Bioelectric Control of Bioluminescence in the Dinoflagellate Noctiluca

I. Specific Nature of Triggering Events

Abstract. *The flash of* Noctiluca miliaris *occurs only in response to a characteristic all-or-none action potential, the polarity of which is opposite to that of metazoan action potentials, whether recorded internally or externally. Mechanical stimulation evokes a slow, generator-like graded potential which can give rise to the flash-triggering action potential. The flash is all-or-none; it facilitates, summates, and exhibits fatigue, each independently of changes in the amplitude of the action potential.*

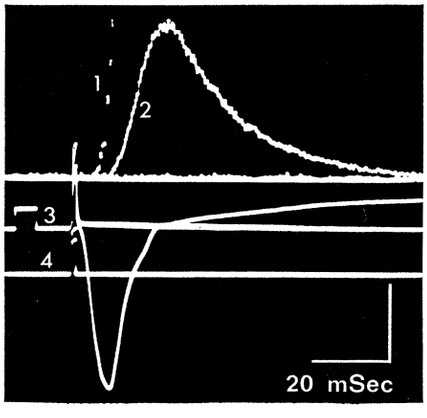
Bioluminescence occurs in certain dinoflagellate species as a brief (50 to 100 msec) emission in response to stimulation (*1–3*). A typical flash of the unarmored dinoflagellate *Noctiluca miliaris* (Fig. 1) reaches maximum amplitude in 10 to 20 msec and decays to 50 percent in about the same period. Two aspects of this phenomenon are especially noteworthy: (i) the stimulus results in light-producing reactants presumably involving reactants already present in the cell, and (ii) within a period of 100 msec these reactions are caused to terminate. These events are examples of elementary biological control phenomena; they are related in concept to the familiar problem of excitation–contraction coupling in muscle.

Marine phosphorescence was first ascribed to dinoflagellate luminescence in 1810, when Suriray noted that *Noctiluca miliaris* was responsible for phosphorescence in the English Channel (*4*). Ethel Browne Harvey noted in 1917 (*1*) that stimuli other than mechanical (electrical, chemical, caloric, osmotic) were also effective in evoking luminescence. Electrically stimulated flashes of *Noctiluca*, when investigated later (*2*) with a photomultiplier, exhibited several phenomena common to striated muscle twitches, namely threshold, summation, facilitation, and fatigue.

Intracellular electrical studies on luminous specimens (*5*) from Japanese waters, and dark specimens (*6*) from Puget Sound showed similar stimulus-evoked action potentials, although the two reports differed regarding certain other major bioelectric features (*7*). Hisada (*5*) did not correlate luminescence with the action potential; however, on the basis of data from other excitable and responsive cells, it seemed probable that the bioluminescent reactions are triggered not directly by stimulus energy but secondarily by events related to an active membrane response initiated directly or indirectly by the stimulus. I now offer experimental evidence that the reactions leading to light emission are, in fact, triggered by a component event of the action potential.

A dividing culture of *Noctiluca miliaris* was established in August 1962 with specimens from a luminescent population in the North Sea (*8*). The organisms are kept in autoclaved, unenriched natural sea water at 17° to 18°C, and are fed at intervals with the marine alga *Dunaliella*, which is grown in an enriched sea water medium (*9*).

Noctiluca (*10*) is a single-celled organism, subspherical in shape, and it ranges in diameter from 300 to 600 μ under the stated culture conditions. Below the pellicle there is a thin layer of



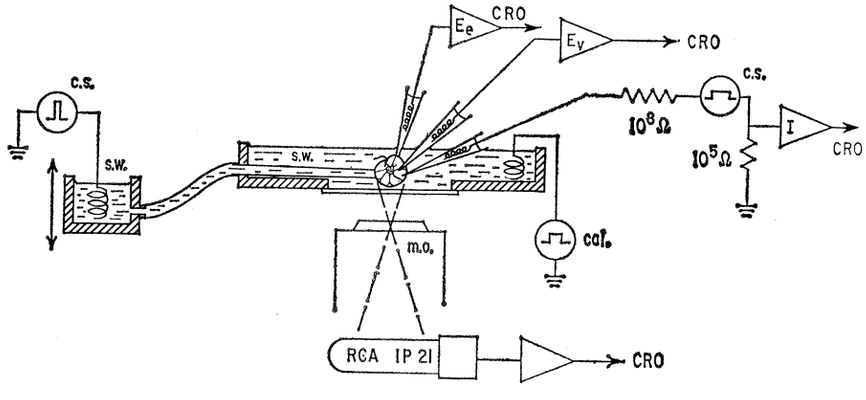


Fig. 1. (Left) The luminescent flash and the flash-triggering potential recorded from a single specimen of *Noctiluca miliaris*. Two sweeps of four traces each, one sweep with external stimulating current (monitored on trace 4) below threshold and a second sweep with suprathreshold current level. Trace 3 displays the potential recorded with microelectrode from interior of the major vacuole. Calibration pulse at beginning of this trace is 10 mv and 5 msec. Conventional polarity (downward is negative) is observed in all intracellular and extracellular recordings. Trace 2 displays the instantaneous rate of photon emission. Trace 1 is superimposed on trace 2 and displays the same signal at 20 times the gain of trace 2; 24°C. Calibration mark: trace 1, 9×10^5 photon/msec; trace 2, 1.8×10^7 photon/msec; trace 3, 32 mv; trace 4, 4×10^4 amp. The maximum emission rate of this flash was 3.6×10^7 photon/msec, while the quantum content of the flash, determined by graphical integration, was 9.7×10^8 photons. (Right) Basic experimental set up. *Noctiluca* specimen is held to end of stimulating pipette by adjusting water level of reservoir to several millimeters below that of bath. All electrodes Ag–AgCl; *c.s.*, current source; *s.w.*, sea water; *cal.*, calibrator; E_e, external potential recorded with differential capacity-coupled preamplifier; E_v, vacuolar potential recorded with high-impedance neutralized capacitance preamplifier; *I*, polarizing current monitor; *m.o.*, microscope objective.

stranded cytoplasm enclosing a single vacuole which occupies most of the cell volume; this vacuole contains a clear fluid having a specific gravity somewhat less than that of sea water. The peripheral cytoplasm is continuous, via fine radial strands, with a central mass of cytoplasm containing the nucleus. In the radial dimension the peripheral cytoplasm varies from a small fraction of a micron to several microns in thickness and is bounded by an outer membrane just under the pellicle and an inner membrane at the vacuolar interface. It contains several organelles, some familiar (such as mitochondria, Golgi bodies) and some unfamiliar (*11*). Light is emitted from multitudinous punctate sources found primarily in the peripheral cytoplasm. This was first noted visually (*1*) and more recently photographically by the image intensifier technique (*12*) used to obtain the cover photograph of a *Noctiluca* flash. The identity of the luminous sources is now under investigation.

Electrophysiological investigations were performed at room temperatures of 20° to 25°C on single specimens held in artificial sea water (*13*) at the end of a small suction pipette over the objective of an inverted-type compound microscope (Fig. 1). Light emitted by the specimen was optically projected onto the photocathode of an RCA 931A or 1P21 multiplier tube, and was displayed oscillographically with a total time constant of less than 1 msec. The system was calibrated to measure total photon flux from a luminous object within the field of the objective by an adaptation of the method of Hastings and Weber (*14*). Stimulating current was delivered either externally through the holding pipette or internally with a polarizing microcapillary electrode (about 0.5 μ in tip diameter). Electrical recordings were obtained with internal KCl microcapillary electrodes, whereas external recordings were obtained from the surface of the cell with macrocapillary electrodes (15 to 25 μ in tip diameter) filled with sea water. Internal electrodes, whether for recording or passing current, were always positioned with tips in the large vacuole that occupies most of the cell interior, because reproducible electrode placement in the thin, motile peripheral layer of cytoplasm is extremely difficult. Stimulus current amplitudes, action potentials, and light fluxes were displayed on a multitrace cathode-ray tube and photographed.

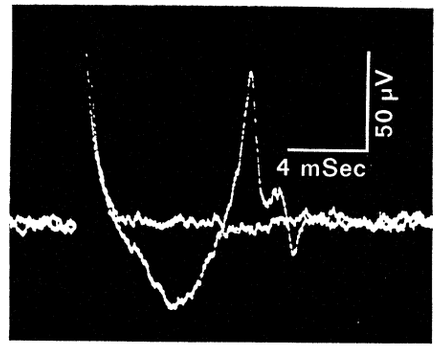


Fig. 2. Action potential recorded externally from *Noctiluca* with capillary electrode applied to pellicle. Two sweeps, one with subthreshold stimulus. Stimulus artifact resulted from current supplied by external anode (holding pipette) approximately 180 deg from recording site. The initial negative deflection is interpreted (*16*) as result of inward source current for approaching action potential; the positive-going spike as outward active membrane current, and the final negative wave as primarily passive inward current.

Introduction of the microelectrode into the vacuole occurs abruptly as the tip "pops" through the pellicle and peripheral cytoplasm. Penetration of the vacuole is generally accompanied by a negative drop of several millivolts in the recorded d-c potential. The vacuolar potential then gradually grows to a steady level as great as −40 mv, but more typically −15 to −30 mv. During this time there is a gradual increase in both the *IR* (*I*, current; *R*, resistance) potential and *RC* (*C*, capacitance) time constant recorded in response to intracellular current pulses delivered with a second capillary electrode. The increase in membrane resistance, capacitance (?), and potential may represent recovery from damage due to stretching of the membrane which accompanies dimpling of the tough pellicle prior to electrode penetration. Data was recorded only after sufficient time had elapsed for membrane recovery.

If the organism was left unstimulated, spontaneous negative-going potentials of relatively slow time course generally appeared after the recovery period. The spontaneous potentials were always correlated with quasi-rhythmic movements of the food-gathering tentacle, and were unrelated to luminescence (*15*). Hence, they are not considered in this report.

A short (0.1 to 1.0 msec) inward current pulse of at least 10^{-4} amperes applied through the suction pipette holding the cell evokes an all-or-none action potential (Fig. 1) with a different shape and faster time course than the spontaneous potentials. A similar action potential can be evoked by a long pulse of inward current passed with the aid of a cathodal intravacuolar capillary electrode. As reported by Hisada (*5*) and Chang (*6*), the polarity of this action potential as recorded from the vacuole is unconventional, driving

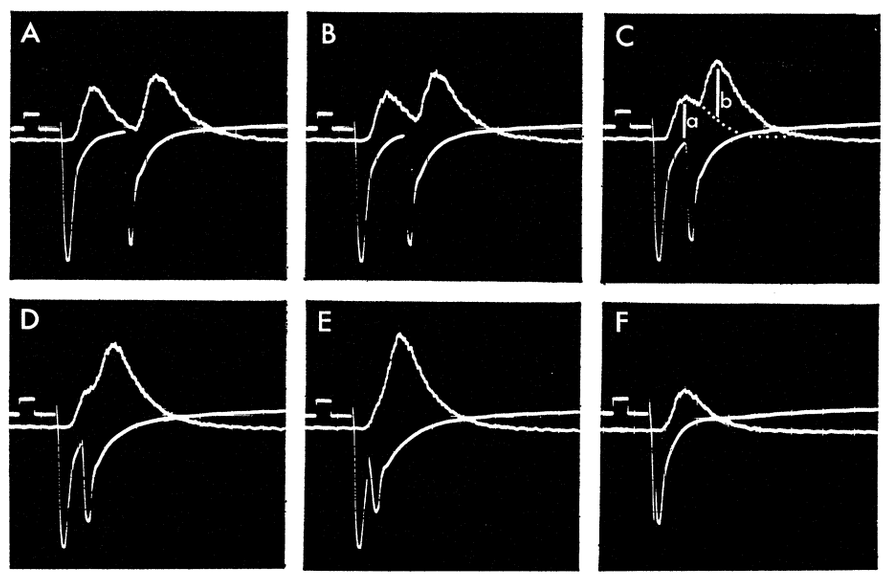


Fig. 3. Facilitation and summation of luminescent flashes from a single *Noctiluca* specimen. Upper trace: intravacuolar potential with 10 mv, 10 msec calibration. Lower trace: light flux, uncalibrated. The double stimulus interval was made progressively shorter from *A* through *F*. In *F* the second stimulus pulse fell within the absolute refractory period of the first action potential. Summation is evident whenever a second flash begins before complete decay of the first flash. Inspection of *C* shows that flash *b* was facilitated.

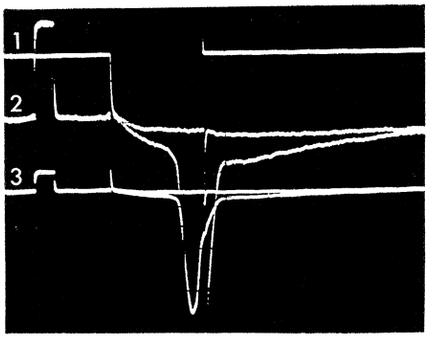


Fig. 4. Flash-triggering action potential arising from slow potential elicited by tactile stimulation of *Noctiluca*. Two sweeps, one with subthreshold stimulus. Interruption of trace 1 indicates duration of current applied to piezoelectric crystal of tactile stimulator. Traces 2 and 3 display intravacuolar 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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8. I thank Dr. Otto Kinne, Director of the Biologische Anstalt, Helgoland, for providing *Noctiluca* specimens; Dr. Luigi Provosoli, Haskins Laboratories, for cultures of *Dunaliella*; Dr. Robert Guillard, for subculturing *Noctiluca* at the Woods Hole Oceanographic Institution; Constance Quinn and Francine Brady, for technical assistance and for care of the cultures.
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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