

Morphometry of Myenteric Neurons in Stomach

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ABSTRACT

The wall of the gastrointestinal tract presents extensive plexuses of nerve fibres and neuronal cell bodies responsible for the modulation of the rhythmic gastrointestinal peristaltic activities, among other functions. One of the most developed ganglionated plexuses of the gastrointestinal tract is the Myenteric plexus located between the inner circular layer and outer longitudinal layer of the smooth muscle tunica. The musculature of fundus, body and pyloric parts of stomach are differently disposed and they perform different functions. Thus the present study was conducted to study the myenteric plexus of all parts of stomach by counting the number of collections of neurons, number of neurons in each collection, diameter and area of the neurons of the plexus. The stomach walls of 1 cm in size were taken from 5 cadavers of medical post mortem cases from Postgraduate Institute of Medical Sciences & Research, Chandigarh and were processed for paraffin sections. 5 and 10 μ thick sections were stained with haematoxylin and Eosin and examined under light microscope. Randomly selected sections were photomicrographed using digital camera and morphometrical analysis was done using Image-Pro Express software. Number of collections of neurons was maximum in fundus with an average of 4.521 and each collection on an average contain 5.27 neurons ranging from 1-31, while body had 3.292 collections containing 1-19 neurons (mean: 3.198), pylorus had 3.883 collections of neurons which contained 1-16 neurons (mean: 4.411). The neurons were classified as small, medium and large according to the size of the area of their cell bodies. In this way, 11.3% neurons were found to be small, 69.5% medium and 19.1% large in fundus, 8.7% small, 80.6% medium and 11.2% large in body and 11.1% small, 74.3% medium and 14.5% large in pylorus.

Keywords: autonomic ganglia, smooth muscle tunica, collection of neurons, size and number of neurons

INTRODUCTION

The wall of the gastrointestinal tract presents extensive plexuses of nerve fibres and neuronal cell bodies responsible for the modulation of the rhythmic gastrointestinal peristaltic activities, among other functions even in the absence of the control from CNS. These plexuses compose the Enteric Nervous System (ENS). One of the most developed ganglionated plexuses of the intestinal wall is the myenteric plexus (or Auerbach's plexus) located between the inner circular layer and outer longitudinal layer of the smooth muscle tunica.¹ The musculature of fundus, body and pyloric parts of the stomach are differently disposed and they perform different functions. The muscles of the upper region of stomach exert a maintained moderate tonic contraction on its stored contents whereas the muscles of lower part are much more motile and peristaltic.²

Hence the morphometrical study was planned to study the myenteric plexus of fundus, body and pyloric parts of stomach by counting the number of collections of neurons, presence of neurons in each collection, diameter and area of neuronal cell bodies to find out if the neuronal population of these parts differs according to their function.

MATERIALS AND METHODS

One cm piece from the same region of fundus, body and pylorus containing entire wall was taken from 5 adult cadavers of medical post mortem cases from Postgraduate Institute of Medical Sciences and Research, Chandigarh and were processed for paraffin sectioning. 5 and 10 μ thick sections were stained with: (1) Hematoxylin and Eosin (H and E), and (2) Marsland, Glees and Erikson's silver stain. Consecutive 5 sections of 10th H&E stained slide of each block were photographed using digital camera and morphometrical analysis was done using Image Pro Express software. The number of collections of neurons, presence of neurons in each collection, diameter and area of neuronal cell bodies were tabulated and statistically analyzed. The neurons were classified on the basis of their area into small, medium and large. The neurons with areas smaller than the mean minus its standard deviation were considered small, those above the mean plus its standard deviation were considered large, and those intermediate to these values were considered medium neurons.¹

RESULTS

Myenteric plexus of stomach contains 1-9 ganglia having multipolar neurons of various sizes and shapes with eccentric nucleus scattered through out the organ (Fig. 1-3).

- In fundus, large elongated ganglia having neurons packed in a characteristically tight fashion in the connective tissue surrounded by satellite cells were observed (Fig. 1). 1-3 collections were present in 47.6% of the sections (Table 1) having 5.270 ± 5.684 mean number of neurons (Table 2). Mean area was found to be 99.47 ± 35.739 with a range of 10.263-254.994 (Table 3). The neurons were of various sizes and 69.5% neurons were of medium size (Table 4).
- In body, the ganglia were elongated and triangular present in the connective tissue surrounded by satellite cells (Fig. 2). 1-3 collections were present in 63.4% of sections and 7-9 collections were found only in 2.4% of sections (Table-1). The mean value of number of neurons in these collections was 3.198 ± 2.588 (Table-2). Mean area of neurons was 127.547 ± 43.929 with a range of 14.184-317.527 (Table3). Of the total, 80.6% of neurons were observed to be of medium size (Table 4).
- In pylorus, the ganglia appeared compressed in most of the sections, the long axis of which lay perpendicular to the circular muscle layer (Fig. 3). 1-3 and 4-6 collections of neurons were found in 50.0% and 45.2% of sections respectively (Table-1). The mean value of neurons was found to be 4.111 ± 4.287 in these collections (Table-2). Mean area of neurons was 131.431 ± 35.962 ranging from 65.111-259.660 (Table3). 74.3% neurons were of medium size whereas only 11.1% neurons were of small size (Table-4).

The difference of size of neurons between fundus and body ($p < 0.001$) and fundus and pylorus ($p < 0.05$) was found to be statistically highly significant. The number of collections of neurons and number of neurons in those collections were found to be more in fundus but size was less as compare to body and pylorus. The maximum number of collections in body was 1-3 and was found in 63.4% of sections. Largest neuron having area $317.527 \mu\text{m}^2$ was found in body of the stomach.

The observations from the present study show that the myenteric plexus of stomach contains large number of collections of nerve cells of different sizes.

DISCUSSION

The collection of neurons in the connective tissue between the inner circular and outer longitudinal layers of smooth muscle tunica was present in all the sections of the specimen. Bowen³ described these complex amalgamations of neurons as ganglia which utilize a wide variety of neurotransmitters. While⁴ stated that these neurons in ganglia are gathered into long cords which fuse into one another lacking a clear demarcation into ganglia. All these complex neuronal networks are embedded in the wall of gut and are described as enteric nervous system.^{5,3,6} The number of enteric neurons probably exceeds that of the remainder of the peripheral nervous system and is at least equal to that of the spinal cord.^{7,8} They also stated that the complexity and size of the ENS reflects its ability to mediate reflexes in the absence of input from the brain or spinal cord. Partner⁹ has described ENS as integrated brain in its own right and has over 100 million neurons more in number than in the spinal cord which informs the brain regarding the danger of infected food by inducing nausea and abdominal pain. In the present study, the number of collections of neurons and number of neurons in these collections were found to be more in fundus but neuronal size was less as compare to body and pylorus. The difference of size of neurons between fundus and body, fundus and pylorus was observed to be statistically significant. In the present study, the size of the neurons varied from 10.263-317.527 μm^2 and neurons were surrounded by satellite cells. Schofield¹⁰ and Jessen¹¹ studied these satellite cells ultrastructurally and stated that they resemble those of Schwann cells and are derived from neural crest. Natali¹ divided the neurons into small, medium and large because of the presence of large range of neuronal size and also stated that the large neurons make up the major neuronal population. Gabella¹² observed large neurons make up larger neuronal population in duodenum of rat. Though in the present study, the neurons were similarly described as large, medium and small using the same methodology but the medium size neurons were more in number than large. Large and medium size neurons of the present study were multipolar neurons which are described to control gastrointestinal motility; secretion and possibly absorption and considered to be a part of sympathetic and parasympathetic nervous system.³ There are two secretomotor neurons in myenteric plexus, one are cholinergic and the other non-cholinergic neurons.¹³

The small neuron was $8.75 \mu\text{m}^2$ in body, $11.11 \mu\text{m}^2$ in pylorus and $11.34 \mu\text{m}^2$ in fundus in the present study which are probably sensory neurons as described earlier which respond to mechanical, thermal, osmotic and chemical changes. Though some of these small neurons are described to be interneurons and are largely responsible for integrating information from sensory neurons and providing it to enteric neurons.³ Out of total neurons, 7.0% are interneurons.^{14,15} Reflex function of bowel independent of the central nervous system arises because of the coordinated activity in their sensory neurons, interneurons and excitatory and inhibitory motor neurons. The neuronal mechanisms that coordinate gut function rely on a complex interplay between many neuroactive mediators and their receptors.^{3,1} Natali¹ also stated that probably number of smaller neurons was more but they remained unstained. The size of neuronal population may depend upon the eating habits of an individual as¹ described the reduction of the cell body size and an increase in the population of small neurons on the rat subjected to hypoproteic chow. Cellular hypertrophy, including in neurons, can be considered as a basic response mechanism in instances where the cells are under aggression, such as decrease of nutrient supply or of necessary stimuli for their functioning. As a result the cells get adapted, with decreased

metabolism, leading to a decrease in the turnover of their own structures and then to a reduction in their volume.¹⁶

The variation in the neuronal population between fundus, body and pylorus may be probably due to different functions of each part of stomach. As the function and disposition of smooth muscle tunica in these three parts of the stomach are different as described in most of the text books.^{2,17}

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Table-1: The number of collections of neurons in myenteric plexus of stomach

Part of stomach	Mean (Range)	% of collections (1-3)	% of collections (4-6)	% of collections (7-9)
Fundus	4.521 (1-9)	47.6	42.8	9.5
Body	3.292 (1-8)	63.4	34.1	2.4
Pylorus	3.883 (1-9)	50.0	45.2	4.7

Table 2: The number of neurons present in collection of myenteric plexus in stomach

Part	Mean	Range
Fundus	5.270±5.684	1-31
Body	3.198±2.588	1-14
Pylorus	4.111±4.287	1-16

Table 3: Area of neuron (μm^2) in each part of the stomach

Part of stomach	Mean area of neuron	Range
Fundus	99.472 \pm 35.739	10.263-254.994
Body	127.547 \pm 43.929	14.184-317.527
Pylorus	131.431 \pm 35.962	65.111-259.660

Table 4: Cell population according to size of neuron in each part of stomach

Part	Small (%)	Medium (%)	Large (%)
Fundus	11.3	69.5	19.1
Body	8.7	80.6	11.2
Pylorus	11.1	74.3	14.5

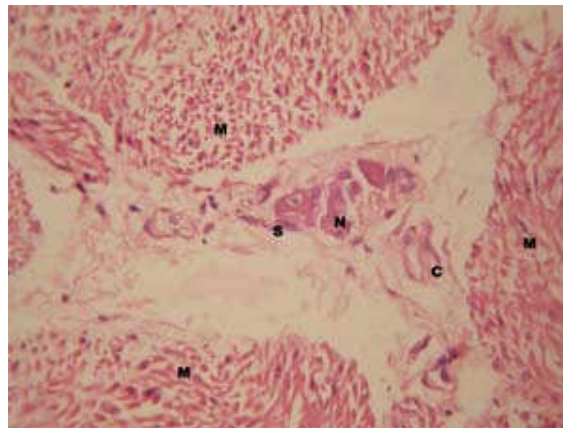


Fig.1. Photomicrograph of fundus of stomach showing collection of neurons in muscularis propria (m); neurons (n), connective tissue (c), satellite cells (s). (H and E, X650).

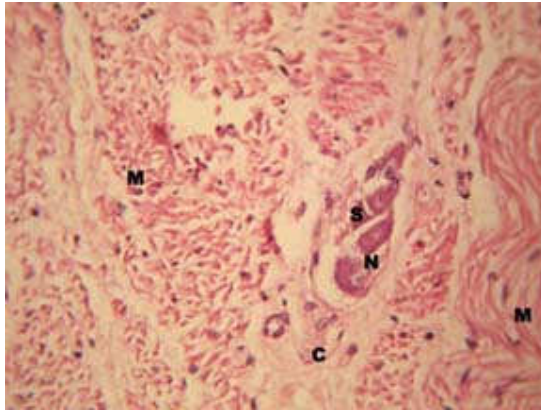


Fig.2. Photomicrograph of body of stomach showing collection of neurons in muscularis propria (m); neurons (n), connective tissue (c), satellite cells (s). (H and E, X650).

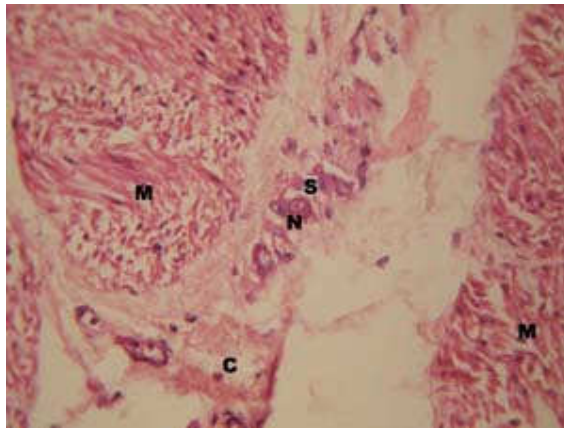


Fig.3. Photomicrograph of pylorus of stomach showing collection of neurons in muscularis propria (m); neurons (n), connective tissue (c), satellite cells (s). (H and E, X650).