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BEHAVIOURAL PHYSIOLOGY OF THE COLONIAL HYDROID *OBELIA*

III. CHARACTERISTICS OF THE BIOLUMINESCENT SYSTEM*†

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INTRODUCTION

Bursts of bioluminescent flashes from individual photocytes in *Obelia geniculata* are apparently under the control of a conducting system, the luminescent potential (LP) system (Morin & Cooke, 1971*b*). This excitation system responds to electrical stimulation by producing bursts of non-decrementing, constant-amplitude potentials (LPs) which show non-polar spread. A LP precedes each luminescent flash. The flashes in a burst show an initial rapid increase of light intensity and then a decline (a summary of these observations is given in Morin & Cooke, 1971*b*, fig. 5).

Facilitation within this excitation-effector system occurs at several sites. There is facilitation (1) at the point of stimulation of the LP excitation system (Morin & Cooke, 1971*b*), (2) within the excitation system itself (see below), and (3) at the junction between the excitation system and the luminescent effector (Morin & Cooke, 1971*b*). This paper will describe some of the quantitative aspects of these various types of facilitation and will further characterize the physiology of the bioluminescent system of *O. geniculata*.

Facilitation within coelenterate excitation (conducting) systems (which includes interneural facilitation) has been studied in the actinian *Calliactis* (Pantin, 1935*a*), a number of corals and octocorals (Horridge, 1957), the hydroid *Cordylophora* (Josephson, 1961*b*, 1965), and a few other hydroids (Josephson, 1961*a*).

The problem of neuromuscular facilitation in coelenterates has been extensively examined in actinians (Pantin, 1935*a–d*; Ross, 1952; Josephson, 1966; Robson & Josephson, 1969). It has also been studied in the Scyphozoa (Bullock, 1943). Facilitation of luminescent effectors has been examined in the pennatulid *Renilla* (Nicol, 1955*a*, *b*) and in the hydromedusa *Aequorea* (Davenport & Nicol, 1955). There appear to have been no studies of excitation-effector facilitation (which would include neuromuscular facilitation) attempted on the Hydrozoa.

MATERIALS AND METHODS

The materials and methods used were the same as those described previously (Morin & Cooke, 1971*a*, *b*).

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

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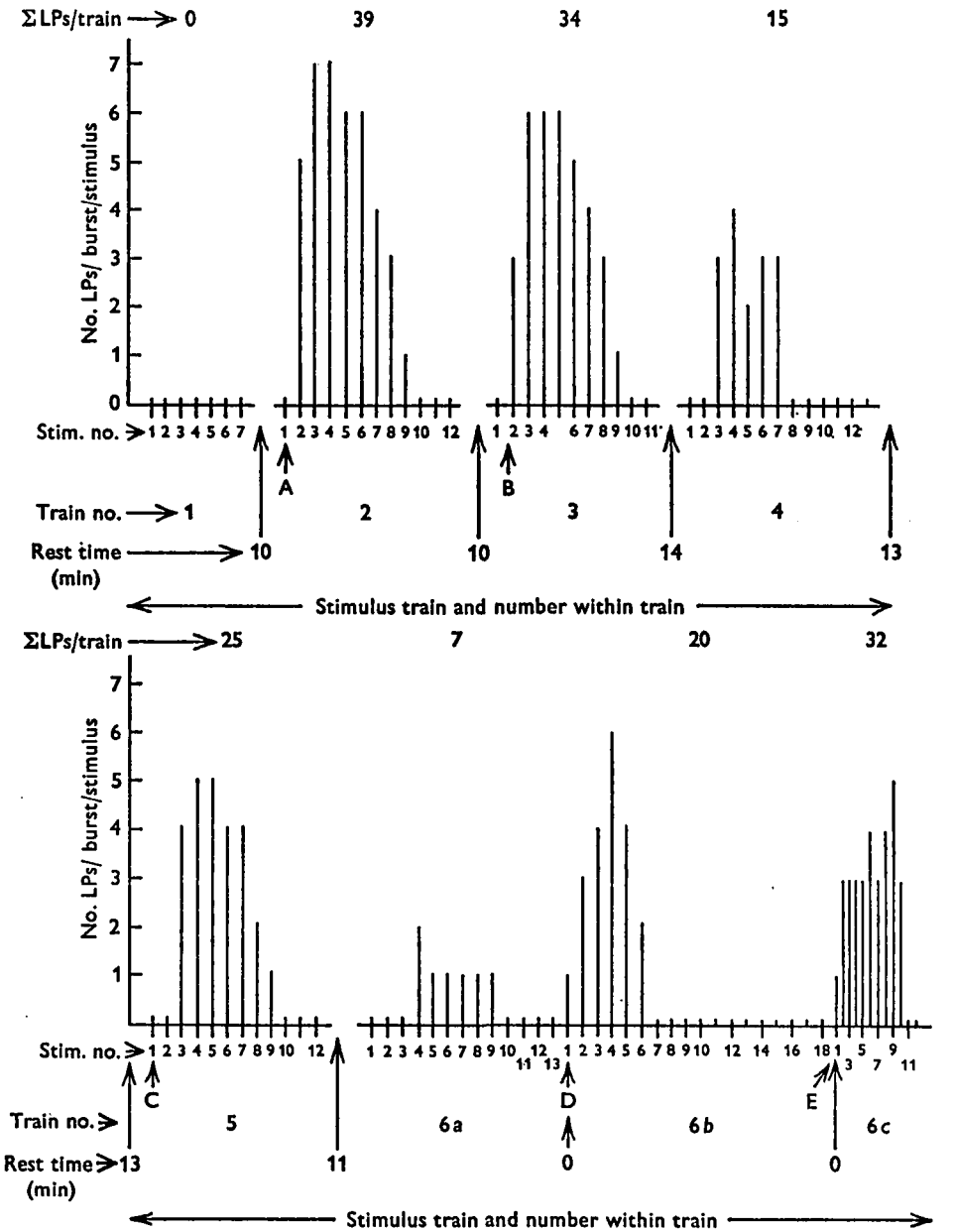

Fig. 1. Number of LPs per burst for each stimulus during successive stimulus trains. Time is represented on the abscissa and number of LPs per burst is represented by each vertical bar. The rest time between stimulus trains is given below the arrows. LPs/train indicates the sum of all the LPs in response to all stimuli of that train (LPs per burst per stimulus per train). A, increase in stimulus strength. B, recovery following a period of rest. C, increase in stimulus strength. D, increase in stimulus duration. E, increase in stimulus frequency. Trains 1–6*b* stimulus frequency at 1/2 s, 6*c* at 1/s.

RESULTS

(I) *Stimulus-excitation system facilitation*

Because of the presence of facilitation within the LP excitation system the number of LPs recorded at some distance from the point of stimulation may be less than the number of LPs initiated at the point of stimulation (see below). However, the number of LPs initiated can be accurately monitored if the recording electrode is very near the point of stimulation. Facilitation within the LP system at these sites of successive electrical stimulation is shown (1) by the failure of the system to respond with LPs until after the second or third stimulus, (2) by an increase in the number of LPs following the first few stimuli in a train, (3) by the decrease in latency to the first LP following the first few shocks, and (4) by the appearance of LPs after fewer stimuli when a second train of stimuli is applied after a period of rest (Morin & Cooke, 1971*b*).

Fatigue and adaptation of the LP system are suggested during continued stimulation by (1) the decrease in the number of LPs per burst, (2) the increase in latency between the stimulus and the first LP of a burst, and (3) the decrease in the total number of LPs per stimulus train during subsequent stimulus trains. Fig. 1[A] shows that there is a definite threshold for LP-burst firing. Adaptation to stimulation by a rise in threshold after the first few stimuli of a train is indicated by the recovery of LP responses after a period of rest between trains (Fig. 1[B]), or by the recovery of LP responses following an increase of the stimulus strength (Fig. 1[C]), duration (Fig. 1[D]), or frequency (Fig. 1[E]). In the cases where the stimulus intensity was increased (Fig. 1[C–E]), the total number of LPs per train (i.e. the sum of the LPs in each burst per train) was greater than that in the immediately preceding train. Usually the maximum number of LPs per burst (indicated by the vertical lines) was also increased. Because the first suprathreshold train of stimuli produced the greatest total number of LPs per train and the greatest maximum number of LPs per burst, it is reasonable to conclude that fatigue within the conducting system was also occurring along with the adaptation. Fatigue became more pronounced in the later trains of stimuli and eventually the LP system did not respond at all, even to a 10-fold to 20-fold increase of one or more of the stimulus parameters.

The combination of the relatively rapid adaptation and fatigue within the LP excitation system and the variable responses from one colony to the next made it difficult to determine whether the number of LPs in a burst was proportional to the stimulus strength and/or duration. The existence of a definite threshold and the recovery of the LP activity with increased stimulus intensity suggest that the number of LPs in a burst is related to the stimulus intensity. More information is required before the absolute relationship can be determined.

(II) *Facilitation, through-conduction and conduction velocity within the luminescent potential system*

Multiple light guides and suction electrodes placed a few millimetres to several centimetres apart, yielded information about facilitation, through-conduction and conduction velocity of the LPs. Fig. 2 demonstrates three spatial features: (1) the initial bursts or initial portions of a burst are not always represented at the more

distant electrode and light guide. (2) If invasion occurs at both sites, the terminal LP intervals are the same at both points. (3) LPs at the far electrode appear later than the corresponding LP in the near electrode.

Bursts were compared at two sites by placing one recording electrode near the stimulating electrode and another at varying distances from the first, either on the same upright or on a different upright. The responses to stimulation were highly labile so that comparisons of bursts could be made at only a single location of the two electrodes before the system no longer responded reliably to stimulation. A new colony had to be used to compare bursts for a different electrode distribution. This method shows that if the second recording electrode is on the same upright as the one near the stimulating electrode, almost all of the LPs will appear at both sites with only a short

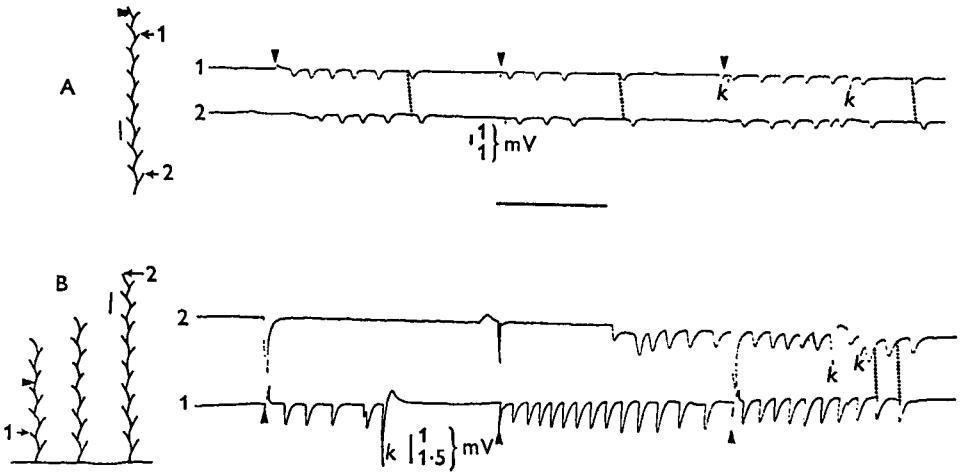

Fig. 2. Records of LP activity from two recording sites (1, 2) during stimulation (arrows). A, electrodes separated by a small distance. B, electrodes separated by a large distance. Delay between corresponding potentials is indicated by dotted lines. (These lines are not vertical because of the curvilinearity of the recording chart paper.) The vertical bars of the recording traces represents millivolts as indicated, the horizontal bar indicates 1 s, and the vertical bar of the hydroid drawings indicates 1 mm.

delay between them (Fig. 2A). As recordings are made at progressively more distant uprights with the second electrode, fewer and fewer of the LPs which appear in the near electrode appear in the far electrode (Fig. 2B). At a great enough distance entire LP-bursts fail to appear at the far electrode. When LPs do appear at the far site, the LP intervals match when progressively earlier intervals, from the last toward the beginning of each burst, are compared. This indicates that the initial LPs fail to invade parts more distal from the point of stimulation, but once LP invasion is achieved the LP-burst patterns are maintained and the system is through-conducting to that point.

These observations suggest that there are a series of facilitation sites distributed within the conducting system. As each LP-burst, travelling down the conducting system, encounters a facilitation site the first LPs will fail to be propagated beyond that site, but they will enable succeeding LPs to do so. The net effect is a loss with distance of the first LPs in a burst, but a through-conduction of the later potentials. As has been noted above whole bursts are prevented from reaching a distant point

by the necessity for such facilitation in the distance of spread. A single point in the colony displays temporal facilitation and several such points distributed along the conducting system result in a facilitation in the distance of spread. Since the luminescent effectors of this system are distributed along the pathways, the net result is an incremental spread of bioluminescence throughout the colony with successive stimuli.

By knowing the distance between the recording electrodes and by counting the number of LPs lost between the two electrodes, it should be possible to determine the approximate distance between the facilitation sites. Three assumptions must be made: (1) each facilitation site blocks only one LP and then allows the others to pass, (2) the

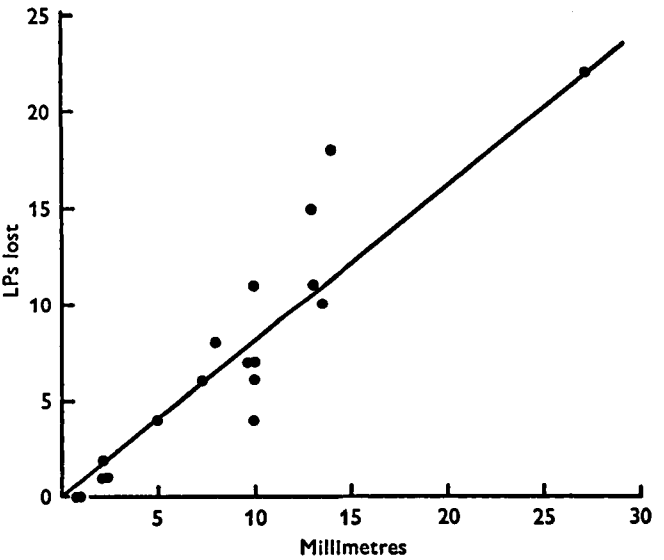

Fig. 3. Plot of the number of LPs lost between two recording electrodes with respect to the distance between the two electrodes. The line is drawn from the calculated least-squares regression line.

facilitation sites are evenly spaced throughout the colony, and (3) the temporal aspects of the facilitation at a site are sufficiently slow so that successive LPs within any given burst are unaffected. The first of these assumptions is probably permissible because the loss of a single LP has been observed between two closely spaced electrodes. The second and third assumptions are possibly over simplifications and may be responsible, at least in part, for the variation described below. A plot of the number of LPs lost between two recording electrodes (with stimulation near one of them) against the distance between the two recording electrodes is shown in Fig. 3 for 18 experiments. The plot is approximately linear over the range examined (the line represents the least-squares regression line). This suggests that the sites are evenly distributed. The average distance per loss of one LP as calculated from this data (and thus the distance between facilitation sites) is $1{\cdot}3 \pm 0{\cdot}5$ mm. This is about two facilitation sites for every three internodes of an upright (the average length from one internode to the next is about 0·8 mm).

The characteristics of the facilitation within the excitation system at any given

point are indistinguishable from the stimulation-excitation system facilitation described above, which suggests that the two sites may be identical.

Conduction velocities can be computed from records made between two points since corresponding LPs, as verified by LP intervals, show an increased delay with increasing distances between the two electrodes (Fig. 2). Recordings have been made at distances of up to 3 cm between the two electrodes. The conduction velocity computed from 103 measurements in 11 experiments showed a range from 13 to 32 cm/s (12 °C) with a mean of 22 cm/s. This figure is slightly higher than the conduction velocity of 15–20 cm/s reported earlier (Morin, Reynolds & Hastings, 1968).

(III) *Latencies and the refractory period of the luminescent potentials*

The latency from stimulation to the initial response of the LP system is difficult to ascertain because of the varying distance between the stimulating and recording electrodes from one experiment to the next. An approximate limit can be fixed by recording close to the stimulating electrode, measuring the delay, and correcting for conduction time. Latencies of 130 to over 600 ms were observed for the first response to a stimulus of a train. The latency dropped to 20–80 ms for the second and third stimuli and then increased to 150–600 ms for succeeding stimuli until the responses ceased (Morin & Cooke, 1971*b*, fig. 5).

The refractory period for the LP was difficult to determine because of the repetitive discharge of the conducting system, but it probably is near the minimum interval between LPs in a burst (slightly more than 60 ms at 20 °C and 150 ms at 12 °C). This value for the refractory period is suggested by two observations. (1) The LP system will not follow frequencies of stimulation much above 5/s at 12 °C. (2) At stimulation frequencies of 2 or more per second a burst will normally not have terminated before the succeeding shock occurs, and the first LP of the new burst does not occur earlier than about 60 ms from the last LP of the preceding burst at 20 °C or 150 ms at 12 °C.

The latency between the onset of excitation (the LP) and the onset of the effector response (the luminescent flash) was estimated by measuring the delay and correcting for conduction time between the luminescent site under observation and the suction electrode. The light guide field and the suction electrode were 0·25–1·0 mm apart. Adding the correction time of 3 ms for the average recording distance of 0·7 mm gave a minimum latency of 5 ms and an average latency of about 20 ± 9 ms for 20 measurements.

(IV) *Luminescent potential intervals and facilitation between the LP system and the luminescent site*

The intervals between successive flashes are a faithful reflexion of the intervals between the LPs within a burst (Morin & Cooke, 1971*b*). The latency between the luminescent flash and the LP does not change appreciably for a given recording position. Therefore, the interval characteristics of a LP-burst can be determined using either electrical or luminescent responses. A statistical analysis of the LP intervals was made by comparing progressively earlier intervals from the last toward the beginning of the burst as described above. LP intervals were examined in this way at 12 ± 1 °C and 20 ± 1 °C, and at stimulation frequencies of 5/s, 1/s, 1/2 s and 1/5 s.

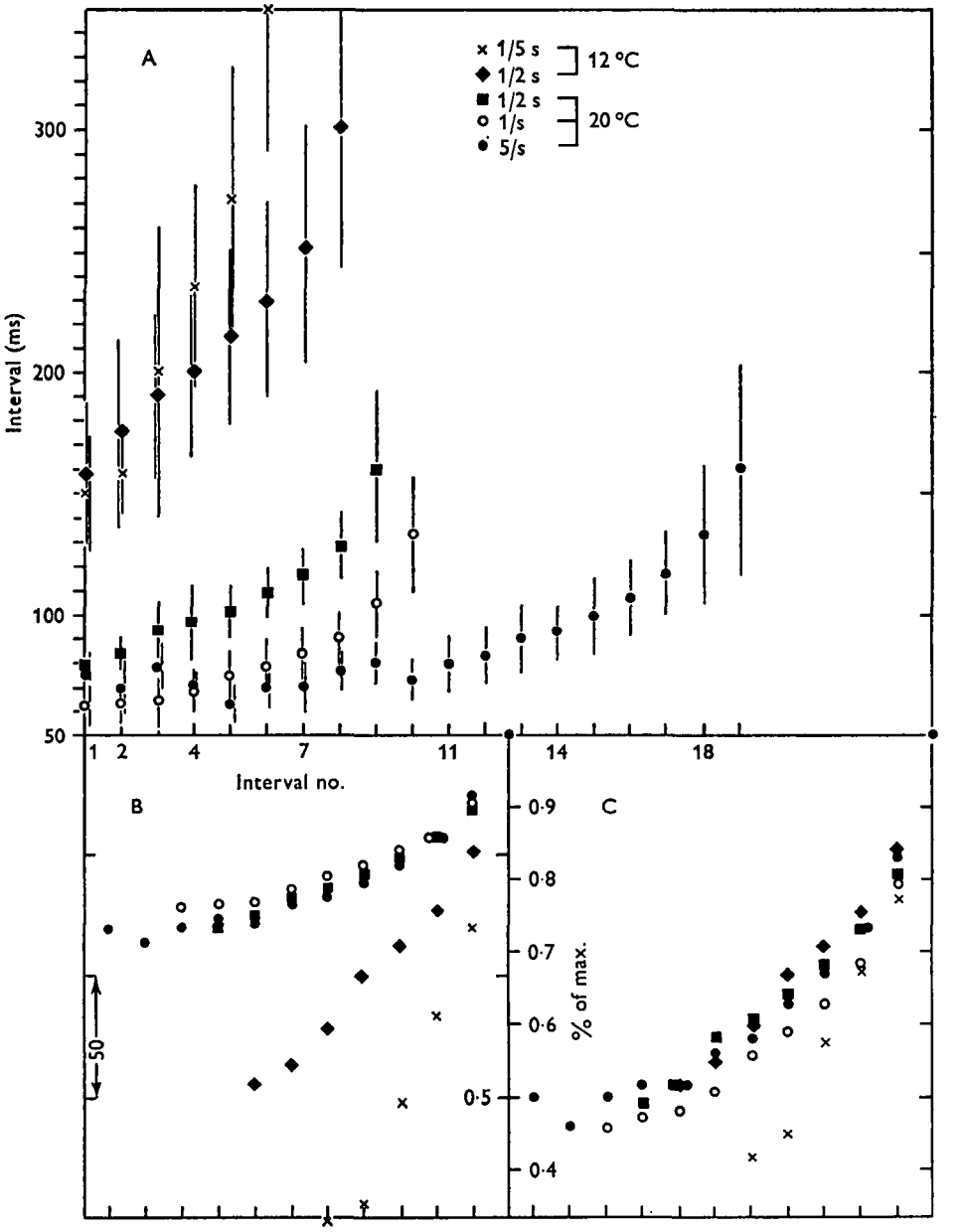

Fig. 4. Average intervals between successive LPs at 20 ± 1 °C and 12 ± 1 °C and at stimulation frequencies of 5/s, 1/s, 1/2 s and 1/5 s. Vertical bars indicate standard deviations. A, absolute interval characteristics: 20 °C, 5/s, $N = 3$; 20 °C, 1/s, $N = 61$; 20 °C, 0·5/s, $N = 21$; 12 °C, 0·5/s, $N = 63$; 12 °C, 0·2/s, $N = 19$. B, curves from A all translated so that the final interval is set to a common arbitrary number. Ordinate units (50) are 'difference interval' in ms. C, curves from A all converted into a percentage of the maximum (i.e. final) interval of each curve.

The usual pattern shows a gradual increase in LP interval with successive LPs (Fig. 4A). Successive bursts within a train show similar interval characteristics although the number of LPs may vary. Thus the equivalent intervals of many bursts were averaged together. A decrease in temperature increases the LP intervals; increasing the frequency of stimulation increased the number of LPs per burst, but had no apparent affect on the rate of interval increase.

The nearly constant interval pattern regardless of the stimulus frequency is shown by translating the last interval of each curve of Fig. 4A to an arbitrary number and adding the difference between this number and the real number to each succeeding interval (Fig. 4B). Two distinct sets of curves were obtained; one at 12 °C and the other is at 20 °C. This shows that the LP-burst sequence runs an apparently predetermined, temperature-dependent course which is unaffected by stimulation frequency. The mechanism determining this LP-interval sequence is not known.

Temperature changes the interval characteristics of a burst (Fig. 4A), but the proportional changes are not altered. This can be shown by setting the terminal interval of a burst to unity and computing the remaining intervals as a percentage of that interval (Fig. 4C). The curves at the two temperatures are nearly identical and indicate that the relative ratios of successive intervals to the final interval are independent of both temperature and stimulation frequency, while the absolute intervals are temperature dependent but independent of stimulus frequency.

The functional significance of this apparently predetermined sequence is not entirely clear, although the time course and the number of LPs appear to play a role in the facilitation mechanisms operating at the LP system-luminescent site junction and possibly at the facilitation sites within the LP system as well.

Successive flashes within the first part of a burst show facilitation of intensity while the LPs initiating these flashes are all-or-none (Morin & Cooke, 1971*b*, fig. 1). The flashes usually do not summate; in most cases the duration of a flash is less than the interval between flashes. Occasionally, the first two or three flashes of a burst at 20 °C may summate to a small degree since the flash duration (about 75 ms) is slightly longer than the first two or three LP-intervals (Fig. 4A). However, as the intervals exceed the flash duration, summation ceases. Facilitation is rapid but variable in response to the first three to five LPs. The usual method of measuring facilitation is to express the facilitation (f) as a percentage of the first response

$$f = \frac{v - v_0}{v_0}, \tag{1}$$

where v is the mean amplitude (intensity in this case) of the response and v_0 is the mean amplitude (intensity) of the initial response (Mallart & Martin, 1967, 1968). Because the facilitation within the LP-system produces a variable first flash depending on the distance of the luminescent site from the stimulus and on the previous stimulation history it was not possible to use equation (1). Thus, facilitation was determined by using the flash with the maximum intensity (I_{max}) as the reference point

$$f = \frac{I}{I_{max}} \times 100, \tag{2}$$

where I is the mean intensity of the flash response for each flash in a burst. Facilitation is expressed as the intensity of a percentage of the maximum intensity. As with the

interval measurements, the facilitation for each flash in a total of 12 bursts was determined from the last flash working toward the beginning of each burst (Fig. 5).

Toward the end of a burst there is an apparent fatigue or adaptation of luminescent flashing. There is evidence that suggests that this decrease in intensity from I_{max} is causally related to the increase of the LP interval and a rapid decay of facilitation. A single shock sets off a battery of LP responses. These responses probably act upon the luminescent effector as multiple electrical stimuli normally act upon the sphincter

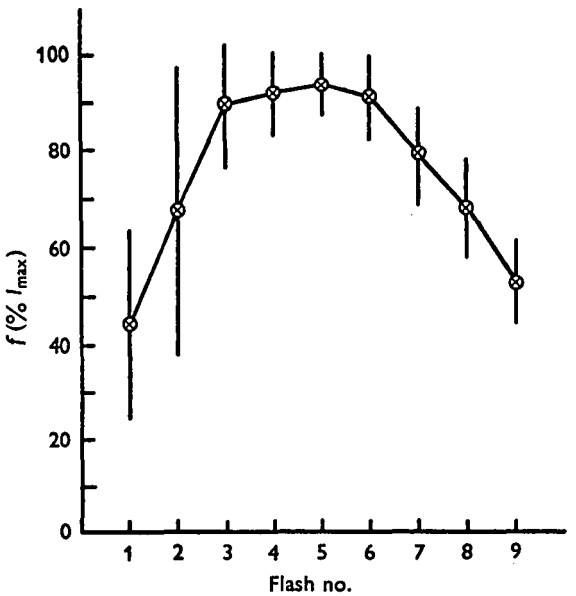

Fig. 5. Facilitation (f) of flash intensity with respect to the flash number within a burst. Facilitation was computed as a percentage of I_{max}. Vertical lines show the standard deviation as determined from 12 bursts.

muscle of *Calliactis* (Pantin, 1935*a–d*); that is, each LP produces facilitation at the effector junction in *Obelia* just as the individual impulse in response to a single shock produces neuromuscular facilitation in *Calliactis* (Josephson, 1966; Robson & Josephson, 1969). A facilitation versus stimulus-interval curve can be constructed for *Calliactis* by double-shock experiments of varying intervals. Such an experiment cannot be carried out on *Obelia* since the effective stimulus to the luminescent site, namely the LP system, occurs in bursts. However, the interval between successive LPs increases within the burst (Fig. 4). Thus, assuming that only the preceding LP and no other affects the facilitation response of the luminescent effector, a facilitation curve can be constructed by re-plotting Figs. 4A (1/s, 20 °C) and 5 as facilitation against LP-interval (i.e. the period of time which elapsed since the preceding LP) (Fig. 6A). This facilitation curve can be extended by measuring the height of the first flash in the succeeding burst (if it occurs with the first LP of that burst) as if it were part of the preceding burst. The interval preceding the flash of the succeeding burst is a function of (1) the stimulus frequency, (2) the termination time of the preceding burst with respect to the succeeding stimulus, and (3) the latency between the succeeding stimulus

and the first LP and its accompanying flash. Thirteen different bursts with varying intervals to the first flash of the next burst were measured and plotted in order to produce an extension of the first facilitation curve (Fig. 6A) in Fig. 6B. The plot shows a facilitation curve with a rapid rise between 60 and 80 ms, a fairly rapid decay between about 80 and 200 ms, and then a long residual decay for the next several hundred milliseconds. At the present time there is not sufficient data to allow the mathematical treatment used by Mallart & Martin (1967, 1968). We can conclude,

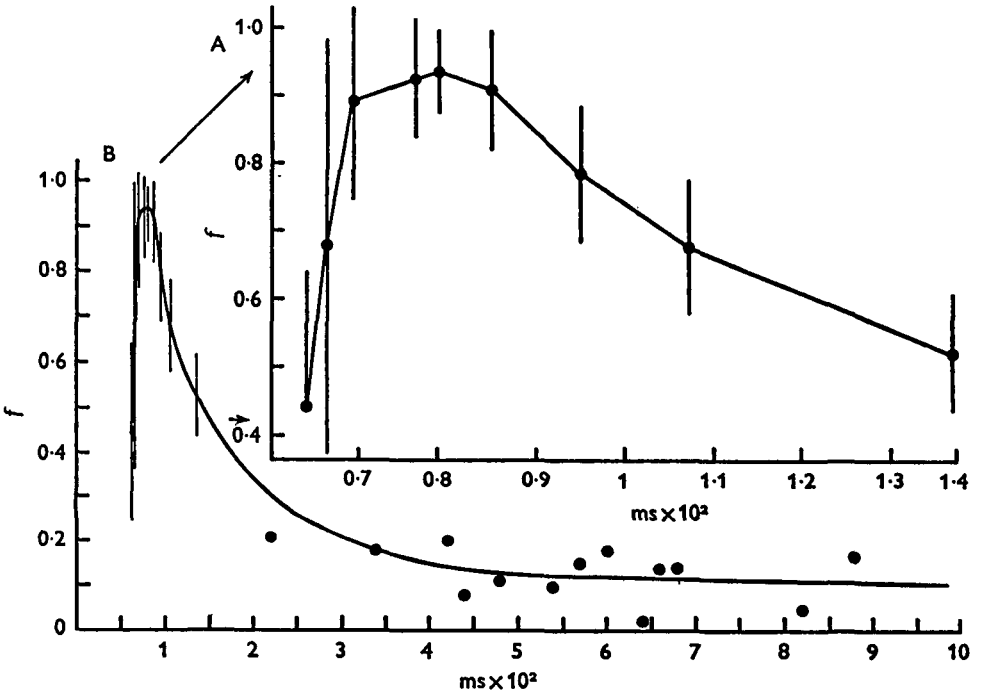

Fig. 6. Facilitation of flash intensity with respect to the interval between flashes. A, facilitation curve derived from Figs. 4 and 5. Vertical bars represent standard deviation. B, same curve as A but showing the extended 'tail' of the curves as derived from the first flash taken from 13 bursts following a previous burst (see text for details).

however, that declining facilitation is responsible, at least partially, for the decrease of the flash intensity toward the end of a burst. As the intervals increase within a burst, the facilitatory response drops rapidly and thus successive flashes decrease in intensity.

The above data constitute physiological evidence for a junctional component between the LP excitation system and the luminescent effector: (1) there is a latent period between a LP and a flash, and (2) there is facilitation of the flash intensity in response to successive LPs. Anatomical information on the nature of this junction is lacking at present.

DISCUSSION

By removing a kelp frond, covered with *Obelia geniculata* from the sea at night and prodding a colony with increasingly greater pressure at one point, the keen observer will see a progressive invasion of bioluminescent light throughout the colony; the

maximum intensity and flashing of light occurs nearest the point of stimulation, and there is an increase in threshold and a gradual adaptation to the stimulus. These observations can be understood in the light of the various types of facilitation, adaptation and other characteristics presented here. The luminescent system shows facilitation between the stimulus and the LP-system, within the LP-system and between the LP-system and the luminescent effector. The first two behave similarly and, therefore, may involve similar sites and have an identical mechanism. All types show temporal facilitation at a fixed point while facilitation in the distance of spread (a spatial form of facilitation) is also shown in the excitation (LP) system between two or more points which show temporal facilitation.

A stimulus input, whether it be mechanical or electrical, is transduced into a burst of potentials which is a function of duration, frequency and probably strength of the stimulus as well as the recent history of the conducting system. If the stimulation is repetitive the bursts of potentials also shows facilitation of LP number at the site of stimulation. As the LPs are conducted through the *Obelia* colony they are subjected to additional integration by the facilitation within the conducting system. At any point this represents a temporal integration of the repetitive potentials. The result is an increase in the number of LPs in successive bursts passing beyond the facilitation junctions with time. The net effect of a number of such integrative junctions is a decrease in number of the all-or-none LPs with increasing distance away from the point of stimulation. However, once the whole colony is invaded by the LPs, the conducting system is, for a short time, in a state of total through-conduction. This occurs only during periods of strong stimulation; total invasion does not normally occur with milder stimulation. Thus the conducting system shows the conversion of a temporal input into a temporo-spatial output; the extent and number of LPs is an expression of the applied stimulus.

The input signal is further modified at the LP-luminescent effector junction where facilitation both decreases the number of effective potentials and increases the intensity of successive flashes. These flashes are also modulated by the LP intervals and the refractory period of the LP-system.

A conducting system such as the LP-system in which the distance of spread increases with increasing number, frequency and strength of stimuli has been termed an incrementing system by Josephson (1965). Conducting systems in which the distance of spread is independent of the stimulus parameters were termed through-conducting systems. However, these terms are not entirely adequate to define the LP-system since it does show through-conduction under some circumstances. The only other incrementing conducting system which has been described from electrophysiological evidence is found in the stolon of *Cordylophora* (Josephson, 1961*b*). Pantin (1935*a*) was the first to present evidence from visual observations for such an incrementing system in his description of interneural facilitation in the oral disk of *Calliactis*. Subsequent visual observations have shown other incrementing systems in certain hydroids (*Hydractinia*, *Cordylophora* and *Pennaria*) (Josephson, 1961*a*) and in corals and octocorals (Horridge, 1957).

The major question still unanswered concerning the bioluminescence of *Obelia* is what function is served by this comparatively sophisticated integration of input signal into a dynamic display of output response. Speculation is premature at this time and

must be deferred until more is known about the general ecology and *in situ* behaviour of *O. geniculata.*

SUMMARY

1. The bioluminescent system of *Obelia* responds to stimulation by producing a burst of excitatory potentials (luminescent potentials, LPs) with each stimulus except the first. These LPs initiate flashes from the coupled luminescent effectors.

2. There is temporal facilitation between the applied stimulus and the LP-system; the LP-system shows adaptation to stimulation by a rise in threshold as well as fatigue.

3. There is facilitation within the LP-system that is temporal at a given point and spatial (in the distance of spread) between two or more points. The result is a limited spread of the first potentials and decreasing numbers of potentials with increasing distance but often a through-conduction of the final potentials.

4. The conduction velocity of the LPs is about 22 cm/s at 12 °C; the refractory period is about 60 ms at 20 °C and 150 ms at 12 °C; the latency from stimulation to the first LP in a stimulus train first decreases from 130–600 ms to 20–80 ms and then increases again to 150–600 ms at 20 °C. The latency between LP and the flash is about 20 ± 9 ms.

5. The intervals between LPs in bursts evoked by stimuli applied at 1/s gradually increase from about 65 ms to about 135 ms at 20 °C. The absolute LP intervals are temperature-dependent (the relative ratios are not) but independent of the stimulus frequency. The number of LPs in a burst is frequency dependent.

6. The facilitation curve of the LP-system-luminescent effector junctions, as determined from flash intensity, shows a rapid rise between 60 and 80 ms, a fairly rapid decay between 80 and 200 ms, and a long residual decay which lasts for several hundred milliseconds.

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