NERVOUS REGULATION OF LUMINESCENCE IN THE SEA PANSY *RENILLA KÖLLIKERI*

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INTRODUCTION

Recent investigations of the sea pansy *Renilla*, carried out independently by Buck and myself, have shown that this animal is particularly favourable for the study of luminescent responses. Under tactile and electrical stimulation the sea pansy produces distinct luminous waves which run over the entire surface of the rachis from the point of stimulation. Luminescence is under the control of a diffuse, unpolarized nerve net, and the course and speed of the luminescent wave are indices of direction and velocity of propagation of excitation in the nerve net.

Luminescence in *Renilla* is intracellular, and the light appears from evenly distributed points all over the upper surface of the rachis. Buck (1955) has pointed out that the point-spots of light arise from three loci, viz. the siphonozooids, and the body and tentacles of the autozooids. The luminous wave which sweeps over the colony results from the glowing of successive ranks of zooids (Parker, 1919, 1920).

In correspondence with results obtained on neuro-effector functioning in other coelenterates, it has been found that the luminescent responses of *Renilla* display marked facilitation. From various lines of evidence it has been deduced that facilitatory processes occur terminally, in the region of the photocytes. In contrast, through-conduction (1:1 transmission) appears to be the normal condition in the nerve net.

Each luminescent wave, including the first, invades the whole rachis in untreated fresh animals. However, evidence for interneural facilitation under special conditions has been secured by partially transecting animals, and studying transmission across the tissue junctions so produced. By this means it has been possible to produce preparations in which additional stimuli are required to bridge the junction between the partially separated segments of the animal. Such junctions are also subject to fatigue. Interneural facilitation, thus artificially revealed, provides functional evidence for synapses and multiplicity of conducting neurones in the nerve net (Buck, 1955; Buck & Coyle, 1955; Nicol, 1955).

The present paper presents further information about the luminescent responses of the pennatulid *R. köllikeri*. Neuro-effector functioning is analysed in more detail, and several additional aspects of the luminescent response have been explored by more refined methods, especially transmission velocity and response parameters.
Methods of recording and other experimental conditions are described in a previous paper (Nicol, 1955). These can be summarized in the statement that the animals were stimulated with condenser shocks (4 \( \mu \text{F} \)), and the luminescent responses were recorded by means of photomultiplier and oscilloscope.

**Facilitation**

The evidence now available shows that facilitation in *Renilla* takes place terminally, in the region of the neuro-effector junction (Buck, 1955; Buck & Coyle, 1955). When an animal is stimulated electrically, by a burst of shocks, facilitation manifests itself in two ways. First, several stimuli are required to elicit a response. Secondly, the consecutive responses in a series increase in intensity. These two aspects of facilitation, viz. initial flash dependent on several impulses, and increase in intensity of subsequent flashes, separately provide means of investigating and characterizing the phenomenon.

**Determination of Number of Impulses Necessary to Evoke the First Response at Low Frequencies**

Observations on this aspect were presented in a previous paper, in which it was shown that as the stimulus-interval was lengthened, more pulses were required to produce a flash (Nicol, 1955). A curve was presented (Fig. 2, 1955), showing that the number of stimuli preceding a flash increases in an exponential manner with lengthening interval until, when the interval exceeds 3 sec., some 15 pulses intervene before the first response appears. These results can be interpreted in terms of build-up and decay of a facilitatory state. At moderate and fast frequencies (> 1/sec.), facilitation reaches threshold level for the luminescent response after 2–3 impulses. But when the frequency is low (< 1/sec.), facilitation largely decays between pulses, and more impulses must intervene before effective level is reached.

**Intensity of Response as Affected by Stimulation Interval**

Previous observations have shown that the intensity of response is affected by interval between stimuli. With bursts of shocks, the net increment of consecutive responses is greater at higher rates of stimulation (see Figs. 1 and 3, A–D in my paper of 1955). In these records, obtained from the whole animal, the intervention of summation at higher frequencies obscures, in some measure, the extent to which facilitation is influencing the intensity of each response. To obtain more precise information about the course of facilitation at restricted loci, recordings have been obtained from narrow slits, some 3–5 mm. in width (Fig. 1). Some typical records obtained in this manner are shown in Fig. 6A, B.

The extent to which successive responses increase in intensity at different frequencies of stimulation is brought out in Fig. 2. The curves in this figure show the net increment in intensity of consecutive responses when the stimulation-interval is varied from 0.33 to 4 sec. The former interval approximates to the refractory period in this animal and, in fact, only the first two stimuli were effective,
later stimuli falling into the refractory period so that only each alternate stimulus was excitatory. There is practically no increment of consecutive responses at stimulation intervals of 2-4 sec., but at shorter intervals the intensity of response rises rapidly, and is obviously related to frequency of stimulation.

To investigate the temporal characteristics of build-up and decay of facilitation, experiments were carried out in the following manner. Specimens were stimulated with 2 or 3 shocks, just sufficient to produce a single flash, which was recorded. After a period of 5 min., stimulation was repeated with a different interval between shocks, and so on until a wide range of stimulus intervals had been explored.

Data obtained in this manner have been used to plot a facilitation/decay curve (Fig. 3). This figure is based upon the responses of a specimen which flashed on the second shock at frequencies about 15/min. In this preparation two shocks were effective when separated by intervals as long as 4 sec. At higher frequencies the animal ceased to respond to 2 shocks separated by an interval of 0.2 sec. Facilitation is little in evidence with intervals longer than 1 sec.; at shorter intervals, intensity of response, and therefore facilitation, increase steeply and linearly, up to a maximum at 0.2 sec. If Fig. 3 be compared with Fig. 2, it will be seen that in both instances facilitation becomes pronounced at intervals shorter than 1.5 sec.

With two impulses, therefore, the facilitatory state lasts about 3 sec. With more than two impulses, facilitation shows far longer duration. This is brought out by the following experiment.

EFFECT OF SUCCESSIVE BURSTS ON INTENSITY OF RESPONSE
Specimens were stimulated with repeated bursts of shocks, each burst consisting of the same number of shocks at the same frequency. In some, but not all specimens, when the interval between bursts is short, responses to successive bursts gradually increase in intensity. There is, of course, much variation from animal to animal,
depending on its previous history and level of excitability. Augmentation of response in successive bursts is illustrated in Fig. 4. The effect has been observed for as many as 10 successive bursts, after which other factors often supervene, leading to after-discharge or fatigue.

Facilitation induced by a burst of stimuli persists for several minutes in some specimens (at least 10 min., the longest interval studied). It appears from these results that repetitive stimulation results in progressive augmentation of facilitation, which takes a long time for complete decay: small residual levels may persist for several minutes, thus influencing subsequent responses. A similar persistence of
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Fig. 3. Facilitation-duration curve. The specimen was stimulated with pairs of shocks, the pulses in each pair separated by intervals ranging from 0.2 to 9 sec. Ordinates represent the intensity of the flash (a single flash to 2 shocks). Abscissae show interval between stimuli. Temp. 18° C.

Fig. 4. Facilitation shown in successive periods of stimulation. The specimen was stimulated with bursts of 10 shocks at 1/sec., each burst separated from the preceding one by an interval of 1 min. Each vertical line represents the intensity of the flash evoked by the 10th stimulus in each burst. Temp. 17° C.
small residual levels of facilitation has also been observed in muscular responses of sea anemones (Ross & Pantin, 1940; Pantin & Dias, 1952).

In these experiments, making use of repeated bursts of stimuli, facilitation manifests itself in yet another way. Initially, the first response appears on the 3rd stimulus; in later bursts with continued stimulation, a response appears on the 2nd or even the 1st shock. When fatigue sets in, and the response intensity declines, the first overt response appears again on the 2nd or 3rd stimulus. In these instances it appears that residual amounts of facilitation, when added to that produced by 1 or 2 shocks, raise the level of facilitation to threshold and cause premature flashing. In a similar manner, the facilitated retractor response of the sea anemone *Bunodactis* regularly appears on the 2nd stimulus, but in a repeatedly excited animal small responses to a single stimulus are encountered (Pantin & Dias, 1952).

![Fig. 5. Comparison of electrical and mechanical stimulation. A: response to a burst of 10 shocks at 1/sec. Dots below represent nervous impulses. The 1st flash appeared on the 2nd pulse. Time scale 14/min. B: responses to mechanical stimulus (dropping a weight on the rachis). Straight line below: duration of mechanical stimulus. Dots below: successive nervous impulses. In addition to propagated waves, there is some localized glowing which contributes to the deflexions in this record of mechanical stimulation. Time scale 72/min. All recordings made through an aperture 2 cm. in diameter.](image)

**FACILITATION IN MECHANICALLY INDUCED RESPONSES**

Facilitation is also observed in responses induced by tactile stimulation. By dropping a weighted point on the rachis of an animal, it is possible to evoke one or several luminescent waves. As the weight continues to press on the animal the waves, succeeding each other rapidly at first, decrease in frequency and amplitude and die away. This is probably due to a process of sensory adaptation, which is also visible on repeated tactile stimulation, when waves become fewer, less intense, and finally give way to a localized glow. Fig. 5B shows a series of flashes induced by tactile stimulation, compared with a series arising during electrical stimulation (Fig. 5A). Since the first flash in this animal is coming up on the second impulse, it is possible to estimate the succession of nervous impulses underlying the luminescent discharge during tactile stimulation. Flashing shows an interval of about 0.25 sec. at first; this increases to 1 sec., when the discharge ceases. Facilitation of the first three responses is observed when intensities of response are measured, viz. 5, 8, 10.5, 6, and 1 mm. deflexion. Intensity of response falls off as interval between impulses lengthens.

Under tactile stimulation the intensity of successive flashes is governed initially by facilitation, but sensory adaptation soon intervenes, rendering the animal insensitive to further stimulation. The degree of response to tactile stimulation
Nervous regulation of luminescence in Renilla shows much variation among different animals. Nevertheless, we may conclude that a propagated luminescent wave is the result of a battery of impulses evoked by sensory stimulation. The luminescent response of Renilla, in this respect, closely resembles sphincter contraction of Calliactis, in which tactile stimulation releases a series of impulses and produces a facilitated response (Pantin, 1935a).

LATENT PERIOD, DURATION OF RESPONSE, AND RATE OF CONDUCTION

Latent period and rate of conduction were determined simultaneously by a recording method making use of two slits (Fig. 1). One slit lay immediately under and in advance of the electrodes; the other several centimetres away. Each slit was about 3 mm. wide and 15 mm. long, with the long axis perpendicular to the line of advance of a wave-front. A few shocks at a slow frequency (c. 1/sec.) were administered and records obtained. Under these conditions latent periods of 0.12 sec. are usually encountered (17–19°C). The latent period so measured is the sum of latencies of transmission systems (nerve fibres and neuro-photocyte junctions), and of the photocytes.

Examination of the records shows that the latent period of the response is affected to some extent by the frequency of stimulation. In the majority of specimens, the latent periods are fairly constant at frequencies of 1/sec. or less. Moreover, the latencies of consecutive responses usually remain constant during a burst of stimuli at any one frequency below 2/sec. At temperatures of 17–20°C, the mean latency is 0.12 sec. At a higher frequency, 3/sec., it is found that the latent period of successive responses usually increases some two- or threefold during a burst of 10 shocks. There is much individual variation, however, and in some animals the increase in latent period may be as much as tenfold in a series of 10 shocks at higher frequencies. This is brought out in the measurements, shown in Table 1, of latent periods of successive responses from a specimen stimulated at different frequencies (cf. Fig. 6A, B).

The increase in latency which occurs at high frequencies could be taking place at one of three loci: at the synapses of the nerve net, at the neuro-photocyte junctions, or in the photocytes themselves. A change at either of the first two loci seems more probable, but specific data are not at hand to resolve this point. It is suggested that prolongation of latency may be the result of fatigue of the transmitter mechanism at high frequencies.

Conduction speed measured over distances of 35–41 mm. varied from 6.66 to 10.15 cm./sec., with a mean of 8.77 cm./sec. (15.9–17.2°C). There is a certain amount of variation from one animal to another, but the rates obtained from any one animal tend to be reasonably constant. The values obtained are of the same order as those found by other workers for Renilla, viz. 6.5 cm./sec. at 15°C., and 7.39 cm./sec. at 21°C. (Parker, 1920), and 4.5 cm./sec. at 21°C. (Buck, 1955). They conform to slow transmission speeds found in other coelenterates, viz. 12–14 cm./sec. for transmission of muscular excitation in Metridium (21°C.), and 4–120 cm./sec. in different pathways of Calliactis (18–20°C.) (Parker, 1920; Pantin, 1935b).
Temporal characteristics of the luminescent response at one locus were investigated by slit-recording (Fig. 6A, B). Measurements of response parameters from oscilloscope records have provided the following data. At a frequency of 1/sec., the duration of the weak 1st response is 0.25 sec. With continued stimulation the response increases in amplitude and apparent duration. After a series of 10 shocks

<table>
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<th>Interval (sec.)</th>
<th>Response</th>
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<tr>
<td>1.4</td>
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<td>7</td>
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The apparent lengthening of response with increase in amplitude may, in part, be an artificial effect determined by sensitivity of the recording apparatus. That is to say, the weak initial flashes are not reaching threshold sensitivity of the recording apparatus until some time after they have actually commenced. Buck (1955), however, points out that the autozooid calices produce a less intense but longer lasting
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glow than the siphonozooids. Moreover, the glowing of the autozooids appears, from visual observation, to be brought up by continued stimulation. There, is then, the additional possibility that apparent lengthening of the response with continued stimulation may be real, and result from differences in the excitability and activity of different luminous centres.

OBSERVATIONS ON HIGH-FREQUENCY STIMULATION
REFRACTORY PERIOD

Slit recordings of animals stimulated at high frequencies bring out certain additional features not evident in recordings from the whole animal (Nicol, 1955). Beginning with a frequency of 1/sec., and becoming more pronounced at higher frequencies, there is some summation of consecutive responses, i.e. each flash fails to decrease to zero before the next one begins (Fig. 6B). The increase in response-intensity secured by higher frequencies, up to 2-3/sec., results, therefore, from a combination of summation and facilitation at each locus of response (photo-emissive area). Despite some basal level of summation, the separate responses remain distinct and are imposed on a constantly glowing background.

At higher frequencies the intensity of response falls off (Fig. 7). At a frequency of 2/sec., initially at least, there is 1 flash for each stimulus beginning with the 3rd shock. With continued stimulation the exact correspondence ceases, and the animal gives only 6-7 flashes for 10 shocks, i.e. towards the end of each burst the animal is no longer responding to each stimulus. With higher frequencies this effect becomes more pronounced, and the animal responds only to every 2nd or 3rd stimulus. The refractory period lies around 0.2-0.5 sec, the exact value varying with the condition of the specimen. Consequently, the fall-off in intensity which is observed when specimens are stimulated with bursts containing the same number of shocks, but at increasing frequencies, is due in part to reduction in number of responses (Fig. 7). Thus, with a stimulation interval of 0.5 sec., there are 8 flashes to 10 shocks; with an interval of 0.3 sec., 5 flashes; and with an interval of 0.18 sec., 3 flashes. Now, facilitation is manifested in consecutive flashes, and depends on number of preceding impulses, and impulse interval. The increase in refractory period which occurs at higher frequencies results in fewer stimuli being effective, and these at longer intervals. The over-all result will be a decrease in facilitation and intensity of response.

These observations show that upper levels of facilitation are limited by the refractory period. Whether the refractory period thus made evident belongs to the excitatory system (nerve net and neuro-effector junctions) or to the photocytes is not resolved by the character of the experiment. By stimulating from two loci, Pantin (1935a) has defined quantitatively the refractory period of the nerve net of the sea anemone Calliactis. In this animal the relative refractory period lasts 0.5 sec., while the absolute refractory period is 0.05 sec. This value for absolute refractory period appears to be far shorter than the limiting intervals discovered in Renilla, but the relative refractory periods in the two animals appear to be similar. In so far as data from one animal can be used to interpret the responses of another, it may be
supposed that the relative refractory period of 0.5 sec. in *Renilla* is a property of the nerve net.

The duration of the refractory period is affected by fatigue, just as are certain other features of excitation. It has been observed that continued stimulation at high frequencies lengthens the refractory period. In one specimen, for example, which was stimulated with bursts of 15 shocks at 3/sec., the animal responded with 1 flash per shock for stimuli 2 to 8, and thereafter only to alternate shocks. On repeating

![Diagram](image-url)

**Fig. 7.** Influence of frequency of stimulation on intensity of response. Each point on the curves represents the intensity of response to the last shock in a burst of 10 pulses; the numbers beside the points indicate sequence of stimulation. Solid line, a run with increasing frequencies; broken line, a return run with decreasing frequencies. Arrows and figures above refer to number of responses produced by a burst of 10 shocks at different frequencies. Since the 1st response appeared on the 3rd stimulus, the maximal possible number of responses with 10 shocks, excluding after-discharge, was 8. Recording from a narrow slit. Temp. 15–17°C.

with another burst at the same frequency, the animal now responded only to alternate shocks. In *Calliactis* Pantin (1935a) notes that the threshold rises when batteries of more than two stimuli are administered, the intervals between the stimuli lying within the relative refractory period. It is suggested that this may be owing to lengthening of the absolute refractory period, but the effect may be complicated by local sensory fatigue.

Previous work has shown that strong electrical stimuli, several times threshold strength, produce very bright and prolonged responses, which come up on the first shock (Nicol, 1955). Several characteristics of the response are worth examining at this point. The bright response produced by a strong shock is usually a uniform glow covering the entire rachis, and it may last as long as ½ min., in contrast to the
Nervous regulation of luminescence in Renilla short flashes evoked by threshold stimuli. It is sometimes followed by long trains of successive waves, quickly decreasing in frequency and amplitude.

The precise characteristics of responses to strong shocks vary among different animals, but two facts suggest that the response itself is produced by repetitive discharge in the nerve net, viz. an occasional preliminary small wave, and the frequent train of successive waves. To secure further information bearing on this hypothesis, a study has been made of the latent period of responses induced by strong shocks. This turns out to be 0.3-0.4 sec., i.e. 2 or 3 times normal latency. The long latency, thus revealed, excludes direct stimulation of the photocytes as an operative factor. Since normal latency is only some 0.12 sec., there is a further interval of some 0.15-0.25 sec. during which one or two impulses could succeed the first to produce a strong facilitated response.

If repetitive discharge is occurring, initially at least, it must be taking place at such a rate that fusion of consecutive responses takes place. It may be that the high level of facilitation built up by the rapid discharge of a few impulses is sufficient to maintain a sustained glow in the photocytes. Certainly, the bright even glow produced by strong electrical stimulation is quite different from the trains of waves induced by threshold stimulation. It closely resembles the bright glow produced by strong tactile stimuli, and both phenomena may have a similar neural causation.

AFTER-DISCHARGE AND MAINTAINED RHYTHMIC FLASHING
Under certain conditions some animals continue flashing for some time after stimulation has ceased. This after-discharge is evoked by tactile and electrical stimulation. Specimens, however, vary greatly in excitability. In some animals a single tactile stimulus or a few electrical shocks evoke a post-stimulatory series of waves; in others repetitive or prolonged stimulation is required. After-discharge varies greatly in duration, from one or a few waves to prolonged flashing lasting many minutes. Initially, at least, the waves in a post-stimulatory discharge arise from the area of stimulation, and run regularly over the surface of the rachis. When a specimen is highly excited, by prolonged electrical or tactile stimulation, the whole surface of the rachis flashes continuously in a shifting and confusing pattern.

After-discharge is obviously a condition dependent on excitability of the nerve net, and it is possible to explore its characteristics by controlled stimulation. The following conclusions are based upon the examination of many animals.

When a short burst fails to elicit post-stimulatory discharge, a long burst of stimuli frequently will do so. Again, when a single short burst is ineffective, a series of similar bursts will often produce post-stimulatory flashing. The effective interval between bursts for production of after-discharge is at least 7 min. (Fig. 6C, D).

The number of flashes in an after-discharge depends on the number of stimuli. Whereas, with a short burst, only one or a few post-stimulatory flashes may appear, a long burst may give rise to a prolonged period of post-stimulatory flashing. Similarly, with repeated bursts there is an increase in the number of post-stimulatory flashes, following each burst of shocks. Thus, in one animal stimulated by bursts of shocks (5 pulses at 1/sec.), with 5 min. between bursts, the first burst produced no
after-discharge, the second produced 8, and the third 14 flashes after stimulation had ceased.

After-discharge is readily induced by high-frequency stimulation. Thus, it has been frequently observed that when a burst of shocks at a low frequency (1/sec. or less) is ineffective, a burst of an equal number of shocks at a higher frequency (3/sec.) results in much post-stimulatory flashing (Fig. 6E, F).

In post-stimulatory discharge induced by tactile and electrical stimulation, the frequency of flashing is usually rapid at first, and shows a tendency to decline. Initial periodicity is of the order of 0.5–1 sec. and increases to 2–3 sec. after 15–30 flashes (Figs. 6C, F, 8). These are approximate values to give some idea of the magnitudes involved. As the interval between consecutive flashes increases during after-discharge, there is a corresponding decline in the intensity of consecutive flashes (Fig. 6C). This results in large part from decay of facilitation as the interval between impulses lengthens.

Repetitive flashing, like facilitation, is subject to fatigue. After a period of prolonged after-discharge, induced by a burst of electrical shocks, similar stimulation may be ineffective in producing after-discharge, or may elicit only one or a few post-stimulatory flashes (Fig. 6D). Nevertheless, by increasing the number of stimuli, or frequency of stimulation, post-stimulatory flashing may be revived or prolonged in such fatigued animals.
THE EFFECTS OF CERTAIN DRUGS ON THE LUMINESCENT RESPONSE

Several drugs were tested on *Renilla* in order to determine whether they would evoke luminescence or affect the facilitatory process. These drugs were adrenaline, acetylcholine, eserine, curare, and nicotine. With the exception of nicotine the tests were negative, and gave no information about transmitter action in this animal. Nicotine in high concentration (1/1000) greatly reduced the response and caused irregular and weak flashing. It appears to have a twofold effect, excitatory on the nerve net, and depressive on the photocytes.

DISCUSSION

This paper has been concerned with three aspects of the luminescent response of *Renilla*, namely a finer resolution of response parameters, facilitation, and the prolonged excitatory state manifested in after-discharge. Various lines of evidence indicate that each electrical stimulus gives rise to a nervous impulse which traverses the entire nerve net of the rachis. The progress of the luminescent wave across the rachis is an index of transmission of excitation in the nerve net, and measurements of conduction velocities obtained by this means give a value of about 9 cm./sec. (15.5° C.).

Earlier work has shown that the nerve net of *Renilla* is unpolarized and that transmission occurs readily in all directions (Parker, 1919; Buck, 1955). There is indirect evidence that the nerve net consists of a meshwork of short distance neurones (Nicol, 1955). Although histological studies on *Renilla* are wanting, it seems likely from our knowledge of neurones in other coelenterates (medusae, sea anemones) that these nerve cells make contact with one another by synaptic junctions (discussion by Pantin, 1950, 1952). Normally each impulse successfully bridges these synapses and invades the whole nerve net, but under certain experimental conditions decremental conduction can be induced, whereby several impulses must intervene in order to force a crossing from one region to another, owing to intervention of synaptic resistance (Nicol, 1955). It has been concluded that facilitation of the luminescent response, observed in these studies of *Renilla*, occurs terminally at the level of the effectors (Buck, 1955; Nicol, 1955).

Facilitation of the luminescent response of *Renilla* shows essentially the same characteristics as those observed in the quick muscular responses of actiniarians and Scyphomedusae (Pantin, 1935a; Bullock, 1943). The question arises, where is the facilitatory process taking place? Pantin (1935a, b) has argued that facilitation of the marginal sphincter in *Calliactis* is a neuro-muscular effect such that each stimulus in a series reaches more and more individual muscle fibres (recruitment of effector cells). He has found that a single stimulus applied directly to the muscle causes it to contract, whereas several impulses are required to evoke contraction when the muscle is stimulated via the nerve net. From this it is concluded that the muscle itself is capable of responding to the first excitatory impulse that reaches it. Since each impulse invades all regions of the nerve net, it follows that the facilitatory process occurs at the neuro-muscular junction (Pantin, 1935c).
Now, the same argument can be fairly applied to the luminescent responses of *Renilla*. First of all we may note that under repetitive stimulation a tiny spot of light appears directly beneath the electrodes on the first stimulus, although several shocks are usually required to give rise to a propagated luminous wave. The photocytes, therefore, are capable of responding directly to a single excitation. Now, conduction of each impulse is widespread, involving the whole nerve net, although two or three impulses must intervene before a luminescent wave appears. It follows, therefore, that the facilitatory process is taking place at the neuro-effector junction, between the nerve fibres and the photocytes. This conclusion would appear to dispose of the possibility that facilitation results from triggering and augmentation of some chain of reactions leading to light emission within the photocytes.

We find that several impulses are required to bridge the gap between nerve fibres and photocytes, thus exciting the latter. The subsequent increase in height of successive responses depends on recruitment of additional photocytes as facilitation builds up. This thesis presumes that there is variation in the threshold of different photocytes, or possibly variation in activity levels of the transmitting mechanism at different nerve fibre terminals. Evidence for variation in the threshold of different photocytes is forthcoming from visual observations. When the eye is dark-adapted, and *Renilla* is given a series of pulses at a low frequency, it is observed that the first few stimuli, although generally ineffective, sometimes cause a few isolated points of light to appear at widely separated places all over the rachis. A related effect is observed in animals stimulated at very low frequencies (interval > 3 sec.). Under these conditions not every stimulus is effective, and there may be gaps of 3, 4 or more shocks before the next flash or transmitted wave appears. But during these dark periods it frequently happens that odd scattered points of light develop on the rachis in response to a stimulus. It seems that these few zooids which are responding are below the threshold of the majority, and similarly, within each zooid, there is possibly variation in the threshold of individual photocytes.

Some previous workers, who have studied muscular responses of actiniarians, have discussed the nature of neuro-muscular transmission in this group (Ross & Pantin, 1940; Ross, 1945). From various lines of evidence they advance the hypothesis that neuro-muscular transmission in anemones involves two distinct processes, viz. a process of excitation; and a process of sensitization of the neuro-muscular junction whereby excitation of the muscle by the nervous impulse can become effective. Among the evidence cited are the following facts. (1) A single stimulus produces no response, and the response takes place on the second stimulus if it occurs at all. (2) When the interval between stimuli is lengthened, the response to the third stimulus in a series disappears at the same time as the response to the second, and it is not possible to obtain two apparently ineffective stimuli before the first response. The observations just cited have been interpreted as evidence against a transmitter hypothesis. They have been selected for consideration because comparable data are available from *Renilla*.

Evidence has been presented for *Renilla* which shows that a luminescent response usually occurs on the 2nd or 3rd shock; subsequent responses increase progressively
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The intensity of response depends on the interval between stimuli, i.e., facilitation builds up and decays in a predictable manner. These observations are compatible both with a theory of a chemical transmitter, and with a theory of sensitizer involved in excitation. There is one condition, however, in which luminescence appears on the first stimulus, viz. shortly after a previous period of stimulation. It thus appears that a sufficient level of facilitation can persist from a previous period of stimulation to make a single shock effective. Moreover, we have seen that when the stimulation-interval is progressively lengthened, the response, instead of coming up on the 2nd or 3rd shock, now appears after an increasingly greater number of shocks. Together, these various lines of evidence strongly suggest that neurophotocyte transmission involves release and decay of a facilitatory agent. The evidence at hand, for Renilla, does not demand the postulation of a sensitizer separate from the excitatory agency. Under normal conditions it appears that several impulses must intervene before facilitation reaches a level sufficient to excite the photocytes; but when some residual facilitation is already present, a single impulse may release enough facilitatory agent which, when added to that already present, will excite a response. In this connexion it is noteworthy that residual traces of facilitation may persist for at least 10 min. Moreover, when the interval is lengthened an increasingly greater number of impulses must intervene before sufficient facilitatory agent accumulates to excite a response.

Whereas the increment in height of successive responses depends on facilitation at the neuro-effector junction, repetitive discharge is a property of the nerve net. It is manifested in interpolated waves which appear during a burst of electrical stimuli. And it occasions the prolonged bouts of post-stimulatory flashing seen in some specimens following electrical or mechanical stimulation.

Repetitive discharge apparently originates in the neurones of the nerve net. At low levels of excitation, one or a few extra waves appear at the site of stimulation. There are instances, however, where rhythmic flashing may continue for some time from the site of stimulation after the stimuli have ceased. Evidence for after-discharge, of course, is indirect, and is derived from observation of the character and course of post-stimulatory flashing. It appears as if certain neurones in the nerve net, when sufficiently excited, continue to discharge rhythmically after stimulation has ceased. They can be primed, so to speak, to higher levels of rhythmic activity by increasing the frequency or duration of stimulation. With time, their excitability declines, and rhythmic discharge, as manifested in flashing, fades away.

Studies now available provide information for regulation of three effector systems of coelenterates, viz. muscular systems, luminescent systems and cnidae. Of these, the muscular and luminescent systems are under nervous control, whereas cnidae are independent effectors. Contractile tissues are universally present among coelenterates; luminescent tissue, on the other hand, occurs sporadically and probably has been acquired independently on many occasions in this group. There are certain striking similarities in neuro-effector regulation between the luminescent responses of sea pens and the muscular responses of medusae and anemones. There is some reason for believing, therefore, that the mode of regulation of the luminescent
response has been determined by the functional organization of a nervous system already concerned with phasic and tonic muscular activity. To this nervous system has been added regulation of the luminescent response, apparently with little change in functional characteristics.

SUMMARY

1. Luminescence has been studied in the sea pansy *Renilla kollikeri*, by means of photo-electric recording, and the mode of nervous regulation investigated.
2. The luminescent response is under control of a non-polarized nerve net and is subject to facilitation which occurs terminally, at neuro-photocyte junctions.
3. Facilitation is analysed in detail, and a facilitation-decay curve presented (Fig. 3). Between successive bursts of stimuli, facilitation may persist for some 10 min.
4. Certain response parameters were measured. Latent period is 0.12 sec.; the response lasts 1 sec., and maximal intensity is reached in 0.22 sec. Conduction speed is 9 cm./sec. at 16–17° C.
5. The refractory period of the response, as determined by high frequency stimulation, is 0.2 sec. It is affected by fatigue and increases under repetitive stimulation.
6. Under repetitive and prolonged stimulation, the animal passes into a hyper-excitatory state and luminous waves continue to arise long after stimulation has ceased. Conditions affecting this post-stimulatory discharge are examined.
7. Comparison of facilitatory processes in quick muscular responses of sea anemones and luminescent responses of sea pens shows that they are essentially similar. From visual observations it appears that facilitation operates by recruitment of photocytes. Luminescence is of sporadic occurrence among coelenterates, and has been independently evolved on many occasions. It is suggested that its mode of regulation has been determined by the characteristics of the nerve net primarily concerned with control of muscles.

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REFERENCES

Nervous regulation of luminescence in Renilla