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Morphoquantitative characteristics of myenteric neurons of the terminal segment of the intestine of *Cyprinus carpio* (Linnaeus, 1758) (Osteichthyes, Cyprinidae)

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ABSTRACT. Considering the importance of the myenteric plexus in fishes and the scarcity of data about it, research was undertaken with the purpose of contributing to the characterization of the myenteric plexus in fishes. The terminal segments of intestines of ten adult *Cyprinus carpio* underwent routine techniques of transverse histological sections of 6 and 10μm in thickness, stained with Weigert-Van Gieson, as well as whole-mount preparations stained with the Giemsa method. The myenteric plexus composed of isolated neurons and ganglia containing groups of two or more neurons was analyzed. Percentages of small, medium and large neurons were 48.8%, 47.6% and 3.6% respectively. Neuronal density in 6.64mm^2 of intestinal segment was 5,099 ± 384.35.

Key words: *Cyprinus carpio*, fish, intestine, myenteric plexus, neurons.

RESUMO. Características morfoquantitativas dos neurônios mientéricos do segmento terminal do intestino de *Cyprinus carpio* (Linnaeus, 1758) (Osteichthyes, Cyprinidae). Considerando a importância do plexo mientérico e a escassez de dados sobre o mesmo em peixes, realizamos o presente trabalho, com a finalidade de contribuir com a caracterização do plexo mientérico nesses animais. Para tanto, o segmento terminal do intestino de 10 exemplares adultos de *Cyprinus carpio* foi submetido a técnicas de rotina para obtenção de cortes histológicos transversais de 6 e 10μm de espessura, corados pelo método de Weigert-Van Gieson, e de preparados de membrana corados pelo método de Giemsa. Observamos o plexo mientérico constituído por neurônios isolados e por gânglios contendo grupos de dois ou mais neurônios. As porcentagens de neurônios pequenos, médios e grandes foram 48,8%, 47,6% e 3,6%, respectivamente. A densidade neuronal em 6,64mm^2 de segmento intestinal foi 5.099 ± 384.35.

Palavras-chave: *Cyprinus carpio*, intestino, neurônios, peixes, plexo mientérico.

Although descriptions of the stratigraphy of the digestive tract of fish highlight the presence of blood vessels and nerves, the presence of nerve cells and, sometimes, myenteric ganglia are merely mentioned (Kobegenova and Verigina, 1988; Williams and Nickol, 1989; Verigina, 1990).

The arrangement of the myenteric plexus varies according to size, shape and number of nerve ganglia in different regions of the digestive tract (Furness and Costa, 1980) and in different animal species (Irwin, 1931). Gabella (1990) commented about the plasticity of form and structure of enteric ganglia in relation to diameter and length of the intestine. Recent research in rat intestine showed the presence of small, medium and large neurons with varied cytoplasmic basophily and peripheral nucleus (Leite-Mello *et al.*, 1997). Quantitative studies revealed the presence of 100 to 200 neurons/cm^2 on the intestine of *Salmo trutta* without the formation of true ganglia (Burnstock, 1959). Souza *et al.* (1982) did not observe ganglia in *Pimelodus maculatus* and they found 82,857 neurons/cm^2 on the rectum of this fish.

Similar analyses of the rectum and colon of rats and cats were carried out by Irwin (1931), Leaming and Cauna (1961), Gabella (1971) and Barbosa (1973). Considering that the motility and secretion of the intestinal tract influence the digestion and absorption processes, and are themselves controlled by the enteric nervous system, we have chosen to carry out the morphological and quantitative analyses of neurons of the myenteric plexus of the terminal segment intestine of *Cyprinus carpio*.

Material and methods

Ten adult *Cyprinus carpio,* male and female, weighing 1,274g ± 141.4 were used. After they were killed by spinal cord destruction, the terminal segments of the intestines were removed and grouped according to following techniques:

Group A. composed of five segments, washed in physiologic solution, fixed in 10% formaline solution and subjected to histological treatment. 6 to 10μm transverse sections were stained with Weigert-VanGieson method to show the location of the myenteric plexus.

Group B. composed of five segments with 2cm in length, washed, stretched and fixed with Giemsa solution. Material was dissected under stereomicroscope to obtain whole-mount preparations stained with Giemsa method (Barbosa, 1978) and subjected to dehydration and diaphanization for mounting on glass slide.

Morphological analysis. Morphology of 500 neurons from group B was analyzed under light microscope fitted with 40X objective and micrometer disc attached to 10X lens. The sum of major longitudinal and transverse axes was obtained for each neuron, which was used to calculate the mean and standard deviation of the measurements. Procedure allowed the classification of the neurons in small, medium and large. The cell body shape, cytoplasmic basophily and nucleus position were observed.

Quantitative analysis. Whole-mount preparations obtained from group B were analyzed under light microscope with 40X objective for the neuronal counting by sampling. 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. Results consisted of 40 fields for each whole mount-preparation. The mean number of neurons/6.64mm^2 of the intestinal segment was calculated from the counts and from the area of 0.166mm^2 covered by each microscope field.

Results

The myenteric plexus was located between the circular and longitudinal layers of the muscular tunica. Isolated neurons and diffuse groups of two or more neurons were surrounded by connective tissue with bundles of collagen fibers, forming ganglia which extended throughout all the circumference of the intestinal segment and even protruding among the fibers of the longitudinal muscular layer (Figures 1 and 2).

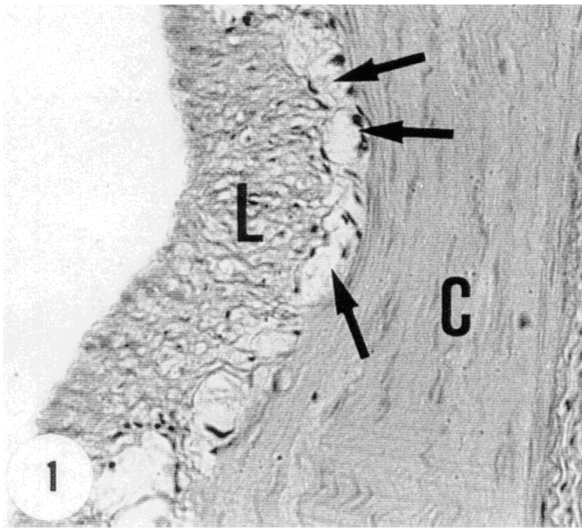

Figure 1. 6μm cross section of terminal segment of the intestine showing ganglia of the myenteric plexus (arrows) located between the longitudinal (L) and circular (C) muscle layers. HE, 246.9X

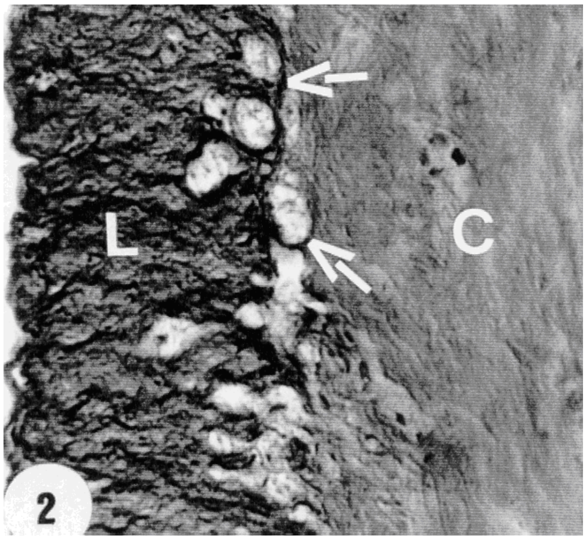

Figure 2. 10μm cross section of terminal segment of the intestine showing collagen bundles (arrows) surrounding the myenteric ganglia. Longitudinal (L) and circular (C) layers of muscular tunica. Weigert-Van Gieson, 242.8X

Analysis of the whole-mount preparations revealed the presence of small, medium and large neurons (Figure 3). The sum of major longitudinal and transverse axes of each neuron varied from 6.57 to 65.75μm. The mean and standard deviation was 39.45 and 10.52, respectively. Large neurons were included in the interval between 49.98μm and 65.75μm; medium size neurons were observed to be between 28.93μm and 49.97μm and small neurons between 6.57μm and 27.92μm. Percentages of large, medium and small neurons were 3.6%, 47.6% and 48.8%, respectively (Table 1 and Figure 3).

The morphology of the neurons varied, independently of their sizes. Neurons with triangular, stellate, oval, round and sometimes without definite shape were observed .

Table 1. Percentage of frequency, cytoplasmic basophily intensity and nucleus position in small, medium and large myenteric neurons of the terminal segment of the intestine of *Cyprinus carpio*

Neurons	F(%)	Basophily (%)			Nucleus position (%)		
		S	I	W	Pe	Ce	Po
small (< 28.93 μm)	48.8	42.6	40.1	17.2	44.2	49.0	6.8
medium (28.93 - 49.97 μm)	47.6	73.0	26.0	0.9	65.5	21.1	13.4
large (> 49.97 μm)	3.6	77.7	22.3	-	70.1	22.3	7.6

S =intense; Pe = peripheral; I = intermediary; Ce = central; W = weak; Po = polar

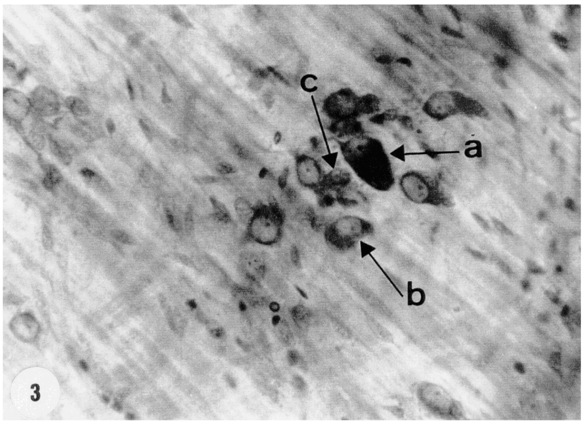

Figure 3. Whole-mount preparation of intestinal terminal segment showing a myenteric ganglion with (a) a large neuron with intense cytoplasmic basophily and a polar nucleus, (b) a medium neuron with intermediary cytoplasmic basophily and a peripheral nucleus and (c) a small neuron with weak cytoplasmic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

The spherical cell nucleus displayed several positions on the neuron without any correlation to its morphology. Position was considered polar when the nucleus was at one end of the neuron, with cytoplasm displaced to the opposite end (Figure 3). When the nucleus was near one of the cell's margins and the cytoplasm evenly distributed between the cell's ends, position was considered peripheral (Figure 3)., Nucleus position was considered central when cytoplasm was regularly distributed around the nucleus (Figure 3).

Data concerning cytoplasmic basophily and nucleus position are presented in Table 1.

The cytoplasm of most of the large neurons stained strongly, indicating intense basophilic affinity (Figure 3). Nuclei were almost always peripheral in these neurons.

Among the medium neurons intense basophilic cytoplasm predominated (Figure 3). Nuclei were peripheral, although central and polar nuclei had been observed less frequently.

Cytoplasmic basophily among small neurons varied from intense to intermediate, with a few of the neurons displaying weak basophily. Nucleus position of these cells was peripheral, central and polar with predominance of the first two (Figure 3).

The mean of the neuronal number was 5,099 ± 384.35 in 6.64mm^2 of the intestinal segment (Table 2).

Table 2. Myenteric neuron frequency in 6.64mm^2 of Giemsa-stained whole-mount preparation of the intestine terminal segment of the *Cyprinus carpio*

Whole-mount preparations	myenteric neurons
Fish 1	6,138 ± 23.80
Fish 2	4,609 ± 18.23
Fish 3	4,764 ± 29.80
Fish 4	4,581 ± 22.66
Fish 5	5,403 ± 29.29
Mean	5,099 ± 384.35

Discussion

The location of the myenteric plexus between the circular and longitudinal layers of the muscular tunica, observed on the terminal segment of the intestine of *Cyprinus carpio* was reported for other fishes by Burnstock (1959), Kobegenova and Verigina (1988), Williams and Nickol (1989) and Verigina (1990). In our samples this plexus extended throughout all the circumference of the organ.

The presence of isolated neurons and of bundles of collagen fibers were observed involving diffuse groups of two or more neurons and thus constituting ganglia. Burnstock (1959) reported that on the intestine of the *Salmo trutta* true ganglia do not exist, because he observed that only one to three nerve cells were present on the ganglia. Nevertheless, Gabella (1971) described ganglia on the rat intestine surrounded by collagen fibers containing few neurons. The presence of large ganglia in some species of Blennioidei and Loaches was commented by Kobegenova and Verigina (1988) and by Verigina (1990), respectively, without any mention of the number of neurons on these ganglia. However, Souza *et al.* (1982) described the myenteric plexus with isolated neurons with no ganglionar formation on the large intestine of *Pimelodus maculatus.*

The presence or absence of ganglia on the fish intestine may be related to the diversity of the digestive tract of these animals as a function of their alimentary habits, as reported by Verigina (1990) for other adaptations verified on the digestive tract of fishes. 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 the formation of ganglia in the myenteric plexus with ascending scale of zoological complexity.

In our samples, small and medium neurons predominated, independently of their shape, in contrast with the observations by Leite-Mello *et al.* (1997) on the colon of rats, where large and medium neurons are the most abundant. According to Gabella (1971), small neurons would represent the intrinsic portion of the myenteric plexus responsible for motor activity and are found in small numbers on the rectum of rats. The same author comments that medium and large neurons would be related to the extrinsic control. However, Burnstock (1959) suggests that large neurons would have sensitive function related to peristaltic reflex arcs.

Agreeing with Leite-Mello *et al.* (1997), we observed that peripheral nuclei predominate on the large and medium neurons. Among small neurons, nuclei show peripheral and central positions. Neurons of the myenteric plexus with polar nucleus were few.

Intense basophilic cytoplasms with a percentage of 77.7% and 73%, respectively, were found among large and medium neurons. This fact was observed by Leite-Mello *et al.* (1997) in 77.3% of colon neurons in rats.

The cytoplasmic basophily of small neurons was intense in 42.6% of the neurons and intermediate in 40.1% of them. Few neurons showed weak basophily; the greatest incidence occurred among small neurons (17.2%). Similar proportions were mentioned by Leite-Mello *et al.* (1997) on the colon of normal rats. According to Junqueira and Carneiro (1995), the presence of rough endoplasmic reticulum and free ribosomes would be responsible for cytoplasmic staining. According to Leaming and Cauna (1961), the different staining reactions mean temporary changes on the neurons, depending above all on their state of activity and enzymatic content.

Neuronal density in $6.64 mm^2$ was $5,099 \pm 384.5$, or $76,792/cm^2$, a smaller value than that found by Souza *et al.* (1982) on the rectus of *Pimelodus maculatus* ($82,857$ neurons/cm^2) and greater than that mentioned by Burnstock (1959) on the intestine of *Salmo trutta* (100 to 200 neurons/mm^2). According to Gabella (1990), the neuronal number is dependent on factors such as diameter and length of the intestine, thickness of the muscular tunica, the body weight, among others. These parameters in fish may range according to feeding habits; *Salmo trutta* is carnivorous, while *Pimelodus maculatus* and *Cyprinus carpio* are omnivorous, besides being of different species.

Considering that the intestinal segment studied represents only a small region of the digestive tract of *Cyprinus carpio*, the continuity of the present studies on the other intestinal segments is necessary to obtain a more comprehensive characterization of the density and morphology of the myenteric plexus of the digestive tract of this fish.

We may conclude that on the terminal segment of the intestine of *Cyprinus carpio* the myenteric plexus is composed of isolated neurons and groups of two or more neurons arranged in ganglia. Small and medium neurons predominate on the plexus, cytoplasmic basophily is intense on most of the large and medium neurons and varies from intense to intermediate on most of the small neurons.

References

Barbosa, A.J.A. Auerbach's plexus of the albino rat. I Quantitative study of the ganglia and nerve cells in the caecum and colon. *Rev. Bras. Pesq. Med. Biol., 6*(5):253-262, 1973.

Barbosa, A.J.A. Técnica histológica para gânglios nervosos intramurais em preparados espessos. *Rev. Bras. Pesq. Med. Biol., 11*(2-3):95-97, 1978.

Burnstock, G. The innervation of the gut of the Brown trout (*Salmo trutta*). *Q. Jl. Microsc. Sci., 100*:199-220, 1959.

Furness, J.B.; Costa, M. Types of nerves in the enteric nervous system. *Neuroscience, 5*:1-20, 1980.

Gabella, G. Neuron size and number in the myenteric plexus of the newborn and adult rat. *J. Anat., 109*:81-95, 1971.

Gabella, G. On the plasticity of form and structure of enteric ganglia. *J. Autonom. Nerv. Syst., 30*:559-566, 1990.

Irwin, D.A. The anatomy of Auerbach's plexus. *Am. J. of Anat., 59*(1):141-165, 1931.

Junqueira, L.C.U.; Carneiro, J. *Histologia básica*. Rio de Janeiro: Guanabara Koogan, 1995.

Kobegenova, S.S.; Verigina, I.A. Structure of the digestive tract of some species of Blennioidei. *Voprosy Ikhtiologii, 2*:266-272, 1988.

Leaming, D.; Cauna, N.A. A qualitative and quantitative study of the myenteric plexus of the intestine of the cat. *J. Anat., 95*(2):160-169, 1961.

Leite-Mello, E.V.S.; Stabille, S.R.; Miranda-Neto, M.H. Effect of maternal protein deprivation on morphological and quantitative aspects of the myenteric plexus neurons of proximal colon in rats. *Arq. Neur. Psiq., 55*:106-113, 1997.

Souza, R.R.; Ferri, S.; Ferraz de Carvalho, C.A.; Paranhos, G.S. Myenteric plexus in a fresh water teleost intestine. I - Quantitative study of nerve cells. *Anat. Anz., 152*:359-362, 1982.

Verigina, I.A. Morphology of the digestive system of some Loaches. *Voprosy Ikht. 30*(2):246-254, 1990.

Williams, J.A.; Nickol, B.B. Histological structure of intestine and pyloric caeca of the green sunfish, *Lepomis cyanellos rafinisque*. *J. Fish. Biol., 35*(2):359-372, 1989.

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