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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Biologia. — *Observations on supramedullary neurons of Labridae* (*). Nota di LUCREZIA MOLA e IVAN BENEDETTI, presentata (**) dal Socio A. STEFANELLI.

RIASSUNTO. — È stata descritta la morfologia dei neuroni sopramidollari di alcune specie di Labridi; tali neuroni mostrano differenze numeriche e dimensionali non solo tra generi della stessa famiglia ma anche tra specie dello stesso genere.

I risultati più interessanti sono emersi dallo studio condotto su esemplari di *C. tinca* e di *L. turdus* di diversa lunghezza e quindi di diversa età. Infatti in *C. tinca* di 11 cm di lunghezza sono presenti circa 30 neuroni sopramidollari; negli esemplari di 25 cm tali cellule sono ridotte a una decina; in esse sono evidenti alcune alterazioni morfologiche e una notevole attività della fosfatasi acida. In *L. turdus* di 18-25 cm i neuroni sopramidollari sono circa 20, mentre negli esemplari di 30 cm sono assenti. Nel midollo spinale degli animali di maggiore età anche alcuni motoneuroni presentano alterazioni morfologiche e una notevole attività della fosfatasi acida. Pertanto l'involuzione e la graduale scomparsa dei neuroni sopramidollari sono stati inquadrati in un processo generalizzato di invecchiamento del midollo spinale.

INTRODUCTION

Supramedullary neurons are elements present in the dorsal region of the spinal cord of various Teleost species. They are sometimes quite large and round or pear-shaped, with abundant Nissl substance and a ventrally oriented axon (Ussow, 1882; Fritsch, 1884, 1886; Tagliani, 1894, 1899; Dahlgren, 1897; Sargent, 1898, 1899).

These neurons were for a long time considered as persisting Rohon-Beard cells (cfr. Ariens Kappers *et al.*, 1960; Nieuwenhuys, 1964). Recently supramedullary neurons have been shown to be a system independent of the early differentiating system constituted by the Rohon-Beard cells (Benedetti e Marini, 1973, 1975; Marini e Benedetti, 1973, 1979 *a*).

Supramedullary neurons have also been submitted to electrophysiological (Bennett *et al.*, 1959 *a*, *b*, *c*; Bennett *et al.*, 1967) and ultrastructural (Nakajima *et al.*, 1965; Marini *et al.*, 1984) studies which have provided interesting contributions.

These neurons often differ notably in size, number and distribution along the spinal cord of different Teleosts. This variability can also be observed

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among specimens belonging to the same family (Marini *et al.*, 1977; Marini e Benedetti, 1979 *a*, *b*). In particular, marked differences regarding the cytology of supramedullary neurons have been observed between the genera *Coris* and *Crenilabrus* (Labridae); this has been interpreted as an expression of different functional moments (Marini *et al.*, 1977). In this context, it seemed of interest to carry out a more in-depth and extensive study, including other Labridae species.

Materials and Methods

The specimens considered in this study were obtained from « Centro Interuniversitario di Biologia Marina » (Livorno, Italia).

The animals were housed in large aquariums containing recirculating artificial seawater at 20-21 °C.

The species considered were:

Crenilabrus tinca (L.): 4 specimens (mean length cm 25);
Labrus turdus L.: 2 specimens (mean length cm 30);
Labrus turdus L.: 3 specimens (ranging in length from 18 to 25 cm);
Crenilabrus scina (Forsk): 2 specimens (mean length cm 11);
Crenilabrus mediterraneus (L.): 2 specimens (mean length cm 11).

Spinal cords from two specimens of *C. tinca* were frozen and cut into 20 μm serial sections in a cryostat; the acid phosphatase reaction according to Burstone (1961) was performed on this material. The spinal cords of the other specimens were fixed in Bouin's liquid, embedded in celloidin-paraffin and cut into 7 μm transversal sections. The histological specimens were stained with toluidine blue, in phosphate-citrate buffer pH 4.6, together with control specimens previously treated with perchloric acid. The reported sizes represent the mean values obtained from all the supramedullary neurons (S.N.) found.

The classification of Šoljan (1975) has been adopted in this paper; the classification according to Tortonese (1975) used in our previous works is reported below:

Crenilabrus tinca (L.) = *Crenilabrus pavo* C.V. = *Symphodus* (*Crenilabrus*) *tinca* (L.);

Labrus turdus L. = *Labrus viridis* L.

Crenilabrus scina (Forsk) = *Symphodus rostratus* (Bloch);

Crenilabrus mediterraneus (L.) = *Symphodus* (*Crenilabrus*) *mediterraneus* L.

Crenilabrus quinquemaculatus (Bl. Schn.) = *Symphodus* (*Crenilabrus*) *roissali* (Risso).

Coris julis (L.) = *Coris julis* (L.).

RESULTS

The spinal cord of *C. mediterraneus* (11 cm in length) had an average of 25 supramedullary neurons, located with a fairly regular distribution in its rostral part. They were generally pear–shaped, with a mean cellular diameter of 40 μm, and had a lobate nucleus containing one or more nucleoli. The Nissl substance was variously represented, appearing as granules and small clumps, and was generally more concentrated in the perinuclear region (Tav. I, A).

The rostral spinal cord of *C. scina* (11 cm in length) presented about forty supramedullary neurons, characterized by a rather irregular distribution: some were spaced μm apart, others were contiguous and at times two neurons were located in the same section. These neurons were round or pear-shaped, and had a mean cellular diameter of 67 μm. The granular Nissl substance was rather uniformly distributed throughout the cytoplasm, but missing, as usual, in the axon hillock. The nucleus was lobate and usually presented a large nucleolus (Tav. I, B).

The rostral spinal cord of *L. turdus* (18-25 cm in length) contained about twenty irregularly distributed supramedullary neurons with a mean cellular diameter of 20 μm. These elements generally had a rounded and vesicular nucleus and one or more nucleoli. Granules and clumps of Nissl substances were present at times, more condensated in the perinuclear region (Tav. I, C). The 25 cm long specimens had more Nissl material, generally distributed throughout the cytoplasm (Tav. I, D). Supramedullary neurons were not present in the spinal cord of 30 cm long specimens of *L. turdus*.

About ten supramedullary neurons were present in the rostral spinal cord of 25 cm long specimens of *C. tinca*. The elements were rounded or pear-shaped. In transverse section their mean cellular diameter was 36 μm. The Nissl substance, generally finely granular, was uniformly distributed, sparing only the axon hillock. The nucleus had an irregular shape, and at times, poorly defined borders with a cloudy content. Only one nucleolus was present (Tav. I, E).

The Burstone acid phosphatase reaction evidenced a marked enzymatic activity throughout the entire cytoplasm in supramedullary neurons of *C. tinca*, except at the axon hillock (Tav. I, F).

This method also demonstrated the presence of enzymatic activity at various other levels, particularly evident in numerous motoneurons.

DISCUSSION

The present observations confirm the variability within the Labridae family since the species of the genus *Labrus* here examined (*L. turdus*) showed only 20 supramedullary neurons, which were markedly smaller than those found in the genera *Crenilabrus* and *Coris* (Marini *et al.*, 1977).

Comparison of the data from *Crenilabrus mediterraneus* and *C. scina* with those from previous observations on *C. tinca* and *C. quinquemaculatus* (Marini *et al.*, 1977) showed that the supramedullary neurons had similar localization and morphology, although supramedullary neurons of *C. scina* were greater in size and more numerous than those in the other three species (see Table I). This demonstrated that supramedullary neurons may differ not only among genera of the same family (Marini *et al.*, 1977) but also among species of the same genus.

TABLE I.

Summary of mean number and cellular diameter of supramedullary neurons in the various species of Labridae examined.

SPECIES	Mean length of specimens (cm)	Mean number of S.N.	Mean cell. diameter of S.N. (μm)
Crenilabrus mediterraneus	11	25	40
Crenilabrus scina	11	40	67
Crenilabrus quinquemaculatus (*)	13	30	48
Crenilabrus tinca (*)	11	30	48
Crenilabrus tinca	25	10	36
Coris julis (*)	18	150	65
Labrus turdus	18-25	20	20
Labrus turdus	30	—	—

(*) Marini *et al.*, 1977.

The most striking data emerged from the study of *C. tinca* specimens of 11 and 25 cm length and *L. turdus* specimens of 18, 25 and 30 cm length.

Supramedullary neurons of 18 and 25 cm long specimens of *L. turdus* had a vesicular nucleus, evident nucleoli and granular and clumpy Nissl material. These elements were absent in specimens of 30 cm length.

Supramedullary neurons of 25 cm long specimens of *C. tinca* were less numerous (about 10) and smaller (mean cellular diameter of 36 μm) than those previously described in young adults of 11 cm length (about thirty neurons, with mean cellular diameter of 48 μm; Marini *et al.*, 1977). Some supramedullary neurons from the longer *C. tinca* specimens also presented cloudy nuclei having poorly defined borders and homogeneous, uniformly distributed Nissl substance. Moreover, these cells had strong acid phosphatase activity.

These data taken as a whole are indicative of process of involution. The absence of supramedullary neurons in 30 cm long samples of *L. turdus* can be explained in this context.

It should be noted that 25 cm long *C. tinca* specimens showed morphological variations and elevated acid phosphatase activity at various levels of the

spinal cord and these were particularly evident in the motoneurons. This indicates a generalized senescence of the spinal cord, of which involution of supramedullary neurons is a part.

Studies being performed on young adults of other Labridae species only sporadically show weak acid phosphatase activity in both supramedullary neurons and motoneurons, supporting the above finding. As soon as material for study becomes available, this research will be extended to young adults of *C. tinca*.

These data give only preliminary indications, but in any case the supramedullary neurons may be of interest for the study of the aging nervous system. They are suitable material due to their peculiar morphology and well defined number in each species.

To further our understanding of these problems, histomorphological, histochemical and ultrastructural studies on the supramedullary neurons of Labridae of different sizes, and thus age, are currently in progress.

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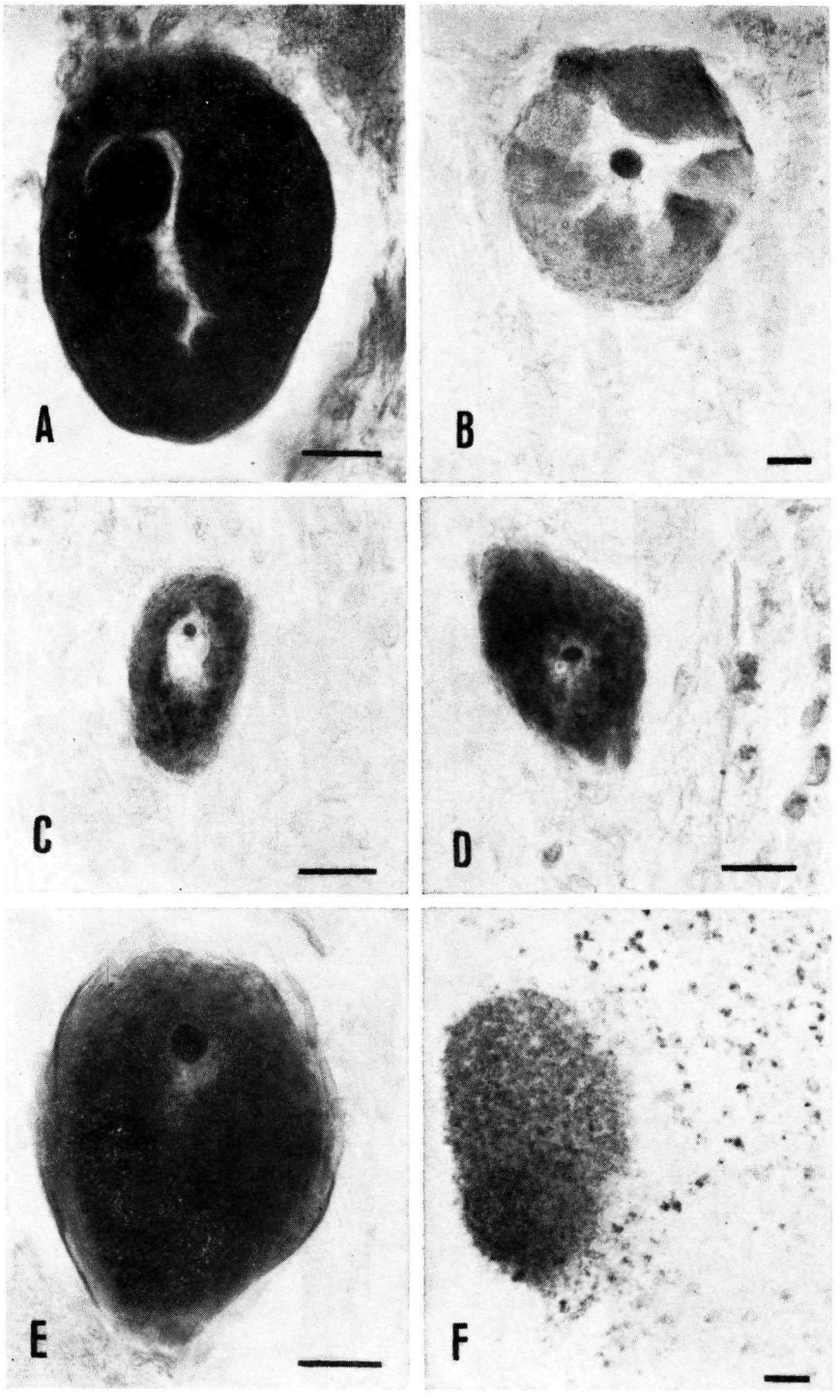

Supramedullary neurons in the spinal cord of: *Crenilabrus mediterraneus* 11 cm long (A); *Crenilabrus scina* 11 cm long (B); *Labrus turdus* 18 cm long (C); *Labrus turdus* 25 cm long (D); *Crenilabrius tinca* 25 cm long (E); toluidine blue stain. The photomicrograph F shows a supramedullary neuron of *Cranilabrus tinca* (25 cm long) after the Burstone acid phosphatase reaction. Bar = 10 μm.