# Photoreceptor Spike Responses in the Hardshell Clam, *Mercenaria mercenaria*

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ABSTRACT Spikes were recorded from single axons in the siphonal nerve of the hardshell clam *Mercenaria mercenaria* which respond to dimming of light. No axons were found to respond to the onset, or increase, of illumination. In a dark-adapted state there is little or no ongoing spike activity. The responsive area of a single axon is a circle of approximately 85 $\mu$m diameter on the inner siphon wall. The number of spikes elicited at the off of constant-duration flashes grows as approximately the 0.4 power of flash intensity. For constant intensity and constant light-time fraction, the off-response increases with increasing duration at least up to 500 s duration. For long durations, the response grows as the logarithm of stimulus duration. Subthreshold light can suppress the off-response from preceding illumination. In a light-adapted state, the off-response is greater and its latency shorter than in the dark-adapted state. The fine structure of groups of cell processes thought to comprise the photoreceptor in *Mercenaria* is described. On the basis of morphological and physiological findings it is suggested that phototransduction occurs in the fine distal processes of the axons from which we have recorded. Axonal processes were found to contain well organized pentalaminar whorls which may be the site of photopigment concentration. The action spectrum obtained from the integrated responses of nerve bundles appears to be that of a single Dartnall pigment having maximal absorption at about 510 nm.

## INTRODUCTION

The quahog, *Mercenaria mercenaria*, exhibits a simple behavioral response to illumination: when light striking the extended siphon is dimmed, the siphon is withdrawn and the shell is closed. *Mercenaria* generally does not respond behaviorally to increases of illumination, nor does it manifest form or color

vision. The dimming response may be a protective reflex, used to withdraw the siphon when the shadow of a predator passes over the animal.

In the experiments to be described here, spike responses were recorded from single axons in the siphonal nerve of *Mercenaria* which convey photic information from the siphon to the visceral ganglia. Presumably motor neurons controlling siphon withdrawal are activated at this ganglion.

Physiological evidence will be presented which indicates that the photoreceptor sites in the *Mercenaria* siphon are physically and functionally isolated from one another. Thus *Mercenaria* is a favorable preparation in that we can ignore the complicating possibility of receptor-to-receptor interactions when interpreting our results.

Our anatomical studies suggest that the transduction from light to neural events may actually be performed in the distal end of the axons from which we have recorded, i.e., it appears that no separate photoreceptor cell occurs distal to the axons. It will also be shown that spikes in these axons are elicited only by decreasing illumination. If our interpretation of the anatomical data is correct, this system provides an opportunity to study "primary inhibition", in which the appropriate stimulus, (light, in this case), inhibits the generation of spikes in the receptor cell, i.e., spikes occur only during or after a decrease in stimulus intensity. (See Discussion in McReynolds and Gorman, 1970 *a*). More knowledge of systems utilizing primary inhibition may provide useful insights to the general area of receptor physiology.

Primary off-responses have previously been reported in several invertebrate photoreceptor systems. Hartline (1938) first showed that the axons of the distal receptor cells in the scallop, *Pecten*, produce spikes only when light is dimmed, whereas those from the proximal layer of the retina produce only on-responses. Recently Land (1966) has presented more quantitative data on spike responses of both the *Pecten* on- and off-fibers. McReynolds and Gorman (1970 *a*, *b*) have recorded intracellularly from both distal and proximal receptor cells of *Pecten*. The distal cells produce a hyperpolarizing receptor potential upon illumination and the proximal cells produce a depolarizing response. Both responses are associated with an increase in membrane conductance. Gorman, McReynolds, and Barnes (1971) report that receptor cells in the eye of a primitive chordate, *Salpa democratica*, respond to light with hyperpolarization and increased membrane conductance. The *Pecten* distal receptors are ciliated whereas the *Salpa* receptors bear microvilli, indicating that qualitatively similar responses can be obtained with either type of structure.

Kennedy (1960) demonstrated that a single axon in each pallial nerve of the surf clam, *Spisula*, responded to the dimming of white light. His data, from experiments using monochromatic light, reveal an excitatory process activated by long-wavelength light and an inhibitory process activated at short wavelengths. Off-responses have been reported in the axons of the photoreceptor

cells in the eye of the cockle *Cardium edule* (Barber and Land, 1967) and in the file clam *Lima*, (Mpitsos, 1969).

Kennedy (1960) mentions that both on- and off-responses were obtained in the long-neck clam, *Mya arenaria*. Light (1930) combined anatomical studies and behavioral studies on specimens of *Mya* with different portions of the siphon removed. He found a correlation between those areas of the siphon whose removal most affected the withdrawal response, and the distribution of large pear-shaped cells. These cells were described as possessing an axon containing numerous fibrils which extended into the cell body, forming a dense network. Also included in the cell body was an "optic organelle" which, in excised cells, was found to focus light onto the network of fibrils. Light implied that this network is the site of photoreception. We have found structures similar to those described by Light in the siphon of *Mercenaria*.

The only previous physiological work on the *Mercenaria* photoreceptor system, of which we are aware, is a brief comment by Kennedy (1960) that axons in the siphonal nerve respond to both the onset and cessation of illumination.

A preliminary report of some of the findings to be presented here has appeared (Wiederhold and MacNichol, 1970).

## METHODS

### A. *Preparation*

Both the anatomical and physiological studies described here were performed on large specimens of the hard-shell clam, *Mercenaria mercenaria*, obtained from the Supply Department, Marine Biological Laboratory, Woods Hole, Mass. More stable physiological recordings were obtained at Woods Hole than when the animals were shipped to Bethesda, Md.

For the anatomical studies, portions of the siphon were dissected from the animal in a seawater bath and fixed by immersion in 6.0% glutaraldehyde in 0.2 M s-collidine buffer at pH 7.4. Sucrose was added to the fixative to bring the final osmolarity to 1200 mosmol and 100 mg of $CaCl_2$ was added to each 25 ml of fixative. Postfixation was carried out in 1% osmium tetroxide in Sorenson's phosphate buffer with sucrose added to bring the osmolarity to 1200 mosmol (Bell et al., 1969). After fixation the tissues were dehydrated in a graded series of ethanols and embedded in Epon (Shell Co., New York) (Luft, 1961).

Sections 0.5 $\mu$m thick were cut on a Porter Blum Mt-2 microtome (Ivan Sorvall, Inc., Norwalk, Conn.) and stained with 1.0% methylene blue and 1.0% azure B in a 1.0% borax solution. Thin sections were obtained from the same blocks, stained with uranyl acetate and lead citrate (Watson, 1958) and examined in a Philips EM-300 electron microscope (Philips Electronic Instruments, Mount Vernon, N. Y.) (Reynolds, 1963).

The physiological preparation consisted of the isolated siphon and a portion of the posterior adductor muscle with the overlying visceral ganglia and siphonal nerves,

maintained in seawater. The siphonal and pallial nerves were cut where they enter the visceral ganglion and the siphonal branch was dissected free for approximately 1 cm to the point where it enters the siphon muscles. (For a general description of the anatomy of *Mercenaria*, see Kellogg, 1892). Fine dissecting needles were used to tease out strands of the siphonal nerve 50–100 μm in diameter. The incurrent siphon was severed along its ventral border and the septum between the siphons was cut. The preparation was secured to a cork block with the inner surfaces of both siphons exposed.

B. *Recording*

The small strands of siphonal nerve were drawn into a polyethylene suction electrode (see Easton, 1965) with inside tip diameter of 50 μm. The suction electrode consisted of a 1 $cm^3$ plastic syringe connected by rubber tubing to the suction tip. Both were filled with seawater. The only metal in contact with the seawater was a platinum wire which penetrated the rubber tubing. In preliminary experiments using silver wire and a syringe with a stainless steel Luer-Lok (Becton Dickinson & Co., Rutherford, N.J.), spike amplitudes were observed to gradually decrease over several hours, whereas the system described above provided stable recording over many hours.

The signal from the suction electrode was amplified with high- and low-frequency cut-offs each determined by a single time constant, usually 0.3 and 9 ms, respectively. The preparation, suction electrode, and first stage of the amplifier were in a light-tight box which also provided electrical shielding.

The amplified electrode signal was fed to a window circuit which produced a standard pulse when the base line-to-peak amplitude of a spike was between two adjustable voltage levels. By adjusting these levels, spikes from one of a number of active fibers in the suction electrode could be counted. It was usually found most convenient to use only the lower level of the window and thus count only the largest spikes.

The standard pulses from the window circuit were fed to a true integrator whose output level increased by one unit of voltage each time a spike was counted. The integrator output reset to zero every time the output reached a preset level. (This level corresponds to a count of 20 spikes in the figures in this paper). The integrator output could also be reset to zero by an externally programmed pulse, e.g., a pulse occurring at the ON or OFF of each flash in a series of repetitive stimuli. The integrator output and a stimulus marker were recorded with a fast, direct-writing recorder. A number of the figures to be presented here are copies of the recorded integrator output. In these figures the integrator output corresponds to the number of spikes of a given amplitude that have occurred since the integrator was last reset; the "slope" of the staircase output is proportional to the instantaneous discharge rate.

This report is based on results from 28 successful experiments. In 20 of these experiments spikes from single axons were isolated; in the other 8, multiple-unit recordings were obtained using either wick or suction electrodes. 29 single-unit recordings were made. For 25 of these, recording was maintained for from 2 to 8 h so that several types of tests could be performed on each fiber.

*C. Light Stimulus*

The photic stimulator consisted of two independent channels of light from tungsten bulbs. Earlier experiments, (See Results, part E), indicate that *Mercenaria* has only one photopigment, maximally sensitive to 510 nm wavelength-light. Thus both channels were filtered with Wratten no. 58 filters (Eastman Kodak Co., Rochester, N.Y.) to have a spectral peak near this wavelength. Infrared energy was filtered out by passing the light through heat-absorbing glass or through 1.2 cm of 4% cupric chloride solution. The spot size of each channel could be varied independently from 0.5 mm to approximately 1 cm, maintaining the same irradiance. Due to light scattering within the siphon, increasing spot size could increase the irradiance at the site of photoreception, even though the flux striking the siphon wall is unchanged. The positions of the two spots could be varied independently. The timing of flashes in the two channels was controlled by independent shutters (fly time of 1 ms), shutter drivers and timing units.

Both tungsten bulbs were energized with direct current, and the intensity of each channel was adjusted by varying the DC voltage from 0 to 6.5 V. Since a narrow-band filter was used in all of these experiments, varying intensity by varying lamp voltage should not change the spectrum of the stimulus appreciably. We do not have an absolute calibration of light intensity, but the relative intensities of the two channels, as a function of lamp voltage, were calibrated with a photomultiplier. Thus, any desired irradiance (relative to an arbitrary standard) could be obtained in either channel by selecting the appropriate lamp voltage. For all figures except Fig. 16, a given intensity (expressed in logarithmic units relative to the maximum intensity available) denotes the same irradiance at the surface of the inner siphon wall. At the minimum intensity used, −4.0 log units, the reflection of the spot on the siphon appeared near scotopic threshold for the exeperimeter.

In measuring action spectra somewhat different methods were used. Small bundles of siphonal nerve were dissected as far as their insertion into the siphonal muscles. One of these was mounted upon a cotton wick protruding from a glass pipette containing seawater and an Ag-AgCl electrode. It was not possible to dissect bundles giving single unit activity. Accordingly the responses of a number of units were "integrated" by full wave rectification and filtering (Beidler, 1953).

Since all fibers appeared to give the same qualitative kind of response to illumination this method of sampling a population appears to be justified.

The optical system consisted of two compact-filament lamps (G.E. 1493, General Electric Company, Schenectady, N.Y.), magnetic shutters, and a lens system which focussed the lamp filament on the shutter apertures, a semitransparent combining mirror, field lenses with adjustable diaphragms and a projection lens. The shutters were programmed by electronic timers (Modified Tektronix 162, Tektronix, Inc., Beaverton, Oreg.).

Illumination was controlled by adjusting the voltage across each lamp, which was read with an accurate mirror-scale meter. Optics Technology (Optics Technology, Inc., Palo Alto, Calif.) interference filters were used in the adapting beam and Wratten gelatin filters in the test beam. With each filter the lamp voltages required to

give various relative energies were calibrated by means of a photomultiplier tube (EMI 9558A, EMI Electronics, Ltd., Hayes, Middlesex, England) using a highly stable high-voltage supply and measuring circuit. The relative sensitivity of the photomultiplier tube was determined at each wavelength from published curves (S-20 photosurface).

In order to keep the preparation well light-adapted both to obtain a vigorous response and to obtain maximal effect from colored adapting lights, measurements were usually made by turning on the adapting beam for 3 or 4 s alternately with the measuring beam which was on for 1 s. The criterion effectiveness of the measuring beam was that intensity which reduced the "integrated" discharge from the nerve bundle to one-half the amplitude obtained when the light was turned off completely during the test period. This method also insured that the adapting effects of the test light would be much smaller than those of the adapting light.

## RESULTS

### A. *Morphology*

LIGHT MICROSCOPE In the light microscope, structures assumed to be photoreceptors (see Discussion) appear as heavily stained round to oval bodies located beneath the epithelium of the inner surface of the siphon. Some of these structures are scattered among the muscle cell layers in the deeper portions of the siphon wall. These structures vary in size from 5 to 15 μm and are identical in appearance with the structures described as photoreceptors by Light (1930) in the siphon *Mya arenaria*.

ELECTRON MICROSCOPE The "photoreceptors" of the *Mercenaria* siphon appear to be identical to those described in the siphon of *Mya arenaria* (Bell and Anderson, 1970). When examined in the electron microscope, each "photoreceptor" unit is found to contain a number of elements.

One component of the "photoreceptor" is a cell of very irregular shape (Fig. 1 A). Processes of these cells interdigitate with other elements of the "photoreceptor". These cells are characterized by round to oval membrane-bounded inclusions which have an average length of 2.5 μm and an average width of 0.5 μm (Fig. 1 A and B). In *Mercenaria* the inclusions are composed of two different types of granular material, one is considerably more electron opaque than the other. The proportion of the dense to light material is usually 1:1, but may vary considerably from this ratio in some of the inclusions. The dense portion of each inclusion appears to have a crystalline substructure (Fig. 1 B).

The irregular cells containing the inclusions are always found associated with a bundle of nerve fibers. The largest nerve processes are 2.5 μm in diameter and the smallest only 0.2 μm in diameter and contain neurotubules, neurofilaments, mitochondria, and smooth-surfaced sacs and vesicles (Figs. 1 A and 2 A). Some of the nerve processes contain clear vesicles 700–1400 Å in

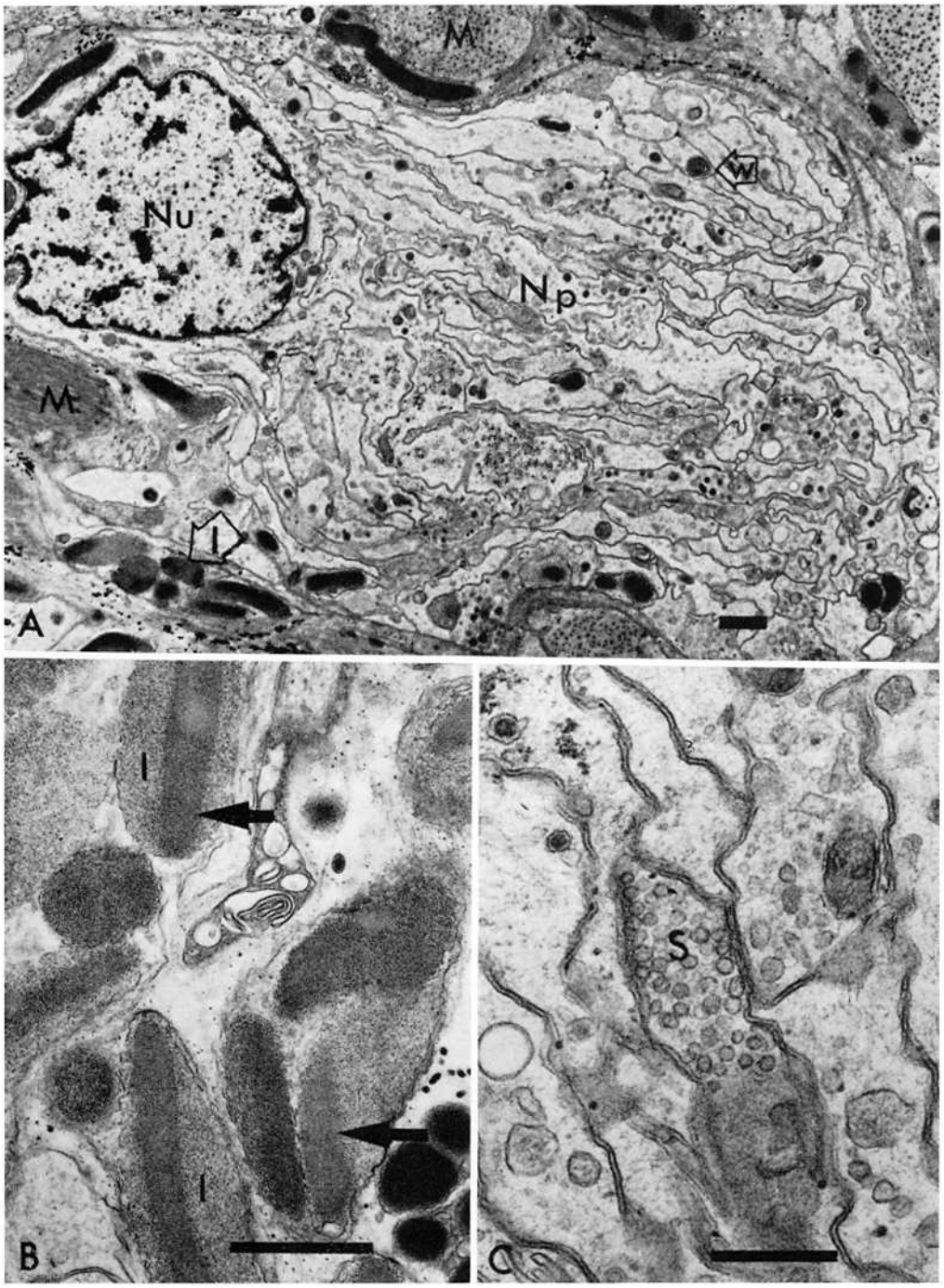


FIGURE 1. (A), A large "photoreceptor" complex consisting of an irregular cell containing a nucleus (Nu) and many processes with inclusions (I) closely associated with a large number of nerve processes (Np). The nerve processes contain clear and dense vesicles and in some regions have their plasma membranes modified into whorls (w). Profiles of the muscle cells (M) of the siphon are seen surrounding the "photoreceptor". Marker equals 1 $\mu$m. (B), High magnification of inclusions (I). These structures are surrounded by a membrane and contain a light particulate component and a denser substance which has a crystalline substructure (Arrows). Marker equals 1 $\mu$m. (C), Detail of one portion of nerve process bundle. Dense cored vesicles are visible in upper left corner and a synapse (S) containing clear vesicles is present. Marker equals 0.5 $\mu$m. Magnification (A), $\times$ 6,480 (B), $\times$19,500 (C), $\times$ 37,600.

diameter (Fig. 1C). Others contain dense-cored vesicles 1000–2500 Å in diameter (Fig. 2A). Occasionally a synapse between two of the nerve processes is found within the bundle of axons (Fig. 1C).

Some of the axons have their limiting membrane arranged in whorled configurations (Fig. 2 A, B, and C). These whorls do not appear to be fixation artifacts commonly referred to as "myelin figures." In each whorl the membranes are arranged in an orderly fashion and some of the adjacent unit membranes are fused, forming pentalaminar configurations (Fig. 2 B and C).

The whole "photoreceptor," consisting of the irregular inclusion-containing cells, the bundle of axons, and nerve terminations, is usually found closely associated with muscle cells of the siphon (Fig. 1 A).

### B. *Qualitative Description of Electrophysiological Response*

A representative recording of spike responses to photic stimulation in the siphonal nerve of *Mercenaria* is shown in Fig. 3. The lower trace shows spikes of two amplitudes occurring after a preceding light is turned off. Only spikes with amplitude exceeding the trigger level (indicated by the dashed line) were counted in this experiment. The upper trace represents the integrator output. The spike amplitude was stable for over 2 h in this case. In other experiments, spikes of constant amplitude have been recorded for 8 h. With this system, spike amplitudes as great as 500 $\mu$V were recorded with a noise level as low as 10–20 $\mu$V. Summated responses of many photoreceptive axons could easily be recorded by laying either the whole siphonal nerve or smaller strands over a cotton wick electrode. It was more difficult to separate the spikes from single axons with wick electrodes and their recordings were less stable than those obtained with suction electrodes.

Several features of the response in Fig. 3 are typical of our observations: (*a*) Spikes appear only in response to dimming the light (turning it off in this case), (*b*) there is a considerable latency to the first spike, (approximately 200 ms here, although this can vary from approximately 100 ms to over 10 s), (*c*) spikes from a single fiber occur transiently (there is little or no ongoing activity if the preparation has been in total darkness for 15–20 min), and (*d*) the spikes occur with irregular inter-spike intervals, seldom exceeding an instantaneous rate of 30–40/s. These points, as well as the dependence of the response on stimulus parameters, will be further characterized in the succeeding figures.

That the response of this system is of the "pure off" type is demonstrated in Fig. 4. Both the fiber producing large spikes, and several giving smaller spikes, discharge only during the OFF period. Although a preceding off-response may persist for some time after a light is turned on, we have never observed a fiber in the siphonal nerve of this animal which responds preferentially to an increase of illumination. Similarly when illumination was increased or decreased slowly by manually changing the lamp voltage, responses were obtained only to decreasing illumination.

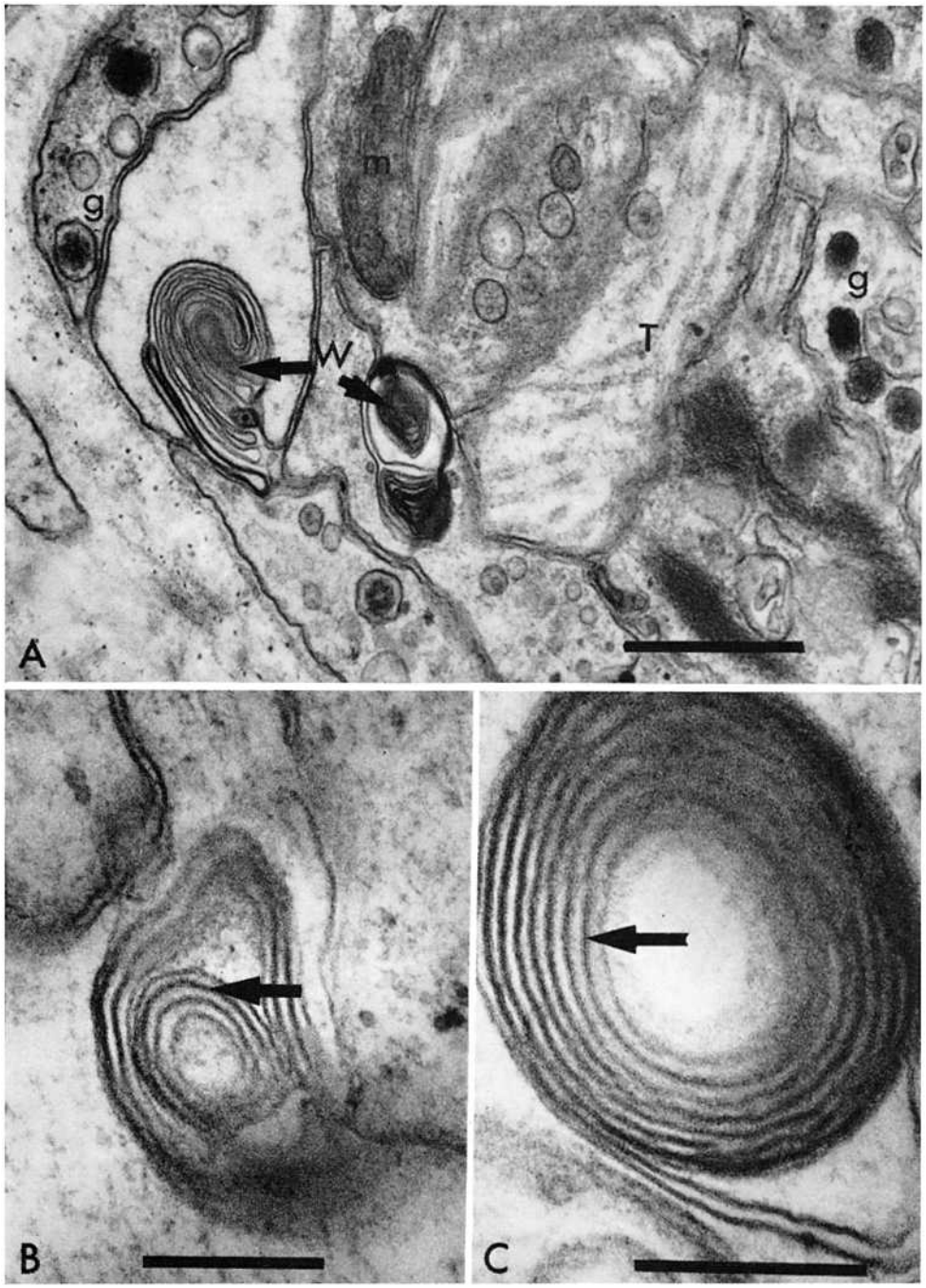


FIGURE 2. (A), Detail of a region of the axon bundle containing two whorled configurations (w). It is clear that the whorl on the left is formed by an invagination of an axon plasma membrane. Also visible in this micrograph are dense cored vesicles (g), neurotubules (T), and a mitochondrion (m). Marker equals 0.5 μm. (B and C), High magnification micrographs of whorls showing the fusion of adjacent plasma membranes to form pentalaminar structures (arrows). Marker equals 0.25 μm. Magnification (A), × 48,800 (B), × 101,400 (C), × 129,600.

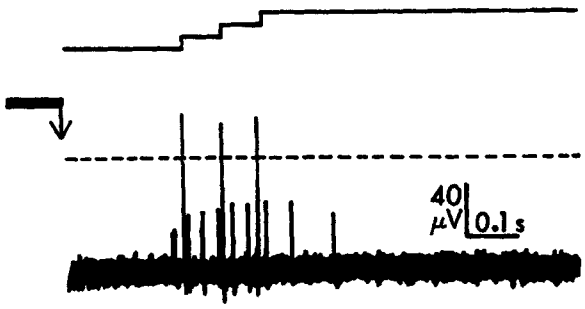


FIGURE 3. Sample recording with suction electrode of spikes from a strand of *Mercenaria* siphonal nerve in response to turning off a spot of light. The original recording has been traced with ink to improve contrast. Lower trace: electrode output showing spikes from at least two fibers. The three largest spikes have nearly constant peak-to-peak amplitude and are presumed to be from the same fiber. Passband: 18–530 Hz. Dashed trace: trigger level. All spikes with positive peak amplitude above this level were accepted by the window circuit and counted by the integrator (the upper level of the window was set well above the highest peak amplitude). Heavy solid trace: stimulus marker indicating when light is on. Arrow indicates when light is turned off (i.e. at beginning of oscilloscope trace in this case). Upper trace: integrator output which increases by 1 unit whenever a spike exceeds the threshold level indicated by the dashed trace (See Methods section). Light stimulus: 1 mm spot, 0 log-unit intensity, white light in this case. Spot on inner surface of incurrent (ventral) siphon approximately 3 mm ventral to septum between siphons. Light on for several seconds before trace begins. Record 5-6-71 I.


FIGURE 4. Similar to Fig. 3 except for different time scale and showing only amplified electrode output (lower trace) and stimulus marker (upper trace). Light spot and recording as in Fig. 3. Light presented repetitively, 3 s ON, 3 s OFF. Record 5-6-71. II.

The effective area on the inner siphon wall, in which reduction of illumination evoked spikes in a single fiber, could be localized to a circular area of about 85 μm diameter. This was determined by moving a small shadow across a constant 1–2 mm spot surrounding the effective area. The sharp localization of effective area indicates that the receptors are not far beneath the surface. No directional sensitivity was observed nor were lateral inhibitory phenomena seen. Increasing the spot size from 0.5 to 1 or 2 mm only increased the off-response. The increased effectiveness of larger spots of the same irradiance

could be due to light scattering as discussed above. These findings seem consistent with the existence of functionally isolated receptors located approximately 100 $\mu$m beneath the surface of a translucent tissue.

Effective or "receptive" areas for single fibers were found in most of the yellow-pigmented regions of the inner walls of both incurrent (ventral) and excurrent (dorsal) siphons. The greatest concentration of receptive areas was found adjacent to the septum dividing the two siphons; more were found in the incurrent than in the excurrent siphon. No receptive areas were found in the black-pigmented distal regions of the siphons or in the tentacles.

### C. *Dependence of Response on Stimulus Parameters*

The first stimulus parameter to be discussed is light intensity. In Fig. 5 responses to repetitive flashes at seven intensities are shown. At the lowest intensity there is no apparent synchrony between spikes and stimulus. At the highest intensities there are more spikes and they occur preferentially toward the end of each OFF period.[1] A response-intensity series constructed from the data of Fig. 5 is shown in Fig. 6. Over the range from $-2.5$ to 0 log-unit flash intensity, the response grows approximately as the 0.3 power of flash intensity. In five such experiments a similar relationship was found with exponents varying from 0.12 to 0.62, the averaging being 0.37.

The effects of varying flash duration were more extensively studied than were those of flash intensity. In the experiment illustrated in Fig. 7, stimulus duration was varied from 300 s down to 1 s.[2] The records show that longer durations elicit both a greater total number of spikes and a higher initial discharge rate when the light is turned off. The latter can be estimated from the initial slope of the integrator output.

The results of Fig. 7 are quantified in Fig. 8. The left-hand portion of the figure plots the total number of spikes vs. duration. It can be seen that the response continues to grow with duration at least to 300 s (5 min), and, for long durations, is nearly proportional to the logarithm of duration. In other experiments this relationship has held for durations as great as 500 s (8 min,

[1] Although, with the resolution of this figure, the spikes may appear to occur at the ON of the next flash, they actually precede the ON. With repetitive flashes such as these, spikes tend to occur late in the period, although not running into the next ON. This phenomenon is more apparent in Fig. 15 and will be discussed further below, as will the lack of response to the first few flashes when the intensity is increased.

[2] As will be discussed in connection with Fig. 12, the response to a transient stimulus is strongly dependent on the preceding time course of illumination. By maintaining equal ON and OFF durations throughout the duration series and thus maintaining equal time-averaged illumination, we have controlled the past history of illumination. By completely dark adapting the preparation between different duration stimuli, interactions between successive responses could be eliminated, but, as will be shown, this system is relatively unresponsive in the dark-adapted state. We have not performed sufficient tests to prove that the paradigm used here is optimal for obtaining reproducible or unbiased results.

20 s). Thus this system is capable of integrating the effect of preceding illumination over quite long times in producing the off-response.[8]

The number of spikes in the first 10 s of each OFF (for durations of 10 s or longer) is plotted in the right-hand portion of Fig. 8. This measure provides an estimate of the initial firing rate. Although this plot shows more statistical variation than the left-hand plot, it is consistent with the conclusion that the response is approximately proportional to the logarithm of ON duration for long durations.

Fig. 9 shows that the relationship between response and ON duration is approximately the same whether the duration is successively increased or decreased. In this case the responses during parts of the descending duration series are greater than those obtained at the same duration in the ascending series. This may be due to residual excitatory effects from the illumination during the ascending series[2] as will be discussed in a later section. The preparation had been in total darkness for several minutes before this series of measurements was started.

A similar growth of response with ON duration was observed in five experiments.

### D. *Time-Course of the Effects of Light*

The decay of discharge rate after light is turned off, can be described as a sum of exponentials. The discharge rate, $R(t)$, in spikes per second, is approximated by the expression:

$$R(t) = \sum_i A_i e^{-t/\tau_i}, \qquad t > 0, \tag{1}$$

where the amplitude factors $A_i$ are in spikes per second and the time constants, $\tau_i$, are in seconds. $t = 0$ is the time when light is turned off. The sum of spike counts up to a given time, $S(t)$, is given by:

$$S(t) = \int_0^t R(t)\,dt = T - \sum_i A_i \tau_i e^{-t/\tau_i}, \qquad t > 0, \tag{2}$$

where $T$ is the total number of spikes contained in a given off-response. Rearranging Eq. 2 we obtain:

$$T - S(t) = \sum_i A_i \tau_i e^{-t/\tau_i}, \qquad t > 0. \tag{3}$$

Fig. 10 illustrates a procedure of separating the individual exponential terms

[8] As will be shown in the next section, the discharge rate decays approximately exponentially during the OFF period and the time constant of the decay is nearly proportional to duration. Thus the percentage measurement error introduced by estimating the total response by counting the number of spikes in an OFF period equal to the ON duration should be constant at all durations.

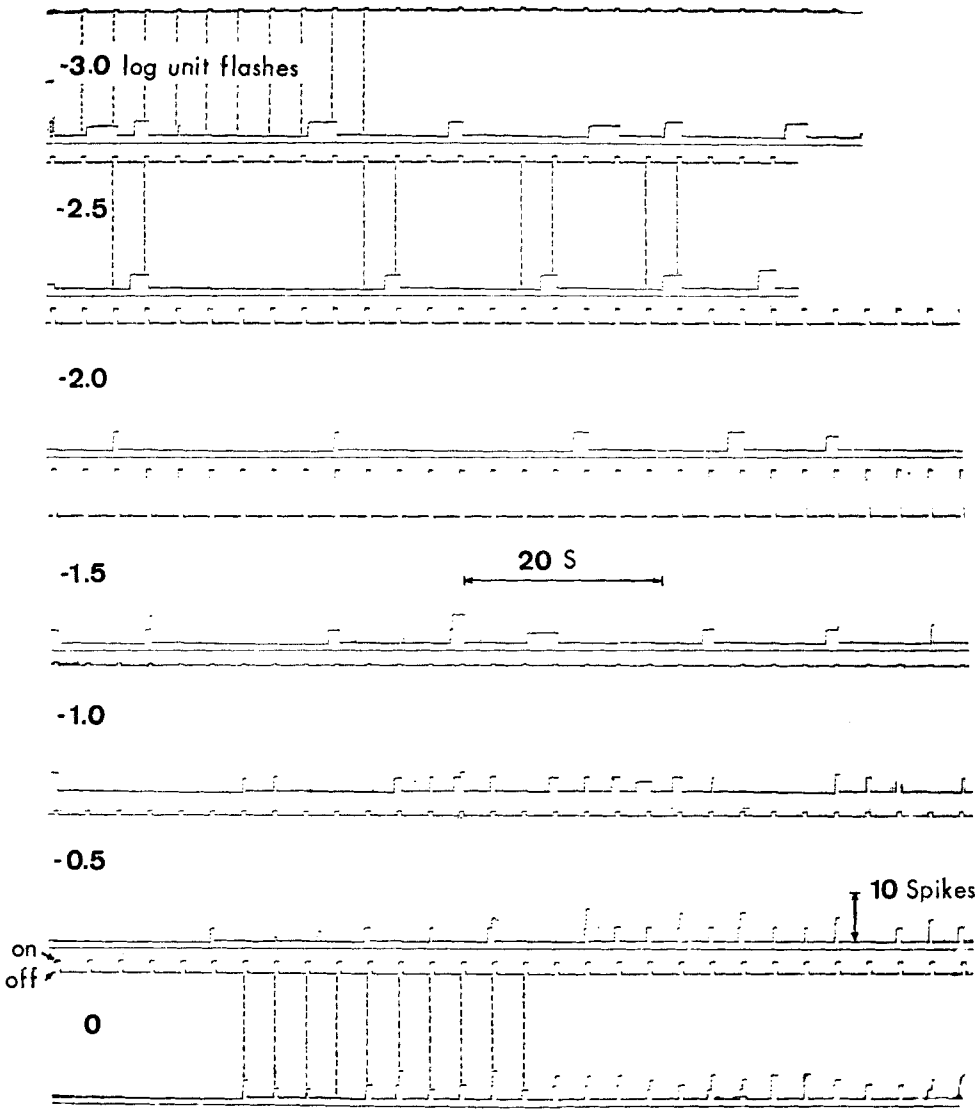


FIGURE 5. Recorded output of stimulus marker and integrator level for seven flash intensities. In the upper record for each intensity an upward deflection indicates light on. The lower record in each pair is the integrator output. The integrator output is reset to zero at the OFF of each flash. Dashed lines added to facilitate comparing time in stimulus and response records. Flashes 0.5 s duration presented 1 per 3 s. Spot diameter 2 mm. Spectral peak of light = 510 nm. Single fiber recording. Record 7-23-70 I.

by the method of peeling of exponentials (cf. Rall, 1969). The data of Fig. 10 represent the 300 s duration stimulus of Fig. 7. The upper points of Fig. 10 are $T\text{-}S(t)$, which, after $t = 40$ s, are well fit by the line satisfying:[4]

[4] The points at the longest times fall consistently below the line indicating an 87 s time constant. This can be accounted for if the exponentials did not reach completion within the finite OFF period. If

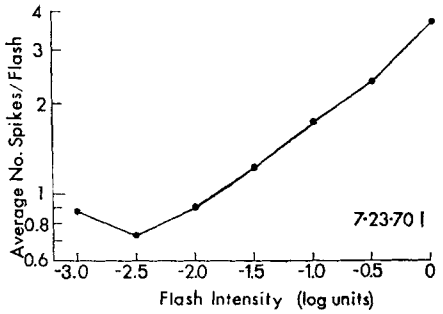


FIGURE 6. Response-intensity series constructed from data of Fig. 5. Average number of spikes per flash computed for all flashes once the response appeared to reach a quasi-steady state. Responses averaged: Last 38 of 43 for 0 log unit, last 51 of 55 for −0.5, last 50 of 55 for −1.0, last 30 of 32 for −1.5, last 43 of 44 for −2.0, last 22 of 24 for −2.5, last 25 of 26 for −3.0. Record 7-23-70 I.

$$T - S(t) = 65e^{-t/87}, \qquad t > 0. \tag{4}$$

The middle set of points are the numerical difference between observed values of $T$-$S(t)$ and the line indicating an 87 s time constant. This second set of points is well fit by:

$$[T - S(t)]' = 17\, e^{-t/20}, \qquad t > 0. \tag{5}$$

For $t < 5$ s a third exponential can be pealed off as indicated by the lower three points and the line satisfying:

$$[T - S(t)]'' = 6\, e^{-t/1.5}, \qquad t > 0. \tag{6}$$

(The estimation of the shortest time constant is less reliable than the other two due to the small number of points available). Thus, for this experiment, the discharge rate is well described by:

$$T - S(t) = 6\, e^{-t/1.5} + 17\, e^{-t/20} + 65\, e^{-t/87}, \qquad t > 0. \tag{7}$$

Since $R(t)$ is the derivative of $S(t)$ we, obtain:

$$R(t) = 4\, e^{-t/1.5} + 0.85\, e^{-t/20} + 0.75\, e^{-t/87}, \qquad t > 0. \tag{8}$$

Fig. 11 shows that the longest time constant, $\tau_3$, is nearly proportional to stimulus duration. The points in this figure represent two different experimental runs, taken several hours apart, on the same preparation illustrated in

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we assume that three more spikes would have occurred in an infinite OFF period and thus add 3 to all of the values of $T - S(t)$, all of the points beyond 40 s are well fit by one straight line. The slope of the improved fit obtained by adding 3 to $T$ in this case was 5% less than that shown in Fig. 10.

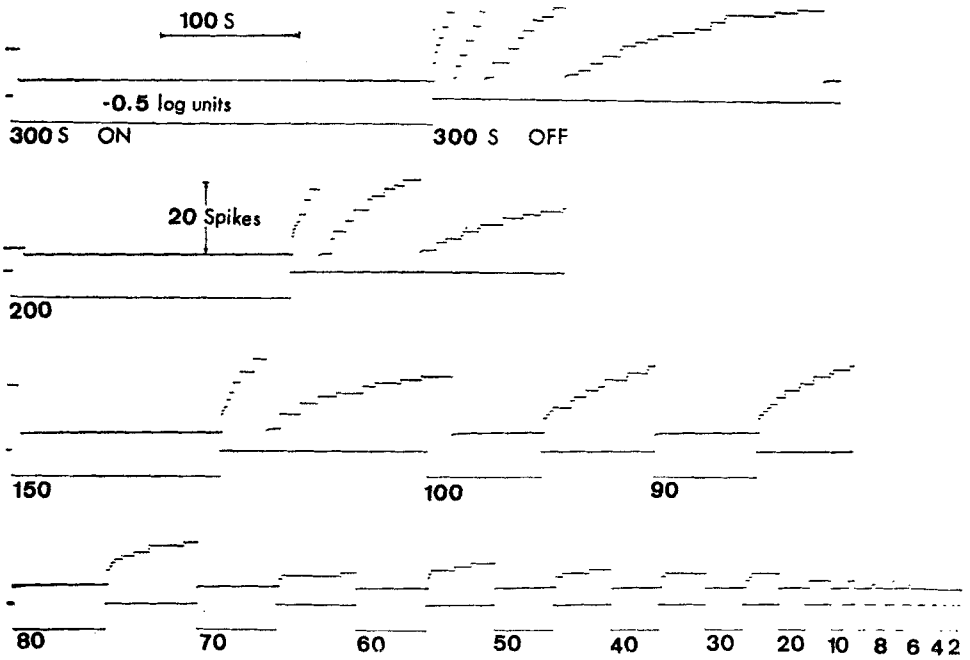


FIGURE 7. Integrator output and stimulus marker for equal ON and OFF durations of −0.5 log-unit intensity, 510 nm spectral-peak light with spot diameter = 0.5 mm. Numbers below each stimulus marker indicate ON and OFF duration in seconds. Stimulus marker trace is down when light is on. Integrator output reset to zero when light turned on and automatically reset to zero whenever count of 20 spikes reached. Single fiber recording. Record 8-6-70 II 69–90.

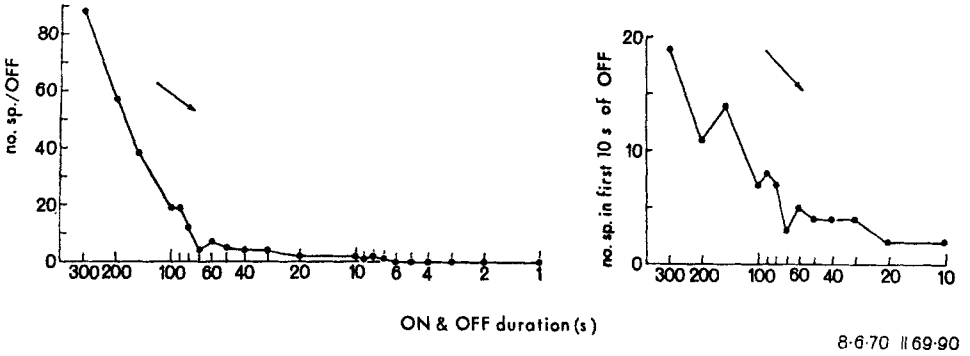


FIGURE 8. Plots of total number of spikes per OFF period (left-hand plot) and number of spikes in the first 10 s of each OFF (right-hand plot) versus ON (and OFF) duration, plotted on a logarithmic scale. Counts made from the data of Fig. 7. Arrows indicate sequence in which durations were presented. Record 8-6-70 II 69–90.

Figs. 7, 8, and 10. The straight line represents the relationship:

$$\tau_s = 0.39\ D, \tag{9}$$

where $\tau_s$ is the longest time constant (in seconds) and $D$ is the stimulus duration (in seconds).

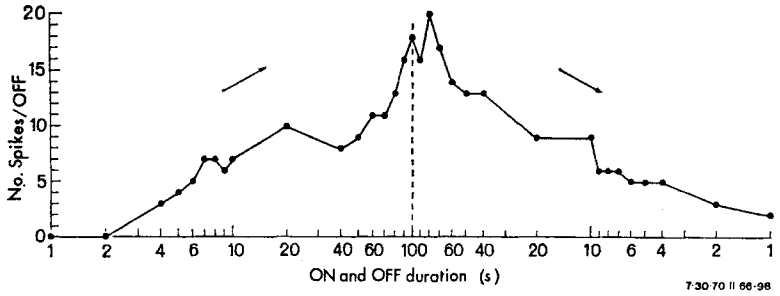


FIGURE 9. Response-duration series similar to the left-hand plot of Fig. 8 (total number of spikes per OFF period is plotted). In this case durations of both ON and OFF were successively increased from 1 to 100 s and then decreased back to 1 s. −0.5 log unit light, spectral peak at 510 nm, 465 μm spot diameter. Single fiber recording. Record 7-30-70 II 66–98.

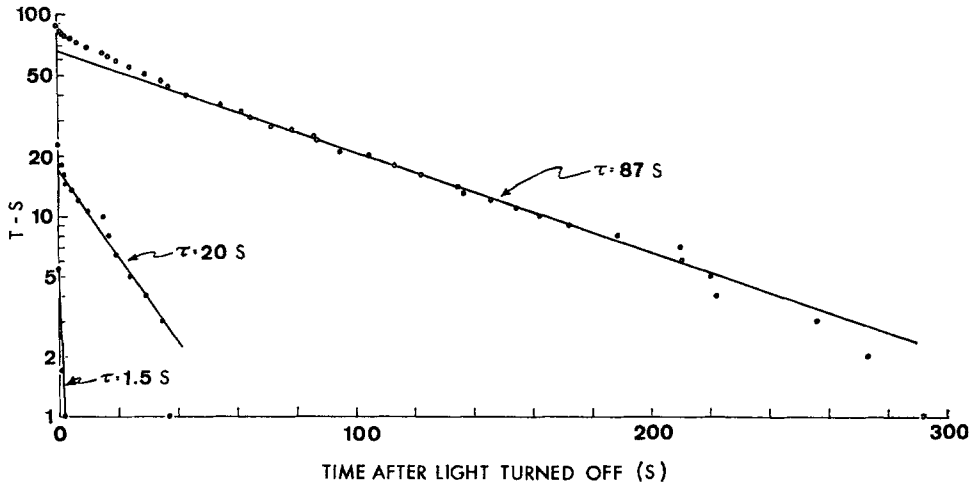


FIGURE 10. Semilogarithmic plot of the decay of discharge rate after light is turned off for the 300 s stimulus of Fig. 7. $T$ is the total number of spikes elicited and $S(t)$ is the sum of the spike count from $t = 0$ (when the light was turned off) to $t$. Upper points are $T$-$S(t)$. See text for explanation of straight lines and lower points.

In two experiments of this type, values of $\tau_3$ have ranged from 27 to 125 s, $\tau_2$ from 12 to 20 s, and $\tau_1$ from 1.3 to 1.5 s, although for the shorter-duration stimuli it was often not possible to estimate $\tau_1$ or $\tau_2$. In general $\tau_1$ and $\tau_2$ increase with increasing stimulus duration, as does $\tau_3$. The Appendix provides an alternative description of the decay of discharge rate after illumination is turned off.

We do not have data of the same form as shown in Fig. 10 for different intensities, but Figs. 12 and 13 present data from a comparable experiment. In this case constant-intensity flashes were presented once every 3 s during and after steady illumination at several intensities. The repetitive flashes serve as test stimuli enabling us to measure the time-course of excitability, although the

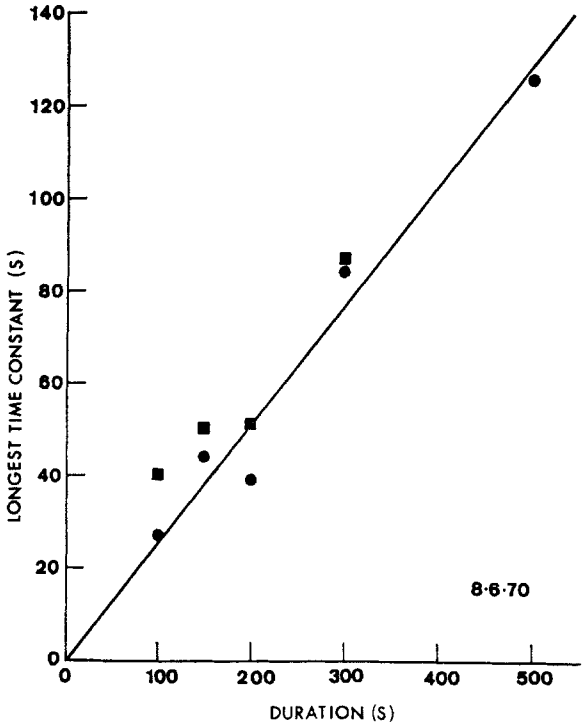


FIGURE 11. Plot of longest time constant, $\tau_3$, (as in Eqs. 1–3, 7, 8) in seconds, vs. stimulus duration, $D$, in seconds, for two experimental runs on the preparation of Figs. 7, 8, and 10. Circles from run taken 2 h after squares. Straight line indicates: $\tau_3 = 0.39\,D$.

responses here are actually a mixture of off-responses to the preceding background and to the repetitive flashes. The number of spikes elicited using this paradigm was greater than that with a totally dark OFF period, so that statistical fluctuations of the data were reduced.

In Fig. 12, responses to the repetitive flashes are almost completely suppressed during all background lights of intensity greater than −2.5 log units. After the background is turned off, the number of spikes occurring after successive flashes is at first large and then decays over several hundred seconds.

The decay of the off-response, after the −0.6 log unit background illumination in Fig. 12, is plotted in Fig. 13. Fig. 13 is plotted in the same manner as Fig. 10, except that here the amplitudes refer to spikes per flash rather than spikes per second. Here there is only one point which does not fit the second exponential, so that the shortest time constant cannot be estimated. The data of Fig. 13 are well described by:

$$T - S(t) = 12\,e^{-t/\tau_1} + 40\,e^{-t/5} + 72\,e^{-t/43}, \qquad t > 0, \tag{10}$$

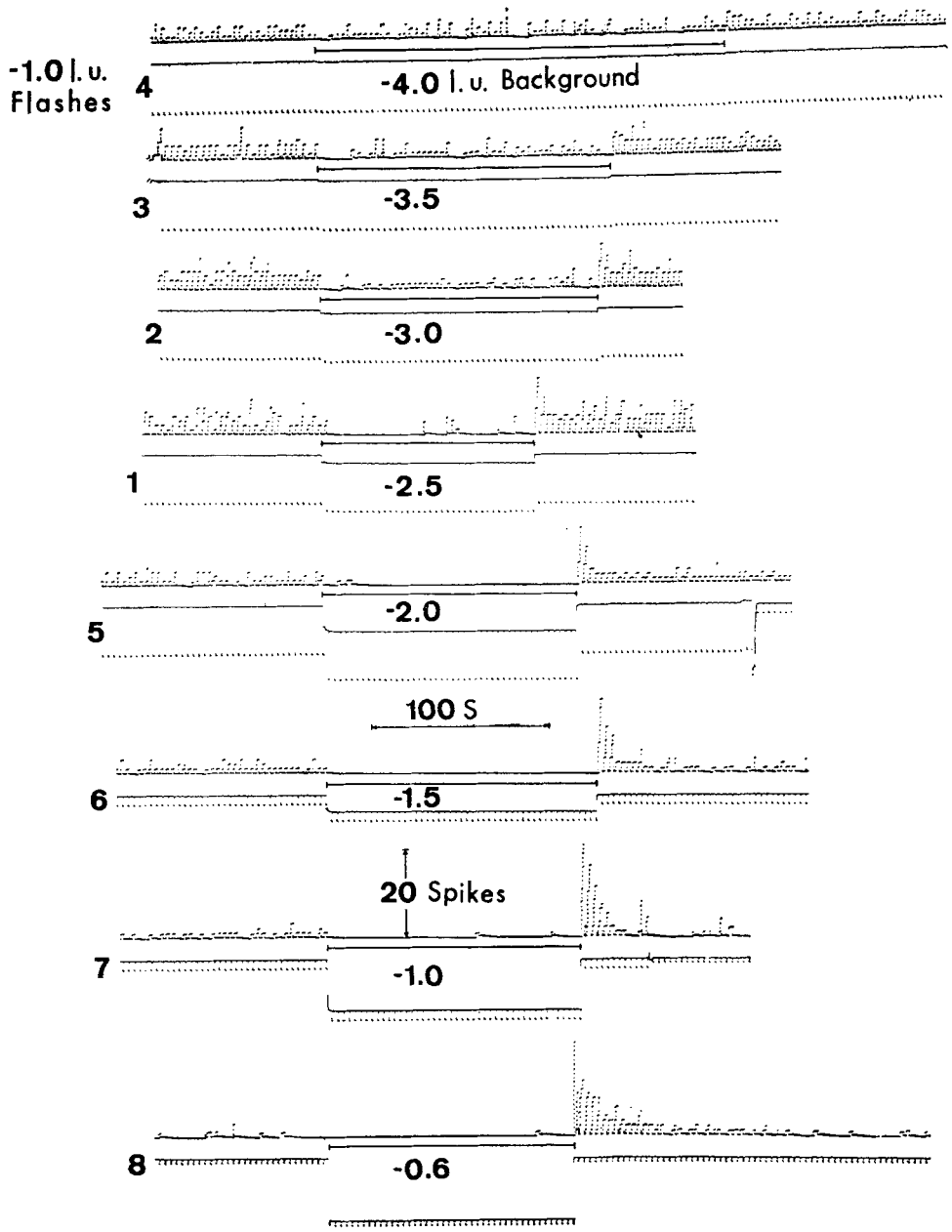


FIGURE 12. Integrator output (upper trace of each pair) and stimulus marker (lower trace-downward deflection indicates light ON) for experiment in which −1.0 log-unit intensity flashes of 0.5 s duration were presented once every 3 s. Integrator level reset to zero at the OFF of each flash. In successive rows steady background lights were on during period indicated by solid bar between stimulus marker and integrator output traces. Both flash and background spot diameters were 650 μm, and were concentric; both had spectral peak at 510 nm. Durations of background lights: 118 s for −2.5 log unit, 140 s for −2.0 log unit, 148 s for −1.5 log unit, 140 s for −1.0 log unit, 134 s for −0.6 log unit. Figure shows records obtained in the order indicated by the numbers at the left of each row. Total duration of figure, 48 min, 40 s. Record 8-7-70 II–III.

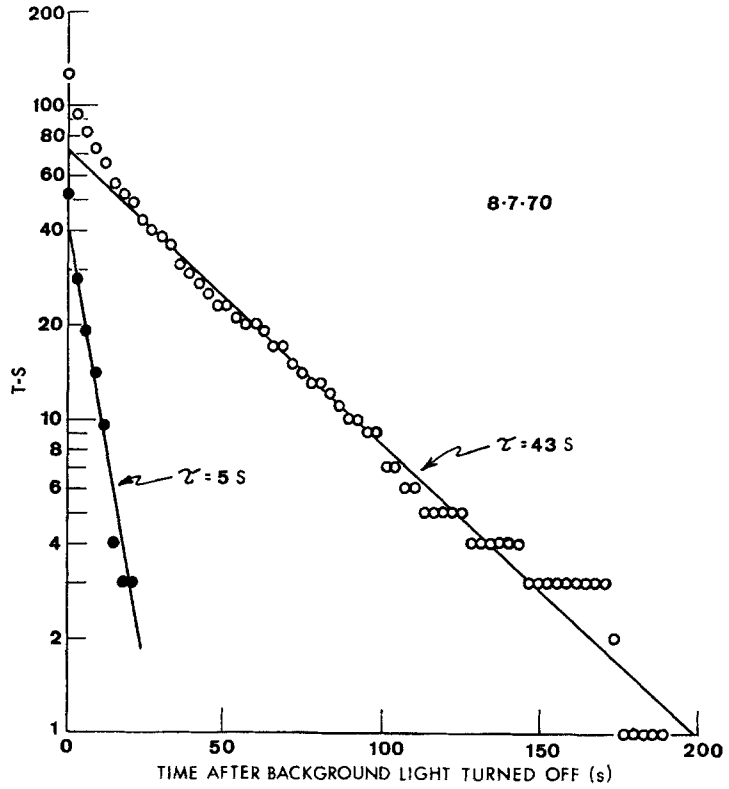


FIGURE 13. Semilogarithmic plot of decay of off-response to 0.5 s test flashes after steady background light turned off. Upper points are $T$-$S(t)$, straight lines and lower points as in Fig. 10 except that here $S(t)$ is expressed as spikes per flash rather than spikes per second (see text). Data of Fig. 12.

and

$$R(t) = \frac{12}{\tau_1} e^{-t/\tau_1} + 8\, e^{-t/5} + 1.67\, e^{-t/43}, \qquad t > 0. \tag{11}$$

Fig. 14 shows that the longest time constant, $\tau_3$, is inversely related to the background intensity. The data illustrated in Fig. 14 are from the same experiment as Figs. 12 and 13. In two experiments of this type, $\tau_3$ varied from 43 to 304 s, $\tau_2$ from 1.5 to 5 s and, since measurements were only made here at 3 s intervals, $\tau_1$ could not be estimated.

The cases in Fig. 12 in which the background light only partially suppresses the flash response enable us to follow the time-course of the inhibitory effect during the background light. For even the weakest backgrounds ($-3.0$, $-3.5$, and $-4.0$ log-unit intensity) the response suppression develops too rapidly to be observed with this method, as the response to the first flash occurring during

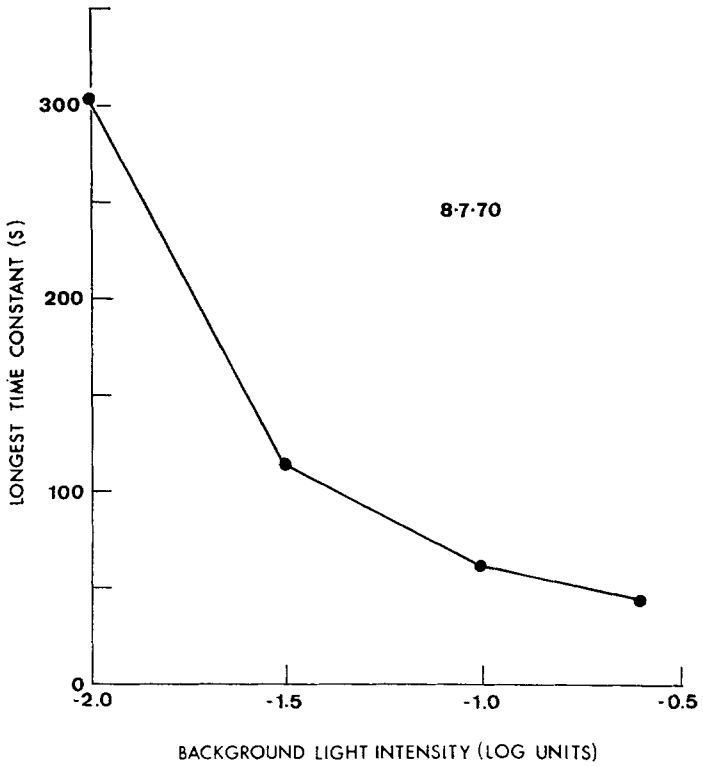


FIGURE 14. Plot of change in longest time constant, $\tau_3$, (in seconds), with intensity of background illumination (in log units). Data from same experiment as Figs. 12 and 13.

the background light is already completely suppressed. We do not have data with sufficient time resolution to describe the time-course of the onset of response suppression when a steady light is turned on. From the data of Fig. 12 it is apparent that suppression can be complete within 3 s even for backgrounds 3 log units less intense than the test flash. (The flash and background spot sizes were equal in this experiment).[5]

After the initial, maximum response-suppression seen in the upper three records of Fig. 13, the responses increase to an approximately steady value, lower than that observed before the background was turned on. Although statistical fluctuations are too great to allow a precise description of this time-course, the growth of the off-response during a background light appears to be approximately exponential. The time-course of the off-response, $R$, for the

[5] In this experiment the physiological equivalence of equal intensities in the flash and background channels was checked by alternating light between the two repetitively and observing the intensity range over which a null in the response was achieved. The center of this null range indicated that two channels were equivalent within 0.1 log units of intensity.

−4.0 log-unit record of Fig. 12 approximately follows the equation:

$$R = \begin{cases} A_1 & t < 0 \\ A_2(1 - e^{-t/\tau}) & t > 0 \end{cases}. \quad (12)$$

$R$ is the number of spikes per flash, $A_1$ and $A_2$ are constants, (2.1 and 1.84 spikes/flash respectively in this case), $\tau$ is approximately 30 s and $t = 0$ is the time at which the background is turned on. $A_1$ was computed as the average number of spikes per flash, averaged over the 20 flashes preceding the background. From Fig. 12 it can be seen that $A_2$ decreases with increasing background light intensity and, in this case, is essentially zero for backgrounds from −2.0 to −0.6 log-unit intensity. Eq. 12 does not describe the development of response suppression (i.e. it assumes the suppression is developed instantaneously at $t = 0$) since we were unable to measure this faster time-course.

In this experiment the threshold for spike responses synchronized to flashes was −1.5 log units. This is 2.5 log units more intense than the lowest background which can be seen to suppress flash responses in Fig. 12.

A response property with a time-course even longer than those described above is evident in Fig. 12. Over the total time of this figure (48 min, 40 s) the response to the test flashes decreases gradually. The average response for the 20 flashes preceding the −2.5 log unit background was 3.4 spikes/flash, whereas the average of 20 responses before the −0.6 log unit background was 0.25. The latter averaging period began 41 min 26 s after the former. This is typical of our experience with repetitive transient stimuli; even when early stimuli in a sequence elicited strong responses, over a period of minutes or hours responses decayed to zero. The response could be restored with long, bright lights.[6]

Preceding illumination also affects the latency and time distribution of the off-response. Fig. 15 shows poststimulus-time histograms of the number of spikes after successive 1 s flashes. The top half of the figure presents data for which the preparation was kept in total darkness for 20 min before the first flash. The data in the bottom portion were obtained after 18.5 min of continuous light. For the first flash given in the dark-adapted state (histogram 1 in the upper portion of Fig. 15), the first spike did not occur until more than 6 s after the flash. In other similar experiments the first spike occurred as late as 12 s after the flash. For the first flash in Fig. 15, the mean of the spike-time distribution (i.e. "center of gravity" of the histogram) was nearly 13 s after the flash

[6] This long-term nonstationary property of the response to repetitive stimuli makes it difficult to average responses sequentially, as would be desirable to elucidate the time-course of the development of response suppression in the experiment of Fig. 12.

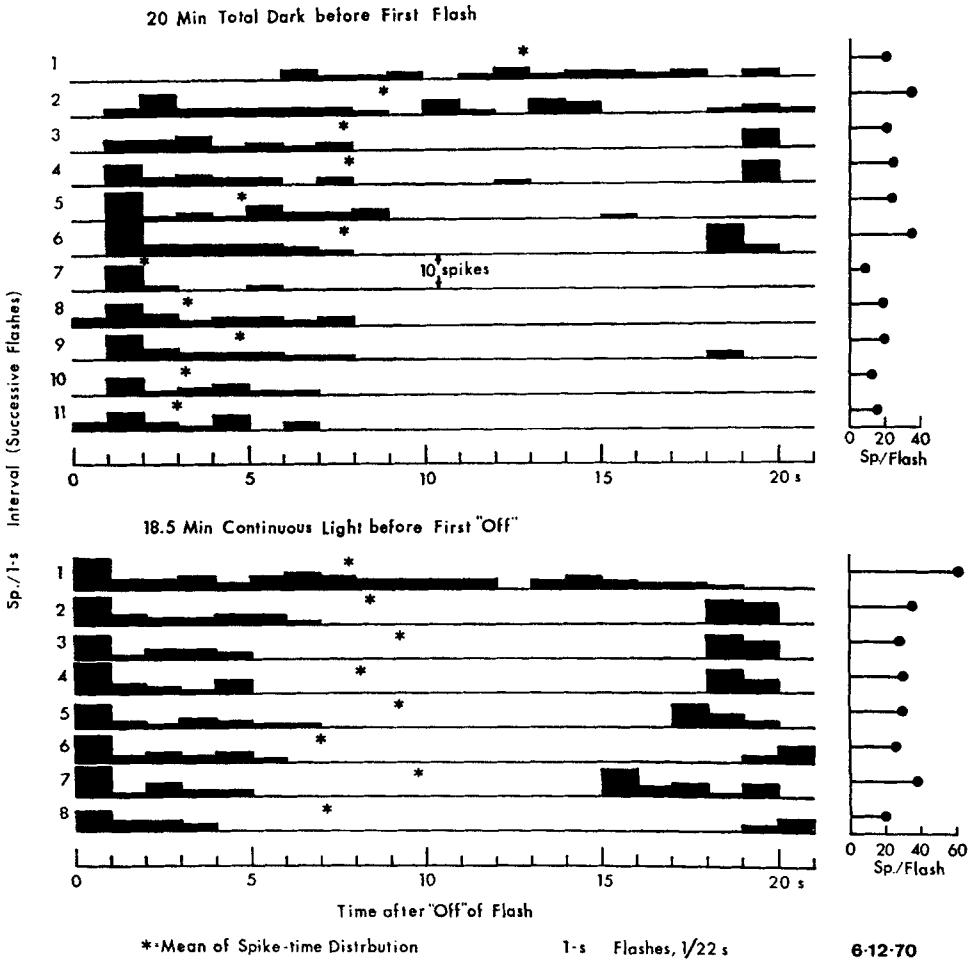


FIGURE 15. Left plots: Poststimulus-time histograms of spike occurrences after successive 1 s flashes presented once every 22s. $t = 0$ corresponds to the OFF of each flash. Bin width = 1 s, * = mean of spike-time distribution. Right plots: total number of spikes occurring after each flash. In the upper portion of the figure the preparation was in total darkness for 20 min before first flash. In the lower portion light was on continuously for 18.5 min before being turned off at the beginning of histogram 1. (Histogram 1 in the lower portion is the off-response to the 18.5 min light; all others are off response to 1 s flashes). Light intensity = −0.7 log units, spectral peak at 510 nm, spot size not measured. Single fiber recording. Record 6-12-70.

ended. During successive flashes the time distribution moved in to shorter and shorter latencies so that by flash number 11 the mean of the distribution was at 3 s and for flashes 8 and 11 spikes occurred within the 1st s after the flash. Presumably this progressive dimunition in response latency was due to the accumulated light-adapting effect of the flashes even though the light-time

fraction was less than 5% here. Note that, in this case, the reduction in latency was not associated with a consistent increase in the total number of spikes in the off-response.

In the lower portion of Fig. 15 every flash elicits more spikes in the first 1 s interval than in any other such interval. Thus the earliest spikes to occur after a flash occur significantly earlier in the light-adapted than in the dark-adapted state.

It can also be seen in Fig. 15 that the responses are greater in the light-adapted state. In the dark-adapted state the average number of spikes per flash was 21.5 whereas in the light-adapted state the average response, excluding the off-response to the 18.5 min illumination, was 29.6 spikes, 38% greater than in the dark-adapted case.

As the latency decreased after successive flashes in the upper portion of Fig. 15, so did the spread in the time distribution. If this finding is typical of all photoreceptor fibers in the siphonal nerve, the increased synchrony of discharges during progressive light adaptation could bias the interpretation of recordings of summed potentials made with gross electrodes. The increased synchrony could produce greater gross-response amplitudes which would not necessarily imply a greater number of spikes.

A rather curious phenomenon which was often observed can be seen in the lower portion of Fig. 15. For flashes 2 through 8 there was a bimodal distribution of spikes with one group occurring directly after the flash and another group coming just before the next flash. When such a repetitive series of flashes was terminated, spikes would typically occur immediately preceding the time when the first omitted flash would have occurred and then no more spikes occurred. A related observation was made several times using 0.5-s flashes presented repetitively at 1/s. The response would "cycle" with an "epoch" of 8–12 flashes: for 4 or 5 flashes no response would occur and then one or two spikes would occur very late in the period. Over the next two or three flashes more spikes would occur with shorter latency and then for two or three flashes the latency would again increase and the number of spikes would decrease until no response was seen, at which point the cycle would repeat. In one case such cycling continued with an epoch of 10 or 11 flashes for over 30 min.

An attempt to measure the dependence on light intensity of the latency to the first spike of an off-response is presented in Fig. 16. Successive points are successive latency measurements and the arrows indicate when the intensity was changed (the direction of the arrow indicates the direction of change of intensity). The long-term nonstationary property, discussed in connection with Fig. 12, here precludes our describing a unique relationship between intensity and latency. In general the latency increased with decreasing intensity, but a true steady state was not reached at any intensity. This was most evident at the

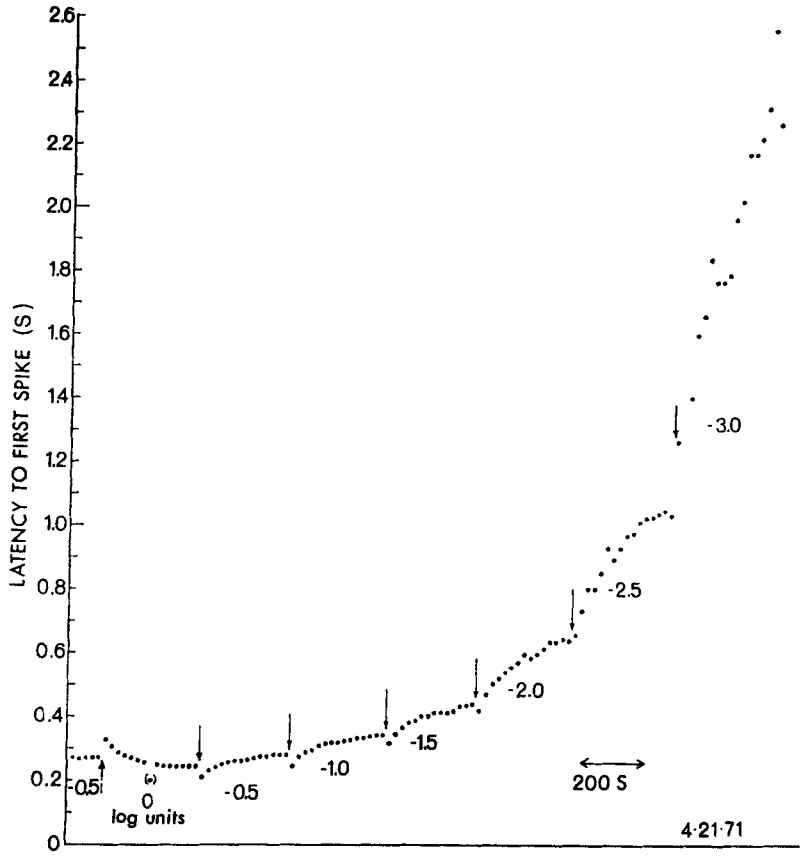


FIGURE 16. Successive determinations of latency to the first spike of off-response to 10-s flashes presented once every 20 s. Flash intensity varied from −0.5 to 0 then in 0.5 log-unit steps to −3.0 log units. Arrows indicate time at which intensity was changed (an upward-pointing arrow indicates increasing intensity, downward-pointing arrows indicate decreasing intensity). Point surrounded by parentheses (0 log units) was a smaller spike, all other points represent the same large spike, (0 log-unit intensity is not the same flux as in the other figures). Record 4-21-71.

lowest intensity (−3.0 log units) where over the course of 15 measurements (5 min) the latency increased from approximately 1.3 to 2.6 s.

Note in Fig. 16 that, at low intensities, each time the intensity was increased, the latency first *decreased* and then over succeeding measurements increased to values greater than those found at the higher intensity. This appears to be related to a phenomenon seen in Fig. 5: when the intensity of repetitive flashes was increased, the number of spikes elicited first decreased and only after subsequent flashes increased. Since the direct effect of light is inhibitory in this system, these transient phenomena may reflect a net inhibitory effect of the increased time-average light intensity when flash intensity is increased.

### E. *Action Spectra*

Flashes of light were presented alternately through a variable wavelength measuring channel and a reference channel (see Methods, part C). The illumination by the reference channel was adjusted to produce an "integrated" discharge of maximal amplitude when followed by total darkness. A criterion response of half the amplitude produced by total darkness was used to adjust the intensity of the variable wavelength measuring channel. This was done to assure that the adapting effect of the reference beam was greater than that of the measuring beam. The lamp currents causing criterion responses were determined at a number of equally spaced wavelengths.

To test for multiple pigments the results with no filter in the reference beam were compared with those obtained with a blue reference (Wratten no. 47 filter, dominant, wavelength 470 nm) and a red reference (Wratten no. 25, cutoff wavelength 600 nm).

Fig. 17 shows raw data obtained under these conditions. If more than one pigment were present it would be expected that there would be significant changes in the shapes of the curves as one pigment or the other was more strongly affected by the reference beam. There was never any evidence of this or of a qualitative change in the discharge pattern of the nerve fibers (for example, an "on-off" transition). Thus unless there are two nearly identical pigments both of which produce a discharge by the same mechanism it can be concluded that only a single pigment is involved.

Further evidence of this is the close fit of the action spectrum of the pigment derived from similar measurements to the spectrum of rhodopsin. Fig. 18 shows the average action spectrum obtained with white reference illumination from twelve sets of measurements on three animals. The current readings were converted to relative quanta and the ratio of the quanta to give a criterion response at the wavelength of maximum sensitivity to the quanta required to give the same response at other wavelengths calculated according to the method given by Dartnall (1962). This curve is plotted on a reciprocal wavelength scale. As Dartnall has shown rhodopsin-like pigments have absorption curves which are invariant in shape when so plotted regardless of the wavelength of maximum absorption. The solid line is the relative absorption of rhodopsin shifted to give the best fit to the experimental points. This curve has a maximum at about 510 nm. It can be seen that there is a reasonably good fit of the experimental points to the rhodopsin spectrum.

Thus it is likely that the photopigment in *Mercenaria* is of the rhodopsin type.

## DISCUSSION

The physiological results described above can be briefly summarized as follows:

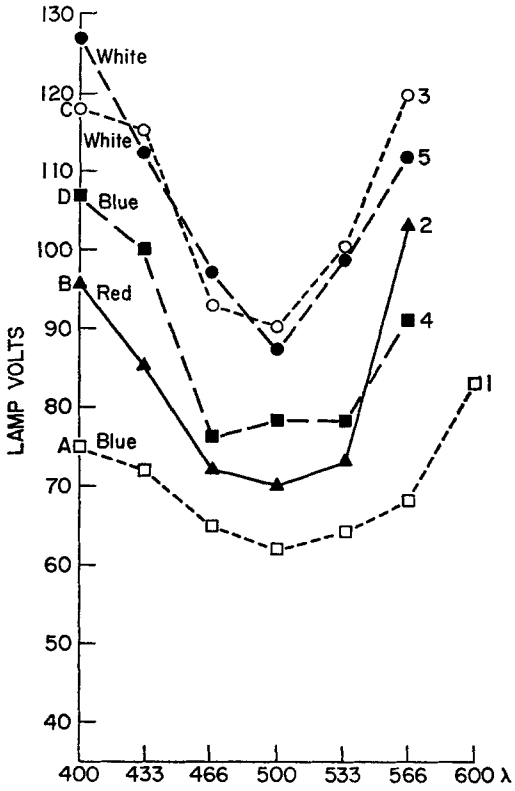


FIGURE 17. Raw data showing the effect of various wavelengths of adapting light upon the responses of the siphonal nerve fibers of *Mercenaria*. A constant response criterion was used at each wavelength. This was the stimulus intensity required to reduce the peak of the integrated nerve activity during the test illumination to one-half of the value obtained when the test light was completely extinguished. Numbers to the right of the curves indicate the sequence in which the tests with different colors of adapting light were carried out.

(*a*) Photoresponsive axons in the siphonal nerve of *Mercenaria* produce spike responses only when illumination is decreased.

(*b*) The responsive area of an individual fiber can be localized to a circle of about 85 $\mu$m diameter on the inner siphon wall.

(*c*) The number of spikes elicited at the OFF of a brief flash grows approximately as the 0.4 power of flash intensity over at least 2.5 log units.

(*d*) The total number of spikes and the initial discharge rate increase with increasing ON duration; for long durations the response increases approximately as the logarithm of duration.

(*e*) The discharge rate and excitability of a fiber decay approximately exponentially after illumination is turned off; the time-course of decay can be described as a sum of exponentials whose time constants vary from 1 to 300 s.

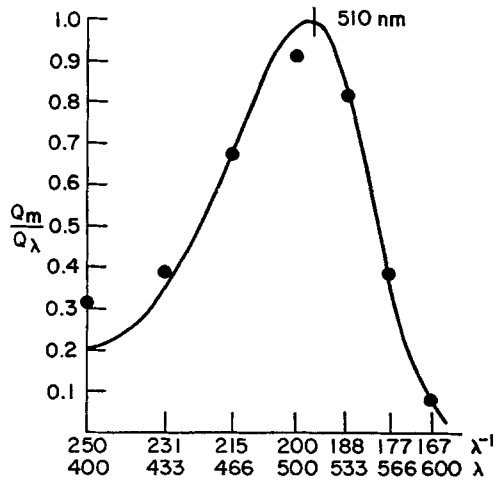


FIGURE 18. Averaged action spectra from 12 experiments. The ratio of the number of quanta required at the wavelength of maximum sensitivity to the number required at each wavelength is plotted as a function of the reciprocal of the wavelength. The solid curve is that of a Dartnall pigment giving the best fit to the experimental points. The test illumination was alternated with a fixed white light and adjusted to give zero response at each transition. White light was used since previous experiments indicated that the spectral composition of the reference illumination has no effect upon the action spectrum.

When a constant background light is turned on, inhibition becomes maximal in less than 3 s and then decays with a time constant of approximately 30 s.

(*f*) For a given stimulus, the response is greater and the latency to the first spike less in a light-adapted than in a dark-adapted state.

(*g*) Cyclic response patterns and paradoxical transient behavior appear to result from interaction between the direct inhibitory effect of light and the increased excitability after illumination.

(*h*) The spectral sensitivity indicates a single photopigment of the rhodopsin type maximally sensitive to light of 510 nm wavelength.

We cannot explain the discrepancy between our finding only dimming-responses and Kennedy's (1960) finding both on- and off-responses. It is possible that we have consistently missed one type of photoresponsive axon. This seems unlikely since we have recorded from over 30 animals and have used wick electrodes recording from either strands of the siphonal nerve or the whole nerve, as well as suction electrodes, with which single-fiber responses can be recorded. In none of these situations have we observed on-responses.

Our results can be compared with both Kennedy's (1960) for *Spisula* and Land's (1966) for *Pecten*. In *Spisula* the off-response using white light grows over the lowest 2 log units of stimulus intensity and then decreases over 2 log units. The difference between these results and our finding of a monotonic

growth of response with intensity are probably attributable to the two-pigment system in *Spisula*. Our results indicate that only one pigment is found in *Mercenaria*. In *Pecten*, a monotonic power function relationship holds between response and stimulus intensity over one log unit, although the exponent is lower than for *Mercenaria*. McReynolds and Gorman (1970 *b*) have shown that the *Pecten* off-receptors have spectral sensitivity indicating a single pigment. In *Spisula* the off-response grows as the log of stimulus duration from 0.01 to 100 s; in *Pecten* a similar growth was observed for durations from 5 to 80 s. Neither Kennedy nor Land describes the full extent of the time-course of the off-response, although Kennedy's plot of the initial decay appears similar to what we have seen (Figs. 10 and 13). Kennedy shows that maximal inhibition is developed within 1 s in *Spisula* which is similar to our observations in *Mercenaria*. As we have observed in *Mercenaria*, subthreshold stimuli can interrupt a long-lasting off-response in *Pecten*. Land (1966) reports a dependence of response latency on the state of adaptation similar to what we observed (Fig. 15). In both *Pecten* and *Spisula* the latency of the off-response is a monotonically decreasing function of stimulus intensity, as it is in *Mercenaria* (Fig. 16).

Since we have no way of tracing the fibers from which we have recorded to their distal ends, we cannot be certain that the cells and their associated nerve fibers described in the anatomy section are instrumental in phototransduction. We must rely on analogy with Light's (1930) conclusions and the fact that there do not appear to be any other innervated structures in the *Mercenaria* siphon which would be likely candidates for photoreceptors. The cells described here appear almost identical in their fine structure to those in *Mya arenaria* which Bell and Anderson (1970) have identified with the cells Light concluded were photoreceptors. If the inference that the cells described do participate in photoreception is valid, we would expect transduction in *Mercenaria* to occur in the distal ends of the axons. The cells containing crystalline inclusions do not resemble any photoreceptors described previously. They appear to be either pigment cells or optical elements serving to concentrate light on the axon endings. If this is the case, the *Mercenaria* photoreceptor system may function as Kennedy (1960) has suggested for *Spisula*, i.e., the photopigment is postulated to be located in the axon itself, and presumably this is where photoreception occurs. The whorled configurations appearing on the axon processes resemble the ciliary portion of the receptor cells in the distal retina of *Pecten* (Barber, Evans, and Land, 1967). However, in *Mercenaria* the whorls do not appear to arise from cilia. While we have no way of determining whether or not these whorls contain the photopigment, we suggest that they be considered as candidates for this role in *Mercenaria*. In *Spisula*, Kennedy reports that photoreception occurs along the axon near the visceral ganglion.

From the experiments using small shadows, we would conclude that the effective stimulus location in *Mercenaria* is at the distal end of the axon near the inner siphon wall.

In view of the similarity between our results and those of Land (1966) for the off-fibers of *Pecten*; it is interesting to speculate that similar cellular mechanisms are involved in the two systems. McReynolds and Gorman (1970 *a*) show that the *Pecten* distal receptors respond with a maintained hyperpolarization as long as they are illuminated. Thus we might expect the distal processes of the axons from which we have recorded to be hyperpolarized by light also.

The most striking features of the data we have presented are the long time constants observed: the off-response to constant-intensity stimuli continues to grow with stimulus duration for more than 8 min and the discharge rate after illumination decays with a time constant of up to 5 min. Although it is difficult to decide which of many possible mechanisms could account for these phenomena, Adelman and Palti (1969) have studied similar long-term excitability increases in squid axons. Using voltage-clamp techniques they demonstrated that the value of the peak inward sodium current was a function of conditioning hyperpolarization over a range of prepulse durations from less than 1 ms to over 3 min. The effect of hyperpolarization in overcoming sodium inactivation increased smoothly with prepulse duration through two plateaus and was just beginning to saturate with prepulses of about 5 min duration (cf. Adelman and Palti, 1969, Fig. 7). On the basis of these experiments, one should expect that posthyperpolarization excitability should also have time constants in the range of many seconds. Indeed such long time constants are associated with a decreased threshold after hyperpolarization as described by Guttman and Hachmeister.[7] Adelman and Palti (1969) attribute these long-term phenomena to the depletion of ionic concentrations in the restricted space surrounding the squid axon (the S-space of Frankenhaeuser and Hodgkin, 1956). The axon processes bearing whorls, shown in Figs. 1 and 2, are located in a constricted space formed by the indentation in the large irregular cell. The currents associated with a long lasting hyperpolarization of the axon processes could slowly change the ionic composition in this restricted space which would lead to increased posthyperpolarization excitability of the photoreceptor axons. Such a process could reconcile the direct inhibitory effects of illumination with the facilitating effect of light adaptation, and account for the long time constants encountered.

## APPENDIX

Fig. 10 indicates that, for times greater than 50 s after illumination is terminated, the decay of discharge rate is well described by a single exponential. However, the

[7] Guttman, R., and L. Hachmeister. 1971. Anode break excitation in space clamped squid axons. In preparation.

earlier decay required the summation of two or three exponentials to adequately describe the data. Fig. A1 (which illustrates the same data as Fig. 10) demonstrates that the early decay is well described by a fractional power of $t$. Fig. A1 is a plot of the summed response up to time $t$, $S(t)$, vs. $t$, plotted on log-log paper. Over the first 50 s, $S(t)$ is described by:

$$S(t) = 5\, t^{0.59}, \qquad t > 0. \tag{1a}$$

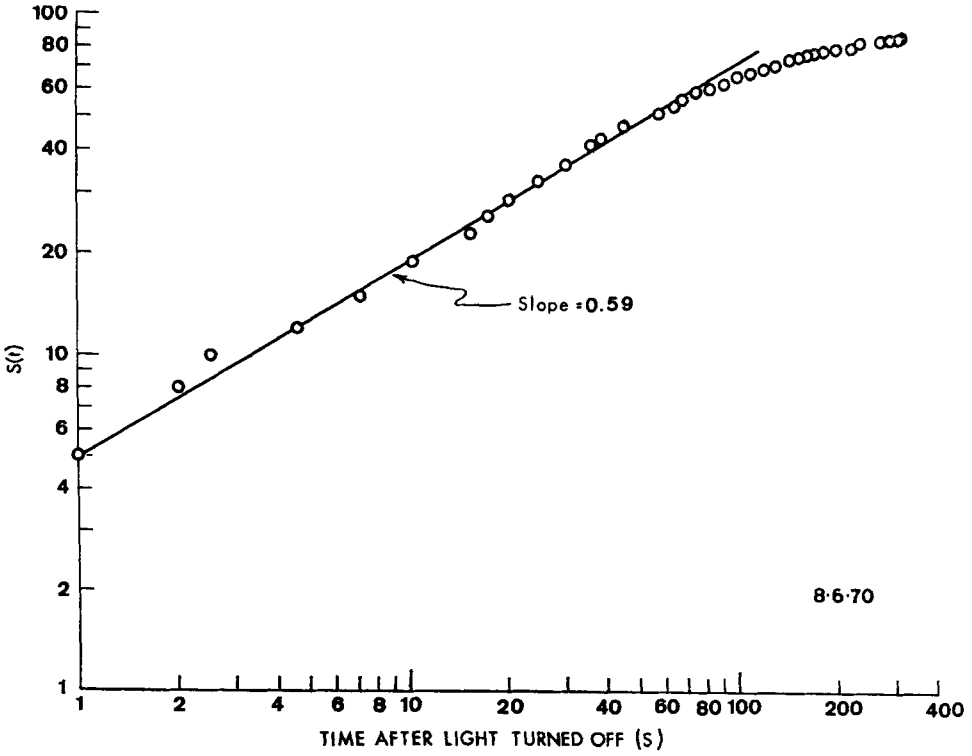


FIGURE A1. Log-log plot of $S(t)$ (the total number of spikes counted up to time $t$) vs. $t$ for times after light turned off. Same data as illustrated in Figs. 7 and 10.

Since $R(t)$ is the derivative of $S(t)$, we obtain:

$$R(t) = \frac{8.47}{t^{0.41}}, \qquad t > 0. \tag{2a}$$

In general, the early decay of discharge rate can be described by:

$$R(t) = \frac{B}{t^{a}}, \qquad 0 < t < \sim 50 \text{ s}. \tag{3a}$$

Fig. A2 illustrates the dependence of the exponent, $a$, on stimulus duration for the

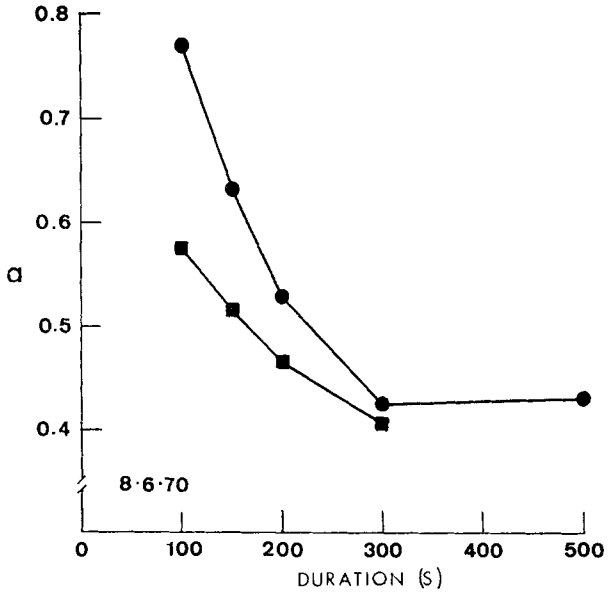


FIGURE A2. Plot of $a$ (exponent obtained by fitting the early decay of discharge rate with $R(t) = B/t^a$) vs. stimulus duration. Same data as Fig. 11, circles and squares as in Fig. 11.

same two experimental runs as illustrated in Fig. 11. In these experiments $a$ varied from approximately 0.4 to 0.8.

Similarly, the early decay of flash response after a steady background illumination (e.g., Figs. 12 and 13) is well described by a fractional power of $t$. In the experiment of Figs. 12 and 13, the appropriate exponent, $a$, corresponding to the formulation of Eq. 3 $a$, increased from 0.3 to 0.5 as the intensity of the background was increased from $-2.0$ to $-0.6$ log units.

Thus the decay of discharge rate after illumination can be described by a negative fractional power of $t$ for the early decay and a single exponential at times long after the stimulus. Whereas the longest exponential time constant, $\tau_3$, increases with increasing stimulus duration, the exponent $a$ decreases with increasing duration. Both of these changes indicate a more gradual decay of discharge rate with increasing stimulus duration. Describing the decay of flash response after a steady background light, $\tau_3$ decreases and $a$ increases with increasing intensity, indicating a more rapid decay with increasing background intensity.

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