Cytoarchitectural Study on the Dorsal Motor Nucleus of the Rat Vagus

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Summary: The dorsal motor nucleus of the rat vagus was studied using the Nissl and Klüver-Barrera methods. This nucleus is elongated rostrocaudally, is about 3-4 mm in length and contains about 5,000 cells. It appears fusiform in shape and is subdivided into oral, middle and caudal regions according to cell distribution. These regions constitute one-fifth, two-fifths and two-fifths of the entire length of the nucleus, and the number of cells in each region is about 10%, 70%, and 20% of the cell total, respectively. The cells of the nucleus are classified into two distinct types, being either small or medium-sized. Small cells are round or oval in shape, measuring less than 20 μm along the long axis and 15 μm along the short axis. This type of cell is distributed throughout all regions of the nucleus and represents approximately 60% of all cells. Medium-sized cells are pyramidal or multipolar in shape and measure 20-30 μm along the long axis and 15-20 μm along the short axis. This type of cell is distributed throughout the central three-fifths of the nucleus and represents approximately 40% of all cells.

The first report on the dorsal motor nucleus of the vagus (DMV) was presented by Stilling (1843). Since then, many reports describing functional and cytoarchitectural investigations of this nucleus have appeared over the years including studies on the cat (Marinesco 1897, Mohiuddin 1953, Kalia and Mesulam 1980, McLean and Hopkins 1981), rabbit (Kimmel 1940, Getz and Sirnes 1949), monkey (Malone 1913, Mitchell and Warwick 1955), dog (Husten 1924), sheep (Bell and Lawn 1955, Welento and Flieger 1974), buffalo (Rao and Sahu 1974), pigeon (Cohen et al. 1970), rat (Lewis et al. 1970, Nosaka et al. 1978, Contreras et al. 1980) and bird (Wild 1981).

In spite of these studies, few reports have appeared concerning the topology and detailed cytoarchitecture on this nucleus. Details of the number, shape and distribution of neurons are therefore still uncertain. The only study to have appeared including the DMV of the rat was presented by Lewis et al. (1970), Nosaka et al. (1978) and Contreras et al. (1980), but only a small part of their reports was pertinent to the present subject. In order to provide a description of the normal cytoarchitecture of the nucleus, the present study on the DMV of the rat has been carried out quantitatively using light microscopic method and the results obtained have been compared with

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the preceding studies. This study is therefore intended to provide a basis on which to pursue further the possible localization of the functional centres which may belong to this nucleus.

**Materials and Methods**

Seven male and female Wistar rats weighing 300–500 g were used in the present study. All animals were anesthetized with 5% sodium pentobarbital (nembutal i. p. 50 mg/kg).

Two brains from the animals used were immersed for a few days in 96% alcohol solution immediately after removal and then processed by the celloidin tissue technique. Serial sections 25 μm in thickness were then cut transversely through the entire brain stem and upper cervical spinal cord. The other five rats were perfused through the left ventricle with 10% formalin. Their brains were then removed and fixed in 10% formalin for three to four weeks, after which they were washed, dehydrated and embedded in paraffin. Serial sections 10 μm in thickness were then cut transversely in four cases and longitudinally in one case. All sections were stained using the Nissl and Klüver-Barrera methods.

The first one of every four sections was selected for observation to provide the number of cells in the different levels of this nucleus. Quantitative observations of the total number of cells were made on all transverse sections through the left and right DMV of four rats. Only the neurons in which nucleoli were clearly seen in the field of vision were counted in this quantitative study. Classifications of neurons were made according to the shape, size, and distribution of Nissl substance.

**Results**

1) The normal topology of the nucleus

In rats, the DMV is located in the typical area immediately beneath the fourth ventricle and between the hypoglossal nucleus and the nucleus of the tractus solitarius. At a more rostral level, the nucleus intercalatus intervenes between the DMV and the hypoglossal nucleus, therefore displacing the DMV gradually into a more lateral position (Fig. 2). At its most rostral pole, this nucleus reaches to the level of the caudal part of the hypoglossal preoptic nucleus. In caudal transverse section, the nucleus is located laterally and sometimes dorsolaterally to the central canal of the spinal cord, dorsally to the hypoglossal nucleus and ventromedially to the nucleus of the tractus solitarius (Figs. 2, 4, 6 and 8).

This nucleus appears fusiform in outline, the oral region being thickly cylindrical, becoming slender in the caudal region. The nucleus elongates rostro-caudally and is about 3–4 mm in length. This is because the number of neurons increases rapidly at a level about one-fifth of the total length from the oral pole, and decreases rapidly at a level about three-fifths of the total length from the oral pole. The nucleus is subdivided into three portions by their two speculative boundaries, namely an oral, two middle and two caudal regions (Fig. 1).

In transverse section, this nucleus has an oblong outline and its maximum dimension is located at about two-fifths of the whole length from the oral pole, just oral to the level of the obex. According to studies of the tissue from the six rats, the long axis measures 600–700 μm in a medio-lateral direction, and the short axis 120–150 μm in a dorso-ventral direction, respectively. In the most caudal transverse section, the nucleus is reduced to a small
ovoïdal or round shape (Figs. 3, 5, 7 and 9).

2) Classification of neurons

It is easy to distinguish the neurons of the DMV from neurons of other nuclei in the sections stained by the Klüver-Barrera method. The neurons of this nucleus are noticeably different from those of the ventro-medial hypoglossal and intercalate nuclei in the size of cell, and the neurons stain more deeply than those of the dorso-lateral nucleus of the tractus solitarius. The constituent cells are either oval, round or pyramidal multipolar in shape. They generally possess a long axis oriented in a medio-lateral direction in the transverse plane.

In this present study, the cells of the nucleus were classified into two distinct types. Small cells were round or oval in shape, being less than 20 μm along the long axis and less than 15 μm along the short axis. These neurons contained relatively large nuclei in relation to cell size, with scanty chromatin granules on the dorsal or ventral borders of the cells. The nuclei were generally oval in transverse section, and located centrally in the cell body (Figs. 10, 11 and 12). Medium-sized cells were pyramidal or multipolar in shape and measured 20–30 μm along the long axis and 15–20 μm along the short axis. The nuclei of these neurons were relatively small in proportion to the cell size and there were more prominent chromatin granules in the cell bodies (Figs. 10 and 11). In addition, a few larger cells were scattered among the small and medium-sized cells in the middle region of the nucleus.

These cells had a long axis exceeding 30 μm in length and a very short axis. The cell size was not as large as that of cells from the hypoglossal nucleus or ambiguus nucleus. They did not form an integral part of the nucleus, because this type of cell was very small in number. It was assumed that they belonged to the medium-sized cell group (Fig. 11).

Results on the distributions of these two kinds of cell indicate that the small cells are distributed throughout the extent of the nucleus, and the medium-sized cells and relatively large-sized cells are only distributed among the small cells in the central and lateral parts of the middle three-fifths of the DMV. The oral and caudal one-fifth regions of the nucleus are composed of the small cells only. The small cells of the caudal region are larger than those of the other regions of the nucleus. In cell composition, the round cells number more than the oval type of cell. The location of these cell types is shown in Table 1 and Figs. 13 to 20.

3) Number of neurons

Literature is scarce concerning the cell count in this nucleus. In this study a quantitative count of neurons in both the left and right nuclei of four rats was performed. The total number of neurons in the nucleus was determined by counting the nucleoli confirmed in such cells in all sections throughout the nucleus. The total
Table 1. Cell numbers in the dorsal motor nucleus of the rat vagus

<table>
<thead>
<tr>
<th></th>
<th>right side</th>
<th></th>
<th></th>
<th></th>
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<th>left side</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>oral</td>
<td>middle</td>
<td>caudal</td>
<td>total</td>
<td>number</td>
<td>oral</td>
<td>middle</td>
<td>caudal</td>
<td>total</td>
</tr>
<tr>
<td>case 1</td>
<td>small</td>
<td>488</td>
<td>2020</td>
<td>606</td>
<td>3114(61%)</td>
<td>408</td>
<td>2240</td>
<td>624</td>
<td>3272(64%)</td>
</tr>
<tr>
<td></td>
<td>medium</td>
<td>1556</td>
<td>410</td>
<td>1966</td>
<td>3922(39%)</td>
<td>1464</td>
<td>328</td>
<td>1792</td>
<td>3699(36%)</td>
</tr>
<tr>
<td>case 2</td>
<td>small</td>
<td>568</td>
<td>1912</td>
<td>784</td>
<td>3264(63%)</td>
<td>536</td>
<td>2347</td>
<td>816</td>
<td>3699(62%)</td>
</tr>
<tr>
<td></td>
<td>medium</td>
<td>1528</td>
<td>336</td>
<td>1864</td>
<td>3298(37%)</td>
<td>1744</td>
<td>440</td>
<td>2184</td>
<td>3699(38%)</td>
</tr>
<tr>
<td>case 3</td>
<td>small</td>
<td>392</td>
<td>1793</td>
<td>585</td>
<td>2770(60%)</td>
<td>462</td>
<td>1688</td>
<td>708</td>
<td>2858(60%)</td>
</tr>
<tr>
<td></td>
<td>medium</td>
<td>1485</td>
<td>344</td>
<td>1829</td>
<td>3274(40%)</td>
<td>1490</td>
<td>353</td>
<td>1843</td>
<td>3699(40%)</td>
</tr>
<tr>
<td>case 4</td>
<td>small</td>
<td>520</td>
<td>1928</td>
<td>592</td>
<td>3040(61%)</td>
<td>563</td>
<td>2060</td>
<td>660</td>
<td>3283(61%)</td>
</tr>
<tr>
<td></td>
<td>medium</td>
<td>1510</td>
<td>408</td>
<td>1918</td>
<td>3036(39%)</td>
<td>1700</td>
<td>360</td>
<td>2060</td>
<td>3036(39%)</td>
</tr>
</tbody>
</table>

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The cell numbers in every region of the nucleus are also represented in Table 1. The numbers of cells in the oral, middle and caudal regions were about 10%, 70%, and 20% of the cell total, respectively. Figures 13 to 20 show distributional patterns of cell numbers at various levels in four left nuclei of rats. These results show that the nucleus has a spindle-shaped form. The highest population of cells lies at a level about two-fifths of the whole length from the oral pole and showed about 50 cells per section. Table 1 and Figures 13 to 20 show the respective cell numbers and proportional patterns of the two types of cell. The small and medium-sized cells made up approximately 60% and 40% of the total cell number respectively, and both cell types showed the highest population of cells at a level about two-fifths of the whole length from the oral pole. In the middle region of the nucleus small and medium-sized cells constituted 50–60% and 40–50%, respectively, while in the caudal region such cells constituted 60–70% and 30–40% of the total number of each region.

Discussion

The same conclusions as earlier investigators about the types and distributional patterns of the cells in the DMV were not drown, even though this nucleus has been known for well over 100 years.

Some authors asserted there were two distinct types of cells within the nucleus. In an early study Molhant (1910) characterized both small and large cells, and declared that the small cells were located in the oral and large cells in the intermediate region, respectively. In the caudal region both types of cells were intermingled with each other. Malone (1913) also reported the same conclusions as Molhant and further suggested that the small cells innervate in smooth muscle, and the large cells in heart muscle. However, Kimmel (1940) found small cells scattered all over the nucleus, while the large cells were confined to the caudal half of the nucleus in the rabbit. Getz and Sirnes (1949) agreed with the
observations of Kimmel and further found that in the oral one-third there were small cells only, whereas in the central region large cells dominated and in the caudal region there was about equal numbers of small and large cells. More recently, McLean and Hopkins (1981) observed medium-sized neurons measuring 18–25 μm and small neurons 9–14 μm in the cat nucleus using the retrograde HRP method. They also found that these two types of cells were represented in all regions of the nucleus.

Contrary to these opinions, Mitchell and Warwick (1955) described that there were three distinct types of cell, namely, small, medium-sized and large cells in the nucleus of the monkey. According to their observations the small cells were ovoid, medium-sized cells were pyramidal or fusiform, and large cells were multipolar in shape. The three types of cell were not segregated in definite zones, except for the large cell, which were located mainly in the middle region of the nucleus. Cohen et al. (1970) also observed three types of cell in the pigeon, but disagreed with the opinion of Mitchell and Warwick (1955) on their distributions. They postulated that the small, medium-sized and large cells formed three distinct patterns of distribution located in the dorsal, intermediate and ventral regions in a ventro-dorsal direction, respectively.

The present result indicates that there are both small and medium-sized cells in this nucleus which are characterized not only by their size also mainly in their distinct shape, the small cells being round or oval and medium-sized cells being pyramidal or multipolar. The two types of cell also differ in the relative size of the nucleus and in the characterization of their chromatin granules. In the present study, although a few cells exceeding 30 μm along their long axis were seen, on the basis of their morphological appearance and for the reason mentioned earlier they can not be considered as large cells. The large cells in the monkey observed by Mitchell and Warwick (1955) and in the pigeon observed by Cohen et al. (1970) were not seen in the rat.

Recently, studies with other methods have also supported this view. Aldskogius (1978) described two types of cells in the nucleus of the rabbit in a microscopic study. He further observed that almost all medium-sized cells showed degenerative changes 8 days following vagotomy, while no small cells were affected. McLean and Hopkins (1982) observed that small cells but not medium-sized cells in this nucleus were labeled after brainstem HRP injections. In addition, the electrophysiological study of Nosaka et al. (1978) also identified two types of cell in the nucleus of the rat.

As far as distribution of the two types of cell is concerned the results of the present study indicate that the small cells are present in all regions of the nucleus. This is in agreement with previous studies. However, medium-sized cells were only seen to be present in the middle three-fifths region of the nucleus. This is contrary to some other observations. In the present study, the small cells in the caudal one-fifth region of the nucleus were larger than the small cells of the other regions, but did not belong to the medium-sized cells classified according to our standard.

Estimates of the total number of cells in this nucleus have so far only appeared in reports of Smolen and Truex (1978) and Mohiuddin (1953). They reported that there were nearly 6,000 cells in each nucleus of the cat. The results of this study indicate about 5,000 cells in each nucleus of the rat. Mohiuddin counted only the first section of every five in the cat, then multiplying by 5 to give a total of about 6,000 cells. Our result, however, was based on 10 μm
sections of all sections through the nucleus of two sides in 4 rats.

In the present study, according to observations from four rats, most of cells are seen to be located at a level of two-fifths of the total length from the oral pole. This is not in agreement with the result of Lewis et al. (1970). They also represented graphically the numerical distribution of cholinesterase-containing cells at different levels in the nucleus by means of a modified chicholine technique. According to their graphs the highest population of cells lay at a level of three-fifths of the total length from the oral pole of the nucleus.

The present study gives the distributions of cells in every part of the nucleus and the proportions of the two types of cell. Only McLean and Hopkins (1981) have previously reported such information. They indicated that small cells only accounted for 20 to 25% of the cell total. This is very different from our result. The difference can perhaps be attributed to differences in the taxonomic standardization of the two types of cell. Cells possessing a long axis exceeding 18 \( \mu \text{m} \) and a short axis exceeding 11 \( \mu \text{m} \) were classified as medium-sized cells by McLean and Hopkins (1981). But in our characterization, only cells possessing a long axis less than 20 \( \mu \text{m} \) and a short axis less than 15 \( \mu \text{m} \) were considered to be small cells. We also found cells measuring 18–20 \( \mu \text{m} \) along the long axis and 11–15 \( \mu \text{m} \) along the short axis which were very common in the nucleus, and were oval in shape. According to our characterization they may be considered to belong to the small cell group.

Acknowledgment

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References


Explanations of Figures

Abbreviations in Figures

| CC   | central canal          |
| CN   | cell number            |
| CP   | caudal pole            |
| CR   | caudal region          |
| DMV  | dorsal motor nucleus of vagus |
| FV   | fourth ventricle       |
| MR   | middle region          |
| NH   | nucleus nervi hypoglossi |
| NI   | nucleus intercalatus   |
| NRP  | nucleus reticularis parvocellularis |
| NTS  | nucleus tractus solitarii |
| OP   | oral pole              |
| OR   | oral region            |
Explanation of Figures

Plate I

Figs. 2, 4, 6, and 8.
Topographical photomicrographs of the dorsal motor nucleus of the vagus. They are arranged in an oral-caudal direction. Crystal violet stain.

Figs. 3, 5, 7, and 9.
Graphic reconstruction of cell distribution in the DMV.
○, medium-sized cell; •, small cell.
Plate II

Figs. 10-12.
Photomicrographs of two types of neuron in the rat DMV. Crystal violet stain, X450.
Fig. 10; small and medium-sized neurons (arrows) from middle region of the DMV.
Fig. 11; relatively large neurons (arrow) among medium-sized neurons from the middle region of the DMV.
Fig. 12; small neurons from the caudal region of the DMV.
Plate III

Figs. 13 to 20.

Comparative differences between regions of the left DMV in four rats.

Figs. 13, 14, 15 and 16; distributional difference of cell numbers of two kinds of neurons

- small neuron
- medium-sized neuron.

Figs. 17, 18, 19, and 20; distributional difference of total neuron number in the DMV of four rats.