

## BEHAVIOURAL PHYSIOLOGY OF THE COLONIAL HYDROID *OBELIA*

### II. STIMULUS-INITIATED ELECTRICAL ACTIVITY AND BIOLUMINESCENCE\*†

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#### INTRODUCTION

The first paper in this series (Morin & Cooke, 1971*a*) dealt with spontaneous electrical and behavioural activity in *Obelia geniculata*. The present study was undertaken in order to (1) describe behavioural and electrically recorded responses of *Obelia* colonies to stimulation (electrical, mechanical and chemical), (2) describe the bioluminescent responses upon stimulation using photomultiplier recording techniques, (3) describe the role of electrical activity in the regulation of luminescent events, and (4) examine coupling between the various types of electrical activity (both spontaneous and evoked).

The effects of applied electrical stimulation to coelenterates have been widely studied especially within the Anthozoa and Scyphozoa. Pantin's (1935*a-d*) classical work on the anemone *Calliactis* showed that muscular responses facilitate markedly to specific repetitive stimulation. This pattern of facilitation has since been shown to be a common feature within the coelenterates (see Bullock & Horridge, 1965). The responses of the Hydrozoa to electrical stimulation have not been extensively examined. Details of behavioural responses to electrical stimulation have been examined in the hydroids *Syncoryne* (= *Sarsia*), *Hydractinia*, *Pennaria* (Josephson, 1961*a*), *Cordylophora* (Josephson, 1961*b*; Mackie, 1968), *Tubularia* (Josephson & Mackie, 1965) and *Hydra* (Josephson, 1967; Josephson & Macklin, 1967). Electrical recordings during stimulation have been obtained from the latter three.

Bioluminescence in certain coelenterates and ctenophores has been elicited by mechanical, electrical and/or chemical stimulation. Repetitive bioluminescent flashing in response to single electrical stimuli has been observed in the ctenophore *Mnemiopsis* (Chang, 1954), the hydroid *Obelia* (Morin *et al.* 1968; Morin & Reynolds, 1969), and under certain conditions in the pennatulid *Renilla* (Buck, 1953; Nicol, 1955*a*). However, *Renilla* responds with single flashes during most of its responses to stimulation (Nicol, 1955*a, b*).

The luminescent hydromedusae *Aequorea*, *Halistaura* and *Phialidium* show a single

\* Preliminary reports of this work have been published (Morin, Reynolds & Hastings, 1968; Morin & Reynolds, 1969).

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flash after each electrical shock (Davenport & Nicol, 1955). It was also shown in that study that the luminescence in *Aequorea* arose from an intracellular mass located in the marginal canals which fluoresced yellow-green in ultra-violet light. A single flash of light, localized at the point of stimulation, appeared in response to a single shock. The light did not spread. Facilitation, summation and fatigue of the luminescence occurred with repetitive stimulation.

The most recent study on bioluminescence of the hydroid stage of the Hydrozoa was carried out by Panceri (1876, 1877). From his attempts to locate the site of the luminescence he concluded that the light emanated from minute points within the epidermis of the hydranths, pedicels, uprights and stolons. Most of the descriptions previous to his indicated only that the luminescent forms were restricted to the Calyptoblastea, particularly the Campanulariidae and that the light was usually seen to flicker throughout the colony when the algae upon which they were growing were rubbed or shaken (Forbes, 1848; Allman, 1871).

#### MATERIALS AND METHODS

*Obelia geniculata* colonies were obtained from the Cape Cod Canal and the vicinity of Woods Hole, Massachusetts. Methods for culturing the colonies and for monitoring electrical activity are described in the preceding paper (Morin & Cooke, 1971a).

The colonies were stimulated using a pair of fine-tipped silver-silver chloride electrodes held about 200–300  $\mu$  apart which were insulated to within 0.5 mm of their tips with flexible latex (Marine Sealant, Dow Corning). Square pulses of 0.5–5 ms duration produced by a stimulator were delivered through such electrodes placed across the perisarc of an upright. The colonies were stimulated mechanically by prodding them with a glass probe.

Bioluminescence was produced by chemical stimulation using the following method: one to several uprights of a colony were placed in a vial, the vial was put into a light-tight chamber situated above a side-window photomultiplier (RCA 1P21-recording was carried out as reported below), and 1 ml of 0.54 M-KCl solution (isotonic to sea water, pH 8) was injected into the vial using a hypodermic syringe which fitted through a small hole in the chamber lid.

Bioluminescence from the colonies produced by electrical or mechanical stimulation was recorded using fine-fibre optics (tip diameter 0.5 mm) as a light guide from the animal to a photomultiplier recording system. The light guide was directed toward the cathode of an end-window photomultiplier tube (EMI 9592B). The photomultiplier was operated between 500 and 1000 V from a stable power supply. The photomultiplier-output anode current was converted to a voltage signal by an operational amplifier (Philbrick P2AU). The output voltage was proportional to the light intensity and the response time of the circuit was well above the recorded flash frequencies. This output signal was led to a low-level d.c. pre-amplifier and was recorded on a polygraph inkwriter (Grass 5B) simultaneously with the electrical responses or displayed directly on a storage oscilloscope (Tektronix 564).

The light guide was brought to the appropriate region of the colony using a micro-manipulator. Luminescent sites (photocytes) were located by illuminating the colony through a 460 nm interference filter which excited the green fluorescence of the photo-

cytes; the fluorescence was observed through a barrier filter (Wratten No. 12) (see Morin & Reynolds, 1971).

## RESULTS

### (I) *Behavioural responses to stimulation*

Two responses may be observed visually upon electrical or mechanical stimulation of a colony of *Obelia geniculata*: (1) bioluminescence and (2) hydranth contraction.

Bioluminescence can be elicited by mechanical, electrical or chemical stimulation. To the dark-adapted eye the luminescence appears as a flickering of minute spots. The luminescence invades more distal parts of the colony from the point of stimulation on successive flashes and stimuli until the whole colony often appears to 'palpitate'. Visual localization of the luminescence to a particular structural area is difficult even with the use of a microscope because of the rapid flickering and relatively weak intensity of the light. The use of image intensification and fluorescence has shown that only the stems, pedicels, and stolons are luminescent (Morin & Reynolds, 1971).

Luminescence within the colony usually disappears after the first few mechanical or electrical stimuli of a train. That is, the threshold rises during stimulation so that the last stimuli in a train become ineffective, but further responses can usually be elicited by increasing the stimulus strength, duration, and/or frequency, or by providing a period of rest (see Morin & Cooke, 1971*b*). It must be stressed that these responses are labile and variable both within the stimulation sequences of one colony and from one colony to another.

The other response that is often observed upon electrical or mechanical stimulation is that of hydranth withdrawal. During stimulation of a single upright, many, but not necessarily all, of the hydranths withdraw. In contrast to the luminescent responses, there is no apparent pattern of withdrawal with respect to the point of stimulation. That is, a hydranth nearer the stimulating electrode or probe is not necessarily more likely to withdraw than a hydranth in a more distal region. Withdrawal contractions of individual hydranths appear similar to spontaneous hydranth contractions. A series of individual contractions take place during withdrawal.

### (II) *Waveform of the bioluminescent flash*

Photometric recordings of the luminescence using fine-tipped light guides verified the visual observations. Following each stimulus except the first, in a train of four to six suprathreshold stimuli given at a frequency of less than two per second, there is recorded a volley of flashes. The amplitude of the flashes initially increases and then decreases toward the end of each burst (Fig. 1). The flashes within a burst usually number from three to ten but may vary from a single flash to as many as 25. The frequency of flashing ranges from 6 to 17/s. These responses can be measured along the colony up to several centimetres from the point of stimulation.

Successive flashes, although of variable amplitude, have similar shapes and durations (Fig. 1). Measurements of 20 flashes at  $20 \pm 1$  °C showed a rise time to peak intensity ( $I_0$ ) of about 22 ms and a 50% decay time of 24 ms (Table 1). The decay is exponential over at least one decade (Fig. 2). This indicates a first-order rate equation for the underlying chemical reaction with a rate constant of  $50 \text{ s}^{-1}$ . This decay is

faster than that for the flash of the hydromedusae *Aequorea* and *Halistaura*, the pennatulid *Renilla*, and the ctenophore *Mnemiopsis*, but is slower than that of the dinoflagellate *Noctiluca* (Table 1) (Davenport & Nicol, 1955; Chang, 1954; Eckert, 1967).

The duration of the flash exhibits a characteristic temperature dependence (Fig. 1 B, C). Measurements of 20 flashes at  $12 \pm 1^\circ\text{C}$  showed the rise time to  $I_0$  to be about 47 ms with a 50% decay time of about 37 ms and the rate constant to be  $29\text{ s}^{-1}$  (Fig. 2). Calculations using the  $k$  values obtained for *Obelia* at 20 and  $12^\circ\text{C}$  give an

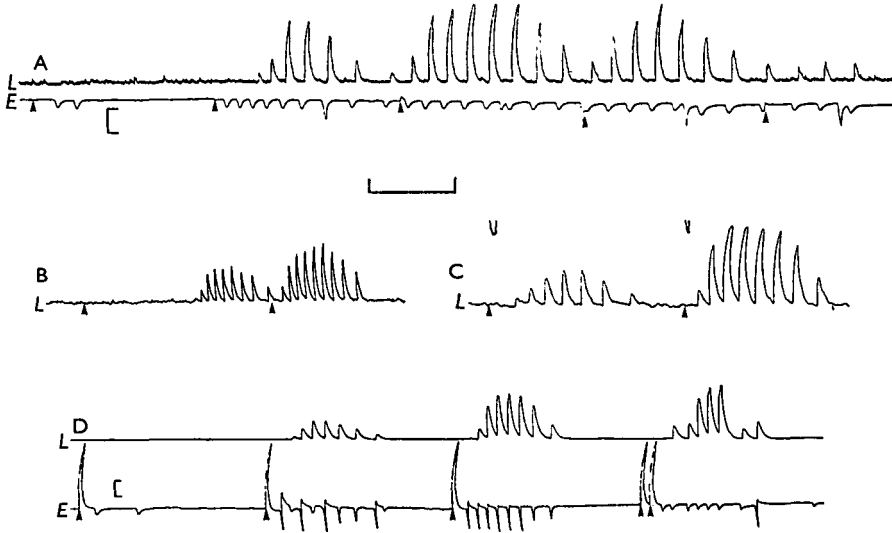


Fig. 1. Luminescent flash ( $L$ ) and luminescent potential ( $E$ ) bursts recorded from an *Obelia geniculata* photocyte and hydranth respectively. A and D show typical responses to a train of applied stimuli at  $1/2$  s. The recording electrode ( $E$ ) shows a volley of luminescent potentials (LPs) following each stimulus (arrows). These potentials are sometimes masked by the larger and more rapid contraction potentials (KPs) as in D (see text for details). The photometric records ( $L$ ) show a volley of luminescent flashes following each stimulus. B and C show luminescent flash bursts to applied stimuli (arrows) at  $20^\circ\text{C}$  (B) and  $12^\circ\text{C}$  (C). The vertical bars indicate 1 mV, the horizontal bar indicates 1 s, and the vertical deflexion of the luminescent ( $L$ ) traces represents light intensity.

Table 1. Luminescent flash characteristics (in vivo) of several luminescent organisms

	<i>Obelia</i>	<i>Aequorea</i> *	<i>Hali-staura</i> *	<i>Renilla</i>	<i>Mnemi-opsis</i> †	<i>Noctiluca</i> ‡
Rise time (ms, $20-22^\circ\text{C}$ )	22	100 (100)	100	56	60	15-20
Half decay time (ms, $20-22^\circ\text{C}$ )	24	140 (95)	375	80	48	15-20
90% Decay time (ms, $20-22^\circ\text{C}$ )	55	360 (260)	1000	200-300	114	ca. 30
$k$ ( $\text{s}^{-1}$ , $20-22^\circ\text{C}$ )	50	7.8	2.6	13	24	88
$k$ ( $\text{s}^{-1}$ , $11-12^\circ\text{C}$ )	29	—	—	—	11	—
$\mu$ (kcal)	12	—	—	—	12	—

\* Calculated from Davenport & Nicol, 1955, except numbers in parentheses which were my determinations.

† From Chang, 1954.

‡ From Eckert, 1967.

activation energy,  $\mu$ , of about 12 kcal, although one must be cautious in accepting such value until additional rate constants are available to corroborate their linearity in an Arrhenius plot. This value is similar to that reported for *Mnemiopsis* (12 kcal; Chang, 1954) and for the *in vitro* aequorin protein of *Aequorea* (11 kcal; Hastings *et al.* 1969).

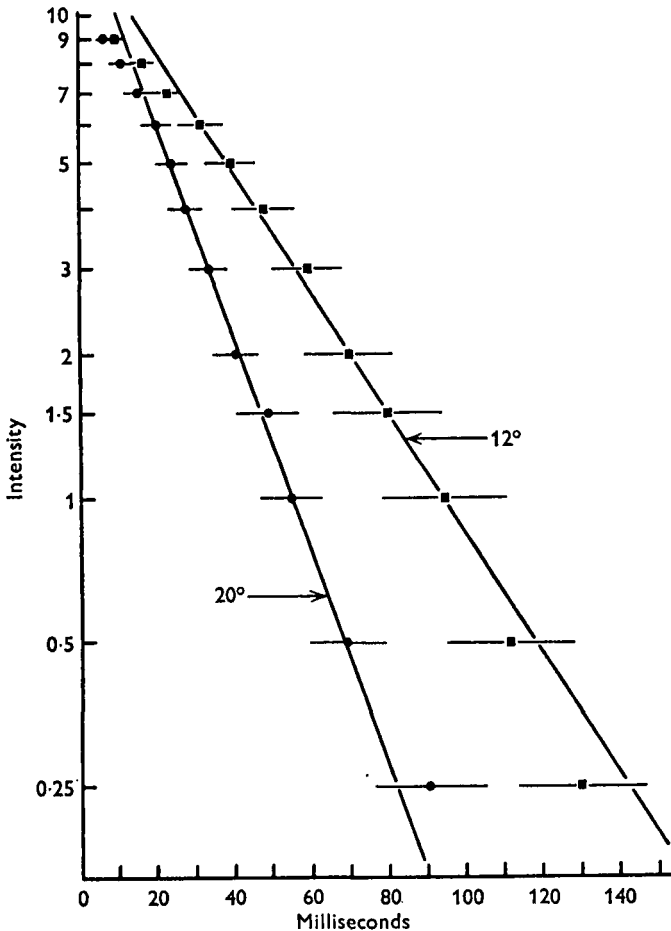


Fig. 2. Average luminescent flash decay curves from *Obelia geniculata*. The average of eight flashes at 20 °C and 12 °C are plotted in a semilog plot. For these calculations maximum light intensity for each flash was set at ten in arbitrary units. Horizontal bars show the standard deviation.

The number of photons emitted during a flash could not be determined accurately from our records because of interfering materials such as other tissues, skeletal perisarc and organisms growing on the perisarc which absorb and scatter light.

The type of stimulation does not affect the waveform of the flash (Fig. 3). Mechanical (Fig. 3A), electrical (Fig. 3B), or chemical stimulation (Fig. 3C) produce similar responses. With KCl the initial flash is often followed by a slow glow (Fig. 3D). As reported by earlier observers (Panceri, 1876) fresh water induces a delayed but long-lasting (up to 1 min) glow. Observations of the tissues through a microscope with artificial illumination during the addition of KCl or fresh water show that the tissues

begin to disintegrate within the first few seconds after addition. Ethanol (70%) induces a bright flash of about 1 s followed by a weak glow (Fig. 3 E). Chemicals such as  $MgCl_2$ ,  $CaCl_2$ ,  $NaCl$ ,  $MgSO_4$  and  $NaHCO_3$  (all isotonic to sea water, pH 8) induce no luminescent responses.

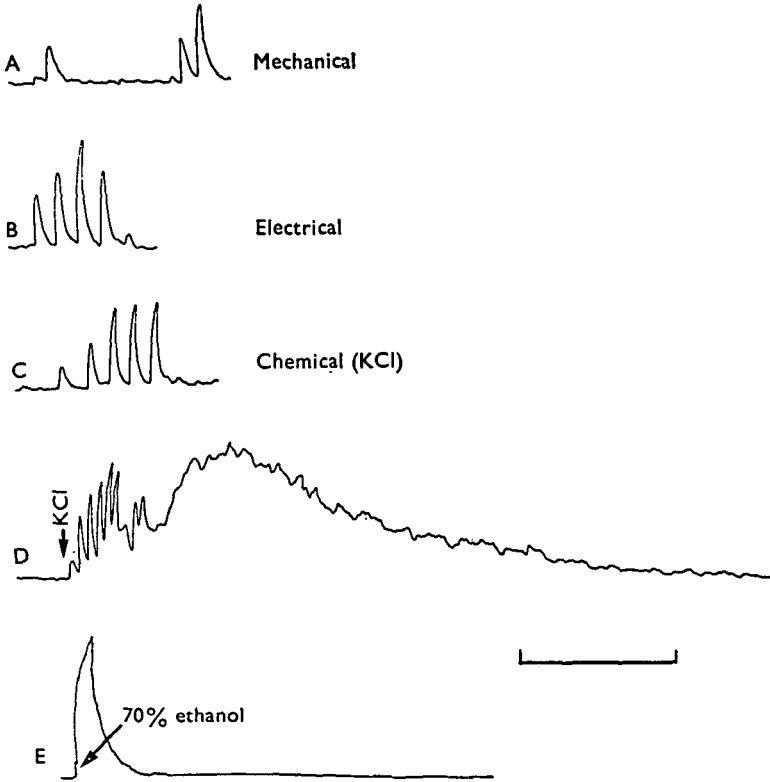


Fig. 3. Effects of different forms of stimuli on the luminescent flashes. A, mechanical stimulation with a glass probe. B, electrical stimulation across the perisarc (see text). C, chemical stimulation by addition of KCl. D, the usual total response to addition of KCl. E, chemical stimulation by addition of 70% ethanol to sample (see text). Horizontal bar indicates 1 s.

### (III) *Electrical activity and bioluminescence*

#### (A) *Luminescent potentials (LPs)*

Potentials which are correlated with luminescence can be recorded from *Obelia* hydranths (tentacles or hypostome). Such potentials are referred to here as luminescent potentials (LPs). Each LP is monophasic, positive (active electrode relative to the bath), and slow (rise time about 35 ms and duration about 120 ms) with a peak amplitude of 0.1–0.7 mV (Fig. 1). No records were made from the hydranth body because of the presence of the hydrotheca. No latency differences were detected when simultaneous recordings were made with two suction electrodes placed on one hydranth, probably because the potentials spread over the limited area of a single hydranth more rapidly than the recording equipment can resolve (about 5 ms). When recording from different hydranths, there are detectable latencies (Morin & Cooke, 1971*b*). Luminescent potentials (LPs) are easily distinguished from contraction,

potentials (KPs), mouth-opening potentials (MOPs), and tentacle contraction potentials (TKPs) recorded from a hypostome (Morin & Cooke, 1971*a*). LPs have a longer duration and smaller amplitude than the KPs and a shorter duration than the MOPs and TKPs (Fig. 4; Table 2).

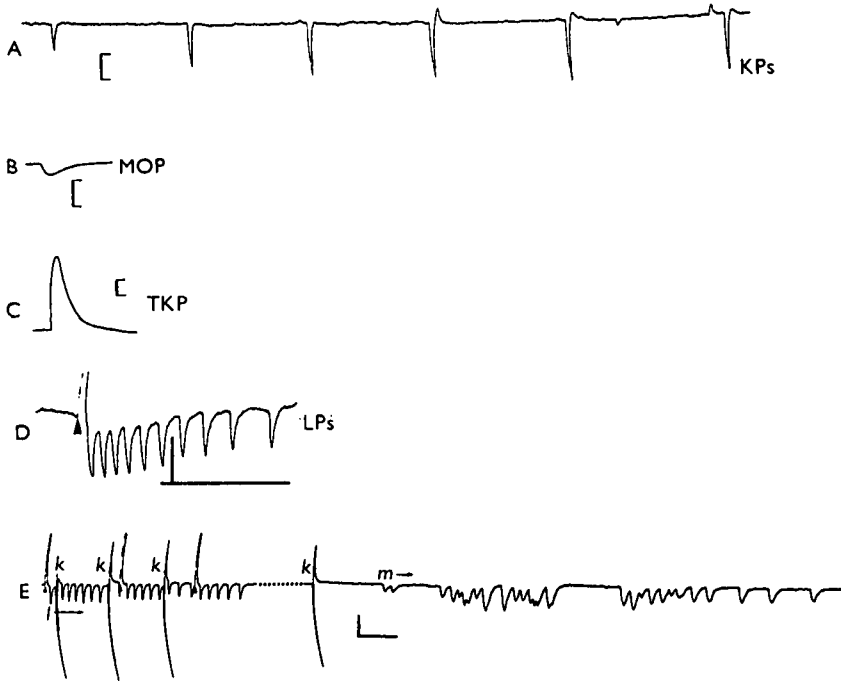


Fig. 4. Examples of electrical activity recorded from the hypostome. A, contraction potentials (KPs). B, a mouth-opening potential (MOP). C, a tentacle contraction potential (TKP). D, luminescent potentials (LPs) in response to stimulation (arrow). E, shows KPs (*k*), LPs (*l*) and MOPs (*m*) on the same record at a slow polygraph speed (dots between records indicate an omission of about 40 s in E). Vertical bars represent 1 mV, horizontal bars represent 1 s (A-D are at the same speed).

Table 2. *Characteristics of electrical potentials from Obelia geniculata*

	(N = 10-25.)			
	LP	KP	MOP	TKP
Rise time (ms)	15 ± 5	15 ± 5	70 ± 20	75 ± 20
Half decay time (ms)	35 ± 15	10 ± 5	115 ± 75	110 ± 50
Duration (ms)	120 ± 50	50 ± 20	550 ± 200	425 ± 100
Amplitude (mV)	0.1-0.7	0.5-1.0	1-10	0.2-0.7
Initial polarity (from hypostome)	+	+	+	-

LPs were never recorded except in response to suprathreshold stimulation. LPs occur in bursts following each stimulus except the first. By stimulating at the base of an upright and recording with one or more suction electrodes on the hydranth(s) of the same upright or other uprights in the same colony these LP-bursts can be shown to be propagated through a colony. A LP-burst can usually be recorded at each electrode site after each stimulus. LP activity evoked by distal (toward the tip of an

upright) electrical stimulation can be recorded from a proximal (toward the base of an upright) hydranth and LP activity evoked by proximal stimulation can be recorded from a distal hydranth. This demonstrates that conduction in the system is non-polar. LPs are conducted through the colonies at about 22 cm/s at 12 °C (Morin & Cooke, 1971*b*). The amplitude of each LP within a burst and in successive bursts is nearly constant. From this evidence it is concluded that LPs are all-or-none, non-decremental potentials (see Morin & Cooke, 1971*b*, for exceptions) that are propagated through a non-polarized conductile system. In these respects the LP system is similar to most of the coelenterate conducting systems which have been described, including nerve nets and epithelial conductile systems (Pantin, 1935*a-d*; Mackie, 1965).

### (B) Luminescent flashes and the LP system

Most of the remaining data were recorded with a light guide situated over a single luminescent site within a pedicel or upright node, a suction electrode attached to the supported hydranth and a stimulating electrode across the upright near this point.

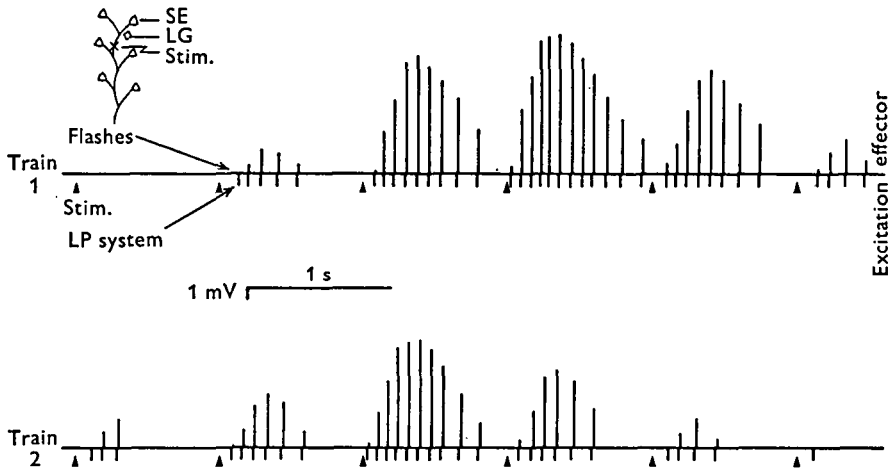


Fig. 5. A diagram of luminescent flashes (*L*) and luminescent potentials (*E*) recorded near the point of stimulation (inset) for two successive stimulus trains (separated by about 10 min). The figure was constructed from many observations such as those shown in Fig. 1*A* and *D*. It is a composite of actual data. Stimuli are indicated by arrows, the vertical bar indicates 1 mV, the horizontal bar indicates 1 s. See text for details.

This arrangement provided the closest possible position for the electrode and the light guide. Because the luminescent sites lie within the stems, pedicels and stolons (Morin & Reynolds, 1971) and are therefore surrounded by perisarc, direct records of the excitatory responses from these sites could not be made with suction electrodes. The closest recording site for the LP system was the non-luminescent hydranth. Latencies between the LP and the luminescent flash responses were therefore corrected by adding a computed conduction velocity (Morin & Cooke, 1971*b*) for the extra distance.

Luminescent flashes are directly coupled to LP activity (Fig. 1). A flash is always preceded by a LP. On the other hand, a LP need not be followed by a flash, although it usually is (Fig. 1). This is clearly demonstrated in studies of light-adapted colonies,



Stimulation of a colony that has recently been transferred to the dark produces LPs (as it does in the light also) but it does not produce luminescent flashes. The luminescence occurs along with LP activity in colonies which have been kept in darkness for a half hour or more. Light-adapted colonies, however, will emit light if placed in 0.54 M-KCl (isotonic to sea water). This demonstrates that light adaptation involves an uncoupling of the LP system from the luminescent effector instead of a blockage of LP transmission or a direct biochemical inactivation of one or more of the components involved in emission.

A diagram of LPs and luminescent flashes recorded near the point of stimulation for two successive stimulus trains (separated by about 10 min) is shown in Fig. 5. The two trains were constructed from many observations such as those shown in Fig. 1 A, D. It is a composite of actual data.

The first shock of a train delivered to a previously unstimulated colony produces no response in either the LP (excitation) system or the luminescent (effector) site (Fig. 5, 1). The second or third shock produces a repetitive discharge of LPs in the excitation system. There is a variable latency (20–600 ms) between the stimulus and the first LP. There is light emission from the effector after the second or third LP of the burst and after each succeeding LP. The latency between the onset of the LP and onset of light is between 5 and 20 ms and is constant. The successive flashes show an initial facilitation and then a decline toward the end of the burst. The intervals between successive LPs increase prior to termination of the burst.

Another burst of LPs follows the next shock (i.e. third or fourth). The latency between the stimulus and the first LP is usually less than that in the previous burst. There are usually more LPs in the burst than in the preceding burst. The luminescent response is similar but often a small flash occurs after the first LP and the maximum intensity ( $I_{\max}$ ) of the brightest flash in the burst is usually greater than the corresponding flash in the previous burst. The next shock (the fourth or fifth) produces another burst which has more LPs and an even shorter latency to the first LP than the corresponding LP of the previous shock. Luminescent flashes reach a greater  $I_{\max}$  than in the previous burst. Responses to succeeding shocks usually show progressively greater latencies to the first LP, fewer LPs per burst, and a lesser  $I_{\max}$  of luminescent responses. After 4–9 shocks the LP system fails to respond unless the stimulus strength, duration or frequency are increased or a period of rest intervenes (Fig. 5).

After a rest period of several minutes, there is often a short burst of LPs in response to the first stimulus of this second train with a facilitating flash sequence commencing on the second or third LP (Fig. 5, 2). The succeeding shocks produce responses similar to those in the first train, but usually with relatively fewer LPs per burst and a lesser  $I_{\max}$  of the flashes per burst. Cessation of LP bursts responding in the second train of stimuli generally occurs after fewer stimuli than in the first train.

These observations show that there is both facilitation of the excitation system at the point of stimulation (no response until after the second or third stimulus and an increasing number of LPs with successive stimuli), and facilitation at the excitation-luminescent effector junction (no light response until after the second or third LP and an increasing flash intensity with successive LPs). These two types of facilitation, along with facilitation within the excitation system, will be considered in more detail in the following paper (Morin & Cooke, 1971 *b*).

(IV) *Interactions of the luminescent potentials with other electrical potentials*

The LPs showed no coupling with mouth-opening potentials (MOPs) or tentacle contraction potentials (TKPs), but LPs did show distinct coupling with hydranth contraction potentials (KPs).

Visual observations showed that stimulation sometimes induced hydranth withdrawals; these are accompanied by KPs. Stimulus-driven KPs are identical to spontaneous KPs in shape, amplitude and duration (Fig. 6A). Only in interval character-

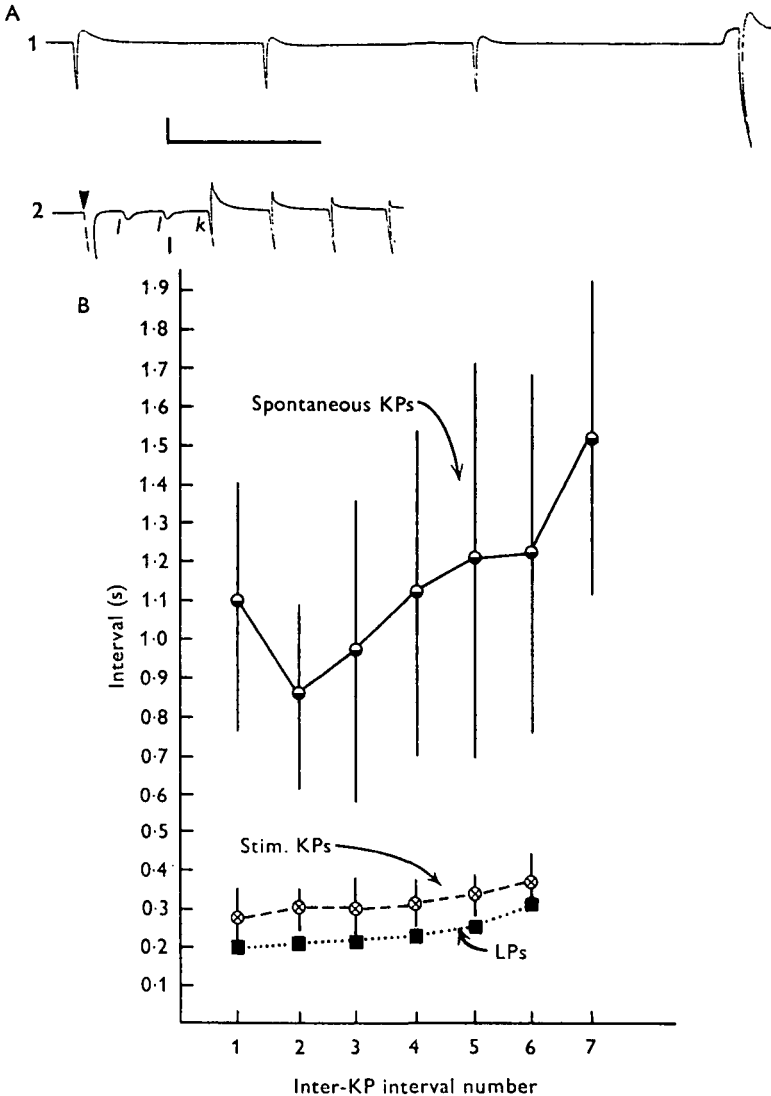


Fig. 6. Spontaneous and stimulus-initiated KP activity. A, high-speed records of a spontaneous KP-burst (1) and a stimulus-initiated burst (2). In (2), LPs are indicated by an *l*, KPs by a *k* (see Fig. 7). B, average interval between successive KPs within KP-bursts: spontaneous KPs; average from 14 such bursts ( $12^{\circ}\text{C}$ ); stimulus-driven (Stim.) KPs; are the average of 12 KP-bursts at 1 stimulus/2 s ( $12^{\circ}\text{C}$ ); LP intervals (LPs) are taken from Morin & Cooke (1971b) and represent the average for bursts at 1 stimulus/2 s ( $12^{\circ}\text{C}$ ).

istics do they differ (Fig. 6B). The stimulus-driven KP intervals plotted in Fig. 6B are the average of 12 stimulus-driven KP-bursts at  $1$  stimulus/2 s ( $12 \pm 1$  °C); the spontaneous KP-burst plot is the average of fourteen spontaneous bursts ( $12 \pm 1$  °C). The stimulus-driven KP-burst interval characteristics are very similar to those of the LPs accompanying the KPs.\* Figs. 6A and 7 show that the KP is usually superimposed on a LP so that the KP is masked. The LP can be assumed to be present

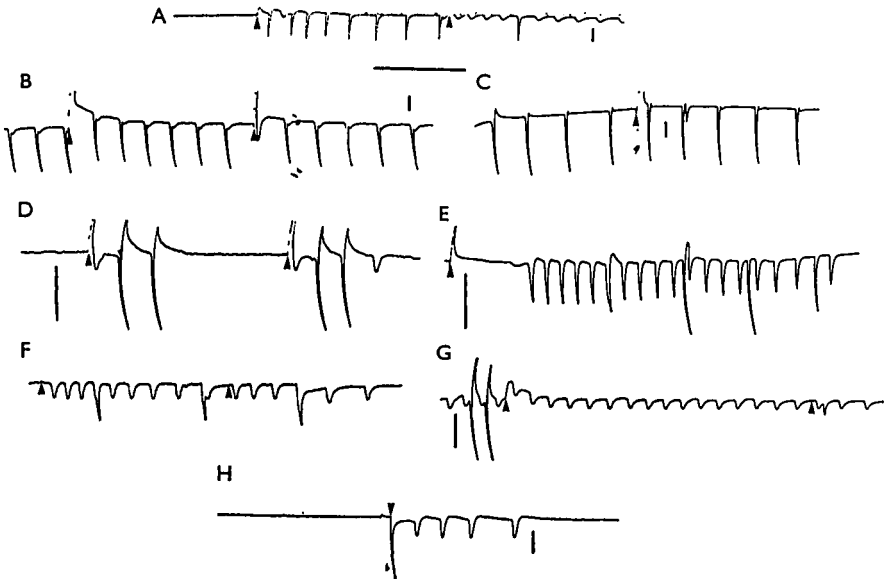


Fig. 7. Examples of LP-KP interactions. They range from tight coupling with complete masking of the LP (part of A) or partial masking (B, C) to no coupling at all (H). Vertical bars indicate 1 mV, the horizontal line indicates 1 s.

from concurrent light emission and the interval characteristics. Coupling is not always tight and does not appear to be a function of stimulus intensity once LP threshold is reached (Fig. 7E, F, G). There are gradations from no stimulus-driven KP-bursts and only LPs to complete coupling of the LPs and KPs. Some records show an incomplete LP-KP interaction with the KP appearing on the rising or falling phases of the LP (Fig. 7B, F, G). Interaction of KPs and LPs does not alter the intervals between the LPs (Fig. 6).

No clear relationship was shown between the proximity to the stimulus and KP-LP coupling over short distances. More distant hydranths sometimes showed more KP coupling than those nearer the stimulus. There was no apparent coupling of KPs between adjacent hydranths except when the individual hydranths' KPs were coupled to the through-conducting LP system. This evidence agrees with the observations that no KP-KP interactions occur between adjacent hydranths in the absence of external stimuli (Morin & Cooke, 1971*a*). There is no predictable threshold difference for the activation of the LP and the KP systems.

This evidence suggests that the LP system can couple with and drive the KPs of a hydranth in some as yet unknown way.

\* The LP interval characteristics are explained in Morin & Cooke, 1971*b*.

## (V) Responses produced by stimulation in other species of the Campanulariidae

We have recorded luminescent flashing following electrical stimulation of *Obelia bicuspidata*, *O. commisuralis*, *O. longissima*, *Campanularia calceolifera*, and *Clytia edwardsi*. The characteristics of the bursts and spread of the luminescence are similar to those described above for *O. geniculata* although the kinetics vary slightly (Morin & Hastings, 1971). These species as well as all of the other luminescent hydroids examined show flashing upon the addition of KCl or electrical stimulation (Morin & Hastings, 1971). Following electrical stimulation, electrically recorded responses similar to LPs occurred in *O. longissima*. These responses were not examined in detail, but the similarities of the luminescent responses and the similar biochemical mechanisms (Hastings & Morin, 1968; Morin & Hastings, 1971) indicate that an LP system that is similar to the one in *O. geniculata* is probably operating in these other hydroid species and possibly in most, if not all, of the luminescent members of the Calyptoblastea.

## DISCUSSION

Individual electrical or mechanical stimuli produce repetitive potentials (recorded with suction or metal electrodes) in *Cordylophora* where the number of potentials increases with increasing stimulus strength (Josephson, 1961*b*). The potentials, termed JPs by Mackie (1968), evoke a contraction of the hydranth. These responses are similar to the LP responses in *Obelia*. Both the JPs in *Cordylophora* and LPs in *Obelia* (1) display repetitive firing to a single shock, (2) occur in numbers which appear to be a function of the stimulus parameters (Morin & Cooke, 1971*b*), (3) couple to an effector response, (4) have similar interpotential intervals which increase toward the end of the burst, (5) display a response threshold which changes, (6) show facilitation in distance of spread with ultimate through-conduction and (7) are non-polar. They differ in their: (1) recorded polarity (LPs are monophasic positive while JPs can be multiphasic with either polarity first), (2) conduction velocities (Morin & Cooke, 1971*b*) (*Obelia* LPs are almost ten times faster than *Cordylophora* JPs: 22 and 2.7 cm/s respectively), and (3) amplitudes (JPs show facilitation in pulse height while LPs are all-or-none). The similarities appear to be of over-riding importance and we suggest that JPs and LPs are homologous. JPs may be a combination of two or more discrete, but coupled, electrical events.

Occasionally, repetitive potentials have been observed in response to single shocks in the sea-anemone *Calliactis* (Pantin, 1935*a-d*; Robson & Josephson, 1969). Such observations suggest that a similar conversion of input stimulus to output discharge may occur under some conditions in *Calliactis*.

It is curious that the hydromedusae do not show repetitive flashing (Davenport & Nicol, 1955). Neither does there appear to be through-conduction in an excitation system that evokes bioluminescence. Although they are fairly closely related to *Obelia* phylogenetically, and the biochemical mechanisms responsible for the luminescence in both *Obelia* the hydromedusan *Aequorea* and are similar (Hastings & Morin, 1968; Morin & Hastings, 1971), they apparently have different mechanisms of excitation.

The pennatulids show a through-conducting excitation system, a facilitating excitation-luminescent effector junction, and occasionally repetitive discharge (Nicol,

1955*a, b*; Buck, 1953). However, usually no repetitive discharge occurs, facilitation within the excitation system is not evident under normal circumstances (Nicol, 1955*b*), and structurally they are considerably more complex (Lyke, 1965) than *Obelia*. These data indicate that the underlying control mechanisms in the pennatulids are probably different from those in *Obelia*; luminescence is probably controlled by a true nerve net in *Renilla* (Ball, personal communication; Lyke, 1965; Nicol, 1955*a, b*) while it is probably controlled by epithelia in *Obelia* (see below).

Light inhibits bioluminescence initiated by electrical or mechanical stimulation in *Obelia* but not the excitation system which normally evokes the luminescence. However, luminescence is evoked from a light-adapted colony by chemical stimulation. This suggests that the light does not directly effect the biochemical machinery responsible for the luminescence (as it does in *Mnemiopsis* (Hastings & Morin, 1968)). These two observations indicate that the inhibition by light must be acting either at the junction between the LP system and the photocyte or within the photocyte itself to physically separate the biochemical components.

The structural elements responsible for conduction of the LPs are unknown, but the evidence from nerve-free epithelia in siphonophores and *Cordylophora* (Mackie, 1965, 1968) and the evidence for transepithelial potentials in *Hydra* (Josephson & Macklin, 1967) suggest that these potentials are epithelial in nature. It is possible that conduction could be neuronal in the uprights and coupled to epithelial conduction in the hydranths. Records within the coenosarc will be required before such possibilities can be resolved. Records obtained from within the stems of *Cordylophora* (Josephson, 1961*b*) indicate that the JPs are conducted in the same way throughout the colony. The structural elements responsible for the luminescence itself will be considered in a following paper (Morin, & Reynolds, 1971).

It is reasonable that coupling should occur between KPs and LPs for protection against predators. Stimulation initiates LP activity and this in turn can drive the KPs with concomitant withdrawal of the hydranth into its hydrotheca. The coupling of the two systems appears to be localized within the individual hydranths since there is no correlation of KP activity between adjacent hydranths during stimulation. It is peculiar, however, that LP-KP coupling is so variable from very tight to very loose even within hydranths on the same upright. It is possible that the condition of the individual hydranths dictates the extent of coupling or the threshold of coupling between these two systems.

#### SUMMARY

1. Electrical, mechanical or chemical (KCl) stimuli produce similar luminescent flashing in *Obelia geniculata*. Hydranth withdrawal also occurs upon electrical or mechanical stimulation.
2. Luminescent flashes in response to individual stimuli occur in bursts. The first few flashes of the burst facilitate in intensity and then decline. Successive flashes within each burst show a constant shape and duration but a variable intensity.
3. The decay phase of the luminescent flash has a rate constant of  $50 \text{ s}^{-1}$  at  $20 \pm 1 \text{ }^\circ\text{C}$  and  $29 \text{ s}^{-1}$  at  $12 \pm 1 \text{ }^\circ\text{C}$ . From these figures an energy of activation of about 12 kcal can be calculated.
4. Upon stimulation, an excitation system, the luminescent potential (LP) system,

initiates the luminescent effector flashing. The LPs are monophasic, positive, and slow (duration about 120 ms) and are distinguishable from contraction potentials (KPs), mouth-opening potentials (MOPs) and tentacle contraction potentials (TKPs). LPs are all-or-none, non-decremental, through-conducting (at least over short distances) and show non-polar spread.

5. The LP systems can drive the KPs at the LP frequency. Coupling varies from none to very tight, and the degree of coupling is determined at each hydranth.

6. Several other campanulariids and probably most, if not all, luminescent hydroids possess similar physiological mechanisms for controlling stimulus-initiated luminescent responses.

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#### REFERENCES

- ALLMAN, G. J. (1871). A monograph of the gymnoblastic or tubularian hydroids. I. *The Hydroida in general*. London: Royal Society, 450 pp.
- BUCK, J. B. (1953). Bioluminescence in the study of invertebrate nervous systems. *Anat. Rec.* **117**, 594.
- BULLOCK, T. H. & HORRIDGE, G. A. (1965). *Structure and Function in the Nervous Systems of Invertebrates*, Vol. 1. San Francisco and London: Freeman.
- CHANG, J. J. (1954). Analysis of the luminescent response of the ctenophore, *Mnemiopsis leidyi*, to stimulation. *J. cell. comp. Physiol.* **44**, 365-94.
- DAVENPORT, D. & NICOL, J. A. C. (1955). Luminescence in hydromedusae. *Proc. Roy. Soc. Lond. B*, **144**, 399-411.
- ECKERT, R. (1967). The wave form of luminescence emitted by *Noctiluca*. *J. gen. Physiol.* **50**, 221-38.
- FORBES, E. (1848). A monograph of the British naked-eyed medusae. London: Royal Society.
- HASTINGS, J. W. & MORIN, J. G. (1968). Calcium activated bioluminescent proteins from ctenophores (*Mnemiopsis*) and colonial hydroids (*Obelia*). *Biol. Bull. mar. biol. Lab., Woods Hole* **135**, 422.
- HASTINGS, J. W., MITCHELL, G., MATTINGLY, P. H., BLINKS, J. R. & VAN LEEUWEN, M. (1969). Response of aequorin bioluminescence to rapid changes in calcium concentration. *Nature, Lond.* **222**, 1047-50.
- JOSEPHSON, R. K. (1961a). Colonial responses of hydroid polyps. *J. exp. Biol.* **38**, 559-577.
- JOSEPHSON, R. K. (1961b). Repetitive potentials following brief electrical stimuli in a hydroid. *J. exp. Biol.* **38**, 579-93.
- JOSEPHSON, R. K. (1967). Conduction and contraction in the column of *Hydra*. *J. exp. Biol.* **47**, 179-90.
- JOSEPHSON, R. K. & MACKIE, G. O. (1965). Multiple pacemakers and the behaviour of the hydroid *Tubularia*. *J. exp. Biol.* **43**, 293-331.
- JOSEPHSON, R. K. & MACKLIN, M. (1967). Transepithelial potentials in *Hydra*. *Science, N.Y.* **156**, 1629-31.
- LYKE, E. B. (1965). The histology of the sea pansies, *Renilla reniformis* (Pallas) and *Renilla köllikeri* Pfeffer with a note on the fine structure of the latter species. Ph.D. Thesis, University of Wisconsin.
- MACKIE, G. O. (1965). Conduction in nerve-free epithelia of siphonophores. *Am. Zool.* **5**, 439-53.
- MACKIE, G. O. (1968). Electrical activity in the hydroid *Cordylophora*. *J. exp. Biol.* **49**, 387-400.
- MORIN, J. G. & COOKE, I. M. (1971a). Behavioural physiology of the colonial hydroid *Obelia*. I. Spontaneous movements and correlated electrical activity. *J. exp. Biol.* **54**, 688-706.
- MORIN, J. G. & COOKE, I. M. (1971b). Behavioural physiology of the colonial hydroid *Obelia*. III. Characteristics of the bioluminescent system. *J. exp. Biol.* **54**, 723-735.
- MORIN, J. G. & HASTINGS, J. W. (1971). Biochemistry of the bioluminescence of colonial hydroids and other coelenterates. *J. Cell Physiol.* (in the Press).
- MORIN, J. G. & REYNOLDS, G. T. (1969). Fluorescence and time distribution of photon emission of bioluminescent photocytes in *Obelia geniculata*. *Biol. Bull. mar. biol. Lab., Woods Hole* **137**, 410.
- MORIN, J. G. & REYNOLDS, G. T. (1971). The cellular origin of bioluminescence in the colonial hydroid *Obelia*. *J. exp. Biol.* (in the Press).
- MORIN, J. G., REYNOLDS, G. T. & HASTINGS, J. W. (1968). Excitatory physiology and localization of bioluminescence in *Obelia*. *Biol. Bull. mar. biol. Lab., Woods Hole* **135**, 429-30.

- NICOL, J. A. C. (1955*a*). Observations on luminescence in *Renilla* (Pennatulacea). *J. exp. Biol.* **32**, 299-320.
- NICOL, J. A. C. (1955*b*). Nervous regulation of luminescence in the sea pansy *Renilla köllikeri*. *J. exp. Biol.* **32**, 619-35.
- PANCERI, P. (1876). Intorno alla sede del movimento luminosos nelle Campanularie. *Rendic. Accad. Sci.phis. matem.* **15**, 193-5.
- PANCERI, P. (1877). Luminous Campanulariae. *Nature, Lond.* **16**, 30.
- PANTIN, C. F. A. (1935*a*). The nerve net of the Actinozoa. I. Facilitation. *J. exp. Biol.* **12**, 119-38.
- PANTIN, C. F. A. (1935*b*). The nerve net of the Actinozoa. II. Plan of the nerve net. *J. exp. Biol.* **12**, 139-55.
- PANTIN, C. F. A. (1935*c*). The nerve net of the Actinozoa. III. Polarity and after-discharge. *J. exp. Biol.* **12**, 156-64.
- PANTIN, C. F. A. (1935*d*). The nerve net of the Actinozoa. IV. Facilitation and the 'staircase'. *J. exp. Biol.* **12**, 389-96.
- ROBSON, E. A. & JOSEPHSON, R. K. (1969). Neuromuscular properties of mesenteries from the sea anemone *Metridium*. *J. exp. Biol.* **50**, 151-68.