

Localization in the dorsal motor nucleus of the vagus in the rat

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Several investigators, including Getz & Sirnes (1949), Mohiuddin (1953) and Mitchell & Warwick (1955) have claimed that it is possible to localize areas within the dorsal motor nucleus of the vagus which are concerned with the innervation of particular thoracic and abdominal viscera. Most of these studies were based on the identification of retrograde chromatolytic changes within the cells of the nucleus, using standard histological preparations following interruption of vagal branches. However, since such changes are reputedly difficult to interpret and are in any case ill-defined in adult animals, especially within motor neurons (Romanes, 1946), it cannot be said that the existence of localization has been satisfactorily proven.

The converse technique of placing lesions in the dorsal motor nucleus and tracing axonal degeneration in the peripheral branches of the vagus has been attempted but with conflicting results. Szentágothai (1952) failed to find any degenerating fibres in the peripheral vagus following lesions of the dorsal nucleus and he suggested that most of the efferent fibres to the viscera did not arise from this nucleus but probably from the nucleus ambiguus and from the nucleus retrofacialis. Calaresu & Cottle (1965), on the other hand, succeeded in tracing degenerating fibres into the vagal cardiac branches following lesions placed in the centre of the dorsal motor nucleus; it must be admitted, however, that their success was limited to two specimens out of a total of five and that investigations in the same laboratory revealed a lack of cardiac response on stimulation of the relevant part of the nucleus (Calaresu & Pearce, 1965), suggesting that the cells giving origin to cardiomotor fibres are so widely dispersed as to escape stimulation by the methods employed.

The principal difficulty in locating the central origin of individual nerves has been the unsuitability of standard histological techniques for tracing degenerative changes, whether these be chromatolysis in the cell bodies or Wallerian degeneration in the peripheral axons. It is comparatively simpler to detect retrograde changes by employing histochemical techniques designed to show those enzymes which are depleted by axonal injury, of which cholinesterases are undoubtedly the most convenient to study as their depletion is clear cut and demonstrable even under the low power objectives of the microscope. Loss of cholinesterase in the cell body following axotomy has been demonstrated at several sites including the hypoglossal nucleus (Schwarzacher, 1958), ventral horn of the spinal cord (Söderholm, 1965), dorsal motor nucleus of the vagus (Navaratnam, Lewis & Shute, 1968) and the superior cervical sympathetic ganglion (Härkönen, 1964). The depletion of enzyme commences 2–3 d following axotomy and persists for up to as long as 12–16 weeks, after which there may be a gradual return of activity if the cells recover.

The motor cells of origin of individual branches of a peripheral nerve trunk may be localized either by interrupting the branches in question and identifying the cells depleted of their enzyme or, alternatively, by cutting the trunk itself at various levels and attributing the cells retaining enzyme activity to branches given off proximal to the level of section. The latter approach is preferable insofar as it is easier to study the distribution of stained rather than of unstained cells. In any case, for practical reasons it is the more feasible approach to study localization in vagal nuclei because some of the vagal branches, particularly those in the thorax, are not easily accessible and others, such as the cervical cardiac nerves, are so fine as to be unidentifiable during operation.

MATERIALS AND METHODS

The present study was carried out on adult albino rats weighing 250–300 g. The levels at which the vagus trunk was divided were as follows:

(a) The nerve was cut unilaterally in the upper part of the neck, proximal to the origin of the superior laryngeal branch. This operation was performed on four animals, two on the right side and two on the left.

(b) The vagus was interrupted unilaterally in the lower part of the neck, below the origin of the superior laryngeal nerve but above the origin of the inferior laryngeal nerve. This operation was performed on six rats, three being operated on the right side and three on the left.

(c) The vagal trunks were cut as they lay round the abdominal part of the oesophagus. At this level there is usually a trunk (sometimes duplicated) to the left side of and anterior to the oesophagus; another trunk, which may also be duplicated, lies behind and to the right of the oesophagus. All the abdominal vagal branches were divided in five animals; care was taken to interrupt all the nerve twigs in the vicinity, including those entering the wall of the abdominal oesophagus. In another four animals the branches on the left side of the oesophagus were interrupted, while those on the right side were cut in a further four.

After a period of 1–3 weeks, the operated animals were killed with ether and perfused with 20% formol-saline. The hind brain in each instance was removed, fixed in 10% formalin at 4 °C for 6–8 h, and sectioned serially on the freezing microtome at 50 μ m. The sections were tested for cholinesterase activity using a modified thiocholine technique, details of which are given in a previous paper (Navaratnam *et al.* 1968). Material from four normal control animals was also similarly prepared.

The cholinesterase-containing cells within the dorsal motor nucleus of the vagus on both sides were counted in each section under the microscope and these counts, as well as their relative rostro-caudal levels, were represented graphically. As the material was sectioned at uniform thickness, the counts obtained were considered accurate enough for the comparative purposes envisaged in this study and no correction was applied to compensate for the appearance of single cells in more than one section.

In view of the suggestion that the nucleus ambiguus might be a source of visceromotor fibres, similar counts were made of the cholinesterase-containing cells in this nucleus in both operated and unoperated animals.

OBSERVATIONS

*Dorsal motor nucleus of the vagus**Normal*

The dorsal motor nucleus of the vagus extends rostro-caudally for approximately 3 mm on the dorso-lateral aspect of the hypoglossal nucleus of the same side. It lies near the floor of the fourth ventricle, very near the mid-line (Fig. 1). Most of its component cells are oval in shape with their long axes in the transverse plane, and

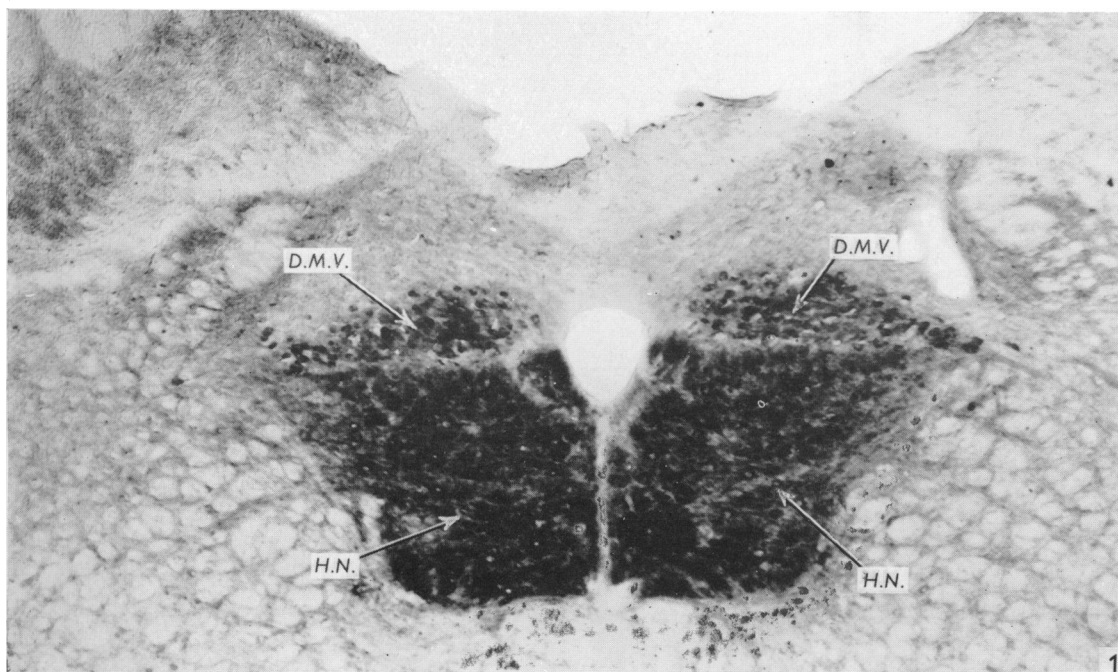


Fig. 1. Section through rat hind brain (control) showing the presence of AChE-containing cells in the dorsal motor nucleus of the vagus (*D.M.V.*) on each side. The adjacent hypoglossal nucleus (*H.N.*) also contains AChE. $\times 55$.

are of fairly uniform size, their maximum diameters measuring 25–30 μm and their minimum diameters measuring 12–20 μm . Scattered among the oval cells are a few rounded profiles measuring about 25–30 μm across; there is no evidence that either type of profile is restricted to particular parts of the nucleus. All the cells in the nucleus contain a high concentration of intracytoplasmic cholinesterase which, judging by the substrate specificity of the staining and its response to inhibitors, comprises both true cholinesterase (AChE) and pseudocholinesterase (ChE), the latter predominating. The neuropil also is considerably stained but here the activity appears to be entirely that of AChE.

The distribution of cholinesterase-containing cells in the dorsal motor nuclei of the four control animals is represented in Fig. 2. Among other features, it shows that the cellular concentrations at various levels are very similar on the two sides in the

same individual and, moreover, that the distributions in the nuclei of different animals are also comparable. The nucleus is characteristically spindle-shaped, the highest population of cells lying more or less at the middle of its rostro-caudal extent.

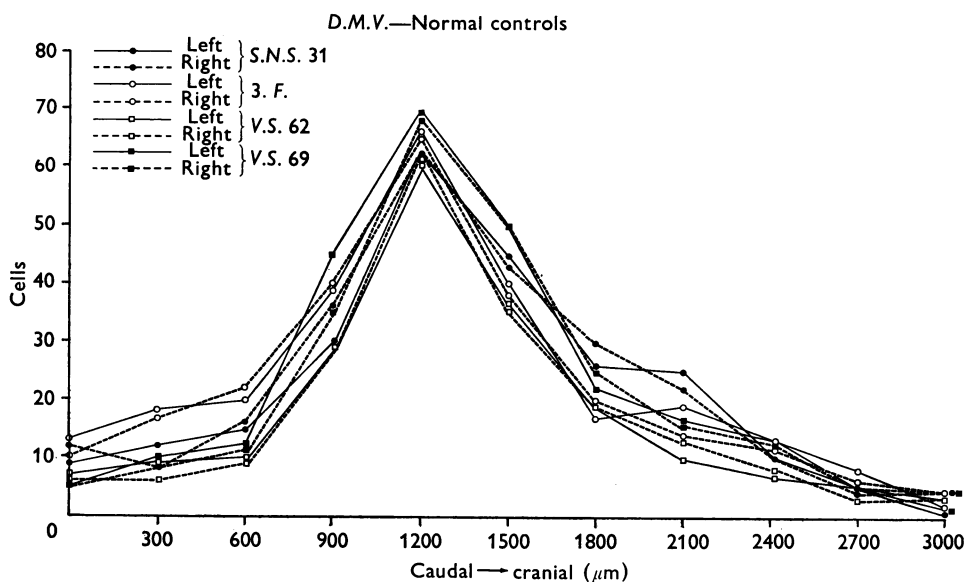


Fig. 2. Graphical representation of the numerical distribution of AChE-containing cells at different levels of the dorsal motor nucleus in four control animals. The distribution of cells in each nucleus is similar to that in its counterpart on the opposite side, as well as in the nuclei of other specimens.

Effects of cervical vagotomy (levels A and B)

Section of either vagus trunk in the neck results in loss of cholinesterase from practically all the cells in the ipsilateral dorsal motor nucleus (Figs. 3, 4). The contralateral nucleus, as far as could be judged by comparison with the distributions in control animals, appears to be unaffected by the lesion. A surprising feature is that no consistent differences exist between the effects of high cervical vagotomy (level A) and those of low cervical vagotomy (level B). In only one specimen of low cervical vagotomy were cells in the ipsilateral nucleus spared to any appreciable extent; however, even here they were very few indeed, not exceeding 20 in all, thus emphasizing the insignificance of cervical vagal branches as far as efferent visceral innervation is concerned.

Effects of abdominal vagotomy (level C)

The qualitative changes in the individual affected neurons of the dorsal motor nucleus following abdominal vagotomy, as well as their time sequence, are similar to those following cervical vagotomy. When the branches on both sides are interrupted, large parts of both nuclei are affected (Figs. 5, 6); about one-third of the cells are spared and the distribution of these residual cells retains the pattern of cell

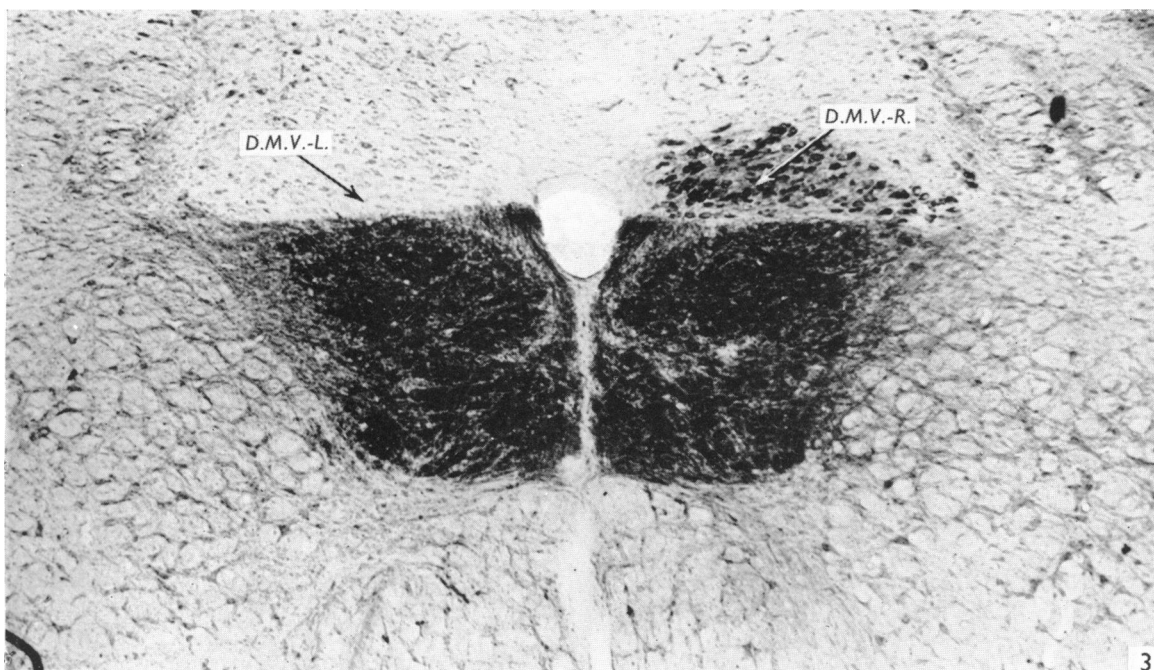


Fig. 3. Section through the hind brain showing AChE activity 2 weeks after left cervical vagotomy. The dorsal motor nucleus on the left side (*D.M.V.-L.*) is depleted of enzyme, whereas its counterpart on the right side (*D.M.V.-R.*) is stained as in normal material. $\times 55$.

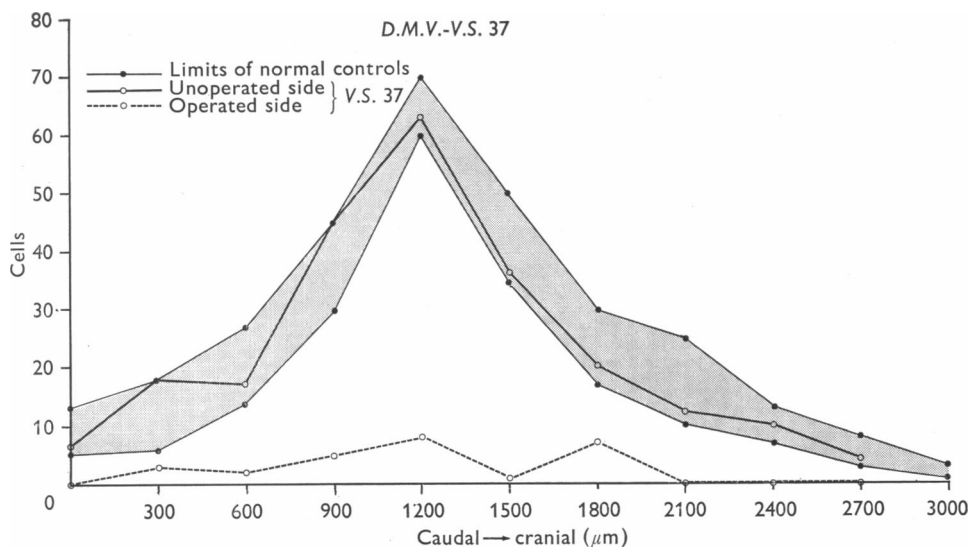


Fig. 4. Crano-caudal distribution of AChE-containing cells in the dorsal motor nuclei of the vagus, 2 weeks after unilateral cervical vagotomy. AChE-containing cells in the ipsilateral nucleus are almost completely absent whereas on the contralateral side their numbers are similar to those in control animals. The limits of distribution at corresponding levels in the four control animals (represented in Fig. 2) are indicated here by the stippled zone.

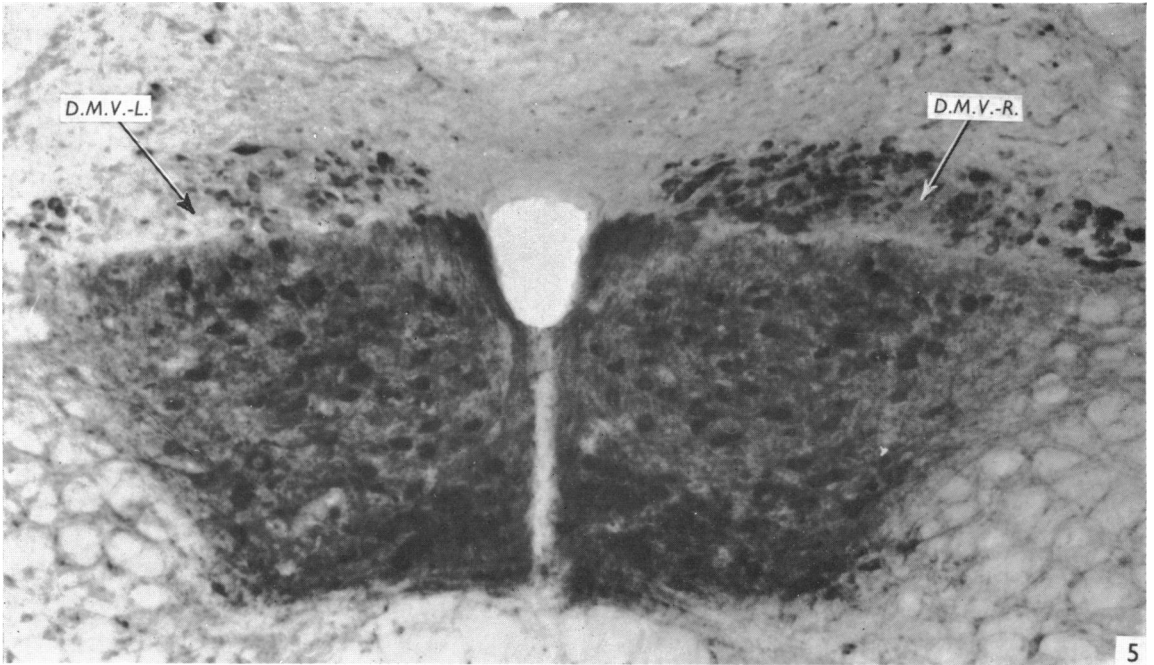


Fig. 5. Section through the hind brain showing AChE activity 2 weeks after bilateral abdominal vagotomy. The dorsal motor nuclei on both sides are partially depleted of enzyme but that on the left side (*D.M.V.-L.*) is more widely affected than its counterpart (*D.M.V.-R.*). $\times 70$.

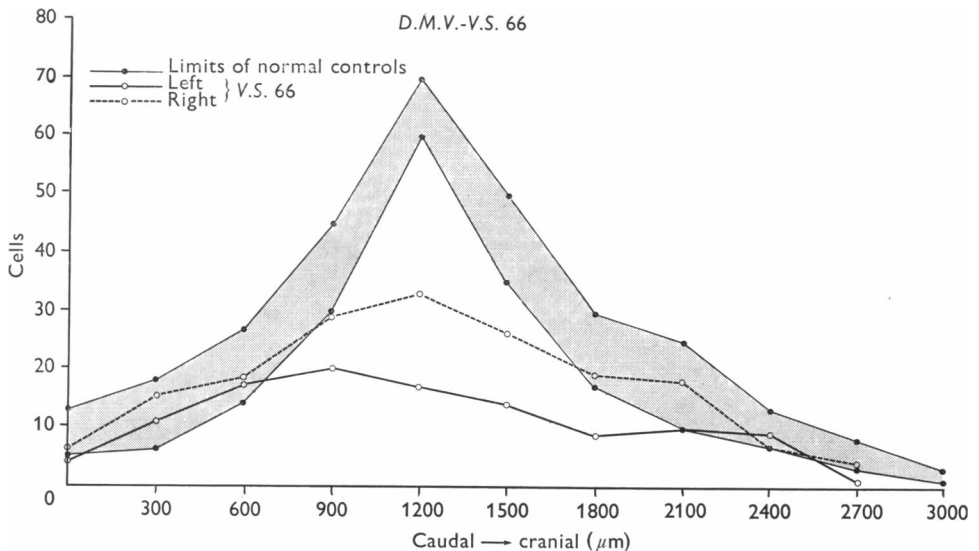


Fig. 6. Distribution of AChE-containing cells in the dorsal motor nuclei of an animal subjected to bilateral abdominal vagotomy, compared to the normal distribution (stippled zone). The nuclei on both sides are partially depleted of enzyme but that on the left side is more thoroughly affected.

distribution of the normal nucleus, though at a lower density. In other words, all parts of the nucleus are affected in proportion to their initial cell population. A consistent feature, however, is that the nucleus on the left side is affected more than its counterpart on the right side. This observation is supported by the results of sectioning a fraction of the abdominal vagal branches. Irrespective of whether

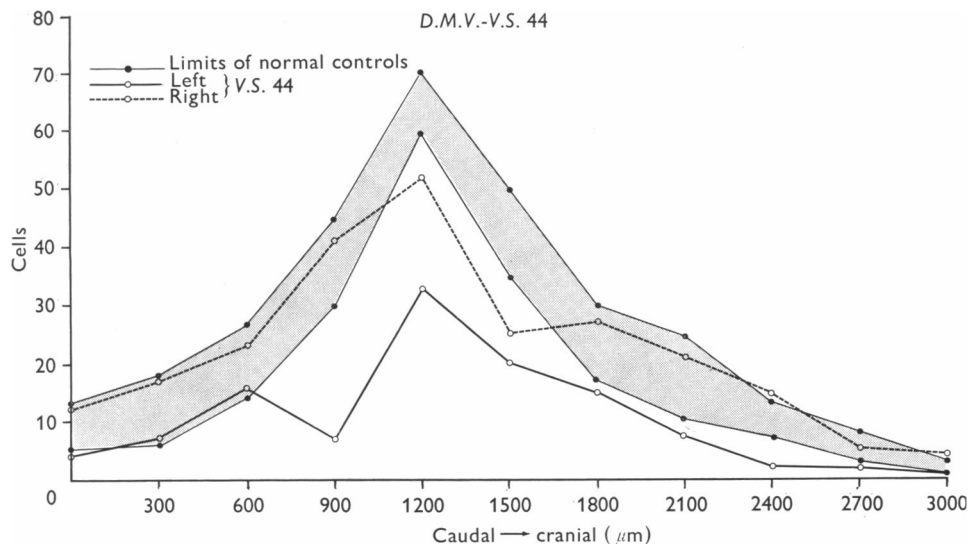


Fig. 7. Distribution of AChE-containing cells in the dorsal motor nuclei, after left abdominal vagotomy. The nucleus on the left side is more widely affected than its fellow.

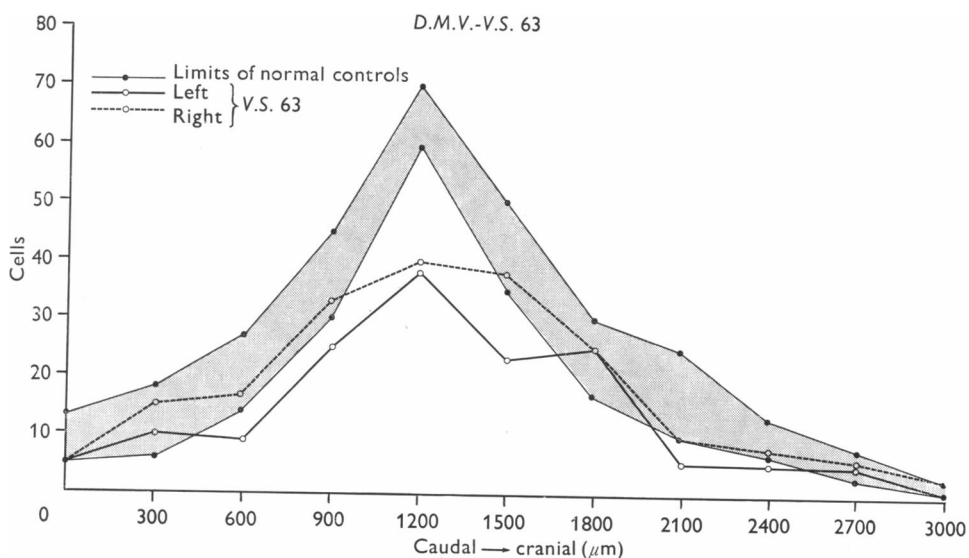


Fig. 8. Distribution of AChE-containing cells in the dorsal motor nuclei, after right abdominal vagotomy. The nucleus on the left side shows more widespread effects than its counterpart.

branches on the left side of the oesophagus or those on its right side are sectioned, the subsequent changes are more widespread in the nucleus on the left side (Figs. 7, 8).

Nucleus ambiguus

Normal control

Unlike the dorsal motor nucleus of the vagus, the nucleus ambiguus lies fairly close to the ventral surface of the medulla oblongata (Fig. 9). It is approximately 2 mm long and the rostral millimetre or so is substantially thicker than the caudal end which overlaps the rostral pole of the dorsal motor nucleus. The nucleus ambiguus

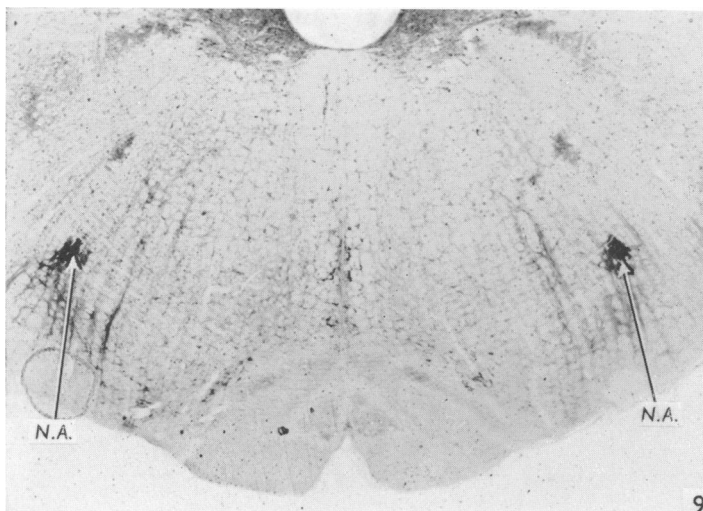


Fig. 9. Section through the normal hind brain showing that the nucleus ambiguus (N.A.) also stains very intensely for AChE. $\times 33$.

stains very heavily for cholinesterase, but unlike the dorsal nucleus, the enzyme in the majority of cells comprises AChE alone; the AChE is principally situated within the cytoplasm of the neuronal somata but the neuropil is also substantially stained. The neurons are irregular stellate-shaped cells of moderate size (approximately 30–40 μm in diameter). Their distribution in control animals is represented in Fig. 10 which indicates that, as in the dorsal motor nucleus, the nucleus ambiguus on the two sides of the same specimen are practically identical in composition and, to a lesser extent, that the distributions in different animals also are comparable.

In the tail of the nucleus ambiguus there is a group of 10–20 cells containing ChE alone. These cells are noticeably smaller than the AChE-containing cells above them.

Effects of cervical vagotomy

Section of the cervical part of the vagus results in loss of AChE activity in a substantial part of the ipsilateral nucleus ambiguus, particularly in its rostral pole (Fig. 11) where over two-thirds of the cells are situated. The narrow caudal part of

the nucleus, including the small group of ChE-containing cells, is unaffected and is stained as intensely as in normal material. As in the dorsal motor nucleus, there is no appreciable difference between the affects of high cervical vagotomy and those of vagotomy in the lower part of the neck.

The contralateral nucleus, as far as can be judged, is unaffected by cervical vagotomy at either level.

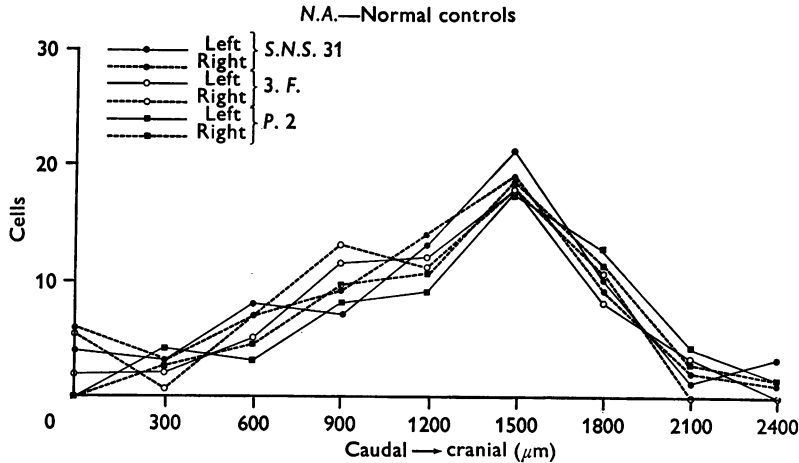


Fig. 10. Crano-caudal distribution of AChE-containing cells in the nuclei ambiguus in three control animals. The cell-composition shows a similar pattern in each of the nuclei.

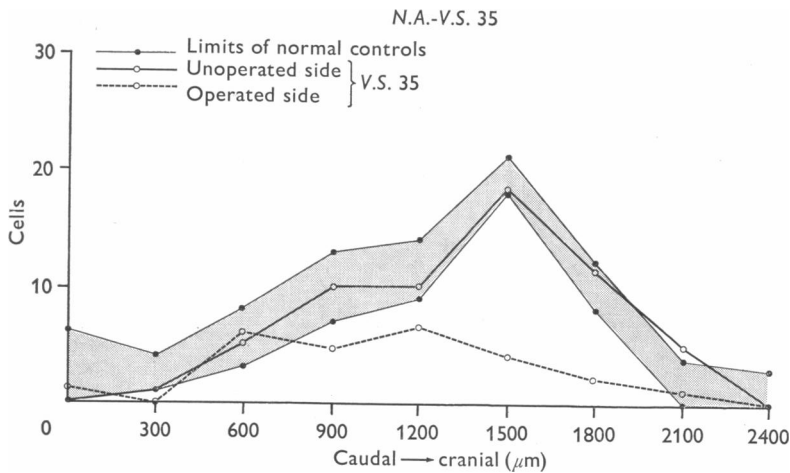


Fig. 11. The distribution of AChE-containing cells in the nuclei ambiguus, after unilateral cervical vagotomy. The ipsilateral nucleus, particularly at its cranial pole, is depleted of enzyme. On the contralateral side, the number of AChE-containing cells is not less than normal. The limits of distribution in the three control animals represented in Fig. 10 are indicated here by the stippled zone.

Effects of abdominal vagotomy

Abdominal vagotomy does not appear to have any effect on the nucleus ambiguus. In all the specimens subjected to this operation, the distribution of cholinesterase-retaining cells lies within the limits of distribution in normal animals. Nor are there changes in any other nucleus, apart from those in the dorsal nucleus of the vagus which have been already described.

DISCUSSION

Although the dorsal motor nuclei on the two sides are almost identical in form and cellular composition, the evidence suggests that there is a lack of symmetry in the peripheral distribution of the nerve fibres arising from them. Before the vagal trunks reach the abdomen, undoubted branching and rejoining has taken place in the thoracic oesophageal plexus, with the result that each of the abdominal branches contains fibres from both right and left vagi. However, section of these abdominal branches, in whatever combination one chooses, affects the left dorsal motor nucleus more widely than its counterpart of the opposite side. Hence, unless a vagal twig containing many more right than left vagal fibres has been inexplicably and consistently missed at operation, it appears that the nucleus of the left side dominates its fellow in the innervation of abdominal viscera. As a corollary, it is likely that the dorsal motor nucleus on the right side is the dominant nucleus in the distribution to thoracic viscera. Such a lack of symmetry of representation, of course, contrasts with the localization in other paired nuclei. However, the dorsal motor nuclei of the vagus are exceptional among cranial nerve nuclei in that they supply unpaired structures such as the heart and the gut, the topographical dispositions of which are anything but symmetrical; indeed, the only paired structures of any magnitude that they supply are the lungs. If this feature is conceded, perhaps it is not so curious to find that the heart is principally innervated by the right vagus and that the stomach and other abdominal viscera owe their supply more to the left vagus than to the right.

Most of the previous investigators tacitly assumed that there is symmetry in the peripheral distribution of fibres arising from the dorsal motor nuclei of the two sides and they concerned themselves with other aspects of localization in the nuclei. Nevertheless, Mitchell & Warwick (1955) do record a single instance 'where the maximal changes apparently occurred in the nucleus on the side opposite to the one operated upon'. Our observations on the effects of right abdominal vagotomy could be expressed in remarkably similar terms. However, Mitchell & Warwick, who dismissed their observation as a possible error in recording, have not specified the site at which that particular vagotomy was performed, so that it cannot be regarded as corroborative evidence for our findings.

Apart from the asymmetry of representation in the nuclei of the two sides there is no evidence of further localization within the dorsal motor nuclei of the vagus. After 'bilateral' abdominal vagotomy, 60–80% of the combined cell population of the nuclei are depleted of enzyme but the affected cells are dispersed throughout the rostro-caudal extent of each nucleus. The remaining 20–40% of the cells, which are

presumably concerned with the innervation of thoracic viscera, are similarly dispersed.

Szentágothai's (1952) suggestion that the thoracic and abdominal viscera owe their innervation more to the nucleus ambiguus than to the dorsal motor nucleus of the vagus has recently received some support from the studies of Kerr (1969), who concludes that the latter nucleus supplies only secretomotor fibres to the viscera whereas the nucleus ambiguus comprises the source of innervation of their musculature. In the present investigation we could find no evidence of any visceral supply from the nucleus ambiguus. Certainly this nucleus plays no part in the innervation of abdominal viscera as it is completely unaffected by abdominal vagotomy. Moreover, the effects of cervical vagotomy on the corresponding nucleus ambiguus are practically identical with those we have observed in two animals in which the inferior laryngeal nerve alone had been interrupted, leaving little which is attributable to distribution in the trunk. Perhaps it is conceivable that the discrepancy between the results reported here and those of Szentágothai and of Kerr are caused by species differences for both the latter investigators worked on the cat. However, such a fundamental species difference would be very curious indeed and the possibility that their lesions had spared parts of the dorsal motor nucleus should also be considered.

SUMMARY

1. The cholinesterase-containing cells in the dorsal motor nucleus of the vagus and in the nucleus ambiguus have been studied histochemically in the rat by means of a thiocholine technique. The cells retaining their enzyme content after cervical or abdominal vagotomy have been counted and their distributions represented graphically.

2. After section of abdominal vagal branches, in whichever combination one chooses, the dorsal motor nucleus on the left side is more widely affected than on the right, suggesting that it is the dominant nucleus in the innervation of abdominal viscera. The affected cells in both nuclei are scattered throughout their rostro-caudal extents.

3. The nucleus ambiguus is unaffected by abdominal vagotomy.

4. After unilateral cervical vagotomy, practically all the cells in the ipsilateral dorsal motor nucleus are depleted of enzyme; it does not appear to make much difference whether the nerve is interrupted high or low in the neck. In the nucleus ambiguus a substantial proportion of cells in the cranial part is affected, an effect similar to that following section of the inferior laryngeal nerve.

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