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Morphological and quantitative analysis of myenteric plexus neurons of intestinal bulb of *Cyprinus carpio* (Linnaeus, 1758) (Osteichthyes, Cyprinidae)

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ABSTRACT. The myenteric plexus shows morphologic and quantitative variability in the alimentary canals of different species of animals. We have quantified and analyzed the morphology of the myenteric plexus of ten adult *Cyprinus carpio* intestinal bulbs, by means of histological cross sections stained with HE and Van Gieson methods, as well as Giemsa-stained whole mount preparations. The myenteric plexus, located between the longitudinal and circular layers of the muscular tunic, is formed by isolated neurons and ganglia. Percentages of small, medium and large neurons were 21%, 63.4% and 15.6%, respectively, with a prevalence of intermediary cytoplasmic basophily and peripheral nuclei. Neuronal density in 6.92mm^2 of intestinal bulb was of 2,040 neurons.

Key words: *Cyprinus carpio,* fish, intestinal bulb, myenteric neurons.

RESUMO. Análise morfológica e quantitativa dos neurônios do plexo mientérico do bulbo intestinal de *Cyprinus Carpio*. (Linnaeus, 1758) (Osteichthyes, Cyprinidae). O plexo mientérico apresenta variabilidade morfológica e quantitativa ao longo do tubo digestório das diferentes espécies animais. No presente trabalho quantificamos e analisamos a morfologia dos neurônios do plexo mientérico de dez bulbos intestinais de *Cyprinus carpio*, por meio de cortes histológicos corados com HE e Van Gieson e de preparados de membrana corados pelo método de Giemsa. O plexo mientérico foi localizado entre os estratos longitudinal e circular da túnica muscular, sendo constituído por neurônios isolados e por gânglios. As porcentagens de neurônios pequenos, médios e grandes foram 21%, 63,4% e 15,6%, respectivamente, predominando neurônios com basofilia citoplasmática intermediária e núcleo em posição periférica. A análise quantitativa revelou a presença de 2.040 neurônios/6,92mm^2 de bulbo intestinal.

Palavras-chave: *Cyprinus carpio,* bulbo intestinal, neurônios mientéricos, peixe.

The arrangement of the myenteric plexus varies regarding size, shape and numbers of nerve ganglia, in different areas of the digestive tube (Sternini, 1988) and among different animal species (Irwin, 1931).

In several species of cyprinid fishes, the morphology of the digestive system varies. Unlike other fishes, cyprinids do not possess a stomach or pyloric caeca, which somewhat reduces the efficiency of the digestive power in these species (Junger *et al*., 1989). In *Cyprinus carpio*, the continuation of the esophagus consists of an area of the intestine with a larger diameter, named intestinal bulb, which may be considered similar to the stomachs of other fish (Junger *et al*., 1989). According to Nikolkaya and Verigina (1974) and Verigina (1990), the feeding habits of fishes are reflexes of the changes in the structure of their digestive system.

Quantitative studies about the myenteric plexus of the intestines of fishes have revealed the presence from 100 to 200 neurons/mm^2 in the intestine of *Salmo trutta*, without the formation of true ganglia, and the total number of neurons is smaller than that

described in mammals (Burnstock, 1959). In *Pimelodus maculatus*, Souza *et al*. (1982) did not observe the presence of ganglia, and in the rectum and duodenum of these fishes 82,857 and 91,142 neurons/cm^2 were observed, respectively. Small ganglia dispersed, isolated neurons and 2,579 neurons/6.92mm^2 were observed in anterior intestinal segment of the *Cyprinus carpio* (Stabille *et al*., 1999). Similar analyses have been accomplished for the intestinal segments of cats, mices and rats by Leaming and Cauna (1961), Gabella (1971, 1990), Santer and Baker (1988), Natali and Miranda-Neto (1996) and Leite-Mello *et al*. (1997).

The motility and secretions of the digestive tract, controlled by the enteric nervous system, influence the processes of digestion and absorption. Considering the complexity and importance of this system, we performed a morphological and quantitative analysis of the neurons of the myenteric plexus of the intestinal bulb of *Cyprinus carpio*, using histological techniques and whole-mount preparations. Through this investigation we increased the knowledge about the morphological characteristics of the myenteric plexus of fishes.

Material and method

In this study, we have used the intestinal bulb of ten adult specimens of *Cyprinus carpio* of both sexes, weighing 1,274g ± 141.4g (mean ± SD), sacrificed by destruction of the spinal medulla under benzocaine anesthesia. Sections from five fishes were submitted to routine histological treatment to obtain cross-sections of 6µm and 12µm in thickness, stained with the hematoxylin-eosin and Van Gieson methods. The sections were analyzed using an Olympus CBB light microscope to locate the myenteric plexus.

Other five intestinal bulbs were washed, stretched and fixed in formaldehyde, glacial acetic acid and sodium chloride solution, and then microdissected under a stereomicroscope to obtain whole mount-preparations. These were stained by the Giemsa method (Barbosa, 1978), dehydrated and diaphanized to be mounted on glass slides.

The whole-mount preparations were analyzed under Olympus CBB light microscope, fitted with a 40x objective and a micrometer disc attached to a 10x lens, to measure and sum the longest longitudinal and transverse axes of the celullar body of randomly chosen 500 neurons. The mean and standard deviation were calculated for the values obtained, estabilishing the intervals that allowed the classification of the neurons with regard to size. In addition to the measurement procedures, the shape, cytoplasmic basophily, position of the nuclei and number of nucleoli of the neurons were also recorded.

For the score of the neurons, by the sampling method, we have used the whole-mount preparations analyzed under Olympus CBB light microscope, with the 40x objective and WF10x lens. Each whole-mount preparation was divided into four equal quadrants. Ten microscope fields were randomly chosen within each quadrant. All neurons in each field were counted, ignoring the half-neurons of one field and including the half-neurons of the other field, resulting in 40 fields for each whole-mount preparation.

The mean of neurons/6.92mm^2 of intestinal bulb was calculated from the counts and from the area of 0.173mm^2 covered by each microscope field. Photographic documentation was performed using a Olympus BX50 photomicroscope and PM 10AK camera with green filter.

Results

In the wall of the intestinal bulb, between the longitudinal and circular layers of the muscular tunic, we have found connective tissue with bundles of collagen fibers surrounding the ganglia of the myenteric plexus. These extended over the entire circumference of the intestinal bulb (Figures 1, 2), and were formed by groups of two or more neurons (Figure 3). There were also isolated neurons among the ganglionic structures.

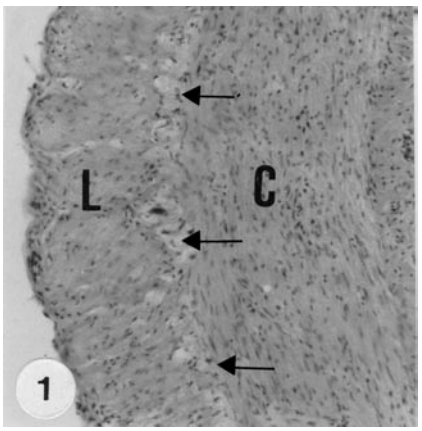

Figure 1. Intestinal bulb showing ganglia (arrows) of the myenteric plexus between the longitudinal (L) and circular (C) muscle layers. HE, 242.9X, 6µm cross section

The sum of the mensuration of the longitudinal and transverse axes of cellular body of each neuron varied from 13µm to 78µm, with a mean of 34.5µm

and a standard deviation of 9.28µm. "Large" neurons were considered to be those with sums from 43.79µm to 78µm (> mean + SD), "medium" neurons varied from 25.22µm to 43.78µm (mean ± SD), and "small" neurons measured from 13µm to 25.21µm (< mean – SD). The percentages of large, medium, and small neurons were 21%, 63.4% and 15.6% respectively (Figure 3; Table 1).

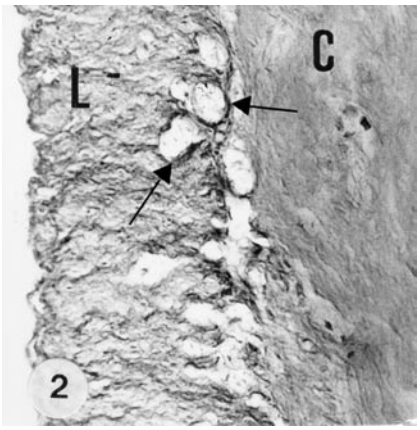

Figure 2. Intestinal bulb showing collagenous fibers (arrows) surrounding the myenteric ganglia between the longitudinal (L) and circular (C) muscle layers.Van Gieson, 244.8X, 12µm cross section

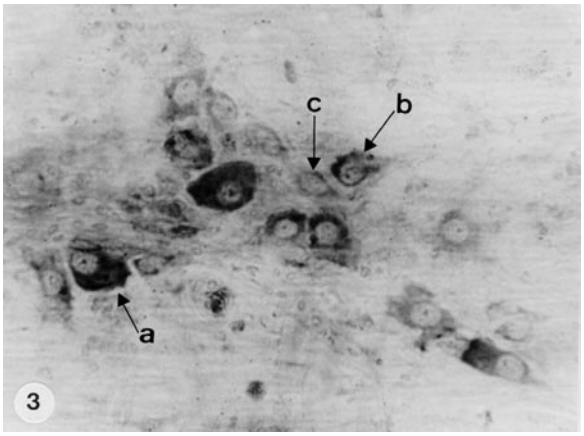

Figure 3. Giemsa stained whole-mount preparation showing a myenteric ganglion with (a) a large neuron with intense cytoplasmic basophily and a polar nucleus, (b) a medium neuron with intermediary cytoplasmic basophily and centrally positioned nucleus, and (c) a small neuron with weak cytoplasmic basophily and a peripheral nucleus. 636.3X

Table 1 Percentages of frequency, cytoplasmic basophily and nuclei position of the small, medium and large myenteric neurons of the intestinal bulb of *Cyprinus carpio*

Neuron	Frequency (%)	Basophily (%)			Nucleus position (%)		
Size		S	I	W	PE	CE	PO
Small (<25.21µm)	15.60	11.53	53.85	34.62	85.90	5.13	8.97
Medium (25.22 to 43.78µm)	63.40	14.83	65.61	19.56	89.27	3.79	6.94
Large (>43.78µm)	21.00	36.19	58.10	5.71	84.76	0.95	14.29

S (intense); I (intermediary); W (weak); PE (peripheral); CE (central); PO (polar)

The neurons morphology was quite varied independently of their size. Neurons were observed with oval, round and elliptical shapes and, sometimes, with no defined shape. The round cell nuclei occupied different positions inside the neurons, with no relation to their morphology.

The cytoplasm of most of the large neurons was stained with medium intensity, indicating an intermediary basophilic affinity. In these neurons, the nuclei were predominantly peripheral (Figure 3; Table 1). The medium neurons had mainly intermediary basophily and peripheral nuclei, although central and polar nuclei were also observed but less frequently (Figure 3; Table 1). The cytoplasmic basophily in small neurons varied from medium to weak, with a small incidence of intense basophily. Nuclei in these cells were peripherally, polarly, and centrally located, with the first category being predominant (Figure 3; Table 1).

Most of the neurons just showed one nucleolus. Two nucleoli were observed in 1.96% of large, 1.90% of medium and 1.30% of small neurons.

The quantitative analysis, after the calculation of the mean, allowed to verify presence of 2,040 neurons in 6.92mm^2 of intestinal bulb, i.e. 29,479 neurons/cm^2 (Table 2).

Table 2 Myenteric neurons frequency in 40 microscopical fields (6.92mm^2) of Giemsa stained-whole mount preparations of the intestinal bulb of *Cyprinus carpio*

Whole-mount preparations	Neurons frequency
Fish 1	2,935
Fish 2	2,370
Fish 3	1,922
Fish 4	2,040
Fish 5	1,782
Mean	2,040 ± 219.12*

* Standard deviation

Discussion

The myenteric plexus location, between the circular and longitudinal layers of the muscular coat in the intestinal bulb of *Cyprinus carpio*, is similar to other species of fish analyzed by Williams and Nickol (1989) and Verigina (1990). We have verified the myenteric plexus, involved by collagenous fibers of the connective tissue, which extended for the entire circumference of the organ. Williams and Nickol (1989) mentioned that the connective tissue between the longitudinal and circular muscular layers acts as a support for the myenteric plexus and blood vessels.

In the myenteric plexus of the intestinal bulb of *Cyprinus carpio*, we observed groups of two or more neurons involved by collagenous fibers, constituting sparse ganglia of different sizes. We also verified the presence of isolated neurons. Similar observations were noticed in anterior intestinal segment of *Cyprinus carpio* (Stabille *et al*., 1999). Burnstock (1959) reported that, in the intestine of the *Salmo trutta*, the myenteric plexus is not composed by true ganglia, since the ganglia that he observed were formed by two to three nervous cells. The presence of large ganglia in some species of Loaches was commented by Verigina (1990), without mentioning to the number of neurons in the ganglia. However, Souza *et al.* (1982) described a myenteric plexus with isolated neurons, without aggregation into ganglia, in the *Pimelodus maculatus* intestine.

The presence or absence of ganglia in the intestines of fishes may be related to their phylogenetic position. Souza *et al.*(1982) noted that the distribution of the myenteric neurons in the intestine of *Pimelodus maculatus* is not uniform, and suggested that this lack of uniformity may indicate a tendency toward formation of ganglia in the myenteric plexus with ascending scale of zoological complexity.

The morphologic variability of the pericaria of neurons had also been mentionated for fish and rats by Burnstock (1959), Natali and Miranda-Neto (1996), Leite-Mello *et al.*(1997). In our morphological analyses, we have verified the presence of small, medium and large neurons, with predominance of medium ones. A similar situation was observed by Stabille *et al.* (1999) in the anterior intestinal segment of carp and by Leite-Mello *et al.*(1997) in the ileum of rat. In the duodenum of rat, Natali and Miranda-Neto (1996) mentioned the presence of 57% of small and 43% of medium and large neurons. Gabella (1971) observed a large number of small neurons in the rectum and stomach of adult mice, with few in the small intestine. Among studies of the myenteric plexus in fishes, Souza *et al.*(1982) did not report the dimensions of the intestinal neurons in *Pimelodus maculatus*, however they related the presence of large neurons at the level of gastroduodenum junction and ileo-rectal valve.

In the intestinal bulb of *Cyprinus carpio*, we have verified that 21%, 63.4%, and 15.6% of the neurons are small, medium, and large, respectively. In the anterior intestinal segment of the same fish, Stabille *et al*. (1999) related the predominance of medium neurons (62.40%). However it should be emphasized that Irwin (1931) and Sternini (1988) described the existence of morphological and quantitative variability in the myenteric neurons present in several gastrointestinal sections of a same specimen and also in different animal species.

Moreover, considering the dimension of the neurons, Gabella (1971) affirmed that small and medium neurons represented the intrinsic nerve contingent of the myenteric plexus responsible for motor activity, and large neurons were of extramural origin. In this respect, Burnstock (1959) commented that the large neurons may have a sensory function, involved in the gastrointestinal reflex arcs of peristalsis.

We have observed that in the neurons of different sizes peripheral nuclei position predominates. Our results agree with those of Cormack (1995) for autonomic neurons of the peripheral nervous system. This author described that the peripheral position of the nuclei should not be confused with the eccentric nuclei of neurons in the chromatolysis stage.

The myenteric neurons showed different dying features, that may be related to the dimentions of the cell body. In medium and large neurons the cytoplasm stains more intensely, while small neurons have less affinity for specific dye (Natali and Miranda-Neto, 1996). In the intestinal bulb of *Cyprinus carpio*, we have detected predominance of neurons with cytoplasm stained with intermediate intensity, independently of the size of the nerve cell in agreement with the observations in anterior intestinal segment of the same fish species reported by Stabille *et al.*(1999).

According to Junqueira and Carneiro (1995), the amount of granular endoplasmic reticulum and free polyribosomes may be responsible for the intensity of cytoplasmic dying of the neurons. Leaming and Cauna (1961) suggested that the different dying reactivities mean temporary alterations in the neurons depending mainly on their state of activity and enzymatic content.

The nucleoli duplicity was found in 1.96%, 1.90% and 1.30% of the large, medium and small neurons, respectively. Similarly, Natali and Miranda-Neto (1996) and Leite-Mello *et al*. (1997) also observed two or more nucleoli in the myenteric neurons of rats.

The mean of the neuronal density/cm^2 of intestinal bulb was 29,479. In the anterior intestinal segment of *Cyprinus carpio*, Stabille *et al.* (1999) related the presence of 2,579 neurons/6.92mm^2 or 37,268 neurons/cm^2. In the duodenum of *Pimelodus*

maculatus, Souza *et al.* (1982) found 91,142 neurons/cm^2, and Burnstock (1959) observed from 100 to 200 neurons/mm^2 in the intestine of the *Salmo trutta*.

Considering the scarcity of data about the myenteric plexus of fishes, we explain the differences found among the morphological and quantitative observations about the myenteric plexus of *Cyprinus carpio*, *Pimelodus maculatus* and *Salmo trutta* as resulting of their feeding habits. Nikolkaya and Verigina (1974) and Verigina (1990) related that the feeding habits reflected the diversity of morphological changes of the digestive system in fishes. Within this diversity variations in the length, diameter, thickness and constitution of the wall of the gastrointestinal tract were included. According to Gabella (1990) these factors influence, mainly, the number of neurons in the myenteric plexus.

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