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Luminescence control of Stomiidae photophores

Jérôme Mallefet*, Laurent Duchatelet, Claire Hermans, Fernand Baguet[1]

Catholic University of Louvain-La-Neuve, Marine Biology Laboratory, 3 Place croix du Sud, 1348, Louvain-La-Neuve, Belgium

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ABSTRACT

Nervous control of light emission from deep-sea mesopelagic fishes has been documented for several species. Studies on the nervous control of photophores from deep-sea luminescent fish, are mainly restricted to a pharmacological approach. For example, the light organs, called photophores, isolated from *Argyropelecus hemygimnus* and *Maurolicus muelleri* show a much higher sensitivity to adrenaline than to noradrenaline. According to these results and other information in different species, catecholamines are considered as main neurotransmitters triggering bioluminescence in deep-sea fishes. The present work is a study of the nervous control of the isolated photophores from two Stomiid fishes, *Chauliodus sloani* (the viperfish) and *Stomias boa* (the dragonfish) with the aim to determine the nature of the nervous control by pharmacological, biochemical and morphological approaches.

Results show that, although the photophores of both species are sensitive to catecholamines, adrenaline is present in larger amount than noradrenaline in the light organs of *C. sloani*. Both catecholamines have different immunoreactive (IR) sites, noradrenaline showing a very diffuse localization as compared to adrenaline in *C. sloani*. On the contrary, only adrenaline is detected in the photocytes chamber and nerves innervating the photophore in *S. boa*. Knowing that the majority of dragonfishes exhibit a luminescent chin barbel, we also investigated the presence of catecholamines in this specific tissue in *S. boa*. Immunohistology reveals the presence of adrenaline within the tissue forming the chin barbel; adrenaline–IR is found in the connective tissue surroundings two group of muscle fibers and blood vessels in the stem but also around the multiple blood vessels located within the barbel bulb.

Our results strongly support the adrenergic control of light emission in bioluminescent stomiid fishes.

1. Introduction

The nervous control of photophores from the epipelagic luminescent fish *Porichthys* is well documented: pharmacological studies have shown that isolated photophores share a similar sensitivity to both adrenaline and noradrenaline (Christophe and Baguet, 1983). The neural distribution of noradrenaline in the photophore and the specific accumulation of [3H]-noradrenaline by nerve varicosities surrounding the photocytes, strongly suggest that noradrenaline should be the natural neuromediator (Lariviére and Anctil, 1986; Mallefet and Anctil, 1992).

Studies on the nervous control of photophores from deep-sea luminescent fish, are restricted to a pharmacological approach: the light organs isolated from *Argyropelecus hemygimnus* and *Maurolicus muelleri* show a much higher sensitivity to adrenaline than to noradrenaline (Baguet and Marechal, 1978; Baguet and Christophe, 1983). More recently immunohistochemical studies revealed the presence of neuromodulatory action of NO in *A. hemigymnus* (Krönström et al., 2005), a mechanism that seems more common since NO was detected in other luminous deep-sea fishes (Krönström and Mallefet, 2010). Localisation of adrenaline in *A. hemigymnus* photophores was documented recently (Zaccone et al., 2011a,b).

The present work is a study of the nervous control of the isolated photophores from the two stomiid luminescent fish *Chauliodus sloani* and *Stomias boa*: the aim is to determine the nature of the nervous control of the light organ (photophore). Moreover, knowing that the majority of dragonfishes exhibit a luminescent chin barbel, morphologically distinct across species (Sutton and Hartel, 2004; Davis et al., 2014), the presence of catecholamines immunoreactivity (catecholamines-IR) was also investigated in *S. boa* chin barbel.

The results show that light organs of *C. sloani*, are sensitive to adrenaline and noradrenaline, while only adrenaline trigger light emission of isolated photophores from *S. boa*. Immunohistochemistry

* Corresponding author at:Marine Biology Laboratory, Earth and Life Institute, Catholic University of Louvain, Place Croix du Sud 3, Louvain-la-Neuve, 1348, Belgium.

E-mail address: jerome.mallefet@uclouvain.be (J. Mallefet).

[1] This paper is dedicated to the professor Fernand Baguet (1939–2017)

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shows that both catecholamines are detected in *C. sloani* photophores while only adrenaline-IR is observed in *S. boa* photophores and in a specific luminous tissue, the chin barbel.

2. Material and methods

2.1. Fish collection

Twelve specimens of *C. sloani* were caught alive with a hand-net in the strait of Messina, during high tide, while deep-water 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 laboratory, where they were transferred to a glass aquarium filled with seawater from the strait. Maintained at low temperature (about 9 °C), many specimens survived for 12 h.

Fourteen specimens of *S. boa* have been collected by trawling between 500–250 m depth at the East of the Kerguelen Plateau region during the LOGIPEV197 campaign. Specimens were either directly used for pharmacological assays or stored in formaldehyde at 4 °C for further immunohistochemical studies.

Animals in this study were collected in accordance with the guidelines of the ethics committee of the French Polar Institute (Institut Paul Emile Victor – IPEV) and the local fish care of Messina Marine Station (Ganzirri) that approved all our fieldwork.

2.2. Dissection of the photophores

Fishes were killed by a rapid cutting head using scalpel blade, after removal of the gelatinous layer covering the ventral area of the fish, series of 4–8 photophores from the ventral or lateral region were cut out from the skin with fine scissors. It was very easy to isolate theses series of photophores with very little surrounding tissues, since photophores are loosely attached to the skin. Muscle and eyes of *C. sloani* were also removed and frozen for further HPLC analysis. Chin barbels of *S. boa* were isolated in order to realize histological and immunohistochemical studies.

2.3. Pharmacology

Pharmacological stimulations were carried out on isolated photophores of *C. sloani and S. boa*. Series of photophores were immersed in Hank's solution (in mM: NaCl 188, KCl 7.4, $CaCl_2{\cdot}2H_2O$ 3.5, $MgCl_2{\cdot}2H_2O$ 2.4, sucrose 120, Tris-HCl buffer 10, pH 7,3). Sucrose is added to increase the solution's osmolarity to a value equivalent to the blood serum of mesopelagic fish (Griffith, 1981). The preparations were placed in small cylindrical perpex chamber filled with 200 µL of Hank's solution. These chambers were placed in a Berthold FB12 luminometer (Berthold Detection Systems GmbH, Pforzheim, Germany) connected to a laptop. The Multiple kinetics protocol (FB12, ver.1.5) enables us to follow 4 preparations simultaneously. The light sensitivity was calibrated with a standard light source (betalight, peaking at 470 nm) held at the place of the organ. Recordings of luminescence were carried out during 25–45 minutes.

Pharmacological *C. sloani* luminescence stimulations were induced by the injection of 200 µL of the following solutions: adrenaline (S.C. Federa); noradrenaline (Fluka, Buchs, Switzerland), isoprenaline (Wintrops S.A., New York, USA), phenylephrine (Sigma, St. Louis, Missouri, USA) hydrochlorides with a final concentration of 10^{-7} and 10^{-4} mol l^{-1} made in a saline (Baguet and Christophe, 1983). *S. boa* luminescence stimulations were only made with adrenaline. Given the varying number of photophores per preparation, the results are standardised per photophore. Adrenaline dose-response curves (10^{-6} to 10^{-3} mol l^{-1}) were measured for *S. boa* with the following variables: total amount of light, maximum intensity of light and latency time to reach the maximum of light emitted.

2.4. High pressure liquid chromatography

Photophores, muscles and eyes were excised from the dragonfish *C. sloani*; tissues were weighed and placed in centrifuge tubes containing 1 ml of 0.1 M perchloric acid (Merck, Darmstadt, Germany). The samples were homogenized and the extracts centrifuged at 47 300*g* and 4 °C for 33 min. The supernatants were stored at −80 °C until screening by HPLC; pellets were resuspended in 1 ml NaOH 1 M for protein content determinations. We used a Bio-Rad 1350 HPLC station (Bio-Rad Labs., Richmond, CA, USA). The detection was accomplished by means of a model 400 EG&G-Princeton electrochemical detector (Princeton, NJ, USA). The electrochemical potential was adjusted to 720–920 mV against Ag/AgCl reference electrode and a glassy carbon working electrode. The sensitivity range varied between 5 and 100 nA, while the time constant was adjusted at 2 s. The retention time of standard and sample monoamine peaks were recorded and identified by spiking and according to their oxidative potentials.

2.5. Immunohistochemistry

Strips of skin containing photophores were excised from fish; fixation was carried out by immersion of photophore series for 1 h in 0.1 M phosphate buffer (pH 7.3) containing 0.5% glutaraldehyde (Sigma) and 4% paraformaldehyde (Sigma) at room temperature, followed by postfixation for 16 h at 4 °C with 4% paraformaldehyde. Tissues were washed for three, 10 min periods in phosphate-buffered saline (PBS) at pH 7.4. They were then immersed in graded series of alcohol 70 to 100. Tissues were embedded in paraffin. Sections of 8–12 µm in thickness were prepared with a Reicher microtome.

Sections were rinsed in PBS for 15 min and then immersed for another 15 min in 0.3% hydrogen peroxide (Sigma) in PBS to inactivate endogenous peroxidases. After a 20 min immersion in PBS containing 0.3% Triton-X100 (Sigma) to facilitate antibody penetration and 5% swine normal serum to eliminate nonspecific reactive sites and to serve as diluting medium for antibodies, sections of photophores were incubated in antiserum against catecholamines.

Antisera against noradrenaline-formaldehyde-albumin (NA-P-1-18) raised in sheep and against adrenaline-formaldehyde-albumin (A-P-2-16) raised in rabbit, obtained from Dr A. A. J. Verhofstad (the Netherland), were used for the immunodetection on *C. sloani*. For noradrenaline, dilution of primary antibody was 1:800 to 1:1200, and for adrenaline, 1:200 to 1:500 (Verhofstad et al. 1983). For noradrenaline immunohistochemistry, rabbit normal serum, rabbit anti-sheep immunoglobulin and sheep PAP (Dakopats) were used. While for the immunodetection on *S. boa*, photogenic organ and barbel section were incubated with polyclonal commercial antibody raised against adrenaline (Epinephrine (EPI) antibody, Rabbit, Catalog No.: PAA858Ge01, lot No.: A20160429569, dilution 1:200, Cloud-Clone Corp., Houston, TX, USA) or noradrenaline (Norepinephrine (NE) antibody, Rabbit, Catalog No.: PAA907Ge01, lot No.: A20160429568, dilution 1:200, Cloud-Clone Corp., Houston, TX, USA). Sections were incubated in the antiserum or the primary antibody in a sealed moist chamber for 18 h at 4 °C, then subjected to three 15 min rinses in PBS at room temperature. The next incubation was in a PBS solution containing swine anti-rabbit serum (diluted 1:50, Dakopats, Copenhagen, Denmark) for 1 h at room temperature. After another washing sequence as above, the peroxidase-antiperoxidase (PAP) reaction was performed with rabbit PAP complex (Dakopats) diluted 1:100 for 1 h in moist chamber. Sections were pre-incubated in 0.05% 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma) in PBS for 15 min, then reacted in DAB solution containing 0.015% hydrogen peroxide for 3–15 min. The immunostained sections were then stained using classical Emalun or Masson's Trichrome before mounting and examination with a Zeiss Diaplan equipped with epifluorescence.

Classical omission of the primary and secondary antibodies was performed for all immunohistochemistry. In order to test antibody specificity primary antibody preabsorption was also done when the conjugated antigen was available. In this case, sections were incubated with adrenaline/noradrenaline antibody preabsorbed overnight at 4 °C with the reconstituted catecholamine. For adrenaline antibody and reconstituted immunogen used for *C. sloani* (provided by Dr. A. A. J. Verhofstad), the specificity was clearly demonstrated in another research on the luminous fish, *Porichthys notatus* (Mallefet and Anctil, 1992); in addition for *C. sloani* preabsorption of the antibody with noradrenaline (Sigma) dissolved in fish saline was performed. While for *S. boa* control, adrenaline (Adrenaline tartrate, solution for injection, Sterop, Bruxelles, Belgium) was commercially obtained in order to proceed to antibody preabsorption.

2.6. Statistical analysis

Statistical analyses (ANOVA followed by post hoc paired student t-tests) were performed with the JMP software Version *13*. SAS Institute Inc., Cary, NC, 1989-2007.

3. Results

3.1. Luminescence in adrenergic stimulated photophores

Photophores were excised from five specimens of *C. sloani* (standard length ranging from 19.5 to 26.6 cm) and from six specimens of *S. boa* (standard length ranging from 16 to 20 cm). The weight of the photophore series were very similar for both species, ranging from 8 to 12 mg.

Isolated *C. sloani* photophores never luminesced spontaneously when immersed in saline or after addition of adrenaline, noradrenaline, isoprenaline or phenylephrine at concentrations lower than 10^{-7} M (data not shown). The normalized light responses for *C. sloani* in presence of adrenaline or noradrenaline 10^{-4} M are presented in Fig. 1; though the emission latency time and the peak of light are similar in both cases, the time course is somewhat different (Table1). The peak of the light emission occurs 822 ± 180 s (n = 16) after addition of noradrenaline. During the extinction phase, the peak of light has been reduced by half of its value in 4.5 min for the noradrenergic response. For the adrenergic response, the maximum of light is reached within 1368 ± 228 s (TLmax; n = 20), extinction rate is much slower: 60% of the max amplitude is still present 20 min later. The amplitude of the response to the adrenergic agonists isoprenaline and phenylephrine 10^{-4} M, is not significantly different from the response amplitude to noradrenaline and adrenaline. Extinction rates are similar for adrenaline and phenylephrine responses on the one hand, for adrenaline and isoprenaline on the other hand.

Table 1
Mean values of parameters characterizing the light emission of *C. sloani*. LT: Latency time; TLmax: time to reach the maximal light emission (expressed in second); Lmax: maximal of light emission (expressed in megaquanta per second per photophores); n: number of different repetitions.

Stim 10^{-4}M	n	LT (sec)	TLmax (sec)	Lmax (Mq s^{-1} photophore^{-1})
Noradrenaline	16	110.6 ± 13.5	822 ± 180	13.2 ± 4.0
Adrenaline	20	136.3 ± 26.0	1368 ± 228	13.9 ± 2.3
Isoprenaline	10	173.8 ± 78.3	955 ± 104	15.9 ± 5.8
Phenylephrine	10	195.2 ± 44.3	792 ± 162	18.3 ± 6.5

For *S. boa* adrenergic dose-response stimulation, a clear positive correlation is shown between light emission and adrenaline concentration. The total amount of light emitted as well as the maximal amplitude of light response occurred in presence of adrenaline 10^{-3} M with a Ltot of 2993 ± 810 Mq by photophore and a Lmax of 3.3 ± 1 Mq s^{-1} by photophore and a TLmax of 600 ± 71.2 s; n = 6 for each concentration except for 10^{-4} M where n = 8 (Fig. 2; Table 2).

3.2. HPLC detection of adrenaline and noradrenaline in C. sloani photophore

The amount of both catecholamines present in isolated photophores, muscle tissue and eyes, has been measured by HPLC on 8 *C. sloani* specimens, Fig. 3 illustrates one original polarogram. The mean catecholamines values are expressed in µg per g of fresh tissue: in photophores, noradrenaline level is barely detectable, 1.0 ± 0.1 µg g^{-1}, but adrenaline is present in much larger amount, 9.1 ± 0.6 µg g^{-1}. In skeletal muscles, contrary to the other tissues, both catecholamines are present in large amount; contrary to the photophores noradrenaline is present in large amount as compared to adrenaline (Table 3). In eyes and other tissues (all other organs pooled), both catecholamines are present in low amounts (Table 3).

3.3. Catecholamines-IR in C. sloani

In a longitudinal section, photophores of *C. sloani* appears under the light microscope, as a bilobate structure (Fig. 4A1-B1) delineated by an

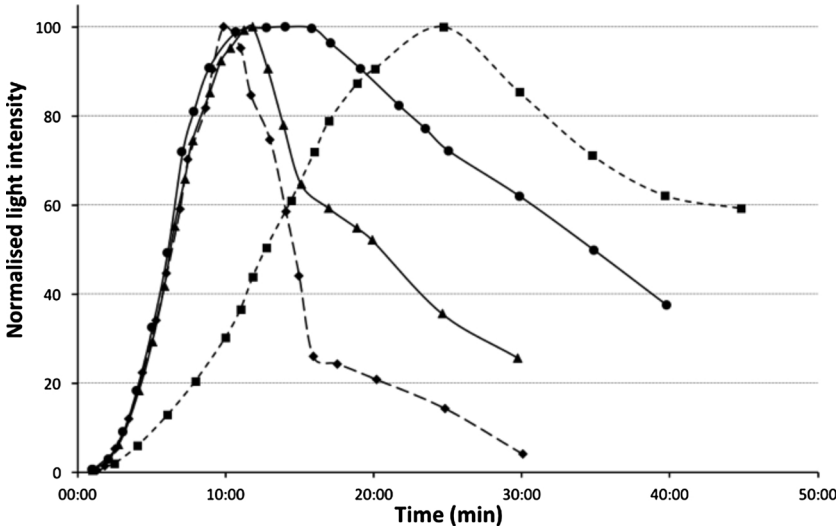

Fig. 1. Normalized light-emission of *C. sloani* isolated photophores after catecholamine stimulations at 10^{-4}M.

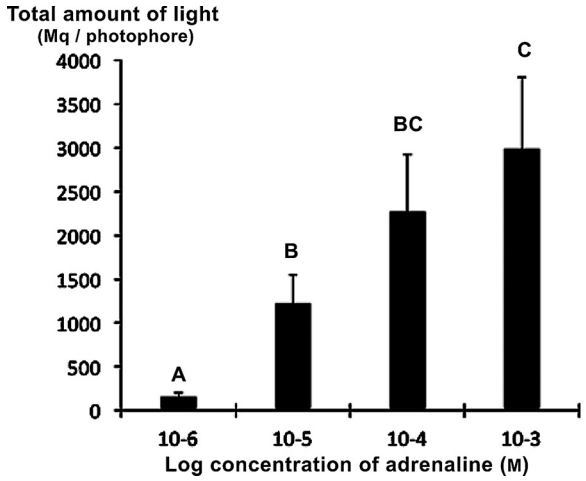

Fig. 2. Dose response of *S. boa* isolated photophores to adrenaline stimulations (10^{-6} to 10^{-3} M). Clustering letters were obtained from post-hoc paired student-t test after ANOVA (statistical difference between letters equal pvalue < 0.05). N = 6 different specimens for each concentration except for 10^{-4} M where N = 8.

Table 2
Mean values of parameters characterizing the light emission under adrenaline stimulation of S. boa. TLmax: time to reach the maximal light emission (expressed in second); Lmax: maximal of light emission (expressed in megaquanta per second per photophores); N: number of fish used. Clustering letters were obtained from post hoc pair wise student-t test (statistical difference between letters equal pvalue < 0.05).

Adrenaline (M)	N	TLmax (sec)	Statistical clustering	Lmax (Mq s^{-1} photophore^{-1})	Statistical clustering
10^{-6}	6	1494.6 ± 231.7	a	0.09 ± 0.03	a
10^{-5}	6	993.3 ± 162.8	b	0.9 ± 0.4	b
10^{-4}	8	716.2 ± 79.9	bc	1.7 ± 0.3	b
10^{-3}	6	600 ± 71.2	c	3.3 ± 1.0	c

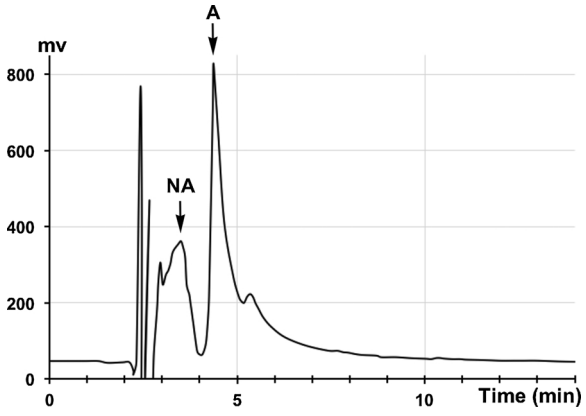

Fig. 3. Original polarogramm of *C. sloani* photophore extract showing adrenaline (A) and noradrenaline (NA) peaks.

external black layer. The external black pigmented layer represent the reflector (r) of the light organ, the smaller lobe shows a mass of radial orientated cells, the photogenic tissue (tp) which is above a larger lobe, the lens area (l) with a spherical mass of different colored cells, the lens filter (f).

This longitudinal section irradiated with UV light (380–410 nm) shows, in the radial orientated cells of the photogenic tissue, bluish fluorescent elliptical vesicles (Fig. 4A2-B2). These radial cells seem to join an eccentric area in contact with the top of the spherical mass of the lens cells of the filter. Comparison of Fig. 4A1–A3, shows that these vesicles appear at some distance from the blue stained external side of the cells. The fluorescent picture reveals a size gradient of these vesicules from external large one to smaller one at the center of the photogenic tissue. Where the spherical mass of cells, of the large lobe, makes contact with the smaller lobe, orange fluorescent cells form a clump which is approximately conical in shape (Fig. 4A2).

Two different types of adrenaline immunoreactive cells are present in the *C. sloani* photogenic organ sections: a first type of brownish colored immunoreactive cells (arrow), forms a continuous sheet of thin flattened cells at the basal pole of the photogenic tissue lobe (Fig. 4A3); alongside the cells present at the periphery of the lens lobe, several sheets of immunoreactive cells are visible forming on each side of the upper part of the large lobe, a dense multilamellar net (Fig. 4A1-A4 arrows).

Two clusters of a second type of brownish colored immunoreactive cells are present on each side of the lens filter spherical mass area (Fig. 4A1-A4). These cells correspond, in the UV irradiated section, to the dark area visible on the external side of the mass of orange fluorescent filter cells (Fig. 4A2).

Although the *C. sloani* sections show a more diffuse IR to noradrenaline through both photogenic tissue and lens lobes, the location of the reactivity is different from that observed for adrenaline. In the photogenic tissue, IR is present in the radial orientated cells appearing as intravesicular large brownish granules. Fig. 4B3 shows at high magnification a granule present in the blue stained basal pole of a radial orientated cell (arrow). At lower magnification, similar intravesicular granules are mostly visible at the periphery of the different cells (Fig. 4B1).

In the lens lobe, emptied vesicles are visible surrounded by a diffuse IR. This IR seems not associated with intracellular organites (Fig. 4B4).

3.4. Catecholamines immunoreactivity in S. boa

S. boa photophores do not differ greatly from those of *C. sloani* (Fig. 5A). The bilobate structure is retained, with a radial arrangement of the cells constituting the photogenic tissue (tp). The lens lobe below display also a radial arrangement of narrow and long cells. The two lobes being surrounded by a narrow black-brownish pigment layer. Nervous like cells seems to connect the smaller lobe to the external tissue of the light organ, crossing this pigment layer (Fig. 5C). Under UV light, the photogenic lobe appears in a diffused bluish autofluorescence, while the lens lobe present a more intense green fluorescence (Fig. 5B)

For the adrenaline immunolabeling of *S. boa* photophore sections, brownish colored IR is localized in the central core of the light organ (Fig. 5C) and in the periphery of the photophore resembling a nerve net like tissue as indicated in the periphery of the lens lobe (arrow).

Table 3
Mean value of the adrenaline and noradrenaline HPLC detection in *C. sloani* various tissues expressed in µg g^{-1} of fresh tissue. A: adrenaline; NA: Noradrenaline; n = 8.

	Photophore		Muscle		Eye		Other tissues	
	NA	A	NA	A	NA	A	NA	A
C. sloani	1 ± 0,1	9,1 ± 0,6	15,6 ± 1	8,3 ± 0,4	2,3 ± 0,5	3,2 ± 1	1,4 ± 0,6	2 ± 0,3

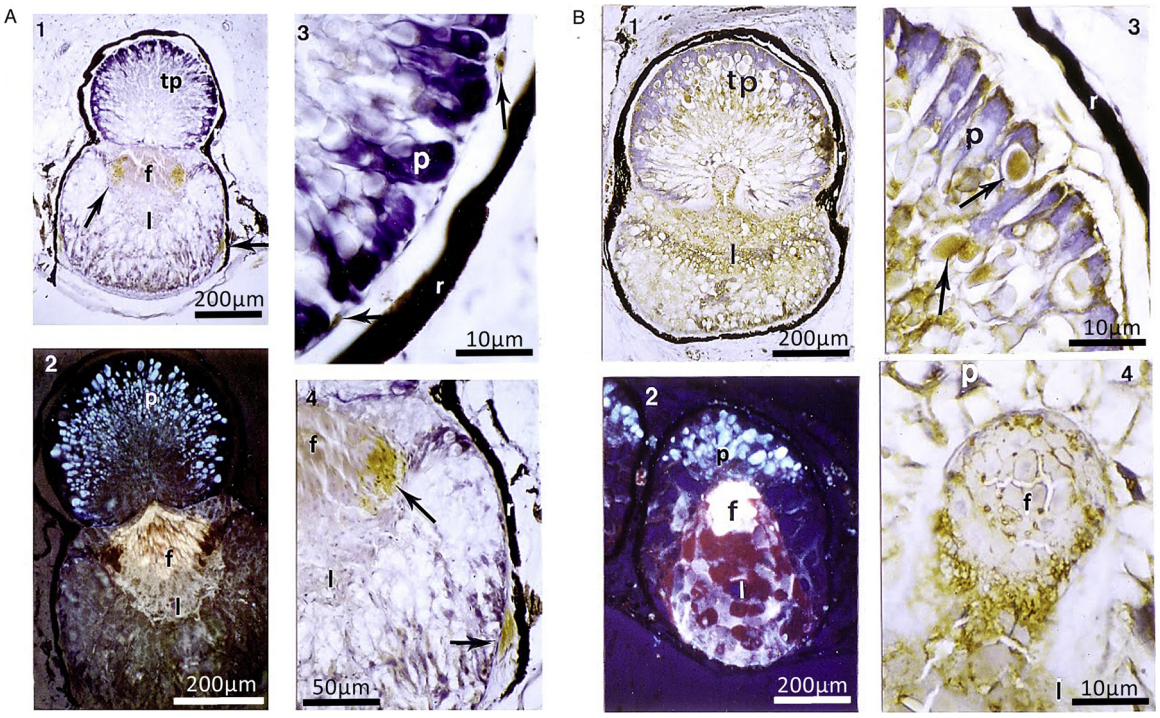

Fig. 4. Histological and immunohistological section across *C. sloani* photophore. A1: Adrenaline immunohistochemical detection in the vicinity of the photogenic tissue; A2: Autofluorescence of the light organ; A3: Close-up of the adrenaline labelling in the tp region; A4: Close-up of the adrenaline labelling in the l region; B1: Noradrenaline immunohistochemical detection over the whole luminescent organ; B2: blue stained basal pole of a radial orientated cell; B3: Close-up of the noradrenaline labelling in the tp region; B4: close-up of the noradrenaline labelling in the l region. Arrows and brown color show the immunostaining of adrenaline (A) and noradrenaline (B). f: filter; l: lens; p: photocytes; r: reflector; tp: photogenic tissue (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

In other hand, labeling for noradrenaline was not found in *S. boa* photophores (data not shown).

Histological analysis of the chin barbel of *S. boa* highlight a long (3–4.8 cm long) cylindrical stem finished by a bulky bulb with three fine and narrow appendages (Fig. 6A). At the base of the chin barbel, section reveals the presence of one isolated photophore (Fig. 6A1, arrow). Transversal and longitudinal sections across the stem show two cylindrical muscle tissues in the center, a blood vessel-like tissue and some nervous fibers between these two muscles mass. All these tissues are embedded in a conjunctive cylindrical matrix edged with a thin epidermis layer (Fig. 6A2–A3). The luminous bulb, in longitudinal section, is composed of a set of interspersed blood vessel-like tissue (arrow) with a radial orientation to the center of the bulb surrounding by a conjunctive then an epidermis layer (Fig. 6A4–A5). The proximal part of the bulb is ended by three small pointed appendages, each composed of one external thick conjunctive and pigmented tissue surrounding a lacunar blood vessel area (Fig. 6A6).

Finally, adrenaline-IR within the tissue forming the chin barbel reveals the localization of this catecholamine in the connective tissue which surround two muscle fibers and blood vessels in the stem (Fig. 6B1–B2). Strong adrenaline-IR is observed at the junction between the stem and the luminous bulb (Fig. 6B3, arrow) while a more diffuse adrenaline-IR is found around the multiple blood vessels located within the luminous bulb (Fig. 6B3) and in the tissue surrounding the lacunar blood vessels of appendages (Fig. 6B4, arrow).

For the control, omission of the secondary antibody does not show any immunoreactivity in any case (data not shown). Moreover, *C. sloani* and *S. boa* sections treated with preabsorbed antibodies, noradrenaline and adrenaline respectively, do not present any labeling (supplemental figure S1).

4. Discussion

Photophores excised from living specimens of *C. sloani*, freshly caught in the strait of Messina, luminesce in the presence of exogenous adrenaline, noradrenaline, phenylephrine and isoprenaline at 10^{-4} M, with a similar maximal amplitude. The photophores from the deep-sea fish *Argyropelecus hemigymnus* and *Maurolicus mueleri*, also luminesce in presence of exogenous adrenaline and noradrenaline 10^{-4} M with a similar intensity to the alpha and beta-adrenergic agonists (Baguet and Marechal, 1978; Baguet and Christophe, 1983). *S. boa* photophores also present a light emission when the light organ is place in presence of exogenous adrenaline at least between 10^{-6} M to 10^{-3} M.

However, the light response of these stomiid species photophores differs from that of *Argyropelecus* and *Maurolicus* photophores in two respects: (i) the *Chauliodus* photophores cannot luminesce longer than 30 min in noradrenaline, but luminesce during several hours in presence of adrenaline. Moreover, *Stomias* photophores seem also to emit light during a long-lasting period under adrenaline stimulation. On the other hand, the response to both catecholamines lasts 3–6 hours *in Argyropelecus* photophore, and in *Maurolicus* photophores, it lasts 30–60 min. (ii) the response of *Chauliodus* and *Stomias* photophore to adrenaline 10^{-4} M is very low: 13.9 ± 2.3 Mq s^{-1} mg^{-1} and 1.7 ± 0.3 Mq s^{-1} mg^{-1}, respectively. For *Argyropelecus* and *Maurolicus* photophore it was 19.99 ± 5.76 Mq s^{-1} mg^{-1} and 79.2 ± 11.7 Mq s^{-1} mg^{-1}, respectively (Christophe and Baguet, 1985).

One might suggest that, contrary to *Argyropelecus* and *Maurolicus*, adrenaline and noradrenaline could cooperate in the control of Stomiidae luminescence: adrenaline should initiate a long-sustained luminescence, while noradrenaline could be involved in the production of a short emission of light. Pinching the tail of a living *Chauliodus* induces a transient luminescence of all the light organs for no longer than 5–10 seconds (personal observation). A short luminescence cannot be evoked in the case of *Argyropelecus hemygimnus* or *Maurolicus muelleri*

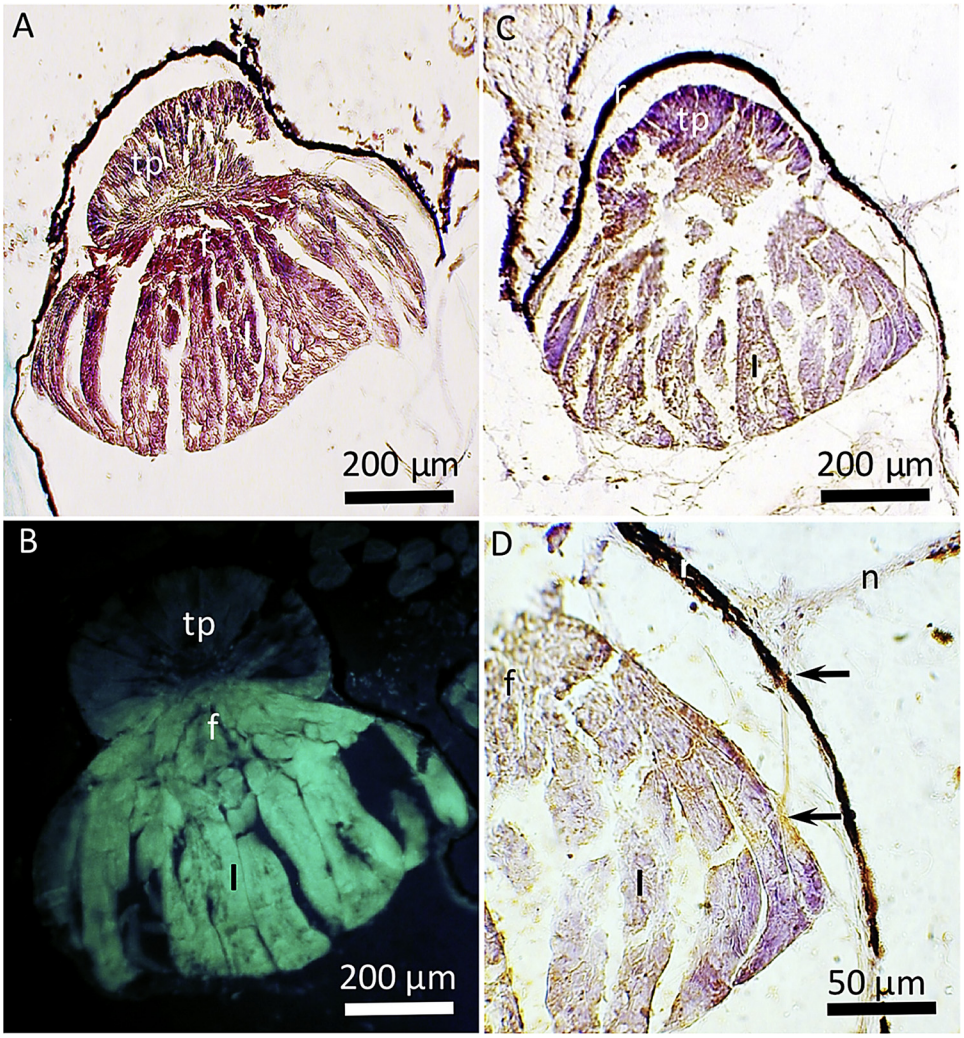

Fig. 5. Histological and immunohistological section across *S. boa* photophore. A: Histological view of the photogenic tissue; B: Autofluorescence of the light organ; C: Adrenaline immunoreactivity in the periphery of the light organ. D: Close-up of the adrenaline immunoreactivity in the periphery of the elongated lens cell cluster and within the nervous cells. Arrows and brown color show the immunostaining of adrenaline. f: filter; l: lens; n: nervous cell; r: reflector; tp: photogenic tissue.

specimens; any mechanical stimulation induces a long-sustained lightening of the photophores (personal observation). It must be pointed out that the capture method (hand-net *versus* trawling at depth) might be responsible of the differential responsiveness of isolated light organs to exogenous application of catecholamines for the two stomiid fishes. It is conceivable that during trawling, *S. boa* might be stressed hence emit already some light on the way to the surface while *C. sloani* is naturally brought to the surface by strong upwelling currents in the strait of Messina.

However, measuring the pharmacological activity of adrenaline and noradrenaline on isolated photophores, is insufficient for determining whether these substances are natural neurotransmitters.

The present HPLC and immunostaining results, reveal not only the presence of significant amounts of endogenous adrenaline and noradrenaline in the photophores, but also the cellular sites of localization of both catecholamines.

Of the two pharmacologically active catecholamines, adrenaline is present in a 10 times larger amount than noradrenaline in the *C. sloani* photophore, in contrast to the muscular tissue where noradrenaline is twice as high in amount than adrenaline.

By analogy with the chromaffin cells of the mammalian surrenal glands, the large excess of adrenaline in the photophore suggests that noradrenaline could be converted to adrenaline by enzymatic pathway involving the phenylamine-*N*-methyltransferase (PNMT). In the photophore of the epipelagic luminescent fish *Porichthys*, part of the noradrenaline released by axonal varicosities and taken up by lens border cells, is converted to adrenaline (Mallefet and Anctil, 1992).

However, the enzymatic conversion hypothesis is not supported by our immunostaining observations: in *Chauliodus* photophores, we observed that the two catecholamines-IR have strikingly different sites of distribution without overlap. The enzymatic conversion hypothesis is thus hardly conceivable in *Chauliodus* since, contrary to what was reported for immunostaining tissues of *Porichthys* photophores, no adrenaline-IR has been detected in the sites showing noradrenaline-IR.

In all the photophores of luminescent fish, a reflector surrounds the mass of photocytes, that is the photogenic cells, located deep in the light organ (Herring, 1985). The photocytes from all the studied organs, show a specific fluorescence when irradiated with UV light. In Stomiidae studied photophore, the photogenic tissue is surrounded by such a reflector (Bassot, 1966; Herring, 1977, 1978). Our results show that the radial orientated cells present in the small lobe shows, on UV light, a bluish fluorescence, for *C. sloani* and *S. boa*, similar to that observed from the lobe in a freshly isolated photophore.

From the observation in *Chauliodus* and *Stomias*, we conclude that the blue fluorescent radial orientated cells can be described as the photocytes containing granules full of luciferin. As described in *Chauliodus* (Mallefet and Shimomura, 1995), coelenterazine might be the luminous substrate of the reaction in Stomiidae.

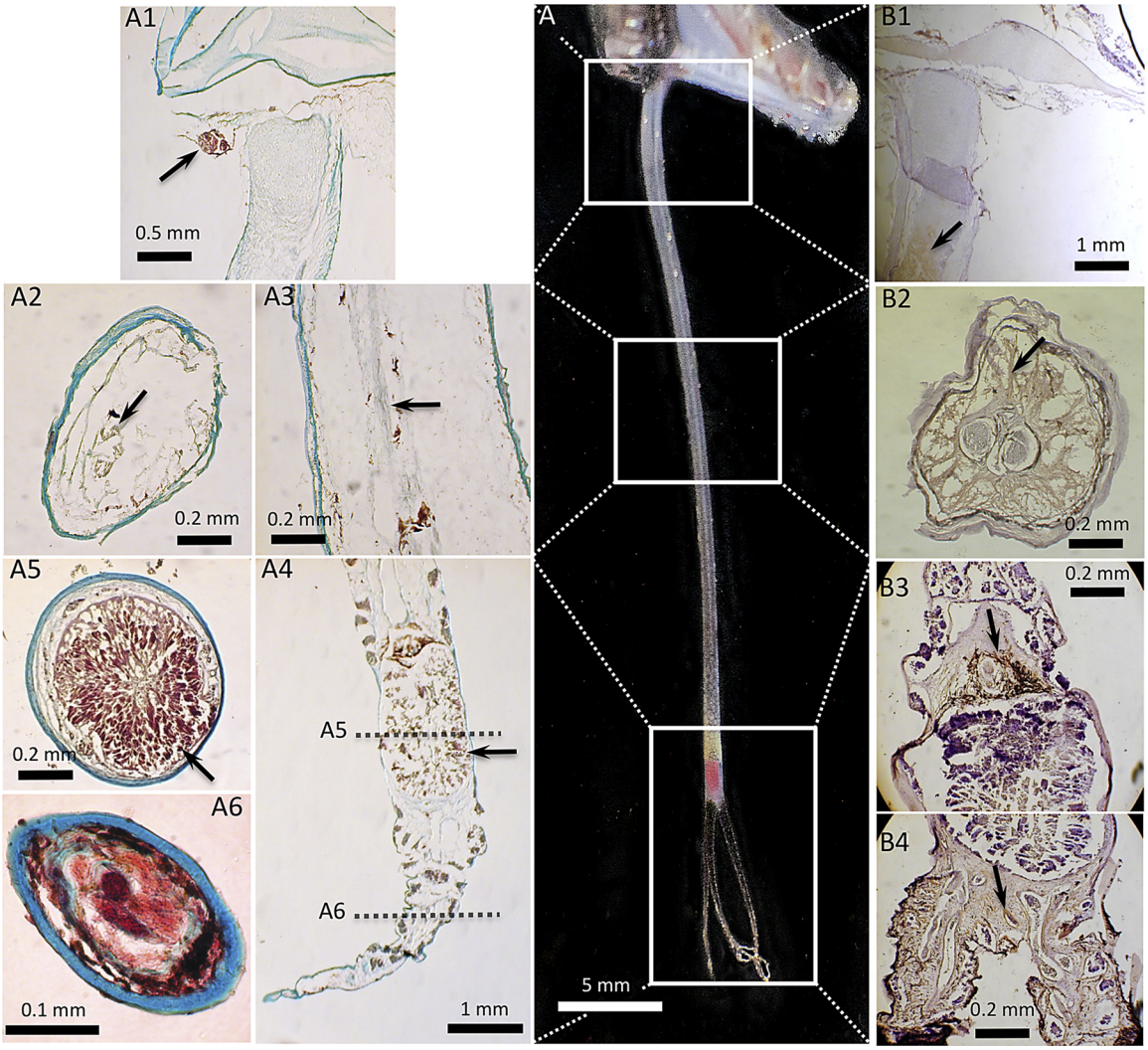

Fig. 6. Immunohistological detection of adrenaline in S. boa barbel. A: external view of a 4 cm long S. boa barbel; A1-A6: Histological sections colored with a Masson Trichrome. A1: Longitudinal section of the proximal portion of the barbel showing the attachment to the jaw (arrow head: isolated photophore). (A2-A3) Transversal and longitudinal section, respectively, of the barbel shaft. Two cylindrical-shape muscle fiber and a nervous fiber are present in this tissue (arrow head). (A4) Longitudinal section across the distal end of the barbel through the luminous bulb. The bulb shows a criss-crossed tissue (arrow head) surrounding by a conjunctive layer and a multilayer cell epidermis. (A5-A6) show, respectively, transversal sections across the bulb and the distal end of the bulb formed by the tree thinkess barbel. (B) Adrenaline detection across the barbel (Epinephrine/adrenaline (EPI) antibody ABIN2120148; 1/200). Immunolabeling (B1) within the proximal portion of the barbel, (B2) in the barbel shaft (B3) in the upper part of the barbel bulb, (B4) in the lower part of the barbel bulb. Arrow head in B1-4 represent the localization of the immunolabeled tissue.

Immunostaining results bring evidence that adrenaline is not present inside the photocytes, but outside the periphery of the cells, the presence of nervous-like cells closely bound to the photocytes; these structures show a specific adrenaline immunostaining.

On the contrary, noradrenaline immunostaining is easily detected into the photocytes of *Chauliodus*: it seems to exist a progressive transition from intravesicular granules in the periphery to extravesicular diffuse material in the central area of the cells. Such noradrenaline staining could not be demonstrated in *S. boa* photocytes.

Studies on electron microscope show that the cytoplasm of the photocytes contains numerous mitochondria, scattered endoplasmic reticulum and various-sized vesicules and vacuoles (Bassot, 1966). Comparison of UV irradiated and noradrenaline immunostained sections suggests that the blue fluorescent vesicles are much more numerous than those containing noradrenaline brown granules in *C. sloani* photogenic tissue. The particular substructural organization of the photocytes presumes an intense secretory activity of noradrenaline as well as a fluorescent material moving from the periphery to a common eccentric zone.

In both, *Chauliodus* and *Stomias* photophores, as in other deep-sea fishes, the photocytes chamber is topped by a structure referred as “lens” covered by a “filter”, names which prejudge their role because their function has never been clearly demonstrated (Bassot, 1966; Herring, 1977, 1978). These structures that form the lens lobe, show a yellow-orange and a green fluorescence in *Chauliodus* and *Stomias*, respectively, when irradiated with UV light. Our results show that in sections, this fluorescence originates exclusively from the spherical mass of cells.

In this lens lobe of *Chauliodus*, the specificity of the adrenaline immunoreactivity is very high as compared with that of noradrenaline; according to our results, adrenaline should be limited to a mass of granular cells that seems to be connected to a very thin network of flattened cells surrounding the external portion of the lobe. On the other hand, noradrenaline seems to be diffusely distributed into the extracellular space. While, for *Stomias*, adrenaline immunoreactivity is present in the nerve cells innervating the photophore in the periphery of and within the lens lobe and no labeling is observed for noradrenaline.

The very localized distribution of adrenaline immunostaining in nervous-like structure in close contact with the basal pole of photocytes, is conceivable with an adrenergic nature of the photophore innervation. Its presence in close contact with the fluorescing cells of the supposed filter, suggests that this tissue is not a passive dioptric structure and could have another function than a passive transmission of light.

On the other hand, the very diffuse presence of noradrenaline immunoreactivity in the *Chauliodus* photocytes chamber as well as in the lens lobe, is hardly conceivable with a neurotransmitter part of this catecholamine. The intravesicular localization rather suggests that noradrenaline could be a product of secretion moving from the basal cytoplasm of the photocyte.

The detection of adrenaline in the connective tissue of the barbel (in the stem and under the bulb) around the muscles and the blood vessels also indicate the likely implication of this catecholamine in the light emission of this organ. The internal structure of the bulb doesn't correspond to an already described bacterial symbiosis lure as in anglerfish where the bacterial luminescence is generally controlled by the blood flow to ensure the alimentation of the bacteria (Hastings and Morin, 1991; Munk, 1999). Therefore, the detection of adrenaline around the bulb also goes against the hypothesis of a bacterial symbiosis and tends to support the intrinsic bioluminescence control by adrenaline. Fluorescence *in situ* hybridization using specific luminous bacteria primers could provide final clarification about the presence of luminous bacteria within this bulb. Finally, the histology observed fully corresponds to the pioneer work of Nusbaum-Hilarowicz (1923) and it might be possible, as suggested by the author, that *S.boa* controls both the motility and the luminescence of its barbel through the detected adrenaline innervation.

Although the immunohistological approach cannot fully elucidate the role of adrenaline and noradrenaline in the control of luminescence, our results strongly suggest differential mechanisms in the two studied fishes. Similarly to the studies developed in the hatchetfish *A. hemigymnus* photophores by Zaccone et al. (2011a,b) it seems necessary to clarify the respective role of adrenaline and noradrenaline in *Stomiidae*. using immunohistochemical labellings of alpha or beta adrenoreceptors antibodies that might reveal precisely the presence of receptors on targeted cells membrane within the photophore or the barbel (lens, photocytes, blood vessel). Radioautographic investigations could be done to determine if exogenous adrenaline and noradrenaline actually share a similar differential distribution, and to identify their selective sites of uptake and retention within the photophore. New pharmacological approach would be devoted to the co-release of these neurotransmitters in order to demonstrate a possible synergic effect of catecholamines while the effects of neuromodulators such as nitric oxide, serotonine or adenosine should also be studied. All these new approaches might help to better understand the functional characterization of adrenergic innervation within Stomiidae photophores.

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Conflict of interest

There are no financial interests or relationships with industry associated with this paper.

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Appendix A. Supplementary data

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Jerome Mallefet:conceptualization, methodology, validation, investigation, resources, writing original draft and review, visualisation and supervision.

Laurent Duchatelet:methodology, validation, investigation, formal analysis, writing original draft and review, visualisation and supervision.

Claire Hermans:investigation, formal analysis, writing original draft.

Fernand Baguet:conceptualization, investigation, resources, writing original draft