

INNERVATION OF THE INTRINSIC MUSCLES OF THE CAT'S
LARYNX BY THE RECURRENT LARYNGEAL NERVE:
A UNIMODAL NERVE

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In the course of a quantitative study of the vagus nerve of the cat (Agostoni, Chinnock, Daly & Murray, 1957), the number, size and function of the fibres contained within the recurrent laryngeal nerve were investigated. The chief interest in this nerve is that it is one of the most characteristic of the 'unimodal' nerves.

The majority of nerves supplying limb muscles are 'bimodal', having a considerable proportion of their fibres below 8μ and above 14μ in diameter: there are relatively few fibres about $10-12\mu$ in diameter (Eccles & Sherrington, 1930), and both the large and the small fibre groups contain afferent as well as efferent fibres. In contrast, the present investigation shows that the branch of the recurrent laryngeal nerve which enters the larynx to innervate the intrinsic laryngeal muscles of the cat has a unimodal fibre-size distribution, with a fairly sharp peak about $10-12\mu$ and relatively few fibres of much smaller or larger diameter. Furthermore, there are few or no sensory fibres in this branch. The evidence suggests that muscles that are supplied by nerve fibres of intermediate size, unimodally distributed, also have little or no proprioceptor supply.

METHODS

The specimens of the recurrent laryngeal nerves mentioned in the present investigation were obtained from the same animals as were used in the previous article on the fibre composition of the cat vagus (Agostoni *et al.* 1957). Identical numbering of animals in the two articles permits of direct comparisons of numbers and sizes of fibres in recurrent laryngeal nerves with those in the vagus trunks from which they have branched.

Three series of experiments involved division of the vagus nerve extracranially above the nodose ganglion, intracranially above the jugular ganglion and caudal to the nodose ganglion. After sufficient time for degeneration of nerve fibres, portions of the recurrent laryngeal nerves were

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obtained from the normal and operated sides at two levels, at the level of the clavicle as the nerve ascends between the trachea and oesophagus, and 2 cm proximal to entry into the larynx to supply the intrinsic muscles.

For study of myelinated fibres the nerves were fixed in Flemming's fluid and stained by a modified Weigert Technique (Gutmann & Sanders, 1943). In staining all axons, both myelinated and non-myelinated, the pyridine-silver method was used (Ranson & Davenport, 1931). For examination of movements of the vocal cords on stimulation of the ipsilateral normal and treated cervical vagus nerves, the thyro-hyoid membrane was incised and direct observations made. Details of the above methods have already been given at length in a previous article (Agostoni *et al.* 1957).

RESULTS

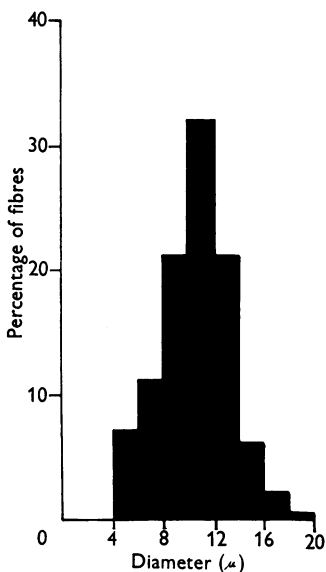
The recurrent laryngeal nerve at the level of the clavicle contains two groups of fibres occupying sharply defined areas (Pl. 1, fig. 1). One area contains large, heavily myelinated fibres and the other small, thinly myelinated fibres. As the nerve ascends between the oesophagus and trachea almost all the small fibres are distributed in tracheal, oesophageal and other branches. Consequently the residual part of the nerve entering the larynx consists almost entirely of large fibres (Pl. 1, figs. 2, 6). This latter part of the nerve will be referred to as the laryngeal bundle of the recurrent laryngeal nerve. In five normal laryngeal bundles measurement of fibre diameter gave a sharply unimodal size-frequency distribution with a close grouping of fibre-size around a peak at about $10\text{--}12\mu$ in diameter (Table 1, and Text-fig. 1). There are about 450 myelinated fibres in this bundle (mean 462, s.d. of mean ± 17). The number of small myelinated fibres passing from the vagus into the recurrent laryngeal nerve is variable. At the level of the clavicle there are about 400–600 fibres (mean 566, s.d. of mean ± 33) and these also have a unimodal size-frequency distribution with a mode about $4\text{--}6\mu$ (Table 1). The great majority of these fibres are less than 8μ in diameter.

The recurrent laryngeal nerve contains relatively few non-myelinated fibres. Counts of non-myelinated fibres in the laryngeal bundles of the five normal specimens given in Table 1 were 67, 81, 48, 56 and 63 respectively. After division of the cervical vagus nerve and a suitable delay for degeneration of nerve fibres (27, 20 and 22 days respectively) there remained in specimens 11G, 15G and 16H of the laryngeal bundle 71, 54 and 65 non-myelinated fibres. Such findings suggest that the majority of non-myelinated fibres in the laryngeal bundle are extra-vagal in origin. Presumably many are post-ganglionic sympathetic fibres (Evans & Murray, 1954).

Interruption of the vagus nerve intracranially or extracranially above the nodose ganglion regularly produces clear-cut changes in the recurrent laryngeal nerve (Pl. 1, fig. 3). Practically all the large myelinated fibres in the laryngeal bundle degenerate, indicating that they are efferent (Evans & Murray, 1954).

However, a small number of myelinated fibres invariably survives. Counts of myelinated fibres remaining in laryngeal bundles in specimens 24D, 29C,

32C and 34C (15, 21, 19 and 28 days after supranodose vagotomy) were 42, 31, 32 and 51 respectively. In the two specimens 39C and 40C in which intracranial vagotomy had been performed 14 and 24 days previously there were 41 and 37 myelinated fibres remaining. In all six specimens, the majority of fibres were less than 6μ in diameter but in all cases there were a few larger, heavily myelinated fibres ranging from 8 to 12μ in diameter (respective counts 12, 11, 6, 14, 17 and 10). On the other hand, there was no histological evidence of degeneration of the small fibre group at the level of the clavicle after supranodose vagotomy. In specimens 24C, 29D and 32D there remained 451, 726 and



Text-fig. 1. Distribution of fibre sizes in the laryngeal bundle of a normal recurrent laryngeal nerve.

TABLE 1. Size-frequency distribution of myelinated fibres in: (A) the laryngeal bundle of the normal recurrent laryngeal nerve near its entry into the larynx, and (B) the small fibre (sensory) bundle of the recurrent laryngeal nerve at the level of the clavicle

| Animal and specimen | Side | No. of fibres in diameter groups of 2μ | | | | | | | | | | Total |
|---------------------|------|--|-----|-----|-----|------|-------|-------|-------|-------|-------|-------|
| | | 0-2 | 2-4 | 4-6 | 6-8 | 8-10 | 10-12 | 12-14 | 14-16 | 16-18 | 18-20 | |
| A | | | | | | | | | | | | |
| 12C | L | 0 | 0 | 15 | 64 | 121 | 104 | 78 | 14 | 6 | 1 | 403 |
| 12H | R | 0 | 0 | 45 | 46 | 125 | 137 | 97 | 31 | 24 | 1 | 506 |
| 12D | L | 0 | 0 | 31 | 52 | 96 | 149 | 98 | 27 | 8 | 1 | 462 |
| 21B | L | 0 | 0 | 27 | 44 | 134 | 145 | 83 | 29 | 5 | 0 | 467 |
| 39H | R | 0 | 0 | 48 | 34 | 112 | 147 | 89 | 21 | 22 | 0 | 473 |
| B | | | | | | | | | | | | |
| 12H | R | 51 | 192 | 288 | 72 | 5 | 6 | 0 | 0 | 0 | 0 | 614 |
| 19D | L | 38 | 112 | 261 | 75 | 5 | 7 | 0 | 0 | 0 | 0 | 498 |
| 39H | R | 41 | 115 | 314 | 61 | 7 | 7 | 0 | 0 | 0 | 0 | 585 |

657 small myelinated fibres at the level of the clavicle. Probably all or almost all the fibres are afferent in nature.

Direct inspection was carried out of movements of the vocal cords on electrical stimulation of the ipsilateral normal and treated cervical vagus nerves. In no case after division of the vagus nerve above the nodose ganglion was movement observed, while it always occurred on the control side. After division of the cervical vagus nerve caudal to the nodose ganglion (Pl. 1, fig. 4), there remained 27, 20 and 22 days afterwards in specimens 11 F, 15 F and 16 G of the laryngeal bundle 30, 32 and 26 myelinated fibres respectively. Of these fibres 10, 16 and 11 were large heavily myelinated fibres between 8–12 μ in diameter and the remainder were small, below 6 μ in diameter. However, after division of the superior laryngeal nerve, as well as the cervical vagus on the same side, there remained 27 and 28 days afterwards in specimens 26 C and 27 C of the laryngeal bundle 10 and 4 small myelinated fibres all below 6 μ in diameter (Pl. 1, fig. 5). Thus there are myelinated fibres in the laryngeal bundle of the recurrent laryngeal nerve which are of extravagal origin, passing up in the superior laryngeal nerve and having their cell bodies in the nodose ganglion. All the large myelinated fibres between 8–12 μ are of this character and some of the small fibres are of similar origin.

DISCUSSION

The fibre content of the recurrent laryngeal nerve in the cat is similar to that in the rabbit (Evans & Murray, 1954). The nerves contain relatively few fibres above 14 μ and below 6 μ in diameter and have a sharp peak of their size-frequency distribution about 10 μ . However, the number of fibres supplying the intrinsic muscles in the larynx of the cat (about 450) is larger than in the rabbit (less than 300). The proportion of large myelinated fibres, above 12 μ in diameter, is also greater in the cat than in the rabbit.

A nervous pathway has been described by which, in the rat, nerve impulses originating in slowly adapting stretch-sensitive endings in the wall of the aorta pass through the recurrent laryngeal nerve, the communicating branch and the superior laryngeal nerve into the vagus (Andrew, 1954). It may be that the small number of myelinated fibres which in the cat pass up via the recurrent laryngeal and superior laryngeal nerves and have their cell bodies in the nodose ganglion are of this nature. Direct evidence on the function of these medium-sized myelinated fibres (8–12 μ) and small myelinated fibres (less than 6 μ) and of the non-myelinated fibres is required.

A unimodal distribution has been found by Häggqvist (1940) in the facial, hypoglossal and sphincter ani nerves, in the abducent by Björkmann & Wohlfart (1936), Häggqvist (1938) and Swensson (1949) and in the accessory nerve by Bergstrand (1939). Rexed (1944) found a unimodal distribution in the phrenic nerve of the rabbit. Fernand & Young (1951), in the rabbit, showed that these

nerves supplied various muscles in the head and neck region including those to the infrahyoid region, e.g. sternothyroid and thyrohyoid and the superficial muscles of the face. They pointed out that the muscles supplied by 'unimodal' nerves show certain common characteristics. Their actions, although no doubt integrated with those of other parts of the body, do not depend to the same extent as do limb muscles on the close and varying integration with the effects of other muscles and of gravity.

It remains to explain why the fibre distribution in 'unimodal' nerves forms such a sharply circumscribed single peak. W. Hayhow and J. G. Murray (unpublished) were unable to demonstrate histologically any spindles in the intrinsic muscles of the larynx of the cat. This is in agreement with the findings of Sherrington (1897). Under favourable conditions, Andrew (1955) could find no functional evidence for proprioceptors in one of the intrinsic muscles, the cricothyroid, in the rat, when recordings were made from the peripheral end of the external laryngeal nerve. The absence of annulo-spiral fibres would explain the absence of large afferent fibres from the recurrent laryngeal nerve. The motor fibres to the intrafusal muscle fibres are small (Ruffini, 1898; Hinsey, 1927; Hines & Tower, 1928; Matthews, 1931; Katz, 1949; Kuffler & Hunt, 1949) and their absence would agree with the absence of small efferent fibres in the nerve. Thus the myelinated fibres in the recurrent laryngeal nerve would consist almost entirely of a single functional group—somatic extrafusal efferent. There is direct evidence for the absence of afferent fibres in the recurrent laryngeal nerve supplying the intrinsic muscles of the larynx. Evans & Murray (1954) found that following division of the vagus central to the nodose or jugular ganglia all the fibres in the recurrent larynx nerve to the larynx in the rabbit degenerated. Similarly, in the present investigation in the cat, there are very few or no afferent fibres in this nerve from the intrinsic muscles of the larynx.

Unfortunately there is little quantitative work on the extent of proprioceptive innervation in other 'unimodal' nerves. Baum (1900) failed to find spindles by histological methods in the infrahyoid muscles of the rabbit. Nevertheless, Andrew (1955) demonstrated a few spindles in the sternothyroid muscle of the rat. He pointed out that there were at least three sources of sensory inflow from the region of the larynx—proprioceptors, from the sternothyroid muscle, the thyrohyoid muscle and the thyroepiglottic joint. In spite of the sensory inflow, it was clear that this did not have a dominating influence on the motor neurones, e.g. those to the sternothyroid muscle. The indications were that the equilibrium of the larynx was determined centrally, by the respiratory centre, with little reference to sensory feed-back. The evidence supports the suggestion of Fernand & Young (1951) that muscles that are supplied by fibres of intermediate size, unimodally distributed, also have scanty or no proprioceptor supply.

SUMMARY

1. The recurrent laryngeal nerve supplying the intrinsic muscles of the larynx in the cat has a unimodal fibre-size distribution with a fairly sharp peak about 10–12 μ and relatively few fibres of much smaller or larger diameter.
2. There are few or no proprioceptor fibres in this nerve.
3. The evidence suggests that muscles that are supplied by nerve fibres of intermediate size, unimodally distributed, also have little or no proprioceptor supply.
4. In the recurrent laryngeal nerve there are a variable number of fibres of extravagal origin. A small number of myelinated fibres may provide a laryngeal pathway for aortic baroreceptor impulses.

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EXPLANATION OF PLATE

All figures are from transverse sections of recurrent laryngeal nerves of the cat and all are stained by the Weigert method.

PLATE 1

- Fig. 1. Normal recurrent laryngeal nerve at level of clavicle. The large and the small myelinated fibres occupy relatively discrete segments of the nerve.
- Fig. 2. Normal recurrent laryngeal nerve near its entry into larynx. Only the large myelinated fibres pass to larynx, the great majority of the small fibres having been distributed in branches to the trachea, oesophagus, and pharynx.
- Fig. 3. Recurrent laryngeal nerve at level of clavicle 28 days after supranodose division. The large myelinated fibres in the laryngeal portion of the nerve have degenerated. The great majority of the small myelinated fibres (*m.f.*) remain intact.
- Fig. 4. Recurrent laryngeal nerve near larynx 27 days after division of the vagus nerve caudal to the nodose ganglion. A small number of medium and small calibre myelinated fibres (*m.f.*) remain intact. They are extravagal in origin.
- Fig. 5. Recurrent laryngeal nerve near larynx 28 days after division of the vagus nerve caudal to the nodose ganglion and of the superior laryngeal nerve. At this magnification, nothing but degenerating fibres can be seen. At a higher magnification, only four small myelinated fibres remain intact, all below 6μ in diameter.
- Fig. 6. A segment of the large myelinated fibres in the laryngeal bundle of a normal recurrent laryngeal nerve at a higher magnification. Although there is a variation in calibre of the fibres, the size-frequency distribution is unimodal.

