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LUMINESCENCE OF *CHAULIODUS* PHOTOPHORES BY ELECTRICAL STIMULATION

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Abstract—1. Photophores isolated from living specimens of the bathypelagic fish *Chauliodus sloanei* emit bright flashes or luminus when they are electrically stimulated.

2. Anodic stimuli of long duration (4–8 msec) and high strength (50 V) evoke quick flashes (10–130 10^6 quanta/sec) that fuse in a erratic way when the frequency of stimuli is higher than 2/sec.

3. Anodic stimuli of short duration (1 msec) and low strength (10 V) evoke slow flashes that fuse together in a "luminus" when the frequency of stimuli increases up to 4/sec.

4. The "luminus" response is characterized by a peak of light developed within 2 sec after the beginning of the electrical stimulation; afterwards, the light decreases to reach a constant level of light within about 10 sec.

INTRODUCTION

Most luminous fish are endowed with photophores generating intracellular light. Extensive studies on the physiological properties of isolated photophores have only been performed on light organs from the mid-water batrachloïd teleost fish *Porichthys*, a hardy fish, easy to capture in good condition and to keep alive in captivity. Recently, Baguet (1975), Baguet & Maréchal (1976, 1978) obtained live bathypelagic luminescent fish from the strait of Messina (Sicily) and reported the first physiological investigations on isolated photophores of *Argyropelecus hemigymnus*.

In the present work isolated photophores from *Chauliodus sloanei*, another bathypelagic fish from the strait of Messina, are electrically stimulated in order to study their excitation mechanism.

MATERIALS AND METHODS

Collection of fish

Specimens of *Chauliodus sloanei* were caught alive with a hand-net in the strait of Messina, during high tide, while deepwater currents violently deflected upwards by the barrier shallow dragged them to the surface (Baguet, 1975). *Chauliodus* was easily located because its head partially emerged from the water while swimming.

After capture, the fish were brought in seawater to the station of Ganzirri, near Messina, where they were transferred to a glass aquarium filled with seawater from the strait, maintained at low temperature (about 9°C) and oxygenated. In these conditions many specimens survived for 12–18 hr.

Dissection of the photophores

The fish is laid on a filter paper wetted with seawater, its head being lighted with a 50 W electric-light bulb. This procedure prevented movements during dissection. After removal of the gelatinous layer covering the ventral area of the fish, strips of 4–8 photophores from the ventral or lateral region (Fig. 1) were cut out from the skin with fine scissors. It was very easy to isolate photophores with very little surrounding tissue, since photophores are loosely attached to the skin.

Measurements of the light emission

The photophores strips were kept in a small vessel filled with air-saturated saline (Baguet & Maréchal, 1976). After 5–10 min, one photophore was cut out from the strip and transferred to a small perspex chamber. It was like a small pouch constricted in the middle region: it was laid upside down, in such a way that the light-emitting area faced the photocathode of the photomultiplier at a distance of 2.0 cm. Two platinum–iridium electrodes (tip diameter 10 μm) were lowered on each side of the photophore in order to stimulate it transversely. The electrodes were connected to a stimulator delivering square pulses. The luminescent response was sensed by a photomultiplier PM 270C (International light) operating with a IL 760 power supply, and displayed on a 20 cm strip-chart recorder. The light production was calibrated with a standard light source (Betalight source, peak at 470 nm) held at the place of the tissue).

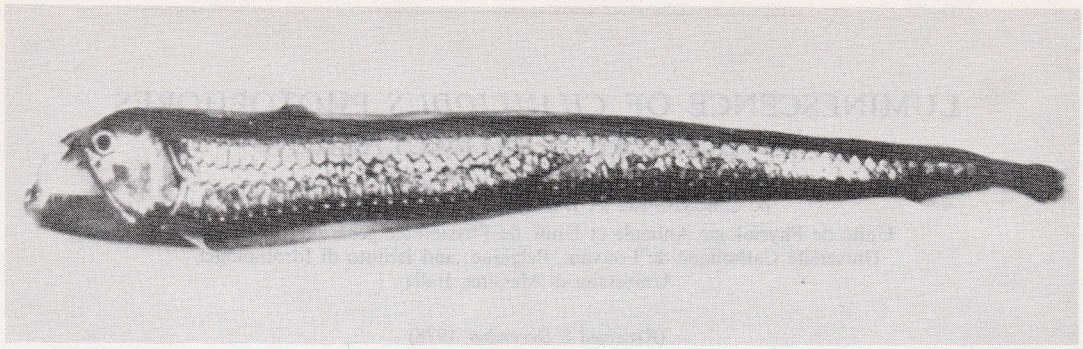
RESULTS

Photophores excised from a living fish never luminesce spontaneously, but they blink in response to single as well as to multiple stimuli. This property does not disappear even if the excised photophores have been kept for 2 days in saline at 5°C.

Types of light responses

a. *Isolated flashes.* Figure 2 shows a series of flashes evoked by anodic stimuli (4 msec, 50 V) applied at 1 sec interval during 65 sec. Flashes of low amplitude are triggered by the first 19 stimuli; afterwards, their amplitude increases progressively but irregularly. In this case, there is potentiation without summation, or inhibition, as each stimulus triggers a flash.

Potentiation is suppressed if the stimulus is increased in duration (8 msec or more) and strength (to 65 V): Fig. 3 shows that in such conditions a lowering of the frequency from 1/sec (series a) to 0.5/sec (series



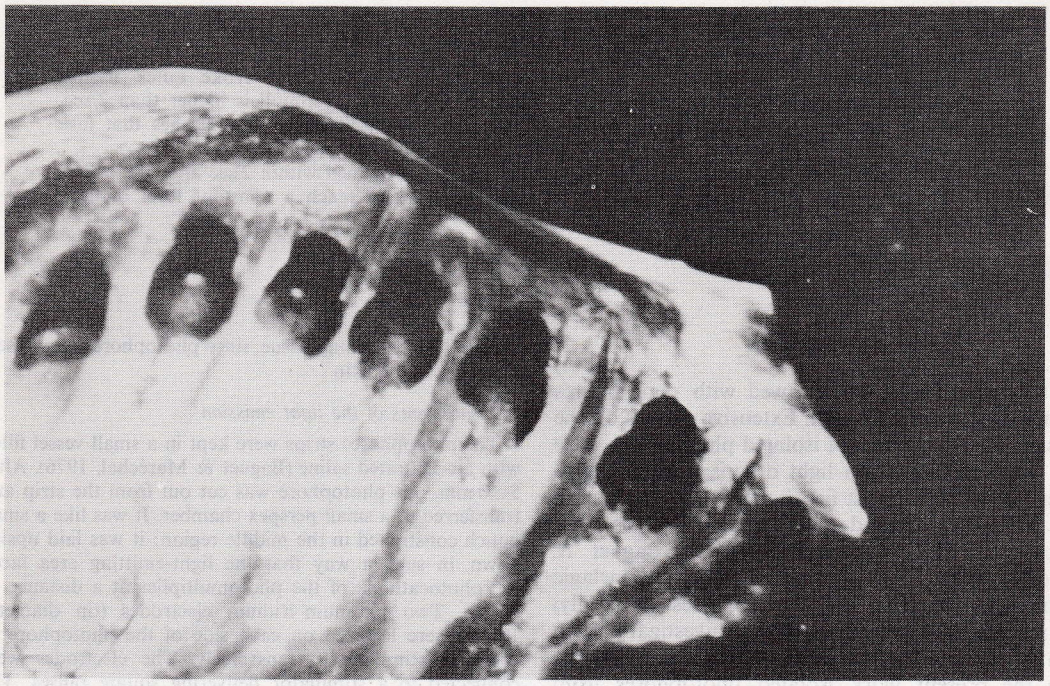


Fig. 1. Photograph of a freshly captured specimen of *Chauliodus sloanei* uppermast showing the lateral and ventral rows of photophores. The length of the entire animal is 17 cm. Detailed view of the lateral photophores.

b) does not restore the potentiation. All the flashes are of similar amplitude from the first to the last stimulus.

On the contrary, partial summation is observed if the stimulus 1/sec is decreased in duration (to 1 msec) and strength (to 15 V), the flashes lasting longer (Fig. 4).

b. *Summation of flashes.* Summation of flashes is obtained for stimulus frequency higher than 1/sec, the exact form of the response being dependent on the stimulus parameters.

For anodic stimuli (1 msec, 15 V) at 2/sec, the summation of the individual flashes is still incomplete and the successive flash responses corresponding to the successive stimuli are clearly visible (Fig. 5a). Their amplitudes increase progressively up to the eighth stimulus. They remain then stable at a maximal value of 6.3 Mquanta/sec until the end of the stimulation. Extinction of the light response is complete in about 1 sec after the last stimulus.

At a frequency of 4/sec (Fig. 5b) the summation is nearly total. The first nine stimuli trigger responses which summate rapidly. The following stimuli evoke a continuous response which oscillates irregularly without obvious link to the stimulus frequency. Extinction occurs in about 1 sec.

At the rate of eight or more stimuli per sec (Fig. 5c) the light emission smoothly rises in about 1 sec to a peak twice as high as that recorded at the frequency of four stimuli per sec. The perfectly fused response is not maintained, however. It decreases rather sharply, during about 3 sec and stabilized at about 60% of the peak; moreover there are oscillations unrhythmed by the stimuli.

For stimuli of longer duration (4 msec or more) and higher voltage (at least 40 V), at 2/sec (or higher), the response is erratic. Figure 6 shows a typical example of the light response evoked by a 5 sec stimulation with stimuli of 4 msec, 45 V, 10/sec. The complex light curve shows a series of partly fused flashes emitted at irregular interval.

The light responses of isolated photophores can summate in suitable conditions like the mechanical response of striated muscles. By analogy with the

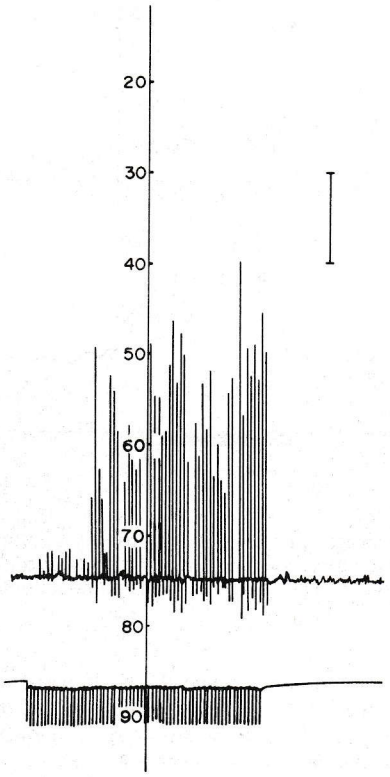


Fig. 2. Original record of flashes emitted by an isolated photophore (upper trace) stimulated during 65 sec with anodic stimuli (4 msec, 50 V) at 1 sec interval (lower trace). Vertical bar 18 Mquanta/sec.

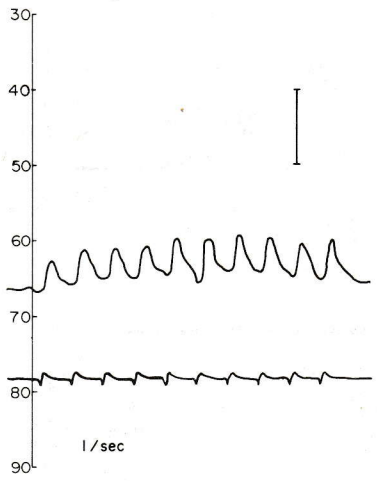


Fig. 4. Time course of successive flashes evoked by 10 anodic shocks (1 msec, 10 V) given at 1 sec interval. Vertical bar 18 Mquanta/sec.

term "tetanus", we call "luminus" a response similar to those shown in Fig. 5. The term was first proposed by Baguet & Maréchal (1976) to describe the light response of epipelagic fish *Porichthys*.

Effects of the stimulus frequency on the luminus

Seven photophores were stimulated with a 10 sec train of 7 msec, 10 V stimuli applied at seven different frequencies: 1, 2, 4, 8, 20, 50 and 100/sec. In order to minimize possible effects of the order of application of these frequencies, the treatments were randomized

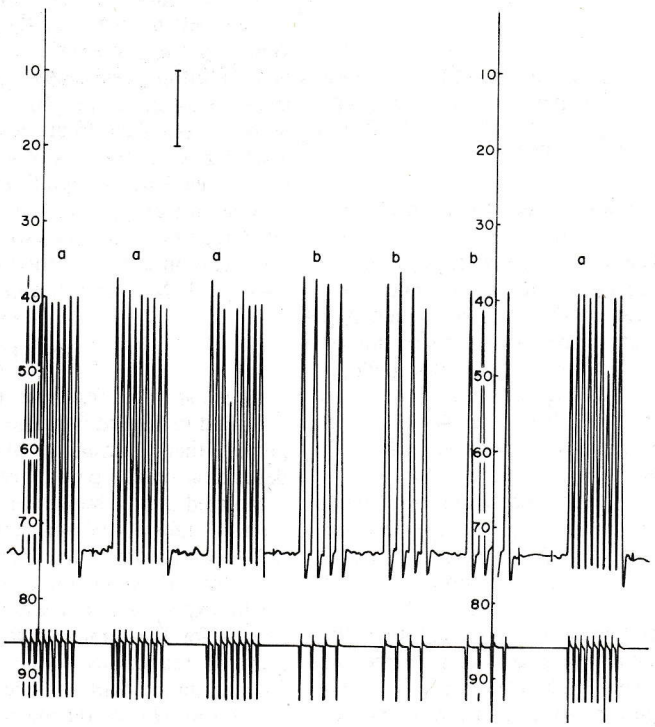


Fig. 3. Seven series of flashes (upper trace) evoked by stimuli (8 msec, 65 V) given at 1 sec (b) and 0.5 sec (a) interval. Vertical bar 42 Mquanta/sec.

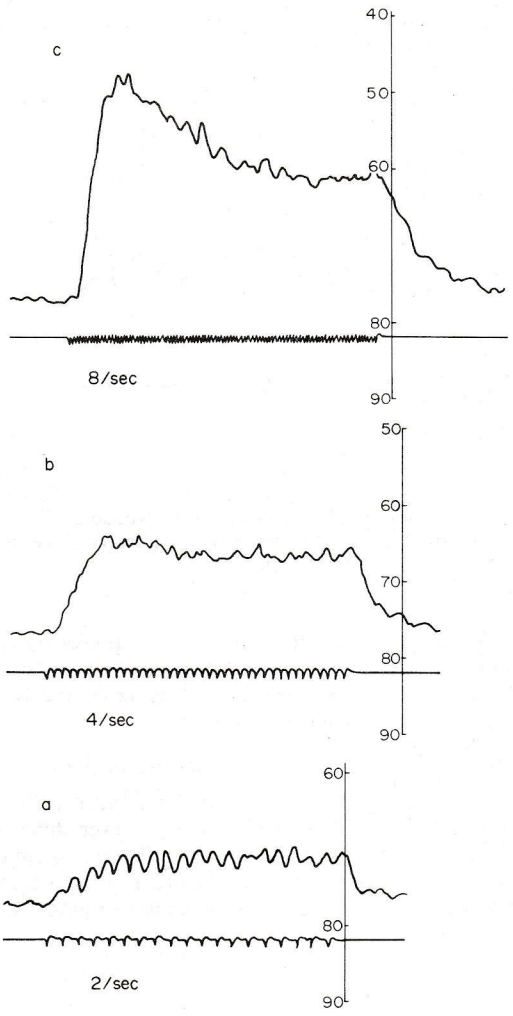


Fig. 5. Time course of three luminus evoked by a 10 sec train of stimuli (1 msec, 15 V) applied on the same preparation at the frequency of 2/sec (a), 4/sec (b) and 8/sec. Vertical bar 18 Mquanta/sec.

according to a latin square design (Cochran & Cox, 1960).

Light production. The luminus peak (L_{max}) increases with the stimulus frequency until a maximal value (28.9 $\pm$ 1.9 Mquanta/sec) observed for a frequency of 20/sec, there being no further increase for higher frequencies (Table 1). The time to reach the peak of light (time to L_{max}) is long at low frequencies (4.5 $\pm$ 0.7 s at 1/sec to 4.0 $\pm$ 0.7 sec at 4/sec). It decreases abruptly to 1.7 $\pm$ 0.7 at 8/sec, where it remains up to 100/sec.

After the peak, the light decreases somewhat exponentially, there being no significant differences between the frequencies tested. It reaches a stable level (L_b) within 8.5 $\pm$ 0.1 sec at 1/sec and 10.0 $\pm$ 0.1 sec at 100/sec (Table 1).

It can be concluded from these results that the lowest stimulus frequency necessary to generate the largest luminus is 20/sec. Higher frequencies do not improve the light performance of isolated photophores.

Extinction. Quite soon after cessation of stimulation, the light production decreases without observable extinction latency time. The half extinction time, i.e. the time for the light to decay to half of the value measured at the end of stimulation period ranges from 0.30 $\pm$ 0.1 sec at 1/sec to 0.84 $\pm$ 0.1 sec at 100/sec (Table 1). The time for complete extinction is three times longer at 1/sec (1.0 $\pm$ 0.3 sec) and about six times longer at the other frequencies (2.7–4.7 $\pm$ 0.3 sec).

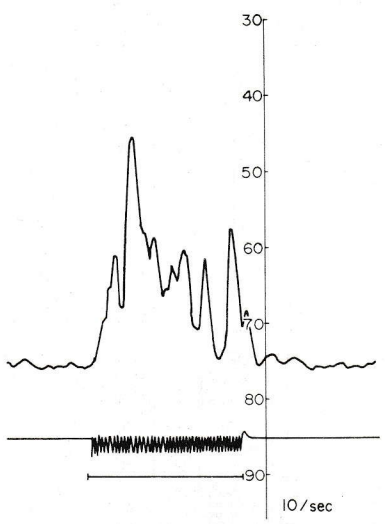


Fig. 6. Typical luminescent response evoked by a 5 sec train of stimuli (10/sec) of long duration (4 msec) and high voltage (45 V). The sensitivity of the photomultiplier is the same as in Fig. 5.

In the case of photophores isolated from mesopelagic fish *Porichthys*, it is known that the extinction time of a luminus is directly related to the luminescence level measured at the end of the stimulation. When all the values of the extinction time of *Chauliodus* photophores are plotted in function of the light level measured at the 10th sec of stimulation (*Lb*), without taking the frequency of stimuli into account, a similar relationship is observed. Regression analysis puts in evidence a significant ($P < 0.01$) correlation between the two parameters. The slope *b* of the calculated regression line gives a quantitative measure of the relationship: in this case, it indicates that 4.44 $\pm$ 0.30 Mquanta disappear in 1 sec of extinction.

DISCUSSION

Chauliodus photophores excised from living animals and immersed in saline (20°C) respond to anodic pulses either by flashes or by sustained luminescences, depending on the parameters of the stimulation.

Isolated anodic stimuli (4–8 msec; 50 V) evoke high intensity (about 10^8 quanta/sec) flashes lasting a few milliseconds, similar to those described by Baguet & Maréchal (1976). Owing to its explosive character we might expect a rapid exhaustion of the substrates needed for the luminous reactions and consequently a rapid reduction of the amplitude of successive flashes. On the contrary, photophores produce flashes of high amplitude during 60 sec or more as long as the interval between the stimuli is over 500 msec.

At frequencies higher than 2/sec, the stimuli evoke sporadic or partly fused flashes, rapidly failing. It

Table 1. Parameters of the luminus with their standard error of mean (SEM)

	Frequency (c/sec) of stimuli							
	1	2	4	8	20	50	100	SEM
L_{max} (Mquanta/sec)	3.1	5.5	10.3	22.7	28.9	26.1	28.1	±2.2
Time to L_{max} (sec)	4.5	5.7	4.0	1.7	1.6	1.5	2.0	±0.7
L_b (Mquanta/sec)	2.9	5.0	8.2	15.0	16.7	15.8	18.2	±1.1
Time to L_b (sec)	8.4	9.3	9.5	9.8	9.9	10.0	10.0	±0.1
$T_{\frac{1}{2}\ extinction}$ (sec)	0.3	0.4	0.6	0.7	0.9	0.7	0.8	±0.1
$T_{extinction}$ (sec)	1.0	2.0	2.8	3.9	4.3	4.2	4.7	±0.3

L_{max} (Mquanta/sec): peak of the light emission (10^6 quanta/sec). Time to L_{max} (sec): time to reach the peak of light in sec. L_b (Mquanta/sec): stable light level (10^6 quanta/sec). Time to L_b (sec): time to reach the stable light level. $T_{\frac{1}{2}\ extinction}$ (sec): time of half extinction in sec. $T_{extinction}$ (sec): time to reach complete extinction (sec.)

seems that the light response to strong stimulus is followed by a refractory period lasting nearly 500 msec, during which the ability to produce light is considerably lowered or even abolished. At the present time it is not possible to decide whether this phenomenon is due to an inhibition of the coupling mechanism between excitation and light production or to an inhibition of some chemical reaction in the luciferine–luciferase system.

On the other hand, short stimuli (1–4 msec) of low strength (5–15 V) evoke flashes with amplitudes about 100 times lower than those produced by strong stimuli: these flashes can summate and fuse together and produce a steady luminescence when the stimulus frequency exceeds 8/sec.

The light emission may then be ten times larger than that produced in a single flash. A further increase of the rate of stimulation does not change significantly the kinetics of the light response. We have proposed to call luminus such a summation (Baguet & Maréchal, 1976).

Chauliodus luminus is characterized by a peak of light which is reached within 2 sec after the onset of stimulation, but it is maintained only for a fraction of a second: the light emission decreases progressively to reach a constant level of about 60% of that measured at the peak. This luminescence which varies from 15.0 to 18.2 Mquanta/sec can be maintained as long as 6 min.

The luminus of epipelagic fish *Porichthys* photophore differs by the following points from the luminus of *Chauliodus* photophore.

(i) Stimuli shorter than 3 msec never induce a luminus at low voltage (5–20 V) whatever their frequency.

(ii) No response is evoked when the photophore is stimulated at a frequency lower than 5 stimuli per sec (Baguet & Case, 1971). At higher frequencies, a completely fused luminus develops slowly after a latency of several seconds. The latency time decreases and the intensity of the luminus increases when the stimulation is repeated.

(iii) The luminus intensity increases at a slow rate during the stimulation: at 20/sec, the maximal light production ranging from 4.6 to 6.2 Mquanta/sec has only been reached 15–20 sec after the beginning of stimulation. Although the magnitude of the luminus in photophores of both bathy and epipelagic fish are similar, the kinetics of the light emission are completely different: *Chauliodus* photophores respond rapidly to electrical stimulation producing light at a high rate while *Porichthys* photophores produce light at a slow rate. The strikingly different response characteristics suggest that *Chauliodus* photophores are controlled by a mechanism which is different from that active on *Porichthys* photophores. It is unlikely that the difference is to be found in the quality of the neurotransmitter, the luminus being inhibited by adrenergic blocking agents in both fish (Baguet, 1977) and triggered by adrenergic activation (Nicol, 1969; Anctil & Case, 1976). This, of course, does not necessarily mean that the triggering mechanisms are the same in both types of light organ. Electron microscopy shows that the cell membranes of *Porichthys* photophores contrary to those of *Chauliodus* photophores have long microvilli extending into surrounding extracellular channels with dense complex membranous whorls (Strum, 1969). Since it is well established that in excitable tissues the cellular membrane is the support of events coupling the extracellular excitation to the intracellular luminescent reactions, it is conceivable that these ultrastructural variations may be required because the triggering mechanisms for luminescence are different.

Moreover, the explosive character of the luminus of *Chauliodus* suggests a simultaneous activation of all the photogenic cells of the photophore and therefore the presence of an efficient synchronizing mechanism.

Extinction begins immediately after cessation of stimulation and is complete in 3.9–4.7 ± 0.3 sec (depending on the frequency of stimuli). In *Porichthys*, extinctions begins later than 1 sec after the end of stimulation; the slope of the calculated regression line for the relation between the extinction time and the light level measured just before the beginning of the extinction period are not similar in *Chauliodus* and *Porichthys*: 4.44 ± 0.30 sec in the first case and 2.1 ± 0.7 Mquanta/sec in the second case. As the extinction rate probably reflects the rate of deactivation of the luminescent sites, we suggest that the mechanism of deactivation is not similar in both photophores.

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