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LUMINESCENCE IN POLYNOIDS

II. DIFFERENT MODES OF RESPONSE IN THE ELYTRA

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(Text-fig. 1)

Earlier studies (Nicol, 1953, 1954) showed that two kinds of luminescent responses are produced by electrical stimulation of isolated polynoid scales, viz. brief flashes and a long-lasting glow, the latter frequently being brighter. The brief flashes were regarded as the normal response of the photocytes to nervous excitation, whereas the long glow might be the result of direct excitation of the photocytes. A prolonged glow can usually be evoked by strong shocks (well above threshold). It is sometimes succeeded by rhythmic flashing (Nicol, 1954, fig. 1B); alternatively, a series of rhythmic flashes may be succeeded by a prolonged glow (Nicol, 1954, fig. 3A).

In the present paper I shall consider the two kinds of responses in more detail.

MATERIAL AND METHODS

The species studied were *Polynoë scolopendrina*, *Lagisca extenuata* and *Gattyana cirrosa*; most observations were made on *Polynoë*. Two kinds of preparations were used. These were isolated elytra, and short sections of worm consisting of a few segments and an elytrum. The material was prepared from worms narcotized with iso-osmotic $MgCl_2$. To induce luminescence electrical stimulation was employed, either condenser discharges or square waves, delivered from an electronic apparatus. The isolated elytrum was placed in a moist chamber over a pair of silver electrodes. To stimulate pieces of the body, silver electrodes were placed on the nerve cord. Luminescent responses were detected with photomultiplier, d.c. amplifier and cathode-ray oscilloscope. Records were made on moving paper. Temperatures were $18° \pm 1°$ C.

OBSERVATIONS

STIMULATION THROUGH THE NERVE CORD

The following observations were made on short sections of worm containing an elytrum, and stimulated via the ventral nerve cord.

Polynoë. A single shock sometimes produces one flash, or causes repetitive flashing; or several shocks may be required to elicit the first recordable response. Fast repetitive flashing occurs at frequencies up to 10/sec.

Consecutive flashes show facilitatory increment. Flashing follows pulse-rate up to about 20/sec, above which there is no longer a flash to each shock. Following several flashes at short intervals, the final flash may pass over into a glow response, having a lengthened decay period (Fig. 1 A). A normal quick flash has a duration of some 120 msec, and rise time of *ca.* 25 msec; decay time is about 100 msec. The decay time of the glow response is variable and prolonged, from 0·25 to 3 sec. When a flash gives way to a prolonged glow, there may be an initial period of fast decay, followed by a secondary rise and slow decay (Fig. 1 A). I would emphasize that the prolonged glow may appear long after the stimulatory shock has been delivered (0·3–0·6 sec).

Gattyana. A single shock elicits either a flash or repetitive flashing. Flashing follows stimulation up to pulse-rates of about 24/sec (Fig. 1 B), above which the scale does not respond to each shock. During a high-frequency burst, repetitive flashing tends to give way to a steady glow, but prolonged glow responses are not otherwise apparent in these records. Consecutive flashes show facilitation. Flashes vary in duration from 54 to 145 msec; rise time is 8–25 msec. In a series of flashes, later ones, of no greater amplitude than the first, may have greater duration and rise time.

Stimulation of the nerve cord in narcotized pieces

Short sections of worm (*Polynoë*) containing nerve cord and one elytrum were narcotized for 4 h or longer in 0·2 % novocaine, and then stimulated via the nerve cord. Weak luminescent responses could only be obtained with very strong shocks (5 × threshold of unanaesthetized preparations). These were glow responses, rather than quick flashes. I assume there was spread of current at the very high voltages to the underlying elytrum, which was excited directly.

ELECTRICAL STIMULATION OF ISOLATED ELYTRA (NARCOSIS-FREE)

Polynoë. In an isolated elytrum a single shock produces either one flash, or a series of flashes, variable in number. Temporal characteristics are similar to those recorded in a previous section (latency, 13–21 msec; duration, *ca.* 100 msec; rise time, 26–30 msec) (Nicol, 1953). Consecutive flashes show facilitation (Nicol, 1954). With bursts of shocks, the scale gives off a train of flashes. At high frequencies (> 20/sec), the scale either follows the rate of stimulation, or flashes to every 2nd or 3rd pulse. Light pulsations at rates up to 64/sec have been observed. The responses at high rates are of low intensity, and when stimulation ceases the scale continues to flash at some lower rate (*ca.* 10/sec) and higher intensity (Fig. 1 C). After a bout of repetitive flashing, induced by one or multiple shocks, there is often a prolonged glow with long decay period (up to 8 sec). Fast repetitive flashes are sometimes superposed on background glow. Strong shocks (2 × threshold) induce rapid repetitive flashing accompanied by a prolonged glow response. Decay of the latter occupies about 2 sec.

Gattyana. Isolated scales of *Gattyana* tend to give the prolonged glow response, even when stimulated at threshold. A single shock evokes responses of the following kinds: (1) initial quick flash followed by a prolonged glow; (2) rapid repetitive discharge passing over into a prolonged glow; (3) prolonged glow, with longer rise and decay time than a quick flash (Fig. 1D); (4) prolonged glow passing into repetitive flashing (Nicol, 1953, fig. 9). In some records the initial repetitive flashes return to base-line (zero intensity), whereas later responses become superposed on a rising background. Latency of the prolonged glow response varies from *ca.* 25 to 100 msec; rise time is 0·25 sec; duration is 4–8 sec.

Lagisca. A shock near threshold usually evokes a flash or a bout of repetitive flashing. When rapid, the latter may end in a prolonged glow response. The maximal rate of rhythmic flashing to a single shock is *ca.* 10/sec. Normal flashes follow repetitive stimulation up to frequencies of about 12/sec. At higher frequencies the scale responds to every 2nd, 3rd, or other stimulus with normal flashes; or closely follows the stimulus-rate with weak pulsations, which give way to bright normal flashes when stimulation ceases (Nicol, 1954, fig. 4C, D). Consecutive flashes show facilitation (Fig. 1E). Flash duration varies from 100 to 200 msec; rise time is 17–50 msec. In a series of flashes later ones tend to be more prolonged. Occasionally, a single shock evokes a glow response, with slow rise (*ca.* 0·3 sec) and long decay (> 2 sec) (Nicol, 1954, fig. 1A). Following rapid flashing, there may be a glow response (Nicol, 1954, fig. 3A).

STIMULATION OF NARCOTIZED ELYTRA

Isolated elytra were placed in solutions of certain anaesthetics, and their responses to electrical stimulation were tested later. Narcotics used were: cocaine, 0·2%; novocaine, 0·2–0·5%; chloretone, 0·05–0·2%; M.S. 222 (Sandoz), 0·025–0·1%; all in sea water. Also employed were iso-osmotic solutions of $MgCl_2$ plus sea water (ratios 1:3 to 1:1).

Evidence, presented elsewhere, has shown that continued rhythmic flashing, after stimulation has ceased, is dependent upon an elytral ganglion. Multiple flashes to a single shock, therefore, have been used as an index of continued nervous functioning. Multiple flashing (two or more flashes) to a single shock was observed in scales immersed for 97 min in 0·1% chloretone, and 54 min in 0·2% novocaine. Immersion times were considered insufficient, and the following observations are based on elytra immersed in narcotic solutions for 4 h or more.

Novocaine 0·2%

Polynoë. Responses of several kinds to single pulses were recorded as follows: (1) quick flash with latency *ca.* 10 msec, rise time 20 msec, duration *ca.* 0·5 sec; (2) initial quick flash followed by a long glow: rise time 20–80 msec, duration up to 10 sec (Fig. 1F); (3) prolonged glow response having a slow rise and long decay period: rise time 0·3 sec, duration 7–8 sec (Fig. 1G). These various kinds of responses are really arbitrary, and I believe there is every gradation in narcotized *Polynoë* scales between a brief flash lasting a half second to a prolonged glow lasting many seconds. Rise time is equally variable. Some records show an initial quick flash in which the decay period is interrupted by a secondary slow rise, succeeded by prolonged plateau and slow decay (Fig. 1H). With repetitive stimulation discrete flashes have been recorded up to rates of 18/sec. In some records there is little or no facilitatory increment in consecutive responses; in other records facilitation is well marked in the first few responses (Fig. 1J).

Gattyana. Only glow responses were observed. These have a latency of *ca.* 27 msec, and duration of 5–9 sec. Rise time is sometimes long, up to 1 sec, but many responses begin with an initial quick flash (rise time some 30 msec) which passes over into a prolonged glow. Occasionally, there is a secondary slow rise after the initial quick flash has started to decay. Facilitation to consecutive pulses is sometimes marked.

Lagisca. Single shocks produced a flash, repetitive flashing, or a prolonged glow. Repetitive flashing was sometimes succeeded by a prolonged glow response. The latency of the glow response was rather long, 0·5–1 sec; rise to maximum was 0·4 sec; total response duration was 3–4 sec. Because of the repetitive flashing observed, it appears that *Lagisca* elytra are not always narcotized by novocaine at 0·2% level.

Novocaine 0·3%

Lagisca. Stimulation produced glow response. Latency *ca.* 20 msec; duration some 10 sec; rise time 0·7 sec. The first two of a series of responses show facilitatory increment.

Novocaine 0·5%

Lagisca. Nil response.

Cocaine 0·2%

Polynoë. Following treatment with cocaine, the following kinds of responses were observed.

(1) Quick initial flash followed by a long glow.

(2) Quick initial flash, with decay period interrupted by a secondary rise.

(3) A quick flash showing rapid return to base-line (Fig. 1K). Latencies are 13–14 msec; rise time is *ca.* 27 msec. Minimal flash duration is about 0·25–0·3 sec; the more prolonged glows last up to 8 sec. With repeated stimulation, the response becomes more prolonged. Facilitation is shown in consecutive flashes (Fig. 1K).

Chloretone 0·05 %

Polynoë. Responses obtained after chloretone show rapid rise (*ca.* 30 msec), and slow decay (2–4 sec). Latency is *ca.* 15 msec. In some records the decay curve is regular; in others an initial decay is followed by a slow secondary rise and long period of secondary decay. There is facilitation to consecutive flashes (Fig. 1 L–N).

M.S. 222, 0·025 %

Polynoë. Responses were a prolonged glow, lasting up to 3 sec, with slow rise time (0·25–0·3 sec). The glow response usually was preceded or accompanied by a few weak oscillations, almost imperceptible in the records. Latency varied in different records from 0·025 to 0·4 sec. The small pulses accompanying the prolonged glow would indicate some residual nervous excitation, and this concentration of M.S. 222 can be considered near threshold.

M.S. 222, 0·05 %

Polynoë. Pulses produce a long glow (duration up to 7 sec). Rise time varies from 0·25 to 1 sec. Facilitation is shown in consecutive responses.

M.S. 222, 0·1 %

Polynoë. Nil response to electrical stimulation.

$MgCl_2$: *sea water* 1:3

Polynoë. Single shocks usually elicited multiple flashing, with responses similar to those of non-narcotized scales. Occasional scales gave the prolonged glow response. Consecutive flashes showed facilitation.

$MgCl_2$: *sea water* 1:2

Polynoë. Pulses evoked quick flashes passing into a prolonged glow, or repetitive flashing sometimes ending in a glow response. Facilitation was evident.

$MgCl_2$: *sea water* 2:3

Polynoë. Single flashes tended to be prolonged. Stimulation sometimes evoked a glow response, on which was superposed repetitive flashing.

$MgCl_2$: *sea water* 1:1

Polynoë. Nil response.

COMMENT

Treatment with each of three narcotics, viz. novocaine, chloretone, and M.S. 222, abolishes repetitive flashing to a single shock. This result is taken to indicate that anaesthesia stops nervous excitation and transmission. The

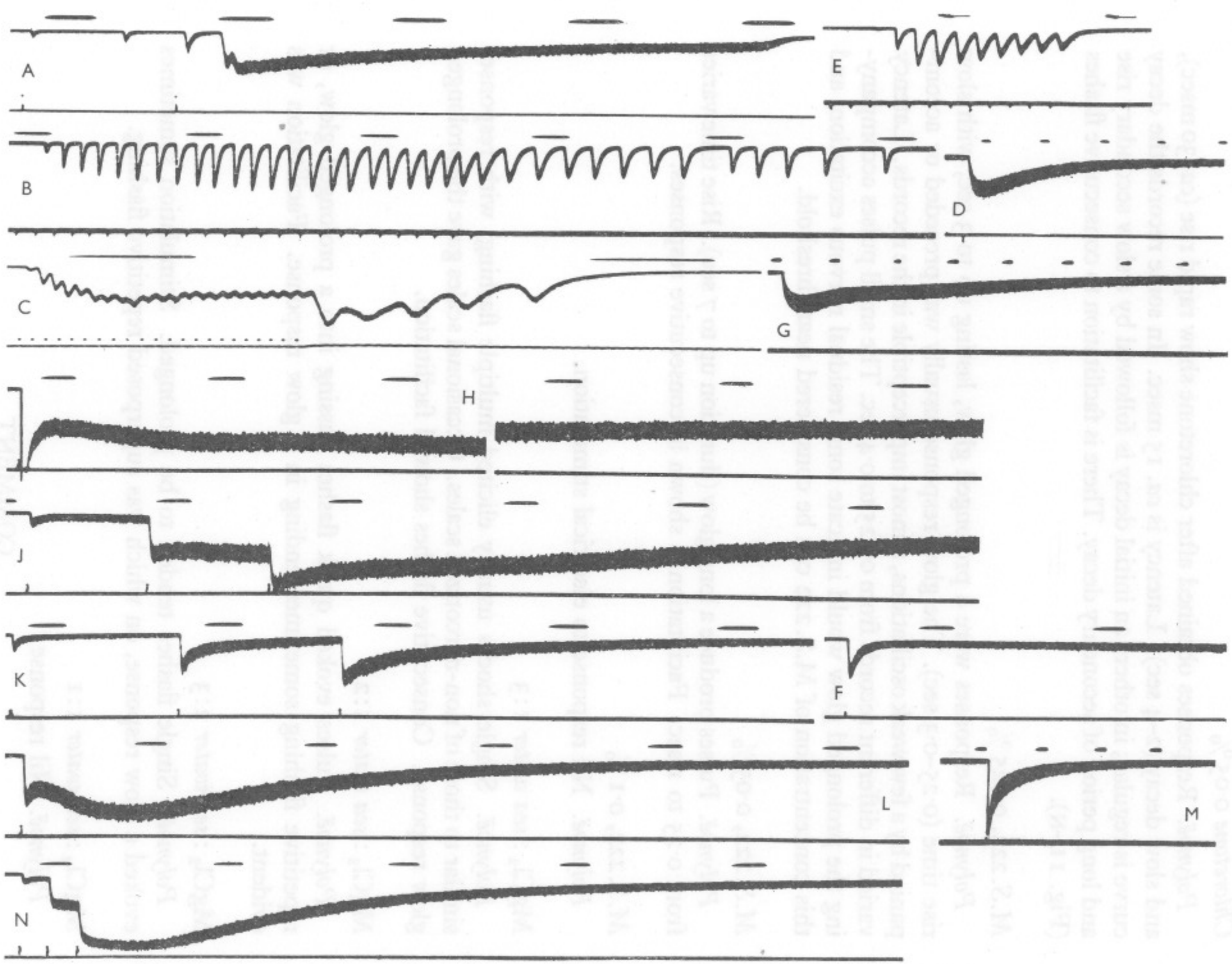

Fig. 1

luminescent responses which are still obtained by electrical stimulation after anaesthesia presumably arise from direct excitation of the photocytes.

Rhythmicity and nervous conduction are admittedly two different phenomena, although the same neurones are concerned with both. A possibility remains, therefore, that nervous conduction is still occurring, even though rhythmic discharge has disappeared. In view of the concentrations of narcotics employed, and the duration of their action, this contingency seems improbable.

Two kinds of responses have been arbitrarily selected, viz. the quick flash (duration *ca.* 100 msec), and the prolonged glow (duration 0·25 sec or longer). The former only appears after nervous stimulation (i.e. in unanaesthetized scales); the latter, following nervous stimulation or direct excitation (i.e. in unanaesthetized and in anaesthetized scales). It has not been possible to produce the quick flash by direct excitation of anaesthetized scales.

There are several reasons for believing that the glow response can be induced by nervous excitation, even under conditions when the isolated scale is stimulated. First, it is produced by stimulation of the nerve cord, when there is no current escape to the scale. Secondly, it succeeds rapid flashing some considerable time after stimulation has ceased. When evoked by indirect excitation, via the nervous system, the prolonged glow is associated with fast repetitive flashing. It seems that the conditions obtaining during fast repetitive flashing create a state favouring prolonged luminescence.

It is not known where the excitation takes place that produces the long glow response. Minimal latent periods of quick flash and glow response are about the same, and provide no help in resolving the question. Conduction time would be very brief in the short distances involved. Since quick flashes

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Legend to Fig. 1

Fig. 1. Oscillograph records

A. Luminescent responses of *Polynoë scolopendrina*. The preparation consisted of a piece of the body containing 3 segments plus 1 elytrum. Electrical stimuli were applied to the nerve cord. Downward deflexions of middle trace are light flashes. Time signal above, 1 sec. Electrical stimuli on lower line.

B. *Gattyana cirrosa*. Repetitive stimulation of the nerve cord. Recording from one elytrum.

C. *Polynoë scolopendrina*. Responses of an isolated elytrum to repetitive electrical stimulation (burst at 42/sec).

D. *Gattyana cirrosa*. Prolonged glow response of an isolated elytrum.

E. *Lagisca extenuata*. Flashing of an isolated scale to repetitive stimulation.

F, G. *Polynoë scolopendrina*. Glow responses of a scale in 0·2 % novocaine, 4 h 20 min.

H. *P. scolopendrina*. Glow response of elytrum in 0·2 % novocaine, 5 h 47 min. Five-second interval between the first and second halves of this record.

J. *P. scolopendrina*. Three consecutive glow responses of an elytrum in 0·2 % novocaine, 5 h 11 min.

K. *P. scolopendrina*. Consecutive responses of a scale in 0·2 % cocaine, 4½ h.

L, M, N. *P. scolopendrina*. Glow responses of a scale in 0·05 % chloretone, 4 h 25 min.

are sometimes superposed on the prolonged glow response, it follows that the transmission of seriated impulses and repeated excitation of the photocytes are not impeded during the lengthy course of the glow response.

The glow response often has a rapid increment, with rise time equal to the quick flash. Decay time greatly exceeds that of the flash, from 0·25 to 10 sec. A secondary rise in intensity, seen in many records, must be of more than fortuitous significance.

After a bright glow response the scale fails to respond, or gives only feeble responses, to further stimuli. The prolonged glow consumes much or all of some substance essential for the luminescent reaction.

Weak stimulation of unanaesthetized scales evokes quick flashes; strong electrical stimulation induces the glow response. Weak shocks excite the nervous system; stronger shocks excite the photocytes directly. These differences need not be one of threshold; they may be due to the geometrical relations of the excitable elements with reference to the cathode.

It has been observed frequently in narcotized scales that repetitive stimulation can engender facilitatory increment (staircase). This is seen most clearly at slow rates of stimulation, when the effect is not obscured by summation. In facilitation some improving effect of a response is carried over to a later response, appearing in a brighter flash. Since the nervous system is excluded, the effect must be ascribed to processes taking place in the photocyte. The facilitatory increment seen in flashes of non-narcotized scales may also depend upon processes occurring within the photocytes, although concomitant neuro-photocyte facilitation is not excluded.

During repetitive flashing later responses may become prolonged. This lengthening of decay period may be due to conditions similar to those responsible for the glow response.

Finally, it has been noted that normal flashing ceases to maintain correspondence with stimulation at rates above *ca.* 20/sec; weak pulsations are sometimes seen at much higher rates, when the isolated scale is stimulated. This problem remains unresolved. It is possible that this very fast pulsation, above that which seems permitted by the refractory period of the nerve, depends upon irregular rotation among a large population of nerve fibres.

I conclude that the prolonged glow response is physiologically possible, and is sometimes produced by fast repetitive excitation from the nervous system. The latter creates conditions favouring a massive luminescent discharge. The duration of a glow response can be greatly exceeded by a bout of protracted iterative flashing; the light, of course, is fluctuating in the latter condition.

Since facilitation or staircase is observed in anaesthetized scales, it appears to depend upon processes taking place within the photocytes.

SUMMARY

Elytra of polynoids show two kinds of luminescent responses, viz. a quick flash (duration *ca.* 100 msec) and prolonged glow (duration 0·25–10 sec). Quick flashes are produced by nervous excitation; prolonged glow responses accompany or follow fast repetitive flashing, or are elicited by strong electrical stimuli. Anaesthetized isolated elytra still give the prolonged glow response when electrically stimulated. Facilitation is also evident in consecutive glow responses. From these various observations it is concluded that the prolonged glow response can be produced by fast repetitive nervous excitation or by direct electrical excitation of the photocytes. The occurrence of facilitation in glow responses of anaesthetized elytra raises the problem whether the same phenomenon in consecutive normal flashes may be due to intracellular changes taking place within the photocytes.

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