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On the "third" sensory receptor of the ascidian tadpoles

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Zoologia. — *On the "third" sensory receptor of the ascidian tadpoles* (*). Nota (**) del Corrisp. GIUSEPPE REVERBERI.

RIASSUNTO. — La presente investigazione concerne la presenza e la ultrastruttura del «terzo» organo sensoriale in larve di diverse specie di Ascidie. L'organo è rinvenuto presente, oltre che in *Ciona intestinalis*, anche in *Phallusia mamillata*, in *Ascidia malaca*, in *Ascidiella aspersa*, in *Styela* sp.: è invece assente in *Molgula impura*. È descritta a forma e la ultrastruttura dell'organo, nelle diverse specie esaminate.

Non viene risolto il problema del significato funzionale di tale singolare recettore sensoriale.

INTRODUCTION

1. It is well known that the tadpoles of most ascidian species possess two sensory receptors: an ocellus for light and an otolith for gravity. They are situated within the cerebral vesicle, and appear, at the ordinary microscope, as two intensively pigmented spots.

Exceptions to this situation, however, also exist: the tadpoles of the Molgulidae, for instance, do not possess the ocellus; those of the Styelidae have a much reduced ocellus, and those of the Botryllidae have a composite sensory organ, a "photolith" [2].

The *ocellus*, situated in the postero-dorsal wall of the cerebral vesicle, consists of an optic cup, a lens system, a few photoreceptor cells. These are unpigmented, and are constituted of the cellular body, the shaft, and the receptoral process; the outer segment of this process is expanded in an array of lamellae derived from multiple infoldings of the ciliary membrane [3, 4, 6, 1].

The *otolith* consists of a cell, which projects into the neural cavity; it is connected with the brain wall by a short stalk, moves freely in the cavity, and its distal cytoplasmic part is occupied by a spheroidal mass of black pigment contained within a vacuole [3, 6].

2. Besides these two sensory receptors, the tadpoles of Ciona also possess another sensory receptor. Its presence was first noticed by Dilly [5], and soon confirmed by Eakin and Kuda [6]. Its function is still disputed: according to Dilly it is a "second" type of photoreceptor; Eakin and Kuda on the other hand consider it as a "pressure" receptor.

It is situated in the dorsal posterior wall of the neural vesicle, and projects into its lumen, and consists of numerous (up to 10) globular or ovoid bodies, which are connected with the neural cells from which they derive, by stalks. Each stalk contains an axoneme of 9+0 doublets of microtubules, arising from a kinetosome: this fact is evidence of its ciliary origin. Each globular

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body has a complicated structure, with a net of tubules of the same diameter deriving, probably, from invaginations of the ciliary membrane. The tubules, which are arranged in parallel arrays appear empty, but occasionally one can see granules, or small vesicles within them [6].

Problem and material

The new sensory receptor has only been described in *Ciona*, but probably it is present also in other ascidian species: a comparative study would facilitate the understanding of its function, which, as already remarked, is disputed.

The present paper reports the results of an investigation on some ascidian species (*Ciona*, *Phallusia*, *Ascidia malaca*, *Ascidiella aspersa*, *Styela sp.* and *Molgula impura*). The last two species were selected, because their tadpoles, as noted above, do not possess the ocellus (*Molgula*), or have an ocellus much reduced (*Styela*).

The tadpoles were fixed for one hour in 2 % osmic acid buffered to pH 7,4 with phosphate; afterwards they were dehydrated in graded alcohols and embedded in DER.

Sections, obtained with glass knives on a Porter Blum microtome, were examined by an electron microscope Siemens Elmiskope I b.

The tecnical help of Gianni Randazzo was much appreciated.

Results

a) *Ciona intestinalis*.

Only a few data can be added to those presented by Dilly [5] and Eakin and Kuda [6].

Plate I *a* is a transversal section of the brain at the level of the ocellus. In the large brain cavity one can note the presence of three small ovoid globules, one of which is attached to a cell of the neural epithelium; the others appear free in the lumen.

The situation is much more evident in Plate I *b*; in the brain cavity five globular bodies are present; two of them are connected with the neural epithelium. A careful examination of one of the globular bodies which are connected with the cells of the neural epithelium shows the presence, in the cell from which it derives, of an "accessory" centriole (Plate II, *b*). Its presence, as well that of the "principal" centriole along the stalk suggests its ciliary derivation. The globular bodies are delimited (Plate II *a*, *b*) by a thick membrane, and their interior is completely filled with tubules, whose wall is also thick.

The tubules which do not seem to be arranged at random are empty. The cells from which the globular bodies depart are cylindrical and their distal regions show an accumulation of mitochondria.

b) *Phallusia mamillata*.

In Phallusia the globular bodies are also present; they do not differ much from those of Ciona: they have a spherical or ovoid configuration and possess circumvoluted tubules (Plate III *a*, *b*) which in some sections are arranged in parallel arrays.

Up to 6 bodies can be found in a single section. Evidence of their ciliary origin was not found.

c) *Ascidia malaca.*

Globular cilia are also present in Ascidia malaca. Their shape is spherical of fusiform (Plate IV *a*, *b*). In a spherical body a very peculiar formation was found (Plate IV *a*): its significance is not clear: possibly it corrisponds to the mouth of the invaginating ciliary membrane.

d) *Ascidiella aspersa.*

The globular bodies are present also in this species. The brain vesicle is much large; the globular bodies are present only in a very limited region of it. Plate V *a–d* are views of the situation. As in A. malaca and in Phallusia the globular cilia are in groups of 6–10 elements.

e) *Styela sp.*

The globular cilia are also present in this species; however they are rudimentary in comparison with those described above.

f) *Molgula impura.*

No globular cilia were found in this species. Only digitations, departing from the neural cells, were found in the lumen of the neural cavity.

CONCLUSION

The investigation on the third sensory receptor in the ascidian tadpoles, shows that this receptor is present, not only in Ciona, but also in Phallusia, Ascidia malaca, Ascidiella and Styela. In the last it is much reduced; in Molgula, however, it is totally absent. The ciliary origin, as asserted by Dilly, as well Eakin and Kuda, is confirmed by the present observations; in Ciona we have noted the presence, in corrispondence with the stalk of the globular body, of an accessory centriole with its typical orientation.

The structure of the globular bodies does not differ much in the species here investigated. They possess a thick double membrane, as well as tubules, which are arranged mostly in parallel arrays.

In Phallusia the tubules are densely packed, and the bodies are larger than those of Ciona and Ascidia malaca.

About the role of these peculiar sensory organs our research does not supply any useful contribution. The fact that they are reduced in Styela, and are lacking in Molgula, does not mean much. Possibly a more extensive investigation will supply more useful information. This, of course, could also be gained by experimental approach: it seems possible to block the differentiation of the cilia destined to give rise to the globular sensory bodies, by treating the developing embryo with appropriate inhibitory substances.

Interesting embryological problems arise from the data above reported. As noted, the globular bodies are transformed cilia; however only a few cilia in the brain evolve into globular bodies; is the number of these cilia definite for each species? Is there in the brain a particular region destined to give rise to these structures? Which is the factor responsible for their differentiation?

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EXPLANATION OF PLATES I–V

Plate I

a) Transversal section of a tadpole of *Ciona* at the level of the ocellus. Within the brain cavity three globular bodies are present; one of them is connected by a short stalk to a neural cell (5,000×); *b*) the section interests the entire brain vesicle: within it one notices six globular bodies (10,000×).

Plate II

Ciliary bodies of *Ciona* at higher magnification; *a*) note the structure of the globular bodies; *b*) a globular body is connected with a neural cell by a short stalk: within the neural cell, at the base of the globular body, one can see the accessory "centriole" which is parallel to the surface of the cell: its presence denotes the ciliary origin of the globular body (20,000×).

Plate III

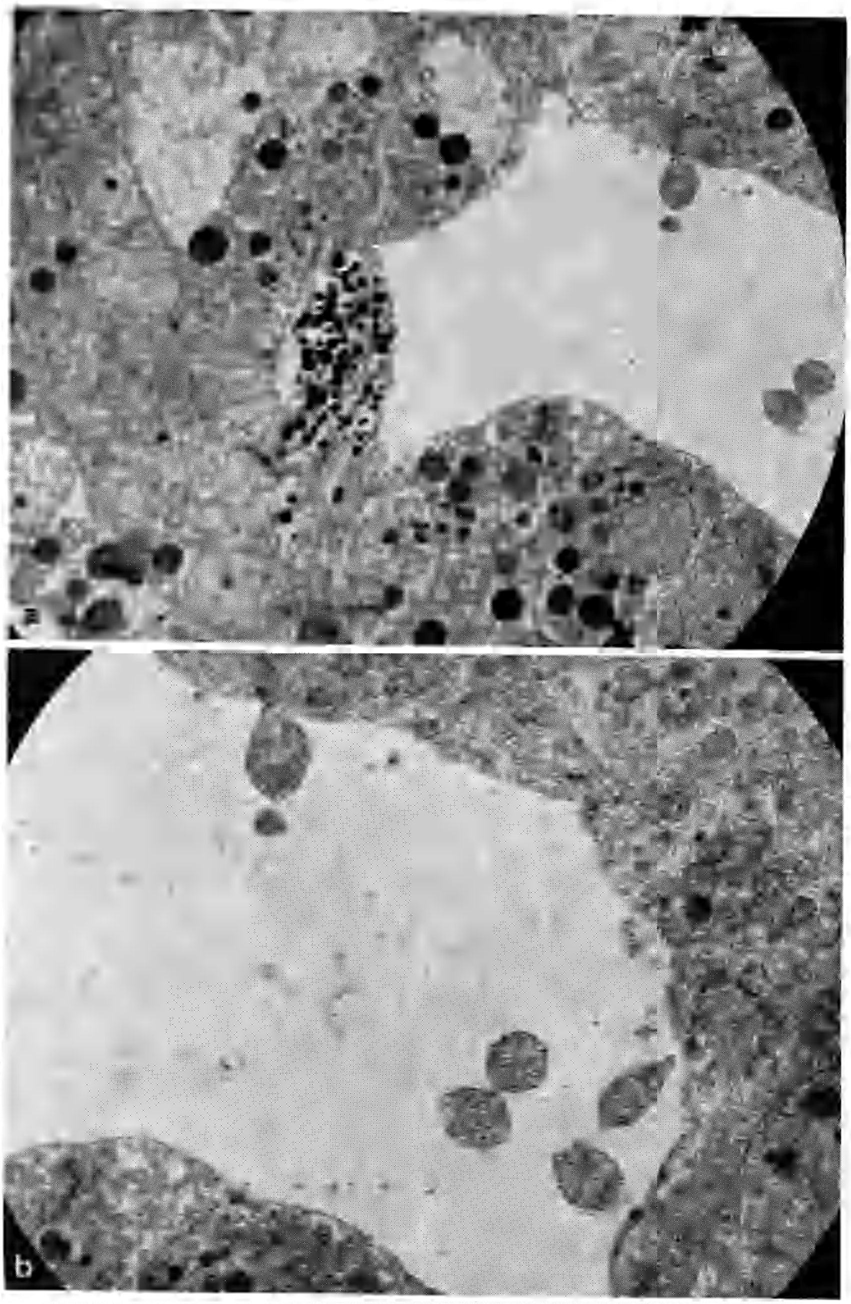
The globular bodies in a larva of *Phallusia*. Notice the arrangement of the tubules within them (a = 13,000×; b = 32,000×).

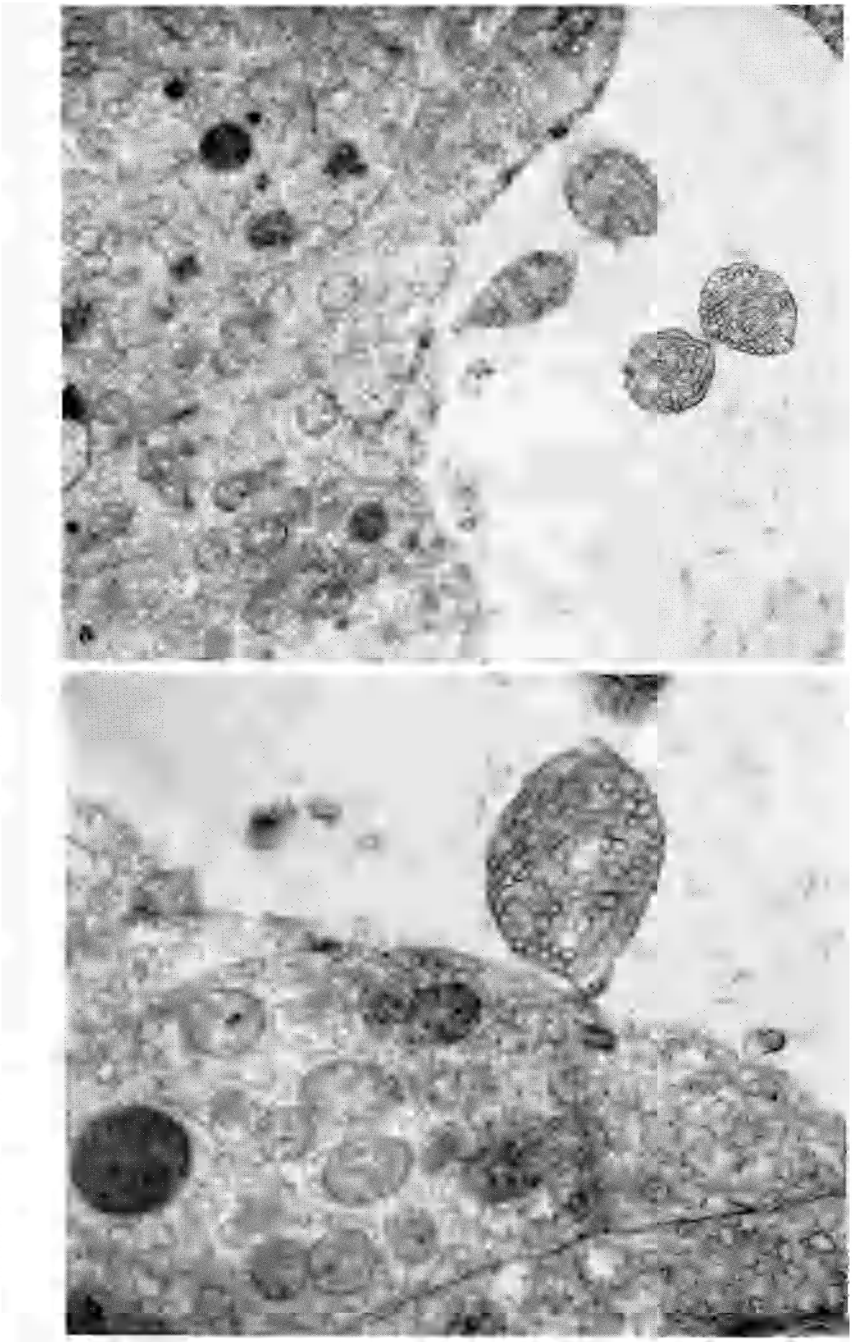
Plate IV

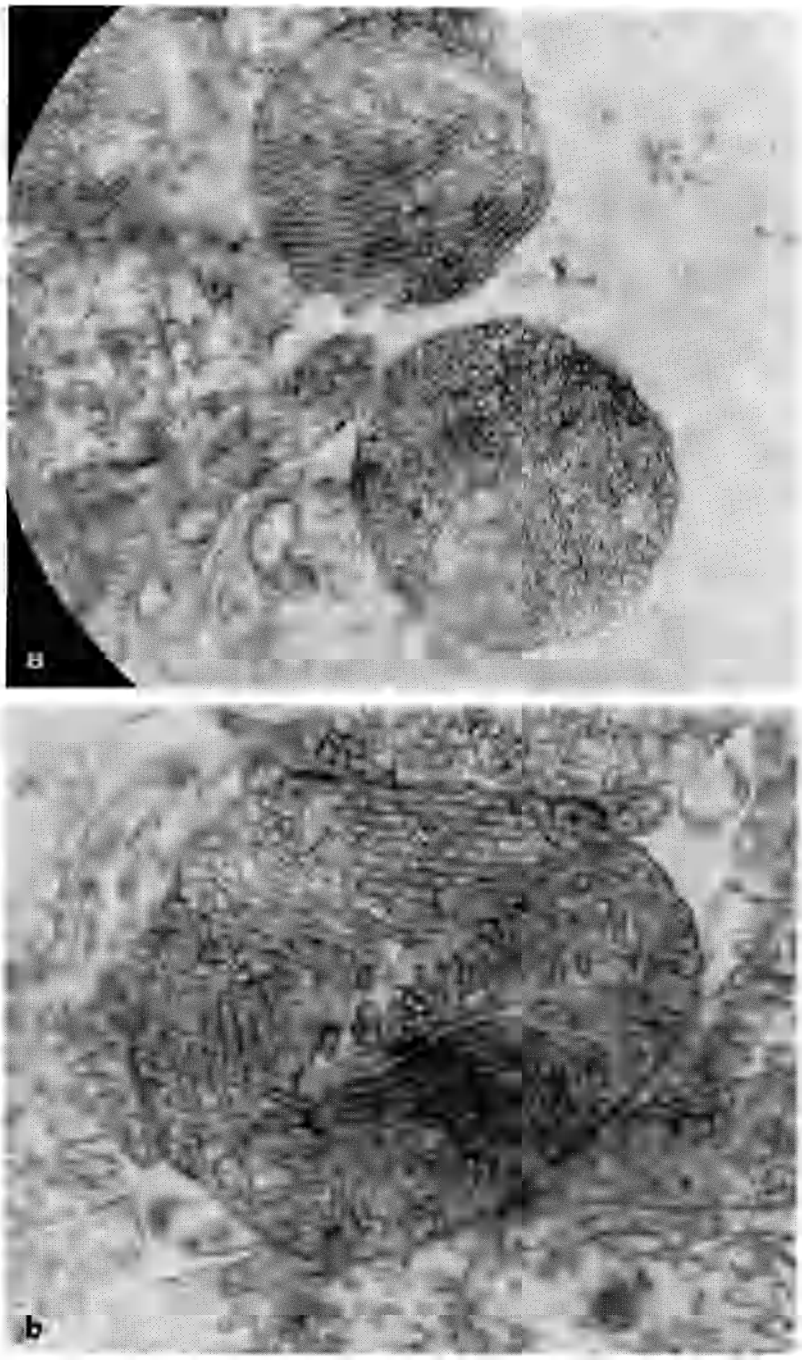
Globular bodies in a larva of *A. malaca*: *a*) the globular body which has an ovoid shape and is limited by a bilaminar membrane shows in the middle a strange feature, which is interpreted as the mouth of the membrane invagination in the transformed cilium (36,000×); *b*) the globular body has a fusiform aspect: it is connected with the cell from which it derives by a long stalk.

Plate V

Globular bodies in a larva of *Ascidiella aspersa*: *a*) (16,000×); *b*) (20,000×); *c*) (18,000×); *d*) (44,000×).


a
b

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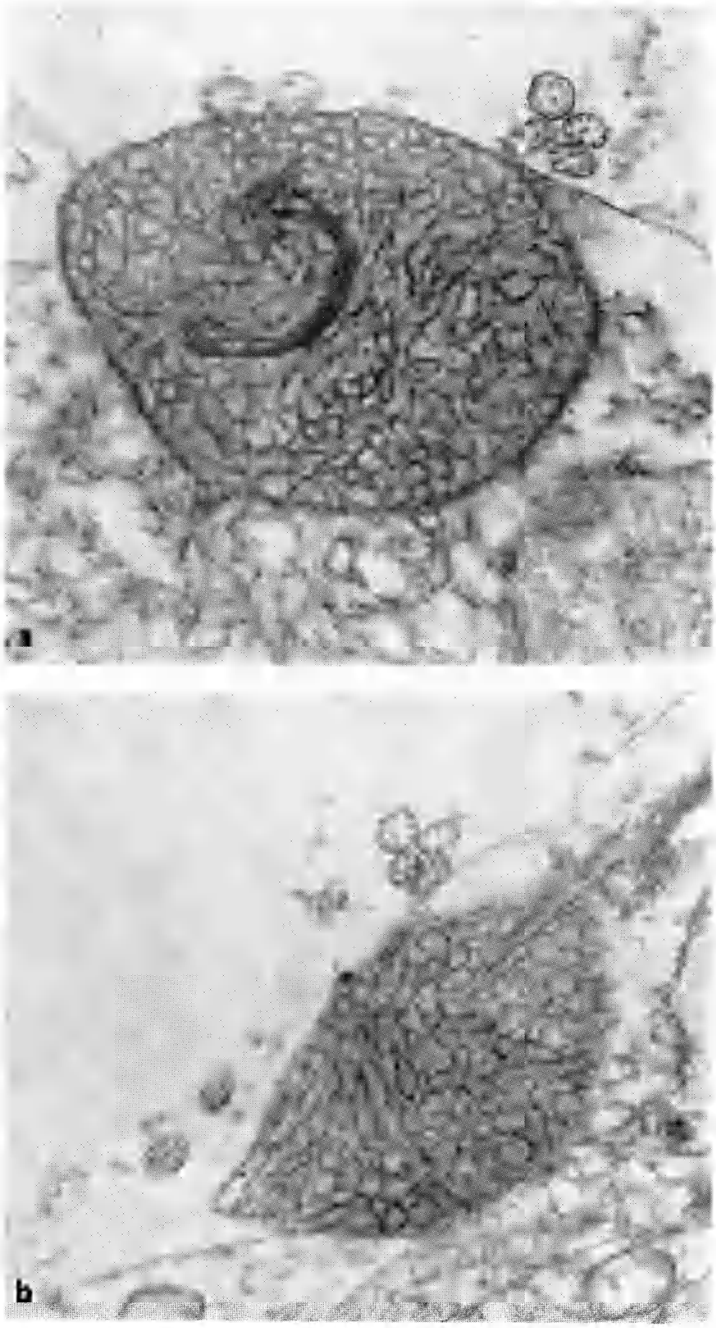
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