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LUMINESCENCE OF ISOLATED PHOTOPHORES AND SUPRACAUDAL GLAND FROM *MYCTOPHUM PUNCTATUM*: ELECTRICAL STIMULATION

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Abstract—1. Isolated photophores of the living bathypelagic fish *Myctophum punctatum* respond to train of weak (10–25 V) and short (2–5 msec) electrical stimuli applied at different frequencies (8–100/sec) by a luminus response.

2. This response is characterized by a short emission latency time, the peak of light develops within about 2 sec after the beginning of the electrical stimulation: afterwards the light decreases to a constant level within about 8 sec.

3. Stimuli of higher strength (60–75 V) and longer duration (8–16 msec) applied at different rates (1–100/sec) evoke brief flashes.

4. The isolated supracaudal gland emits flashes in response to electrical stimulation whatever the strength and the duration of stimuli.

5. The flash of the supracaudal gland differs from the flash of the isolated photophores in three respects: lower threshold, higher magnitude and longer duration.

INTRODUCTION

Myctophids or lanternfishes luminesce either on mechanical pressure (Nicol, 1958, 1960; Edwards & Herring, 1977) or on external application of electric current (Anctil, 1972; Barnes & Case, 1974).

Similar luminescence occurs after intramuscular injection of norepinephrine 10^{-3} M or saline (Barnes & Case, 1974), hydrogen peroxide or peroxidase (Anctil, 1972).

However, any attempt to stimulate luminescence of isolated photophores of myctophids failed, probably owing to the damage to fishes incurred by conventional trawling.

In the present paper, we report the first luminescent responses induced by electrical stimulation of photophores and supracaudal gland, isolated from living specimens of *Myctophum punctatum* captured in the strait of Messina (Sicily).

METHODS

Collection of fish

Specimens of *Myctophum punctatum* (Fig. 1) were caught alive with a hand-net in the strait of Messina, during high tide, where deep water currents deflected upwards by the barrier shallow dragged them to the surface (Baguet, 1975). After capture, the fish were brought in fresh seawater to the station of Ganzirri, near Messina, where they were transferred to a small glass aquarium filled with seawater from the strait, maintained at low temperature (8°C). Those experiments were conducted in January and May 1979.

Dissection of the photophores

The fish was laid on a filter paper wetted with seawater and the photophores were excised without anesthetizing of the fish. Strips of 18 posterior anal photophores (Bini, 1970) were cut and immersed in a small vessel filled with saline (Baguet & Maréchal, 1976). The supracaudal gland of male specimens was isolated according to the same procedure.

Electrical stimulation

The photophores or the supracaudal glands were transferred to a small perspex chamber where two platinum–iridium electrodes (tip dia. 10 μm) were laid at each end of the tissue and connected to a stimulator delivering square pulses. The only way to induce luminescence was to apply a composite stimulus: the square pulse of cathodic current was followed at an interval of 1 msec by a square pulse of anodic current, both pulses having the same intensity and duration. Thus, a "stimulus" means a "double shock" of that type, the parameters (voltage duration) being given for the first pulse only.

Measurements of the light emission

The small perspex chamber with the photophores was laid perpendicularly to a photomultiplier (PM 270 C, international light) in order that the emitting area of the photophores faces the photocathode at a distance of 2.0 cm. The luminescent response obtained from the specimen is displayed on a 20 cm strip-chart recorder. The light sensitivity was calibrated with a standard light source (betalight) held at the place of the tissue (Baguet & Maréchal, 1976).

RESULTS

Isolated photophores or supracaudal gland immersed in saline never luminesce spontaneously but respond to electrical stimulation, even 2 hr after excision from the fish.

Posterior anal photophores

Short duration stimuli. A sustained luminescence is evoked with stimuli of 2–5 msec duration (15–25 V) at a rate ranging from 8 to 100/sec.

Figure 2a shows a typical light response induced by a 10 sec train of stimuli (25 V, 5 msec, 20/sec).

In this particular case, the latency time of the light

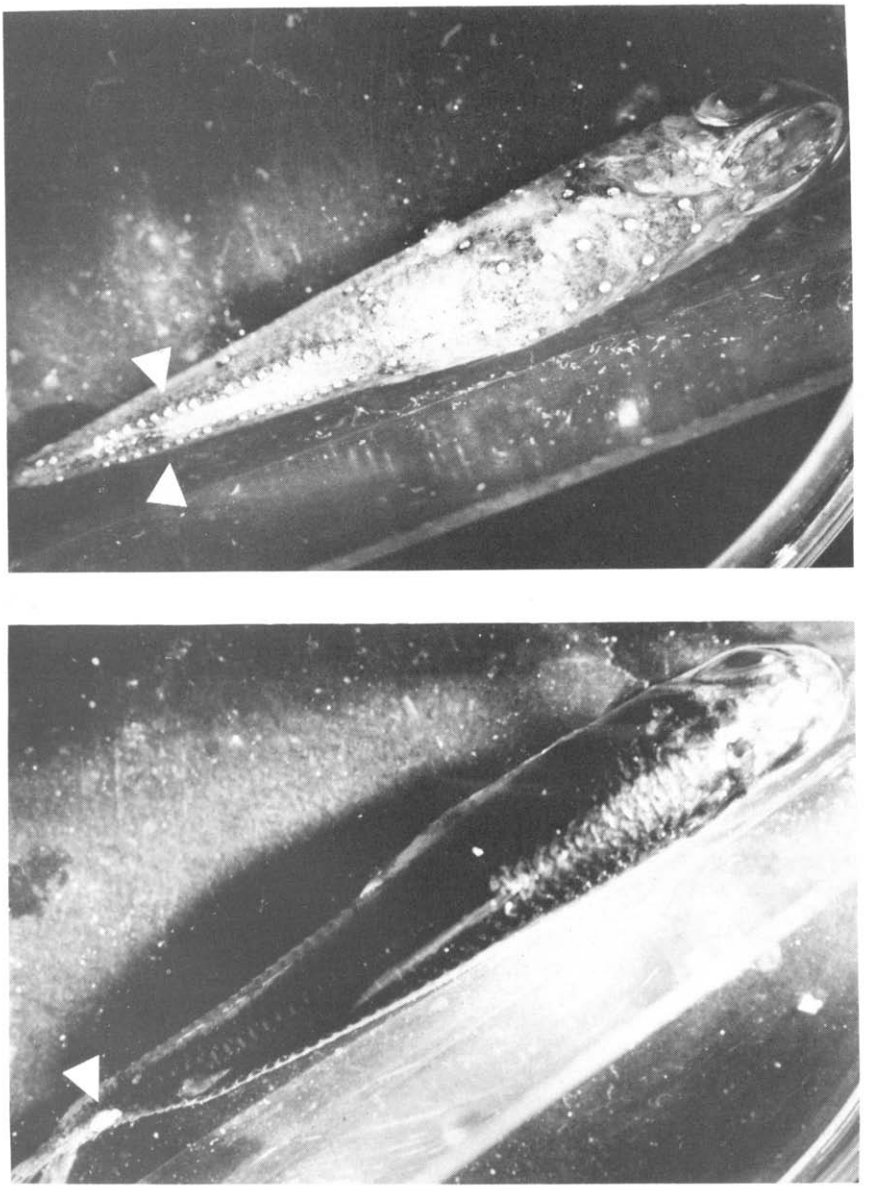

Fig. 1. Upper photograph: ventral view of a freshly captured specimen of *Myctophum punctatum* showing the ventral and the anal photophores (two arrows). Lower photograph: dorsal view of the same specimen showing the supracaudal gland (one arrow).

response is 0.4 sec; after this short period, a peak of light (14.6 10^6 quanta/sec or 14.6 Mq/sec) develops in 2.1 sec. After this peak, the light decreases to reach a stable level (3.6 Mq/sec) after 7.8 sec: this next level of light corresponds to 25% of the peak.

After cessation of stimulation, the light production decreases without any detectable latency time, at least in our experimental conditions. The time for complete extinction, which is 0.9 sec in Fig. 2a, is directly proportional to the level of light measured at the end of the stimulation period. This is illustrated in Fig. 3a which shows a preparation stimulated 6 times at 60 sec interval with a 10 sec train of stimuli (15 V, 2 ms, 8–100/sec). However, this type of light response is labile: we never succeeded to record more than 10 responses on the same preparation, even when the train of stimuli were applied at a longer interval (60 sec). Nevertheless, the photophores remain electrically excitable since another light response is observed on application of stronger and longer stimuli.

Long duration stimuli. Photophores of *Myctophum punctatum* respond to strong (60–75 V) and long (8–16 msec) stimuli applied at different rates (1–100/sec) by brief flashes. When the stimulus is repeated at a low frequency (1–4/sec) the flashes are erratic and vary in size, being at times as strong as 98 Mq/sec and at times so weak as to be barely detectable. For higher frequencies, (5–20/sec) flashes of the same size are produced after a latency time whose duration decreases with frequency (Fig. 2b): at 5/sec the first flash is trig-

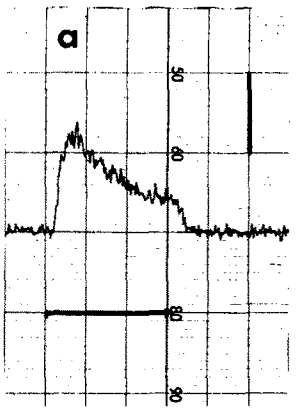

Fig. 2a. A typical sustained luminescence produced by a 10 sec train of stimuli (25 V, 5 msec, 20/sec). Vertical bar: 10.4 Mq/sec.

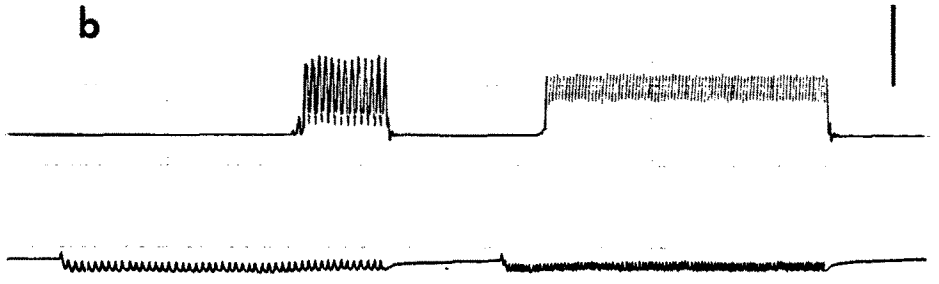

Fig. 2b. Two series of flashes evoked by two successive 10 sec train of stimuli (65 V, 8 msec) applied at two different rates, 5/sec and 10/sec. Vertical bar: 246.4 Mq/sec.

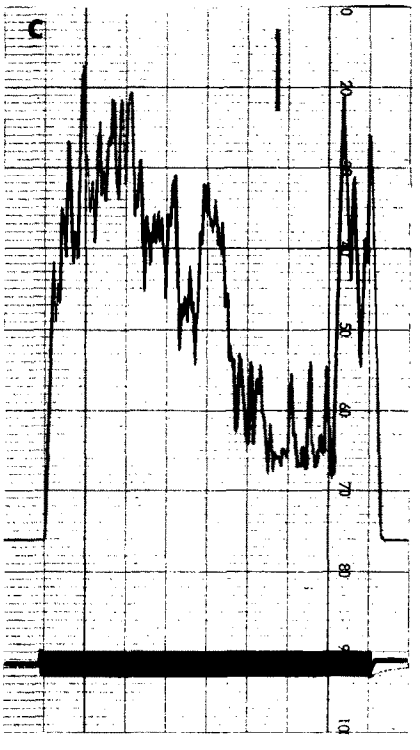

Fig. 2c. Burst of light produced in response to a 10 sec train of stimuli (65 V, 8 msec) applied at a high rate (50/sec). Vertical bar: 246.4 Mq/sec.

Fig. 2. Light responses of isolated photophores to electrical stimulation.

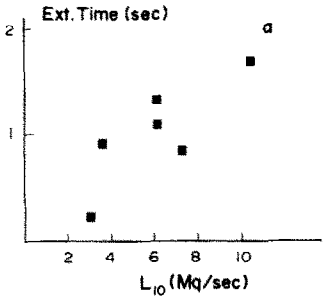

Fig. 3a. Isolated photophores: relationship between the extinction time (sec) and the amplitude of the light sustained at the 10th sec of 6 light responses evoked by 6 trains of short duration stimuli (15 V, 2 msec, 8–100/sec).

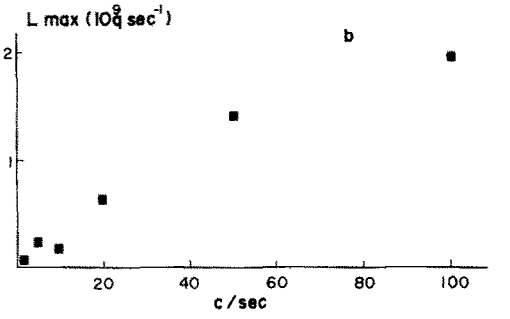

Fig. 3b. Supracaudal gland: Influence of the frequency of stimuli (65 V, 8 msec, 10 sec) on the amplitude of the flashes emitted.

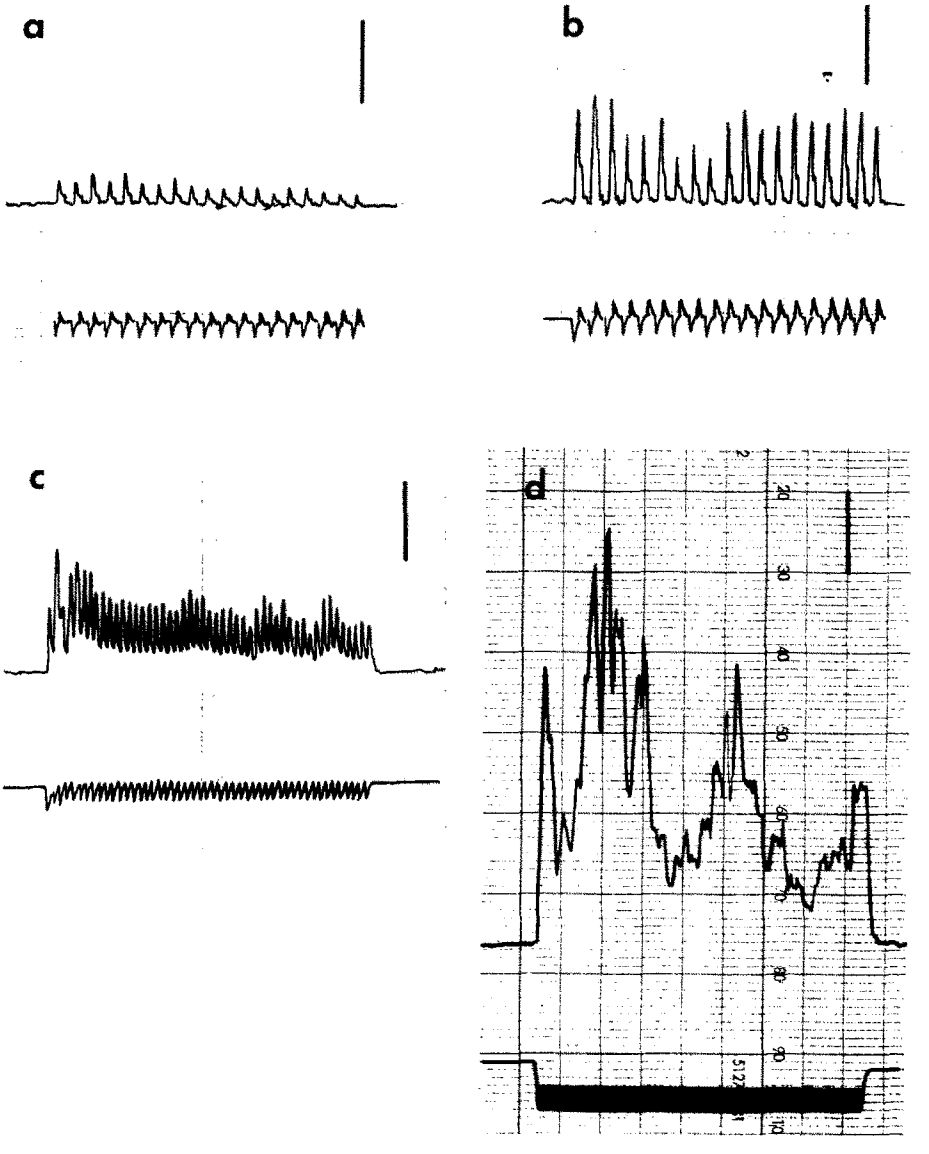

Fig. 4. Flashes emitted by an isolated supracaudal gland in response to four trains of stimuli. Vertical bar, 46.2 Mq/sec. a. and b. 20 stimuli of 1 msec duration applied at 2/sec. Stimulus strength: 15 V (a) and 20 V (b). c. and d. A 10 sec train of stimuli (20 V, 1 msec) applied at 5/sec (c) and 50/sec (d).

gered by the 35th stimulus, i.e. after a latency period of about 6.8 sec. The maximal amplitude of the flashes is 234 Mq/sec. At 10/sec the latency period is reduced to 1.3 sec.

At 20/sec the different flashes fuse completely, while for higher frequencies (50–100/sec) the light curve becomes complex, showing a series of partly fused flashes (Fig. 2c). The magnitude of the flashes depends on the stimulus duration and the stimulus frequency. Curve b of Fig. 3 shows the relationship between the maximum amplitude of the flashes of a preparation stimulated with a 10 sec train of stimuli (65 V, 8 msec) applied at different frequencies: at 100/sec, the flash magnitude is about 30 times higher than the flash evoked at 1/sec.

Supracaudal gland

A single pulse of short (1 msec) or long (8 msec or more) duration applied on the isolated supracaudal gland elicits a brief response lasting 200–400 msec. The amplitude of the flash in response to a stimulus of a given duration increases with voltage: Figs 4a and b show the amplitude and the time course of successive flashes elicited by two series of 20 pulses (1 msec) applied at 2/sec. Although the amplitude of the flashes induced by stimuli of 15 V strength (4.68–18.48 Mq/sec, a) is much lower than those evoked by 20 V (27.7–64.7 Mq/sec, b), in both cases the flash is composite: during the extinction period, when the peak amplitude has been reduced to one half or one third, a second peak of light appears. The individual flashes are still visible when the pulses are applied at 5/sec (Fig. 4c), but at frequencies higher than 10/sec, the response becomes erratic (Fig. 4d): the light curve is complex and shows a series of partly fused flashes emitted at irregular intervals. Contrary to the photophores, we never observed a sustained fused luminescence in response to a train of electrical stimuli.

DISCUSSION

Stimulation of isolated photophores

Photophores isolated from living specimens of *Myctophum punctation* emit flashes in response to electrical stimuli of long duration (8–16 msec) and high strength (60 V) and emit a sustained luminescence in response to stimuli of short duration (2–5 msec) and low strength (10–25 V). By analogy with the photophores of other bathypelagic luminescent fish, namely *Argyropelecus hemigymnus* and *Chauliodus sloanei*, which produce two similar types of luminescence in response to electrical stimulation, we will call the flash an *F* response and the sustained luminescence, an *S* response or luminus (Baguet *et al.*, 1980).

The maximal magnitude of the luminus response corresponds to a light emission of 1.0 Mq/sec per photophore, i.e. a response whose amplitude is similar to that of the *S* response of photophores from both *Chauliodus sloanei* and *Argyropelecus hemigymnus* (3.5 Mq/sec) (Baguet *et al.*, 1980). However the fresh weight of one anal photophore of a male adult specimen of *Myctophum punctatum* is about 0.02 mg; on the other hand the weight of one ventral photophore of an adult specimen of *Chauliodus sloanei* or *Argyropelecus hemigymnus* is 0.8–1.0 mg. Although the weight values do not include exclusively photogenic tissues, it means that the amount of photogenic tissue in a photophore of *Myctophum punctatum* is considerably low. It follows that the maximal light emitted during a luminus is 52 Mq/sec per mg of photophore for *Myctophum punctatum* and only about 3 Mq/sec per mg of photophore in the case of *Chauliodus sloanei* and *Argyropelecus hemigymnus*.

Authors studying the photophore luminescence of lanternfish *in situ*, i.e. on living animals, also reported two types of luminescence: Barnes & Case (1974) studying *Triphoturus mexicanus*, recorded a sustained luminescence originating from all the photophores in response to electrical stimulation delivered through two fine electrodes placed near the spinal cord on the head or the tail of the fish. On the other hand, mechanical stimulation of the head of *Myctophum aspersum*, *Myctophum nitidulum* and *Benthosema glaciale* evokes quick flashes (Edwards & Herring, 1977); bursts of partly fused flashes were reported on *Myctophum punctatum* during mechanical stimulation of the tail of the fish (Nicol, 1958, 1960, Edwards & Herring, 1977). The synchronous luminescence of the photophore observed on these lanternfishes *in vivo* suggests that the light response is controlled by a nervous system. The similitude between *in vivo* luminescence and light responses of isolated photophores suggest that in our preparations, the light responses should be mediated by nerve terminals excited by electrical stimuli. Although there are no descriptions of photophores innervation in the case of *Myctophum punctatum*, there are several morphological evidences showing innervation systems of photophores in other lanternfishes (Ray, 1950; O'Day, 1972). Our results suggest that the flash emission observed *in vivo* should result from a strong stimulation of the nervous excitatory system innervating the photophores of Myctophids, while a sustained luminescence as described by Barnes & Case (1974) should be due to a feeble activation of this system.

Stimulation of supracaudal glands

The supracaudal gland of *Myctophum punctatum* isolated from the fish emits flashes in response to electrical stimulation whatever the strength and the duration of the electrical stimuli. However the flash of the supracaudal gland differs from that of the photophore of *Myctophum punctatum* in two respects:

(i) the supracaudal gland emits flashes from the first stimulus of a train of stimuli, and

(ii) the flash is composite, is higher in magnitude and lasts longer than the flash of the photophore. However, when the light response is calculated per unit of tissue weight, the difference is reversed: the weight of a supracaudal gland isolated from an adult specimen in our experimental conditions is about 1.2 mg, i.e. about 60 times the weight of a caudal photophore. We caculated that the maximal flash amplitude recorded from a supracaudal gland is 229 Mq/sec per mg of tissue and as high as 5407 Mq/sec per mg of anal photophore.

There are no reports on the pattern of light production from supracaudal gland of *Myctophum punctatum in situ*; the only observations refer to the infracaudal

gland of *Myctophum nitidulum*, *Taaningichthys bathyphilus* (Edwards & Herring, 1977) and *Lampanyctus ritteri* (Barnes & Case, 1974). However, the mechanical stimulation of the living fish, or the electrical stimulation of the area adjacent to the caudal gland, trigger in both cases flash emission.

The morphology and organisation of the photocytes in both lanternfish photophores and caudal organs are similar (Anctil & Case, 1977, Herring & Morin, 1978). The complex of nerve terminals is much more elaborate in caudal organs where four to five layers of processes are sandwiched between two photocytes, compared to one to two layers in photophores (O'Day, 1972). Such a dense innervation might be of significance in view of the low threshold, and long duration of the flashes which were obtained from isolated supracaudal gland as compared with isolated photophores. Nevertheless, the absence of luminous response in the supracaudal gland means that the triggering mechanism may differ in the two types of tissue. The success of these first investigations on excised myctophum photophores and caudal gland promises a means by which it is possible to examine neuro-effector control in both luminous tissues.

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