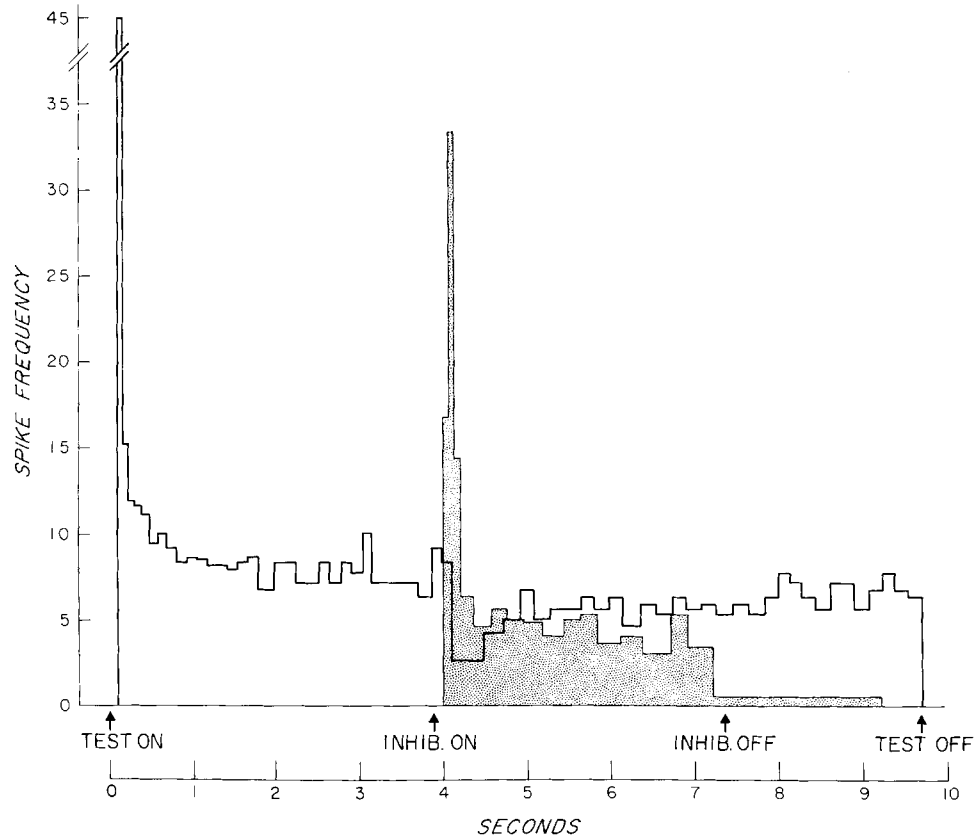


FIGURE 4. Instantaneous spike frequency as a function of time for test receptor response and inhibitory unit response. Shaded region delineates inhibitory unit response.

as the test receptor are recorded simultaneously is a relatively rare one. In the usual case, only the test receptor response can be recorded. There is a way to circumvent this difficulty and still obtain an indication of inhibitory unit onset latency. It can be seen in Fig. 4 that the onset latency and form of the initial test receptor response are very much like the corresponding features of the inhibitory unit's response. In fact, the magnitudes of the latencies are the same although the peak transient frequencies differ. This ought not be surprising since the test receptor and inhibitory unit are neighboring ommatidia within the same eye under identical environmental conditions. Based on this reasoning, the onset latency of test receptor response has been used in this analysis as a measure of the inhibitory unit onset latency. Thus, the measure of relative synaptic delay used here is the time difference between inhibition onset latency and test receptor onset latency in one and the same test receptor under a particular set of experimental conditions.

The temperature sensitivity of relative synaptic delay, defined above, is

shown in Fig. 5. The latency difference, normalized to that at 22°C, and for four test-light intensities, is plotted as a function of temperature. The latency difference can be 6–10 times greater at 9°C than at 22°C and results, for these experiments, in a Q_{10} of approximately 5.

The relative insensitivity of several test receptor (or inhibitory unit) parameters to temperature variations can be shown by isolating the transducer process from the spike encoding and subsequent mechanisms. This is done by intracellular recording of reticular cell response; the results of measuring three

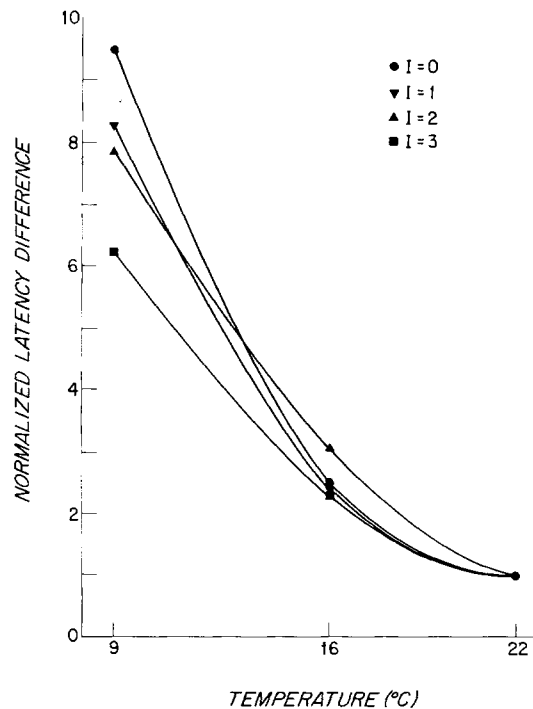


FIGURE 5. Temperature dependence of latency difference between test-response onset latency and inhibition onset latency normalized to difference at 22°C. I -values are log attenuation factors of test-light intensities. Inhibitory light intensities constant for all test-light intensities.

such transducer properties, e.g. onset latency, transient potential, and steady potential, are given in Fig. 6. The lines represent optimum least-square fits to data gathered during a program of cycled temperatures, and illustrate the reversibility of these changes as well as their relative temperature insensitivity.

The temperature dependence of transport delay in axon collaterals cannot be easily measured. In this study, we assume that the thermal sensitivity of spike conduction velocity in directly accessible optic nerve fibers is a reasonable measure of the relevant transport delay properties. Fig. 7 shows antidromically evoked compound action potentials measured *en passant* from a bundle of fibers of the optic nerve. Each trace corresponds to the superim-

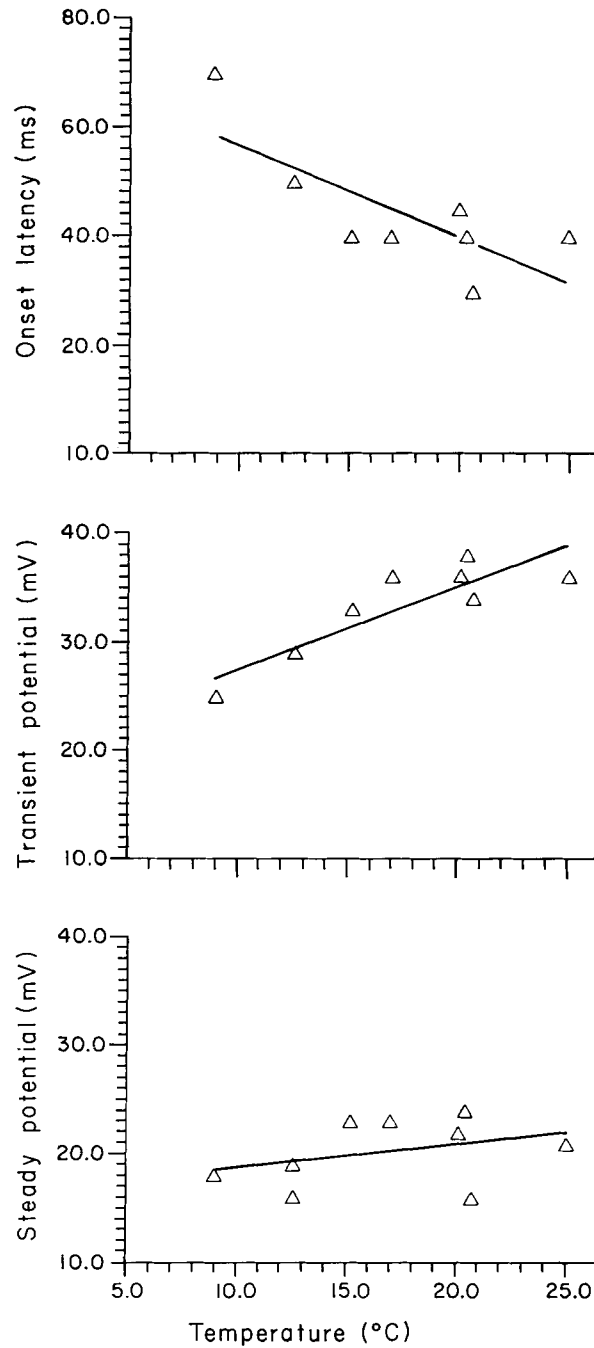


FIGURE 6. Temperature sensitivity of reticular cell response characteristics. Responses to constant intensity light pulses (400 ms duration) at a sequence of temperature starting at moderate level (20°C), to low (9°C), to high (25°C), and return (20°C). The lines are the optimum least-square fits to the points from an ensemble of fits up to third-order. The Q_{10} 's are onset latency, 1.43; transient potential, 1.27; steady potential, 1.12.

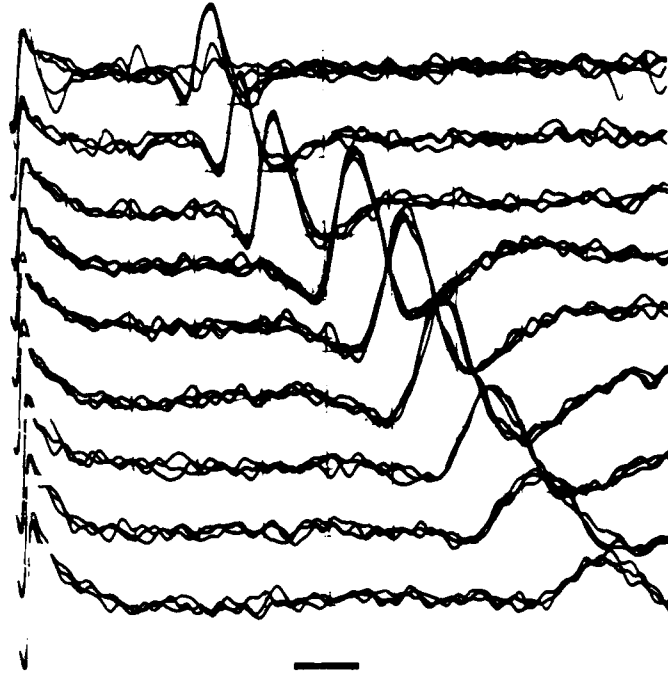


FIGURE 7. Antidromically evoked compound action potentials in the optic nerve as a function of temperature. Each tracing consists of three superimposed responses evoked at 2-s intervals. Stimulus artifacts along left edge. Eye and optic nerve temperatures, °C (from top, downward): 27.2, 23.2, 19.9, 16.0, 13.1, 11.6, 10.2, 9.1, 8.2. Time scale, 1 ms.

posed responses to several stimuli at temperatures ranging from ca. 8°C for the lowest trace to ca. 27°C for the topmost trace. At least two temperature-sensitive features are apparent from the responses. The mean conduction velocity decreases as a function of decreasing temperature, and the spread of conduction velocities of the units in the bundle which compose the compound action potential, increases with decreasing temperature. The Q_{10} for both of these compound action potential features are between 1.7 and 1.8.

Onset latency and transport delay, two inherent factors of relative synaptic delay, are weakly temperature dependent compared to the strong temperature dependence of relative synaptic delay. This suggests that the actual synaptic delay component is also strongly temperature dependent.

The fact that many of the properties of the lateral inhibitory network in the lateral eye retina are temperature sensitive suggests that it may be possible to modify the functional properties of that network by varying eye temperature, i.e., thermally "tune" the lateral inhibitory network.

Fig. 8 shows the response of a group of optic nerve fibers in response to diffuse, intense illumination of the cornea. The response at 20°C (A) shows a

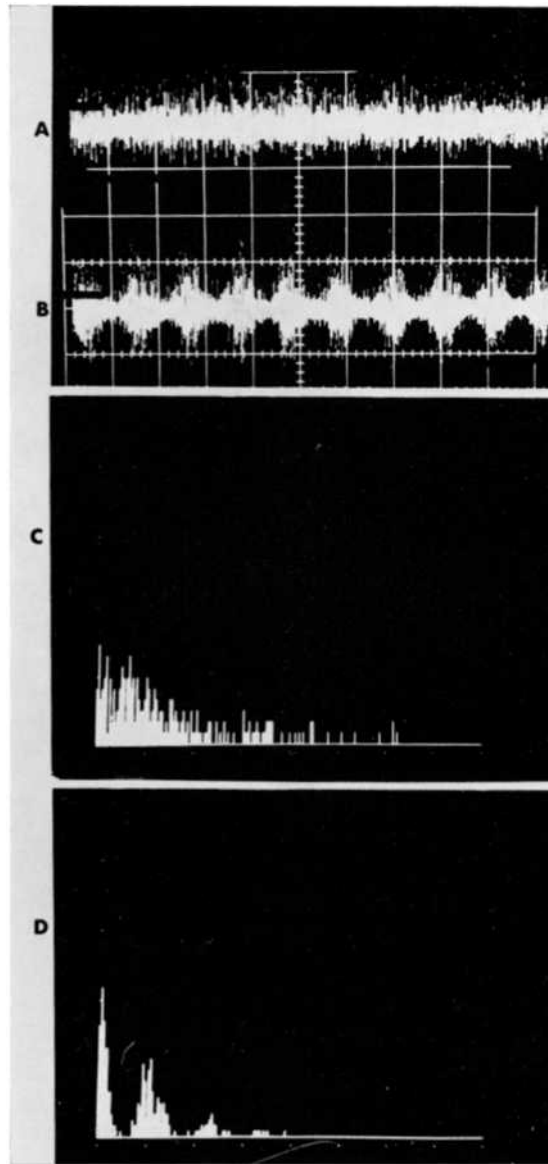


FIGURE 8. Optic nerve multifiber spike output of a group of receptors in response to constant, diffuse, light stimuli. A, response at 20°C; B, response at 25°C; C, spike interval histogram of response in A; D, spike interval histogram of response in B. Time scale, 100 ms per division in A and B, and 100 ms per abscissa scale point in C and D.

random distribution of interspike intervals (C); in contrast to this, the response at 25°C (B) shows regular periodic bursts and this is confirmed by the interspike interval histogram (D). This type of maintained periodic burst response is not due to abnormalities in the responses of the various units comprising

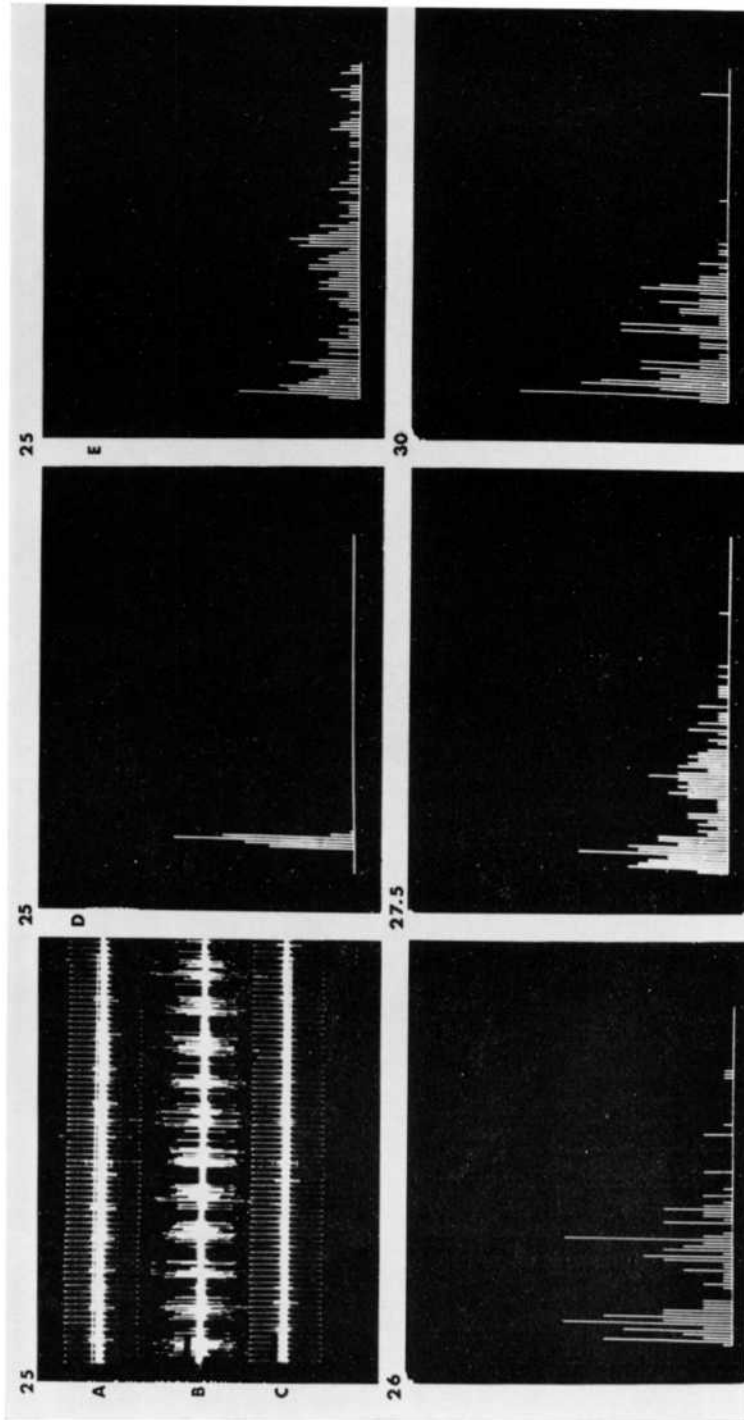


FIGURE 9. Optic nerve spike output in response to constant, diffuse, light stimuli at different eye temperatures. Numbers at upper-left corners of the panels are eye temperatures ($^{\circ}\text{C}$). A and C are responses from a single unit within group producing multifiber response in B. D, spike interval histogram of single unit response at 25°C and E, spike interval histogram of group response at 25°C . Lower three panels show spike interval histograms of group responses at 26°C , 27.5°C , and 30°C . Time scale, 25 ms per division in all panels.

the group, e.g. "galloping," produced by the elevated temperature. The oscillations do not occur in the dark or in response to dim illumination, at the elevated temperature. Also, recordings of responses of single units within groups exhibiting the bursting responses show no obviously abnormal behavior, and this is illustrated in Fig. 9.

The response of a small group of optic nerve fibers is shown in the upper left-hand panel of Fig. 9. In addition to a diffuse and intense stimulus light which activated many receptors within the receptive field of the group's fibers, a small fiber optic bundle was positioned to isolate the response of a single unit within the group. The response of that single unit, before (A) and

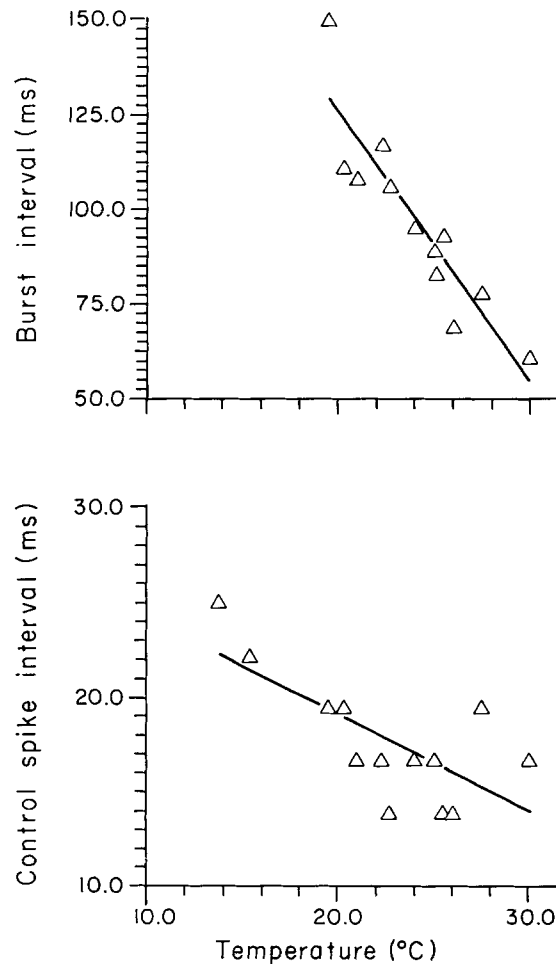


FIGURE 10. Temperature dependence of fundamental burst interval of group response (top) and spike interval of response of single unit within group (bottom). The lines are the optimum least-square fits to the points. Q_{10} of burst interval is 2.4; Q_{10} of single unit response is 1.4.

after (C) activation of the whole group by the diffuse stimulus (B), at 25°C, suggests that interaction among units within the group is necessary to obtain the bursting response. The spike interval distribution of the unit response (D) indicates a simple pattern of rather constant firing rate (a single narrow peak). The spike interval distribution of the group response at 25°C (E) shows a significant difference from the noiselike distribution exhibited by an unpatterned response (Fig. 8 C), and has a marked peak at an interval corresponding to the fundamental burst interval. The lower panels in Fig. 9 illustrate group response interval histograms at other eye temperatures. The peak corresponding to the fundamental burst interval occurs at shorter intervals at increased eye temperatures.

The temperature dependence of group burst interval and spike interval of a single unit within the same group are shown in Fig. 10. The data come from an experiment in which eye temperature was cycled from room temperature (ca. 22°C) to low point to high point and return to room temperature, and illustrates the reversibility of the phenomenon. The lines are optimum least-square fits to the data points. The Q_{10} for the single unit response is 1.4, which is similar to the sensitivity found for control response in the inhibitory decrement experiments reported in the beginning of this section. The Q_{10} for group burst response is 2.4 and is similar to the sensitivity of the inhibitory decrement found in the other experiments.

DISCUSSION

There are several temperature-sensitive factors involved in lateral inhibition as studied in these experiments. First, the sensory transducer-spike encoder processes in the ommatidium: Intracellular recording of reticular cell receptor potentials indicate that at least onset latency and transient and steady potentials are relatively weakly dependent on temperature (Q_{10} of 1.4, 1.3, and 1.1, respectively). Furthermore, control test receptor responses of optically isolated receptors, which include not only the sensory-transducer processes but spike encoding mechanisms as well, are also weakly temperature sensitive (Q_{10} of 1.6). Self-inhibition (Purple and Dodge, 1965) may be of some significance in determining the test response under the latter experimental conditions, however its effect on transducer-encoder temperature sensitivity is not obvious. It cannot be as easily isolated for study as can lateral inhibition; it is usually studied indirectly through its effects on spike afterpotentials, accommodation to current stimuli, etc. In any event, a chain of neural events which may involve self-inhibition, i.e. the optically isolated sensory transducer-spike encoder processes, has a temperature dependence significantly less than have two factors closely related to lateral inhibition, inhibitory decrement and synaptic delay.

Inhibitory decrement, the difference in spike frequency between a control

uninhibited response and the inhibited response, is used here as one measure of the synaptic processes underlying lateral inhibition. Although the transducer-encoder processes of the inhibiting ommatidia are involved in lateral inhibition and so ultimately in the inhibitory decrement, as far as thermal sensitivity is concerned, the controlling factor is something other than the weakly temperature-dependent transducer-encoder processes. It is some process or series of processes with a relatively strong temperature dependence (Q_{10} 2.64 or more), similar to other well-known chemical synaptic processes in frog neuromuscular junction (Q_{10} of 3) (Katz and Miledi, 1965) and guinea pig *vas deferens* (Q_{10} of 2.7) (Kuriyama, 1964).

The thermal sensitivity of synaptic delay has been studied through the effects of temperature on latency differences between onset latency of the test response and latency of inhibition. Onset latency and transport delay in collateral branches of the optic nerve fibers are at least two of the factors contributing to relative synaptic delay. The Q_{10} of onset latency is about 1.4. Spike conduction velocity in *Limulus* optic nerve has a Q_{10} of about 1.7; Bullock and Horridge (1965) indicate that the Q_{10} of conduction velocity in many invertebrates is less than 2. This is considerably less than the Q_{10} ca. 5 for relative synaptic delay in *Limulus* eye.

Variations in certain parameters of an inhibitory neural network such as found in *Limulus* lateral eye, may lead under some conditions to inhibited responses which are larger than uninhibited ones, i.e., amplification (Ratliff et al., 1969). When the appropriate temporal relationships exist among the neural signals in an "amplifying" inhibitory network, long-term instabilities can arise in the form of oscillations of neural output (Hartline et al., 1961). According to Ratliff et al. (1969), a predisposing condition for such instability is narrowing of the spread of times of peak inhibition produced by the ensemble of neighbors inhibiting a test receptor. Among the constellation of effects produced by increasing the temperature of the lateral eye, as described in this paper, may be reduction of the spread of transport delays in axon collaterals from inhibiting neighbors. Perhaps it is possible to tune by means of temperature variations the parameters of lateral inhibition in the eye and thereby produce amplification or oscillation of the optic nerve output.

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