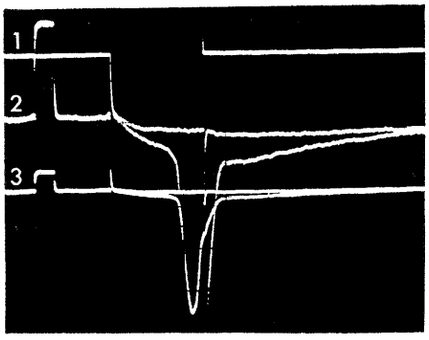

Fig. 4. Flash-triggering action potential arising from slow potential elicited by tactile stimulation of *Noctiluca*. Two sweeps, one with subthreshold stimulus. Interruption of trace 1 indicates duration of current applied to piezoelectric crystal of tactile stimulator. Traces 2 and 3 display intravacuolar potential at two different gains, the calibration pulse on each representing 10 mv and 10 msec. Note graded nature of the slow potential.

the vacuolar potential an additional 40 to 70 mv more negative. Maximum amplitude is attained in 5 to 8 msec. Return to the vacuolar resting potential is slower and typically occurs in two stages, the second phase being the slower. Frequently, the vacuolar potential continues in the positive direction up to 20 mv beyond the resting level for a period of several hundred milliseconds. Since the intravacuolar electrode records across at least two membranes, the true voltages across the active membrane are not known.

Externally recorded, the potential is essentially triphasic (Fig. 2). An initial slow negativity with a maximum recorded deflection of 0.1 mv is followed by a faster, positive-going "spike" (0.3 mv maximum, 1 to 2 msec duration). The spike component is followed by a typically notched negative wave of intermediate amplitude. The form of the external potential is interpreted as the result of a passive-active-passive membrane current sequence (*16*).

The light flash begins early during the rising phase of the internally recorded potential, with a latency of about 3 msec between stimulus and initial signs of emission (Fig. 1). The maximum flux from an unfatigued cell is typically between 0.5 and 1.5 $\times$ 10^8 photons per msec, and the total quantum content of a typical unfatigued flash is 2 to 5 $\times$ 10^9 photons. The emission spectrum has a peak at approximately 470 mμ (*2*).

Flashes are all-or-none (*17*) although summation and facilitation are readily demonstrable (Fig. 3). The action potential is of constant amplitude, except at high stimulus rates when it falls within the refractory period of a previous action potential (Fig. 3). Facilitation, therefore, appears to have its origin in the coupling step or steps between the action potential and the luminescent reactions. Repetitive stimulation at intervals of less than about one minute causes a decrease to a frequency-related steady state of both the maximum flux and the photon content of each flash.

The luminescent response of *Noctiluca* is, of course, normally evoked by mechanical energy. Therefore an examination was made of flash initiation by tactile stimulation with abrupt excursions of a fine glass probe mounted on a piezoelectric phonograph crystal. Subthreshold stimuli evoke graded, slow potentials which are negative-going in the vacuole. Increasing the excursion increases the magnitude of the slow potential to a point where it develops into the all-or-none flash-triggering action potential (Fig. 4). The graded, slow potential is reminiscent of the "generator" potential recorded in mechanoreceptors and most other metazoan receptor cells (*18*). No light emission occurs in response to mechanical stimuli unless an action potential is evoked, regardless of the magnitude of the slow, graded potential. A somewhat similar graded potential gives rise to the action potential when it is initiated by long, threshold-level currents delivered with an intravacuolar polarizing electrode. Again, no emission is seen, even at high photometer sensitivity, unless the graded potential develops into the all-or-none action potential. The relation between luminescence triggering and potential change is apparently discontinuous.

Coupling between the action potential and the flash appears rigid. A single action potential invariably triggers a single flash; conversely, a flash occurs only in response to an action potential, regardless of the nature of intensity of stimulation.

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7 December 1964

II. Asynchronous Flash Initiation by a Propagated Triggering Potential

Abstract. *The action potential of* Noctiluca miliaris *is conducted over the cell, triggering luminescent cytoplasmic organelles as it propagates away from the stimulus site. Local light emission follows local active current with a latency of 1 to 3 milliseconds. Whereds bioluminescence normally occurs over this cell with an advancing front of emission, it can be initiated synchronously by electrical stimulation of the entire cell.*

The stimulus-evoked flash of the bioluminescent marine dinoflagellate *Noctiluca miliaris* occurs only in response to a characteristic action potential (*1*). Hence, it is postulated that a component of this action potential directly or indirectly triggers the reactions leading

to light emission. This report lends further support to the cause and effect relation between bioelectric events and bioluminescence in this cell by demonstrating that: (i) the action potential is propagated from the stimulus locus over the remainder of the cell surface; (ii) the front of light emission moves over the cell in a progressive manner with a conduction time similar to that of the propagated action potential; and (iii) light emission from luminescent cytoplasmic organelles in the cell periphery occurs with a latency of about 2 msec after local active current flow.

The experimental techniques and relevant morphology are described in the accompanying report (*1*). The initial evidence for propagation was obtained when two widely spaced external recording electrodes were placed on the surface of *Noctiluca,* and recorded an action potential asynchronously when the cell was stimulated by inward current delivered through the holding pipette. The possibility of impulse conduction was more carefully examined by placing one external recording electrode near the site of stimulation, a second electrode about 90 degrees around the cell, and a third electrode approximately opposite the site of stimulation (*1*, *2a*, and *3* in diagram, Fig. 1). Although the positions relative to one another of the stimulating and recording electrodes were usually the same, their relation to cellular landmarks differed from one preparation to another. The vacuolar potential was monitored with a capillary microelectrode (*4* in Fig. 1). Simultaneous recordings from the three external electrodes (Fig. 1*A*) consistently showed the electrode nearest the site of stimulation to have the shortest latency, and the electrode opposite the site of stimulation to have the longest latency. The latency difference between the two extreme recording sites ranged from 3 to 6 msec, or approximately 1 msec/100 μ of cell diameter. The intermediate electrode always showed an intermediate latency. Externally recorded potentials did not occur outside the duration of the action potential recorded from the vacuole and were never observed in its absence.

These spatially correlated latencies are believed to reflect the conduction time of a propagated action potential. The triphasic nature of the locally recorded external potential (trace 1, Fig. 2) is therefore interpreted as the result of (i) inward passive current during the approach of the action potential causing

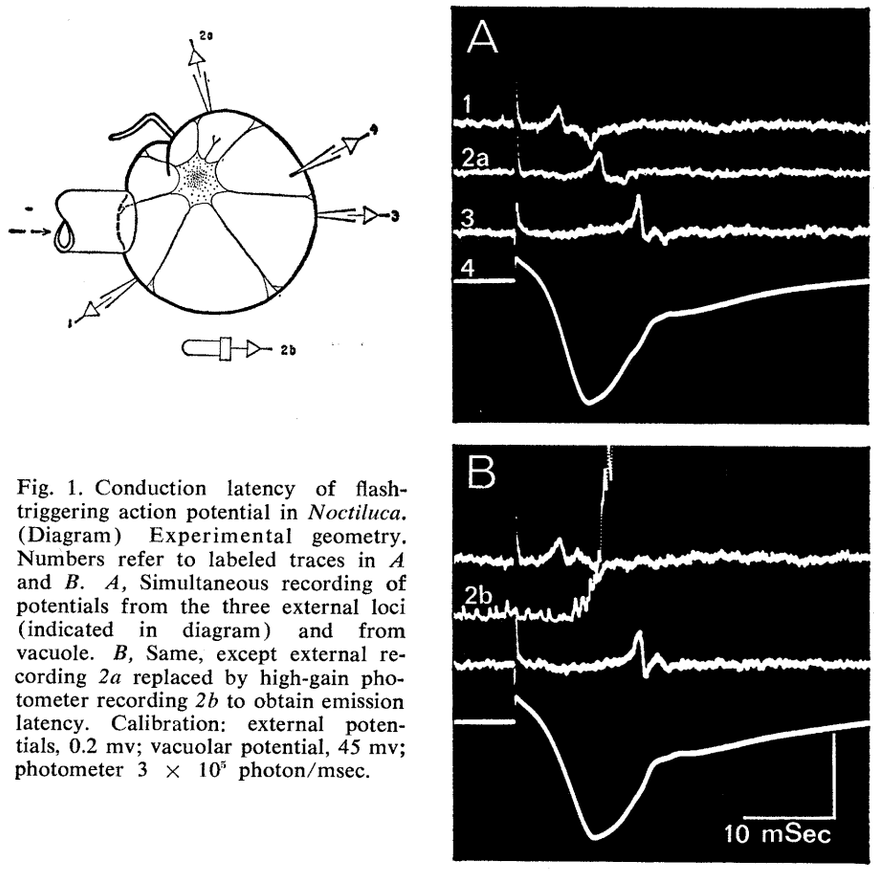


Fig. 1. Conduction latency of flash-triggering action potential in *Noctiluca.* (Diagram) Experimental geometry. Numbers refer to labeled traces in *A* and *B*. *A*, Simultaneous recording of potentials from the three external loci (indicated in diagram) and from vacuole. *B*, Same, except external recording *2a* replaced by high-gain photometer recording *2b* to obtain emission latency. Calibration: external potentials, 0.2 mv; vacuolar potential, 45 mv; photometer 3 $\times$ 10^5 photon/msec.

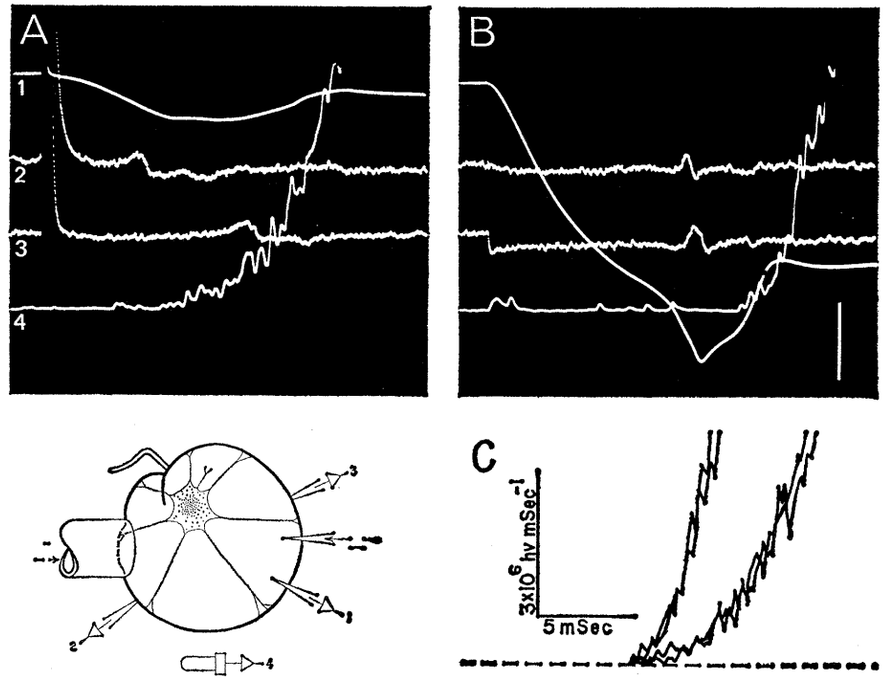


Fig. 2. Asynchrony and synchrony of the flash-triggering system. (Diagram) Experimental geometry. Numbers refer to traces in *A* and *B* of this figure. *A*, Action potential initiated locally by positive current pulse applied through holding pipette. *B*, Action potential initiated synchronously over the entire cell. Note faster rate of rise and greater linearity of initial light emission during synchronous bioelectric activity. *C*, Two tracings each of initial emission rates in response to propagated and synchronous action potentials. Potential calibrations: external, 0.2 mv; vacuole, 80 mv. Photometer and time calibrations in *C* also apply to *A* and *B*.

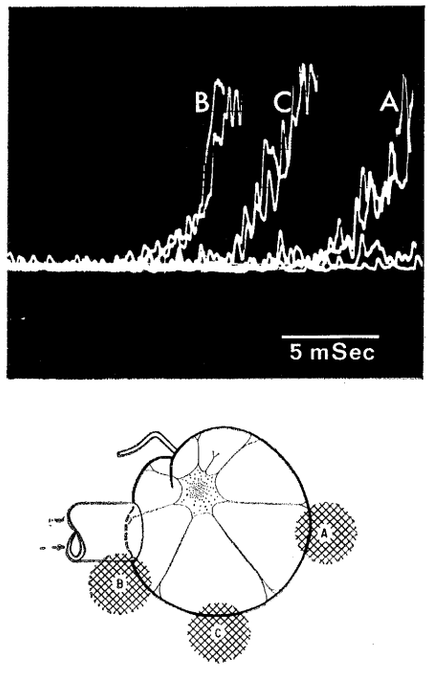


Fig. 3. Emission latencies at selected distances from stimulus site. (Top) Two high-gain photometer recordings from each of the areas labeled in diagram. Stimuli applied coincident with beginning of each sweep. (Bottom) Restricted areas of the periphery were positioned within photometer field in the alphabetic order indicated. Altering this order in separate experiments failed to alter relative latencies.

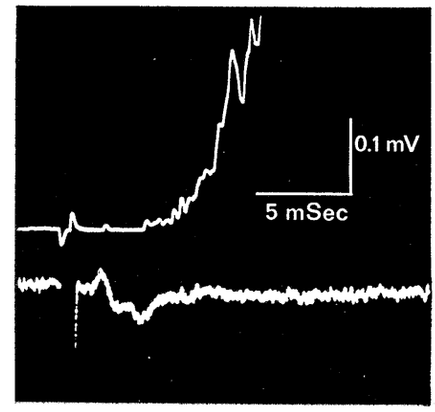
the initial negative deflection, (ii) outward active current causing the positive spike deflection, and (iii) a final passive current deflection. Two observations in particular support these conclusions. First, the size of the spike component is independent of the relative location of the recording pipette with respect to the stimulating pipette. The initial negative-going wave, however, is almost absent near the stimulus site. The converse is true of the final negative wave; it is diminished when recorded 180 degrees from the stimulus site. Second, on rare occasions the positive spike component recorded by one electrode gradually diminishes in amplitude and finally disappears, while remaining concurrently unchanged at other recording sites on the same cell. In such instances, moreover, the negative potentials persist at the blocked site and form a single uninterrupted deflection.

The signs of externally recorded potentials in *Noctiluca* are the opposite of those recorded externally during conduction in excitable cells of the metazoa, and hence, the sequence of current direction must be the reverse (*2*). On the other hand, the unconventional polarity of external potentials is consistent with the negative sign of the internally recorded potential of *Noctiluca*.

Although the ionic basis for the action potential and the identity of the active membrane are uncertain, the following evidence supports the suggestion (*3*) that the membrane at the vacuole-cytoplasm interface is the locus of activity. (i) The action potential responds only slowly to alterations in the ionic composition of the bathing solution. Substitution of propionate for chloride causes a steady but slow decline in the action potential until it is reversibly abolished after about 10 minutes. Replacing major ions other than chloride has little effect on the action-potential amplitude (*4*). (ii) *Inward* current (from sea water through cytoplasm into vacuole) is the effective stimulus for initiating the action potential, even though the vacuolar "resting" potential is negative. If membrane excitation in *Noctiluca* resembles that of orthodox electrically excitable membranes—namely, if it is depolarization rather than hyperpolarization which initiates subsequent membrane conductance changes—it follows that the two membranes bounding the thin cytoplasmic layer must be polarized in opposite directions.

If the cytoplasm is negative with respect to the sea water, then, while the outer membrane has the conventional internally negative polarity, the polarity of the vacuolar membrane is oppositely directed. Stated differently, the two "batteries" are in series but are thought to be opposed, with a large negative drop from sea water to cytoplasm and a smaller negative drop from vacuole to cytoplasm. In this scheme it is the vacuolar membrane which is depolarized by an imposed inward current, and is therefore the probable locus of active current. The developing phase of the action potential, in that case, would have its basis in either the movement of anions from cytoplasm to vacuole, or cation flow in the reverse direction.

Core-conductor theory (*5*) precludes impulse conduction in an internally isopotential cell surrounded by a low-resistance volume conductor, since local circuit current flow cannot take place. For this reason a propagated action potential does not occur in most spherical or subspherical cells. In *Noctiluca*, however, the electrical space relations are complicated by the occurrence of the peripheral cytoplasm as a thin and



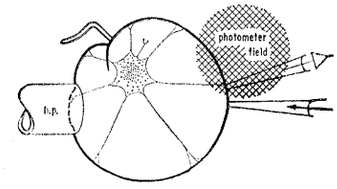


Fig. 4. Latency between local action potential and local emission. (Top) Upper trace monitors local luminescence at high gain, while lower trace displays local changes in the external potential recorded from a locus at which the propagated potential first enters photometer field. (Bottom) Experimental geometry.

tortuous membrane-limited layer between the large internal vacuole and the pellicle (*6*). Hence, the cytoplasm has a high internal resistance and is not isopotential. It is reasonable, therefore, that conduction should occur in this cell, provided the action currents are compelled, during part of their circuit, to flow within the peripheral cytoplasmic layer, parallel to the cell surface. Since the electrical properties of the vacuolar membrane and the plasmalemma are unknown, the distribution of current flow during impulse propagation in *Noctiluca* is uncertain. However, if, as suggested above, it is the vacuolar membrane which is active, conduction probably occurs by a local circuit that begins with local active current flow from vacuole to cytoplasm. Passive flow from cytoplasm back into the vacuole would be distributed according to the electrotonic properties of the cytoplasmic layer. Some current would also be expected to leak across the outer membrane.

What is the relation between the externally recorded action potential, which appears to propagate over the cell from point of stimulus, and the potential recorded from the vacuole? The latter potential has a time course approximately equal to the externally recorded conduction time from one side of the

cell to the opposite side (Fig. 1*A*). Furthermore, it is reasonable to assume that the interior of the vacuole is essentially isopotential and that electrical events occurring locally at one part of the cell periphery are indistinguishable when recorded in the vacuole from similar events occurring at other points. Hence, the vacuolar potential can be expected to reflect the sum of electrical activity at the periphery during impulse conduction.

This interpretation was tested by comparing the shape of the vacuolar potential during normal impulse propagation with its shape during synchronous firing of the entire cell. Two external electrodes were used to monitor activity next to and opposite the stimulus site (*2* and *3* in Fig. 2 diagram) while the vacuolar potential was recorded with a third electrode (*1* in Fig. 2 diagram). When the stimulus consisted of a local inward current through the holding pipette, the external electrodes recorded asynchronous spikes and the internal electrode recorded a typical slow vacuolar action potential (Fig. 2*A*). When the stimulating current was drawn inward through the entire cell surface by an intracellular current-passing electrode, the externally recorded action potential was synchronous, and the vacuolar potential was significantly shortened in time course (Fig. 2*B*). With threshold amounts of current in a variation of the last-mentioned experiment, several degrees of asynchrony were obtained. The degree of temporal compression of the vacuolar potential was then closely related to the degree of peripheral firing synchrony.

Two primary conclusions have emerged thus far from the evidence presented here and in the preceding report. First, the luminescent flash of *Noctiluca* is triggered bioelectrically; and second, the flash-triggering action potential propagates actively in the peripheral cytoplasm.

Taken together, these two conclusions lead to a third, which also is subject to test, namely that luminescence is not initiated simultaneously throughout the cell, but is triggered asynchronously by the conducted action potential. Consideration of Fig. 2, *A* and *B*, trace 4, shows that the emission develops more rapidly and linearly during synchronous discharge of the cell than it does when the action potential is allowed to propagate. Figure 2*C* illustrates the reproducible nature of emission rate differences. During synchronous firing of the active membrane, luminescence appears to initiate simultaneously from all areas, causing a steeper rise of emission rate as recorded from the whole cell.

If luminescence is triggered locally during spread of the propagated potential, luminescence must spread over the cell with an advancing front whose velocity is similar to that of the triggering potential. This was experimentally substantiated by high-gain photometer display of flash initiation at selected portions of the cell periphery, while the cell was locally stimulated with current passed through the holding pipette. A 25× objective was focused to include in its field (and hence on the photocathode of the multiplier tube) limited portions of the cell periphery at the positions shown in Fig. 3. Photometer recordings of flashes from these areas were displayed with the oscilloscope sweep synchronized with the stimulus. Spatially correlated latency differences of emission (Fig. 3), similar in duration to those observed for the externally recorded action potential, were consistently observed.

The latency between local bioelectric activity and local light emission was examined by simultaneous electrical and photometric recording from small areas of the cell surface (Fig. 4). Latencies of emission ranged from 1 to 3 msec when measured from the active portion of the externally recorded potential. No significant local latency differences were found at diverse locations on the organism. This latency presumably represents the time required for the completion of the events which couple the luminescent chemistry to the active current flow of the action potential. It is similar in duration to that of vertebrate twitch muscle fibers (*7*).

Microscopic observations of *Noctiluca* by direct means (*8*) and with the aid of electronic image intensification (*9*) has shown that light emission is from numerous 1 to 2 μ sources distributed primarily in the peripheral cytoplasm (*10*). It is surmised that the action potential triggers luminescence locally at the level of individual organelles, and that the population of luminescent organelles is triggered asynchronously because of conduction latency of the propagated flash-triggering action potential.

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11. Dr. J. W. Hastings first called my attention to *Noctiluca* as a preparation potentially useful for the study of excitation-response coupling. I thank him for discussions during this investigation.
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Body Composition and Coat Color Correlation in Different Phenotypes of "Viable Yellow" Mice

Abstract. *The "viable yellow"* (A^{vy}-) *mouse genotype produces phenotypes with "clear yellow," "black spotted," and "agouti" coat color. "Agouti"* $A^{vy}a$ *mice gain weight at a lower rate and contain significantly less fat and water than "clear" and "spotted"* A^{vy}- *mice but have similar nonfat dry weights. Between 4 and 28 weeks of age, the length of the tail increases at the same rate in all phenotypes.*

Heterozygous "viable yellow" $A^{vy}a$ mice have a variable coat color pattern ranging from clear yellowish orange through various degrees of black spotting to complete agouti which is indistinguishable from mice of the wild-type *A*- genotype. Heterozygous "lethal yellow" $A^{y}a$ mice have a clear yellowish orange coat color and deposit excessive fat in the carcass and liver (*1*). Homozygous $A^{vy}A^{vy}$ mice are viable and fertile (*2*), whereas homozygous $A^{y}A^{y}$ mouse embryos die about the time of implantation (*3*).

"Agouti" $A^{vy}a$ mice appear with a frequency of about 10 percent in litters from all $A^{vy}a \times aa$ and $A^{vy}A^{vy} \times aa$ matings in the VY/Wf stock, derived from (C3H/Di × C57BL/6J) F_1 hybrids (*4*). "Clear" yellow mice are al-